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Diagnosis and New Treatments in Muscular Dystrophies

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DIAGNOSIS AND NEW TREATMENTS IN MUSCULAR DYSTROPHIES

Summary

Duchenne muscular dystrophy (DMD), Becker muscular dystrophy (BMD), and limb girdle muscular dystrophies (LGMD) represent a significant proportion of paediatric and adult neuromuscular neurology practice. The proactive symptom based multidisciplinary team (MDT) management and access to non-invasive ventilation has enabled improved survival into adulthood. Nevertheless the severe disability imposed by conditions such as DMD, poses a challenge for successful transition of care and management for paediatric and adult neurology teams.

DMD is discussed in detail as a paradigm illustrating diagnosis, management and role for different pharmacological interventions to improve survival, but also challenges in adulthood care, and cutting edge therapies.

LGMDs are much rarer than DMD and BMD and in addition there is a significant genetic and clinical heterogeneity, which leads to diagnostic difficulties. The clinical and laboratory diagnostic features of seven LGMD subtypes are summarised and their allelic “non-limb girdle” phenotypes are tabulated to illustrate the theme of one gene causing multiple clinical phenotypes, with the aim of refining the clinician’s diagnostic approach. The lessons learnt from DMD MDT management to improve survival are broadly applicable to LGMDs with severe motor disability / multisystem complications.

Introduction

The muscular dystrophies are an inherited group of disorders characterised by muscle wasting and weakness, and sharing common histological features of “dystrophic” muscle biopsy changes, including variation in muscle fibre size, muscle fibre degeneration and regeneration, and replacement of muscle by connective tissue and fat. The clinical and genetic heterogeneity of these conditions is well recognised: some have prenatal onset while others only affect adults, some are rapidly progressive, while others are associated with prolonged periods of stability; some are associated with multisystem involvement including cardiac and central nervous system. The major

advances over the last two decades have improved diagnostic precision, focused symptomatic management and are increasingly leading to development of cutting edge therapies.

This review article focuses on Duchenne muscular dystrophy (DMD), Becker muscular dystrophy (BMD), and limb girdle muscular dystrophies (LGMD), emphasising diagnosis and new treatments from the clinician's view point, and in particular, the presentations and complications in the adolescent, and early adulthood.

DMD is discussed in detail as a paradigm illustrating diagnosis, the impact of presently available symptomatic managements on survival, challenges in adolescent and young adulthood care, and genetic based therapeutic interventions.

Duchenne muscular dystrophy (DMD)

Introduction

DMD is the most frequent muscular dystrophy variant, affecting 1 in every 3,500 live male births. Common presentation is with abnormal gait, calf hypertrophy and difficulty in rising from the floor between 2 to 5 years of age; speech / global developmental delay may dominate the presentation in a minority of cases.^{1 2} Untreated, progression of muscle weakness leads to complete wheelchair dependence by the thirteenth birthday, followed by scoliosis, respiratory insufficiency, cardiomyopathy and death in the late teens or early twenties. Feeding difficulties and weight loss are common in the late stages of the disease.

Though a curative treatment is not yet available, the multidisciplinary team (MDT) proactive approach³ for optimal surveillance and management of multisystem complications together with pharmacological intervention with corticosteroids has altered the natural history of DMD, so that most of these

individuals can now be anticipated to survive into adulthood, with transfer of care to the adult neurologists.

Genetics and Pathophysiology

A brief review of the molecular genetic basis of DMD is essential to understand the approach to diagnosis and limitations of the various diagnostic techniques. DMD is caused by mutations in the Dystrophin gene at Xp21. The dystrophin locus contains 85 exons and encodes for a large but low abundance protein, named dystrophin.⁴ Dystrophin is a rod shaped protein, which localises at the cytoplasmic side of the sarcolemma: the carboxyl terminal end binds to the dystrophin associated glycoprotein complex at the sarcolemma while the amino terminal end binds to the cytoskeletal actin. Dystrophin is postulated to be essential for protection of the sarcolemma from the stress of repeated contractions by providing an indirect link between the subsarcolemmal cytoskeletal actin and the intermediate filaments in the muscle fibre with the extracellular matrix. Other functions in the control of signalling molecules such as neuronal nitric oxide synthase, and intracytoplasmic calcium homeostasis are also postulated. The mutations in the dystrophin gene, which result in DMD, cause disruption of the reading frame, resulting in a severe reduction or absence of dystrophin in the skeletal and cardiac muscle. This in turn leads to increased intracytoplasmic calcium content, followed by muscle fibre damage and degeneration. Several dystrophin isoforms are also expressed in brain and their deficiency in this tissue is responsible for the mental retardation, which complicates the course of DMD in approximately 1/3 of cases.

