DUCHENNE MUSCULAR DYSTROPHY: CLINICAL CHARACTERISTICS, DIAGNOSIS AND MANAGEMENT

George-Konstantinos Papadimas, Sophia Xirou, Evangelia Kararizou, Constantinos Papadopoulos

Department of Neurology, Brigham and Women's Hospital and Jamaica Plain Veterans Administration Hospital, Harvard Medical School

Abstract

Duchenne muscular dystrophy (DMD) is the most common form of dystrophinopathy, followed by the milder Becker muscular dystrophy and the DMD-associated dilated cardiomyopathy. DMD is inherited in an X-linked recessive manner, caused by mutations in DMD gene encoding for dystrophin, and presents in early childhood with muscle weakness and gait impairment. Respiratory involvement is a major cause of mortality, and the use of steroids and non-invasive ventilation have significantly increased survival. Dilated cardiomyopathy is another big challenge, especially for the older patients with DMD, carrying a poor prognosis. Despite the important efforts and progress that have been made over the last years, curing DMD is still a far-reaching goal. However, strict application of the current guidelines and emerging genetic treatments have decisively improved the clinical course of the disease and provide reasonable hope for a much better outcome in the future.

Key words: duchenne muscular dystrophy, myopathy, dystrophinopathty

INTRODUCTION, EPIDEMIOLOGY AND GENETICS

Duchenne Muscular Dystrophy (DMD) is an X-linked recessive disorder caused by mutations in dystrophin (*DMD*) gene, located in the short arm of the X chromosome. It is the most frequent inherited myopathy and one of the most common debilitating muscular diseases, with a birth prevalence of 15.9-19.5 per 100000 live male births [1-4]. In addition to DMD, dystrophinopathies also include Becker Muscular Dystrophy (BMD), which is a milder but rarer disease than DMD, with one case per 6000-8000 live male births [2, 5], DMD-associated dilated cardiomyopathy (DCM), and the female carriers of DMD mutations, who may occasionally be mildly to moderately symptomatic. The predominant primary presenting symptom in most forms of dystrophinopathies is skeletal muscle weakness. However, cardiac muscle is also very often involved and remains one of the most common causes of morbidity and mortality [6-8]. The predominant primary presenting symptom in most forms of dystrophinopathies is skeletal muscle weakness. However, cardiac muscle is also very often involved and remains one of the most common causes of morbidity and mortality [6-8]. It is noteworthy that there are two hotspots in the *DMD* gene, located mostly in exons 45-55 and secondarily in exons 2-19 [9]. On the contrary, in BMD, deletions are found in 60-70% of patients, duplications in approximately 20% and only 5-10% are point mutations, small deletions or insertions [4, 10, 11]. The predominant primary presenting symptom in most forms of dystrophinopathies is skeletal muscle weakness. However, cardiac muscle is also very often involved and remains one of the most common causes of morbidity and mortality [6-8].

The diagnosis of *DMD* is suspected based upon the clinical symptoms, biochemical findings, especially the CK (creatine kinase) increase and the possible presence of a positive family history, and is finally confirmed by genetic testing. Prenatal diagnosis and counselling in female carriers of a known pathogenic *DMD* mutation is of outmost importance, in order to avoid the birth of an affected boy. However, even with the application of very clear prenatal recommendations and genetic counselling for at risk women of reproductive age, the birth of affected boys cannot be completely avoided, since in one-third of DMD and BMD have a *de novo* mutation with negative family history [12-14]. Finally, it is important to note that the mothers of DMD/BMB children, who are not somatic carriers of a *DMD* mutation, exhibit a higher possibility of birthing another affected boy, due to germline mosaicism [15].

PATHOPHYSIOLOGY

The predominant primary presenting symptom in most forms of dystrophinopathies is skeletal muscle weakness. However, cardiac muscle is also very often involved and remains one of the most common

causes of morbidity and mortality [6-8]. Dystrophin has four important domains: a) the acting-binding domain, which attaches to F actin, providing a linkage between dystrophin and the subsarcolemmal actin network, b) the central rod domain, which contains 24 spectrin repeats and mediates the dystrophin interaction with microtubules, c) a cysteine rich domain, and d) a carboxyl-terminal domain. The latter is the dystroglycan-binding end, providing the connection to the dystroglycan complex within the membrane that is anchored to extracellular matrix [16-18].

The disease starts very early and muscle inflammation can be observed soon after birth, while muscle fibrosis usually starts to develop even within the first year of life. Muscle degeneration and necrosis are the primary features of DMD. Several hypotheses on the pathophysiology of the disease have been elaborated, but according to the most prevailing theory, DMD is caused by a structural of functional defect of dystrophin [19, 20]. The absence of dystrophin results in lack of integrity within the muscle cells causing progressive damage particularly during muscle contraction, while the loss of linkage with the dystroglycan complex (α-dystroglycan and β-dystroglycan) leads to disruption of transmembrane signaling [16, 21]. The integrity of the sarcolemma is dependent on the normal function of the dystrophin-associated protein complex (DAPC). The DAPC disassembly results in weakening of the muscle membrane, which can no longer withstand the strong mechanical stress produced by repeated contraction and relaxation of the sarcomeres, leading to sarcolemma ruptures. Muscle enzymes, such as creatine kinase (CK), aldolase, and transaminases leak through these membrane tears into the bloodstream [22, 23].

A dysregulation of calcium homeostasis is highly implicated in the pathogenesis of muscular dystrophies and particularly DMD. An abnormal increase in calcium influx and intracellular calcium concentration is well known from very early studies in dystrophic animal models and is associated with muscle fiber hypercontraction and myonecrosis [24-27]. The increased intracellular calcium concentration may originate either from an enhanced calcium influx through calcium channels, such as TRPC mechanosensitive voltage-independent calcium channels, which are highly expressed in DMD and plasma membrane calcium ATPases, or from microscopic sarcolemma microtears and sodium-calcium exchangers [4, 28, 29]. Another source of elevated cytocolic calcium is the sarcoplasmic reticulum (SR), which permits calcium release through the defective ryanodine receptors (RYR1) of the dystrophic muscle [30