Approximately 65% of patients with DMD have intragenic out-of-frame deletions and approximately another 10% have duplications of one or more exons of the dystrophin gene. The remaining patients have point mutations or other smaller gene rearrangements (pure intronic deletions, insertions of repetitive sequences, splice site mutations). Typically, out-of-frame dystrophin gene mutations lead to a severe reduction or absence of dystrophin in the muscle resulting in DMD phenotype, whereas in-frame mutations lead to the expression of a partly functional truncated protein, resulting in the milder

Becker muscular dystrophy (BMD). The frame shift hypothesis holds true for over 90% of cases and is commonly used both for diagnosis and for differentiating between DMD and BMD. There are however exceptions to the frame shift rule; in-frame mutations in the gene coding for the crucial domains of dystrophin protein (actin and dystroglycan binding domains) may cause a DMD phenotype, whereas some out of frame mutations are associated with BMD, due to different mechanisms such as compensation by exon skipping or reinitiation codons located in the 5' end of the gene.

DMD is a X-linked recessive condition, but there is a high incidence of new mutations and at least 1/3 of the cases do not have a positive family history.

Diagnosis

The characteristic phenotype of a boy under five with calf hypertrophy, toe / waddling gait and Gowers' sign lends to easy recognition. Global developmental delay is the other frequent early presentation.²

Serum Creatine Kinase (CK) is massively elevated (10-100x normal, since birth) and should be the first investigation when DMD is suspected.

Electromyography (EMG) has no role in the investigation of DMD and should not be requested.

The advances in molecular genetic testing over the last decade have vastly improved mutation detection in the dystrophin gene. As most DMD patients carry deletions in two mutational hot spots of the gene, the screening of only 19 exons, following amplification of genomic DNA, was the mainstay of the diagnosis until recently as it allowed to identify mutations in ~ 65% of cases.⁵ The limitations of this technique include the inability to identify rarer mutations and the breakpoints of some deletions. Other genomic DNA based techniques such as multiplex ligation dependent probe amplification⁶ method, or a combinatorial strategy using the fluorescent multiplex quantitative PCR followed by conformation sensitive capillary electrophoresis (CSCE),⁷ have increased the efficiency of mutation detection close to 100%. Another major advantage of these techniques is their ability to detect mutations in carrier females, allowing a precise genetic counselling.

The molecular genetic documentation of a dystrophin mutation alone allows to predict accurately the phenotype in ~ 90% of cases, while exceptions to the frame shift hypothesis account for 10% of cases. Establishing the precise diagnosis of DMD, especially in young and not fully collaborative children, is therefore best achieved by a combination of clinical observation of the patient's strength and functional abilities, ascertainment of dystrophin levels on muscle biopsy and knowledge of the gene mutation.

A partial dystrophin deficiency is the hallmark of BMD; it is important to recognise that secondary dystrophin protein reduction may occur in LGMDs due to mutations in sarcoglycan genes or, more rarely, in genes responsible for α -dystroglycanopathies.⁸ Western blot analysis is helpful in such cases as the identification of a truncated protein product clearly points towards the primary gene defect, as in BMD.

Management

Physiotherapy and orthoses

Physiotherapy to prevent joint deformities and promote walking remains important⁹ and these principles are applicable to other LGMDs as well. In DMD, rehabilitation in knee ankle foot orthoses (KAFOs) at the time of loss of independent walking is effective in prolonging walking for an average of 18 months to two years. This has been associated with reduced incidence of scoliosis.¹⁰

Glucocorticoid corticosteroids

A recent Cochrane systematic review of the glucocorticoids in DMD is available.¹¹ In randomised controlled trials (RCT), prednisone stabilised strength and function for 6 months to 2 years. Non-randomised studies with daily dose Prednisone (0.75mg/kg) or deflazacort (0.9 mg/kg), have documented prolongation of walking ability, preservation of respiratory function and reduction in the incidence of scoliosis and cardiomyopathy¹² in

Duchenne boys who tolerated long-term daily dose corticosteroids. Not surprisingly, the daily glucocorticosteroid therapy has significant side effects, notably, weight gain in the short-term, and vertebral fractures in approximately a third of the long-term treated patients.¹³ Alternative regimens have been proposed, with the hope to reduce the adverse effects associated with the daily steroid regimes: a six month RCT of Prednisone 0.75 mg/kg/day for the 1st 10 days of every month, demonstrated slowing of functional deterioration.¹⁴ An international RCT to compare daily dose prednisone and deflazacort with intermittent prednisone regime is under consideration. Corticosteroids should preferably be started in all early ambulant cases (4 - 6 years) but otherwise also in the older ambulant children, unless contraindicated. Treatment requires careful monitoring of benefit and management of adverse effects¹⁵. The optimal starting dose of prednisolone 0.75 mg/kg/day is often not tolerated in the long term and, over the course of years, careful dose adjustment is required. Protocols for monitoring, dosing and adverse effects management are available (www.muscular-dystrophy.org/research/northstar/). It is important to promote bone health in corticosteroid treated patients with dietary advice regarding calcium and vitamin D, and supplementation if plasma Vitamin D levels are low.¹⁶ There is currently no evidence that oral bisphosphonates should be used prophylactically in children receiving steroids; however their acute administration is recommended in the treatment of vertebral fractures, where they are very effective.

Management of respiratory complications

Decreasing respiratory reserve in the teenage years leads to sleep disordered breathing, with REM sleep related hypoxemic dips¹⁷ and obstructive apnoeas. The resulting symptoms include morning drowsiness, poor appetite, headaches, nausea, fatigue, tiredness, poor concentration at school, failure to thrive, reduced coughing ability or overt respiratory failure in the course of “minor” respiratory infections.

In the past, the onset of symptomatic sleep hypoventilation signified imminent demise, as the only way to prolong life was mechanical ventilation through

tracheostomy. From its initial introduction into neuromuscular practice in the 1990s, domiciliary non-invasive ventilation (NIV) is now a well established technique and is effective in symptom relief and prolonging survival.¹⁸ The patient's breathing at night is augmented with breaths delivered by a compact, portable ventilator with a snugly fitting facial or nose mask. NIV corrects sleep hypoventilation and affords symptom relief without significant encroachment on living space or restriction of travel. NIV, and if needed, the use of cough assist devices, can extend average survival to mid-twenties¹⁹ and in some cases to the fourth decade. It is common view that denying NIV to hypercapnic DMD patients is unethical.²⁰

Forced vital capacity (FVC) predicts the development of hypercapnia and survival.²¹ Regular monitoring for symptoms of sleep hypoventilation, FVC, and overnight sleep studies when the FVC falls below 50% allow for timely intervention with NIV. Gradual initiation of NIV in the phase of nocturnal hypercapnia but daytime normocapnia,²² without waiting for daytime ventilatory failure, allows the individual to acclimatise to the ventilator and avoids uncontrolled decompensation during chest infections.

Management of cardiac complications

Dilated cardiomyopathy (DCM) occurs in up to 90% of DMD individuals ≥ 18 years. The severity varies and traditionally, cardiomyopathy was considered responsible for death in up to 20% of DMD individuals. This proportion is likely to increase over the coming years as NIV prevents respiratory related mortality. There is some controversy regarding optimal timing of introduction of therapy for DCM.²³ Duboc et al reported that early treatment with the Angiotensin converting enzyme (ACE) inhibitor perindopril delayed the onset and progression of prominent LV dysfunction, and was associated with lower mortality in DMD.²⁴ Some cardiologists suggest that treatment is not necessary for a complication that is often asymptomatic for a long time before deteriorating into clear-cut cardiac failure²³, though this view is at odds with the current evidence on related forms of DCM, in which early treatment is clearly superior compared to late therapy. Indeed considering the invariable

occurrence of DCM in DMD, intervention before progression to severe cardiomyopathy would be logical.

While awaiting the results of RCT, the published consensus documents²⁵ recommend the use of ACE inhibitors and beta blockers in patients with early cardiomyopathy. It is important to look for and treat co-existing nocturnal hypoventilation, which aggravates cardiac function.

Scoliosis management

Scoliosis usually develops in the phase of constant wheel chair dependence, shows rapid progression during pubertal growth spurt and adversely affects respiratory function, feeding, seating and comfort.

Progression of the spinal curve is the indication for surgical spinal fusion.²⁶ The optimum time for making the decision is when the range of the curve's Cobb angle is 20° – 40°. MDT input is essential to ensure that the operation is performed at a time when the FVC is above 30% predicted for height, and the cardiac function, as confirmed by echocardiogram, is good. Spinal surgery can be performed when the FVC is below 30%, but the risks are greater, and is best undertaken in specialized centres.²⁷

Spinal brace (jacket) does not prevent progression of scoliosis, but may be useful in postural management, especially in cases where spinal surgery is contraindicated or not acceptable to the patient.

Nutritional aspects

These include obesity during the late ambulant phase, especially in corticosteroid treated individuals, and severe wasting in the spinal surgery post-operative period and the late teenage years. Regular weight monitoring and dietary advice to avoid obesity should be offered to all DMD patients, especially when treated with daily corticosteroids.

Young adults with DMD may have chewing and swallowing difficulties,²⁸ prolonged mealtimes; episodes of choking on food contribute to fear of eating

and failure to thrive, although frank aspiration is very rare. Appropriate facilities for hoisting and weighing the wheel chair dependent adolescents should be available in the clinics to facilitate regular weight monitoring. Patients with failure to thrive and/or dysphagia benefit by dietetic and speech and language therapist's assessment for nutritional supplementation and advice about postural management, feeding aids or gastrostomy insertion.

Survival, new complications and transition of care

Eagle et al¹⁹ reported a mean survival of 27 years in UK DMD patients who were able to access NIV from 1990s onwards. In a Swiss cohort of 43 patients with DMD²⁹, 22 received long-term mechanical ventilation (17 with mask NIV, and 5 via tracheostomy), and Kohler et al²⁹ estimated the probability of survival to 30 years at 35%. Further prolongation of survival is anticipated as the currently corticosteroid treated cohort matures and accrues the long-term beneficial effects on respiratory function. This change in natural history of treated DMD means that most of these adolescents are now reaching adulthood, and will be under the adult neurologists' care for a number of years.

Maha et al³⁰ reported the parental experience of caring for 15 children with neuromuscular disorders, 3 of whom had DMD. The common parental experience was that of being the "lifeline" for their child's survival and quality of life. The parents wished for more support by health care professionals; their perception of insufficient support from local community agencies, and a shortage of respite facilities were identified.

In a cohort of 25 young adults with DMD, Parker et al³¹ reported that 8 had cognitive / developmental disability, whilst 6 of the 24 boys achieved college or university academic success. Two patients were seeking and one was able to sustain employment. Insufficient external support to the patient and his family, and striking difficulties with provision of practical aids for day to day life were reported. These data identify the challenges in provision of care, both for medical and social services, to maintain quality of the life in this prolonged survival group.

With improved survival, complications only rarely observed before are being reported. Shumyatcher et al³² reported symptomatic nephrolithiasis in 20% of their cohort of 29 DMD patients ≥ 20 years. Parker et al³¹ reported severe additional problems of constipation, dysphagia, gastroesophageal reflux and malnutrition in their adult DMD cohort. Gastric distension is also a rare late complication. These medical and social difficulties underline the need for development of robust protocols for transition of care to the adult medical teams, and in particular, for the drive to enable improvement in rehabilitation, employment, social participation and social services for the adult with DMD.

Genetic therapies for DMD

Research aimed at finding a genetic based treatment has been greatly facilitated by the use of two naturally occurring animal models; the dystrophic golden retriever dog (GRMD), which suffers a fatal clinical course akin to the humans and the *mdx* mouse which has a stop codon in exon 23 resulting in dystrophin deficiency but no overt weakness or reduced life span. The various genetic strategies aimed at restoration of dystrophin in the affected muscle³³ are listed with a basic description and their current status in table 1. These models have allowed two strategies to be firstly developed as proof of concept, and more recently to be experimented in human subjects, as discussed below.

Exon skipping with antisense oligonucleotides:

The strategy behind the use of antisense oligonucleotides (AOs, also known as “molecular patches”) is the modification of dystrophin mRNA splicing. These small RNA-like molecules prevent the normal splicing of the gene by masking crucial areas of the messenger RNA during the splicing process, and induce exon skipping. In DMD patients with out of frame deletions (~ 65% of all cases) the manipulation of exon skipping with the deletions of additional exons can restore the reading frame, with a result similar to what is found in the milder BMD. The early proof of concept studies on the role of this approach was obtained in cell cultures of the *mdx* mouse and subsequently demonstrated in DMD cells. Systemic administration of AOs in the *mdx*

mouse also resulted in appreciable induction of exon skipping which in turn induced dystrophin expression of functional levels in body-wide skeletal muscles, with improvement in muscle function³⁴. There are however several limitations of AOs. Firstly, different deletions will require different AOs and secondly the treatment is not permanent but limited to the period in which the AO persist in the tissue. AO treatment will therefore require repeated administrations for the entire life of the DMD boys.

Two European consortia are testing the safety and efficacy of intramuscularly administered AOs in humans. Van Deutekom et al³⁵ recently reported local synthesis and partial dystrophin restoration at the site of PRO051 AO injection into the tibialis anterior muscle of four patients, without adverse events. In UK, the MDEX consortium also utilises the exon skipping approach (<http://www.mdex.org.uk/index.php>), providing a good example of collaborative efforts of basic and clinical scientists, parent and patient organisations, the governmental funding bodies and pharmaceutical industry. The study of the MDEX consortium demonstrated that the intramuscular administration of a morpholino AO (AVI-4658) was safe; the same antisense oligonucleotide will now be studied in an intravenous dose ranging study with 12 weekly infusions.

Read-through of stop codon mutations

This technique is applicable to ~10% of DMD patients, those carrying dystrophin gene nonsense point mutations,³⁶ causing premature cessation of translation. Aminoglycosides cause misreading of the RNA code at the premature but not the normal termination codons, leading to insertion of alternative amino acids at the site of the mutated codon, transcription and protein formation. Gentamicin was effective in the *mdx* mice,³⁷ but the results were not replicated in a human trial of intravenous gentamicin in 2 DMD and 2 BMD subjects.³⁸

PTC124 is an orally administered investigational compound which allows read through at the nonsense mutations, and showed an acceptable safety profile in 62 healthy adult volunteers in phase 1 study.³⁹ A multinational multicentre RCT of PTC124 is currently underway (<http://clinicaltrials.gov/ct2/show/NCT00592553>).

Becker Muscular Dystrophy (BMD)

The incidence is about one-fifth of that for DMD and the prevalence⁴⁰ in the male population around 14×10^6 . Of interest is the heterogeneity of the presentation including typical hypertrophic, proximal weakness BMD, quadriceps myopathy, X-linked cramps, myoglobinuria, and isolated cardiomyopathy. Cardiomyopathy occurs in up to 72% of BMD patients,⁴¹ and is the determinant of survival in this condition. Rarely, hitherto undiagnosed BMD patients with no/mild weakness may first present with a potentially fatal “malignant hyperthermia like” reaction with rhabdomyolysis, hyperkalemia and myoglobinuria⁴² on exposure to suxamethonium or halogenated inhaled anaesthetic for a surgical procedure. This rhabdomyolytic risk is common to DMD and BMD, and a notable caution in their anaesthetic management.⁴³

Kesari et al⁴⁴ reported a higher rate of exceptions to the reading frame in Becker muscular dystrophy, partly explained by a high incidence of deletions in 5' of the dystrophin gene (a region known for hotspots for exceptions), and due to alternative splicing patterns.

A higher incidence of learning difficulties, behavioural problems, and autistic spectrum disorders has been reported in BMD as compared to the general population.⁴⁵

Manifesting Carriers of DMD / BMD

Approximately 2-5% of female carriers of the mutated dystrophin gene, may have skeletal muscle symptoms and are described as manifesting carriers. Their symptoms range from muscle pain & cramps on exertion to a proximal girdle weakness, which rarely can be severe enough to cause wheelchair dependence.⁴⁶

Female carriers of Duchenne muscular dystrophy have a 10% lifetime risk of developing cardiomyopathy; apart from genetic counselling and antenatal diagnosis, this is a key reason for precise evaluation of the carrier status to institute echocardiographic surveillance and treatment if necessary.

The possibility of carrier of DMD/BMD should be considered in the differential diagnosis of asymptomatic serum CK elevation and/or muscle weakness in females, as it is an important differential diagnosis for LGMD.

Carriers of DMD dystrophy may show a mosaic pattern of dystrophin positive and dystrophin negative fibres⁸ (reduced dystrophin in BMD carriers) on muscle immunocytochemistry. It is important to note that a minority of carriers may have normal dystrophin immunostaining on muscle biopsy,⁴⁷ and dystrophin gene analysis should be performed for all suspected carriers.

Limb-Girdle Muscular Dystrophy (LGMD)

Detailed reviews of LGMD classification, phenotypes, pathogenesis, genetic basis and management are available.^{48 - 53} This review highlights the common phenotypes, specific features associated with some of the LGMDs, providing diagnostic clues, and the variable frequency of multisystem (cardiac respiratory, and others) complications which require LGMD subtype specific surveillance and management protocol.

Clinical features of muscular dystrophies presenting with limb-girdle muscle weakness are summarised in table 2. The table is not all inclusive, but tabulates LGMD phenotypes as paradigms. The allelic “non-limb girdle” phenotypes of some LGMDs are also tabulated to illustrate the theme of one gene causing multiple clinical phenotypes.

The LGMDs are classified into autosomal dominant LGMD1, and autosomal recessive LGMD2 forms. A nomenclature, based on the deficient protein, and the underlying molecular genetic defect is in common usage⁴⁸ (e.g. dysferlinopathy, laminopathy), but a comprehensive classification based on inheritance mode, clinical features, proteins and gene defects, is not yet agreed.

The overall frequency of LGMD is 1 in 15,000⁴⁰. The variable incidence of LGMD subtypes in specific population/country is well recognised, and may

help direct investigations in a particular patient. An example is the LGMD2I, which is the commonest muscular dystrophy in some Northern European countries as compared to a higher incidence of sarcoglycanopathy in Northern Africa, and LGMD2B and LGMD2A in an Australian cohort.⁵⁴

The diversity of the clinical features, the genetic heterogeneity, and the lack of easily available DNA based molecular genetic testing emphasises the need for a methodical approach, based on the clinical features of the individual patient, to prioritise investigations and molecular genetic diagnosis.

Serum CK level may be a pointer to certain LGMD subtypes, but it is not an absolute screening test for all LGMDs, as it may rarely be normal (eg LGMD1B⁵⁵, and in late stages of LGMD with muscle mass wasting) or only minimally elevated.

Specific patterns of muscle involvement on magnetic resonance imaging (MRI) have been reported⁵⁶. The current clinical role of muscle MRI is in specific cases where the histological and immunoanalysis findings are not diagnostic, and the identification of a specific pattern of muscle MRI involvement may direct that particular LGMD's gene testing.

Muscle biopsy is the key diagnostic step. Histology shows variable degree of dystrophic change and may also give diagnostic clues e.g. lobulated fibres in LGMD2A⁵⁷. Muscle immunocytochemistry, and western immunoblot identify the protein / enzyme deficiency, in turn allowing final diagnostic confirmation with appropriate gene studies. Reduction of a particular muscle protein on muscle biopsy may be primary (dystrophin reduction in BMD), or secondary (reduced dystrophin immunostaining in LGMD2I secondary to dystrophin-dystroglycan-sarcoglycan axis disruption by the primary α -dystroglycan deficiency⁸). It is therefore important to utilise a full panel of antibodies for immunoanalysis and that the definitive diagnosis should be obtained by genetic analysis which opens the possibilities for genetic counselling / antenatal diagnosis. Considering the rarity of the individual LGMDs, the plethora of genotypes and phenotypes, multiple protein assays, clinical

acumen, laboratory facilities and expertise required in diagnostic interpretation are considerable; in the UK, this is helped by centralization of this service by the National Commissioning Group (NCG) for LGMD

(<http://www.ncl.ac.uk/ihg/services/muscle/>).

A detailed discussion of LGMDs is beyond the scope of this article. The phenotypic features of some paradigmatic LGMDs are listed in table 2 and pertinent issues summarised below.

LGMD1B (Laminopathy)

This is an allelic variant of the autosomal dominant form of Emery Dreifuss muscular dystrophy (EDMD), a form characterised by predominant scapulohumeral wasting and elbow and Achilles tendon contractures.⁵⁵ The defective gene is the LMNA; when mutated most patients present with typical EDMD features, but a minority show more proximal weakness, and absence of prominent contractures, featuring the LGMD1B phenotype. As in EDMD, LGMD1B patients have a high incidence of cardiac involvement. Mutations in LMNA however can give rise to a much wider spectrum of clinical phenotypes, ranging from children presenting in the first year of life within features evocative of a congenital muscular dystrophy⁵⁸ to isolated cardiac complications. There is a high incidence of de-novo dominant mutations. Experts' guidance²⁵ on surveillance and management of the life threatening the cardiac complications, especially the ventricular dysrhythmias, which can lead to sudden death despite pacing, are available. Recently these advocate the use of defibrillators, and not pace-makers, in individuals with laminopathies.

LGMD1C (Caveolinopathy)

In a series of 10 patients, Aboumoussa et al⁵⁹ reported myalgia as a prominent symptom symptoms of caveolinopathy; this adds to the recognised broad spectrum of presentation including LGMD1C, distal myopathy, rippling muscle disease, and HyperCKemia. Rippling in muscle is present in majority of LGMD1C phenotype patients.

LGMD2A (Calpainopathy):

LGMD2A, one of the most prevalent LGMD, is caused by mutations in calpain-3 gene (CAPN3), coding for an enzyme calpain 3, which binds together different proteins involved in myofibrillogenesis.⁶⁰

The characteristic LGMD phenotype is remarkable for marked involvement of posterior thigh muscles (well visible also on muscle MRI⁶¹), tendo-Achilles contractures and scapular winging, and high CK, but the phenotypic variability is wide. The immunoblot analysis of calpain 3 was considered the mainstay of the diagnostic process but more recently normal protein expression on blot has been reported for a significant proportion of cases, suggesting that an apparently normal western blot does not reliably rule out LGMD2A.^{62 63} Additionally, a secondary deficiency of calpain 3 has been described in several muscular dystrophies, including LGMD2B (dysferlinopathy). Final diagnosis therefore rests on CAPN3 gene mutation confirmation.

Muscle MRI in LGMD2A⁶¹ shows characteristic and early involvement of adductors, semimembranosus, and vastus intermediate muscles in the thigh, and these observations may help direct CAPN 3 molecular genetic studies.

LGMD2B (Dysferlinopathy):

The characteristic phenotype is of presentation in late teenage years, with reduction of previous good motor abilities, with markedly high serum CK.⁶⁴ The early gastrocnemius weakness, with inability to stand on toes is a valuable clinical clue. There may be a phenotypic overlap with allelic Miyoshi myopathy (MM). The diagnosis is reliant on muscle immunoblotting study, and ultimately, the gene study⁶⁵.

Currently, a double blind, placebo-controlled study Deflazacort in LGMD2B/MM) is in progress in Munich (www.clinicaltrials.gov).

LGMD2D (α -sarcoglycanopathy)

LGMD2D⁶⁶ is the most frequent sarcoglycanopathy; these are four conditions due to the deficiency of one of the 4 sarcoglycan (SG) proteins ($\alpha, \beta, \gamma, \delta$ SG,

responsible, respectively for, LGMD2D, LGMD2E, LGMD2C, LGMD2F). The phenotype with regards to severity, progression of muscle weakness, and respiratory involvement is similar in LGMD2C-F but cardiac involvement is rare in LGMD2D. Most neurologists are familiar with the α -sarcoglycanopathy as a differential diagnosis of dystrophinopathies. Muscle immunoanalysis shows reduced expression of one or all of the four sarcoglycans in patients with LGMD2C-F (more frequently in β and δ SG deficiency)(and also, as a secondary phenomenon, in DMD/BMD)⁶⁷. Klinge et al⁶⁸ studied 24 genetically characterised patients with LGMD2C-F and demonstrated that residual ($\alpha,\beta,\gamma,\delta$) sarcoglycan protein expression was highly variable and did not enable an accurate prediction of the genotype.

Rodino-Klapac et al⁶⁹ injected human α -sarcoglycan ($h\alpha$ -SG) into the tibialis anterior muscle of α -SG knock out mice with adeno associated vector (AAV) type 1, using different promoters to augment level of gene expression; they reported robust and sustained α -sarcoglycan gene expression, and restoration of the dystrophin-glycoprotein complex under muscle creatine kinase promoters, without evidence of cytotoxicity. A phase 1 human trial of Gene Transfer of rAAV1.tMCK. Human-Alpha-Sarcoglycan for LGMD2D is in progress in US, with estimated completion in 2012 (www.clinicaltrials.gov).

LGMD2I (FKRP related α -dystroglycanopathy)

This is one of the commonest forms of LGMD in Northern Europe. Phenotypic similarity with DMD or BMD⁷⁰ can be striking and many sporadic cases of this condition were misdiagnosed as a dystrophinopathy in the past.⁷¹ Cardiac⁷² and respiratory involvement is common. The common missense mutation (L2761) is frequently associated with milder severity, and helps expedite molecular genetic diagnosis.

LGMD2I is the most common of the “ α -dystroglycanopathies,” which are defects in glycosylation of α -dystroglycan. The α -dystroglycanopathies⁷³, related to mutations in the six known glycosyltransferase genes, are associated with congenital muscular dystrophies with brain and eye involvement but also with milder allelic LGMD variants. Of these, Fukutin-

related protein (FKRP) gene related forms are the commonest cause of LGMD presentation, accounting for LGMD2I.

Mutations in the other α -dystroglycanopathy genes POMT1, Fukutin, LARGE, POMT2, and POMGnT1, which are usually associated with the more severe Walker Warburg disease or muscle-eye-brain phenotypes, have recently been reported with LGMD phenotypes with onset in childhood.^{74 75} These LGMD phenotypes (LGMD2 K - N) are rare, may have associated learning disability, but ocular or brain involvement is not constant.

The benefit of corticosteroids was reported by Darin et al⁷⁶ in two patients with LGMD2I, and by Godfrey et al⁷⁷ in 3 patients with Fukutin related LGMD2L; this opens a therapeutic approach for these patients.

Although the precise composition of the glycans present on ADG are not known, it was recently demonstrated that the forced over-expression of LARGE, or of a related protein, LARGE2, is capable of increasing the glycosylation ADG in wild type cells.⁷⁸ In addition its overexpression is capable of restoring dystroglycan glycosylation and laminin binding properties in primary cell cultures of patients affected by different genetically defined dystroglycanopathy variants. These observations suggest that there could be a role for therapeutic strategies to overcome the glycosylation defect in these conditions via the overexpression of LARGE. The concept is similar to the one which is being used to upregulate fetal hemoglobin for the treatment in the thalassemia and of utrophin in DMD. This could be achieved either by the identification of compounds active on the promoter of LARGE (and/ or LARGE2); or with compounds which would affect the recycling of LARGE (and/ or LARGE2) from the Golgi⁷⁸

LGMD2J (Titinopathy)

Titin is a sarcomeric protein connecting the Z-disk with the M-line, and considered to contribute to striated muscle development, structure, elasticity, and cell signalling. Tibial muscular dystrophy (TMD), described by Udd⁷⁹ in Finland, is the most common allelic phenotype, and is secondary to

heterozygous dominant mutation in C terminus exons of the TTN gene. LGMD2J is the earlier onset, more severe phenotype of homozygous distal TTN mutations⁸⁰. Carmignac et al⁸¹ reported 1st decade onset myopathy with cardiomyopathy, associated with previously not described homozygous C terminal TTN deletions, in consanguineous Moroccan and Sudanese families, further illustrating the phenotypic and ethnic variability in titinopathies.

Conclusion

With the improved survival of patients with muscular dystrophies, DMD in particular, it is anticipated there will be an increased caseload for the adult neurologist, and calls for a proactive approach in management. The MDT management model for DMD lends well to adult neuromuscular practice for LGMDs with disease progression and multisystem involvement, and allows disease specific monitoring and management protocols to improve survival. The current sub-optimal social support for these patients with disability and complex needs has to be rectified to augment patient and carer's of quality of life.

The patient-lead drives to find curative treatments in DMD and LGMD are now stronger than ever, and require a cohesive approach for integration of clinical and research teams to enable precise diagnosis and translational research. This is anticipated to be facilitated by multinational networks like Treat NMD (www.treat-nmd.eu), and national organisations like the recently established UK MRC Muscle Centre (<http://www.cnmd.ac.uk/>), which is a collaboration between three leading neuromuscular centres, Medical Research Centre (MRC), and Muscular Dystrophy Campaign, the patient representative organisation.

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Table 1. Strategies for gene therapy in Duchenne Muscular Dystrophy

Research Strategy	Action	Effect	Current Status
Adeno-associated virus (AAV) vector	Using AAV as a vehicle to transfer "mini-dystrophin" Gene into the affected muscles	Expression of truncated but partly effective Dystrophin protein in muscle	Pilot study initiated in Ohio (USA) http://genetherapy.unc.edu/ .
Utrophin up-regulation	Utrophin up-regulation in skeletal muscle by pharmacological means	Increased utrophin levels, which has 85% homology with dystrophin protein, binds to the dystrophin-glycoprotein complex, and ameliorates dystrophic pathology in transgenic mice	Future clinical trials are planned with VOXC1100 a drug discovered by a UK company VASTox.
Myoblast transplantation	Direct injection of myoblasts in DMD muscle	Studies in the <i>mdx</i> mice resulted in expression of dystrophin	No significant dystrophin expression in human studies
Stem cell transplantation	Delivery of stem cells to muscle to give rise to progenitor or precursor cells before differentiating to other cells	<i>mdx</i> mice study has shown that human derived pericytes, can differentiate into satellite stem cells	Clinical trials using pericytes are being planned in DMD
Modification of dystrophin mRNA splicing (Exon skipping)	Antisense oligonucleotides (AOs) used to redirect splicing and induce exon skipping	Temporary restoration of reading frame to allow dystrophin production	Two European Consortia tested safety and local efficacy of intramuscular AOs. Systemic AOs trial starting in 2009
Read-through of stop codon mutations	Aminoglycosides and the recently produced PTC124 can cause misreading of the RNA code, allowing insertion of alternative amino acids at the site of the mutated codon.	Transcription and dystrophin protein formation in <i>mdx</i> mouse muscle. Gentamicin not effective in human study	PTC-124, an orally administered Molecule, Technique relevant to ~ 10% of DMD patients who have nonsense point mutations. Multinational RCT of 2 dose levels and placebo in DMD is currently in progress .

Muscular Dystrophy	Protein	Gene	Age of onset (years)	CK Level (x upper limit of normal)	Pattern of weakness	Muscle hypertrophy	Contractures	Respiratory	Cardiac	Clinical clues
Becker MD	Dystrophin	BMD	Late 1 st – 3 rd decade (5 – 50)	5 – 50x	Proximal >> Distal	Prominent feature	Tendon Achilles (TA)	Rare	CM	Cramps, Myoglobinuria. At times learning difficulties. Cardiomyopathy may be severe. Beware of Rhabomyolytic myoglobinuric crisis with halogenated inhaled anaesthetics.
LGMD1B	Lamin AC	LMNA	3 – 30 (earliest case in 1 st year)	N – 10x	Proximal & Distal esp in legs	Often atrophic phenotype	Prominent in elbows, long finger flexors, TA	Yes	CM & Ary	“Atrophic contracted” phenotype often present, leading to description as Autosomal Dominant Emery Dreifuss. Cardiomyopathy in most all by 3 rd decade
L-CMD	Lamin AC	LMNA	1 st year	3 – 12x	Limbs & axial	No	Talipes, spinal stiffness	Yes	CM & ARy	Floppy infant and Congenital MD phenotypes may occur. Cardiac complications may be life threatening.
LDMD1C	Caveolin-3	CAV-3	1 st – 5 th decade	5 – 25x	Proximal (rarely distal onset)	Calf hypertrophy in some cases	No	No	No	Muscle rippling, or, percussion induced rapid muscle contractions. Additional phenotypes: myalgia, myoglobinuria & idiopathic hyperCKemia
LGMD2A	Calpain	CAPN3	1 st – 2 nd decade (2 – 40)	10 – 20x	Proximal > Distal	Some cases	TA contractures & toe walking may be prominent	No	No	Relative preservation of hip abductor strength. Posterior thigh muscle atrophy clinically and on muscle MRI. Toe walking secondary to tenoAchilles contractures
LGMD2B	Dysferlin	DYSF	2 nd – 3 rd decade	10 – 100x	Proximal & Distal, legs > arms	May occur in thighs. Lower leg thinning	No	No	No	Distal weakness in legs, with difficulty standing on toes. “Myositis-like” onset with muscle pain and swelling described
Miyoshi myopathy	Dysferlin	DYSF	3 rd – 4 th decade	10 – 100x	Distal: gastrocnemius & soleus initially	uncommon	Secondary to weakness	No	No	Difficulty standing on toes. Muscle imaging shows early gastrocnemius and soleus and occasionally, paraspinal muscle involvement.

LGMD2D	α Sarcoglycan	SGCA	1 st decade (3 – 40)	10 – 100x	Proximal > Distal	Often prominent	Develop mainly when non-ambulant	Yes	CM	Presentation may be of DMD severity, with calf hypertrophy. Scapular winging.
LGMD2I	Fukutin related protein	FKRP	1 st – 2 nd decade (1-40)	10 – 100x	Proximal > Distal	Prominent feature	Not prominent	yes	CM	Mimics DMD and BMD very closely
LGMD2J	Titin	TTN	1 st decade (5 – 20)	N – 25x	Proximal > distal	\pm Calf & thighs	May occur	No	CM may be fatal	Autosomal Recessive, early onset. Muscle biopsy may show (secondary) calpain reduction.
Tibial MD (Udd myopathy)	Titin	TTN	3 rd – 6 th decade	1 - 4x	Distal >> proximal		Not a primary feature	No	Rare	Autosomal Dominant, late onset. “Hanging big toe”. Distal gene mutation mild, upstream mutations more severe phenotype. Rimmed vacuoles on muscle biopsy in early stages.

Table 2. Muscular dystrophies presenting with limb girdle distribution of weakness, and some of their allelic variant phenotypes.

CM – cardiomyopathy

Ary – Arrhythmia

> - more than

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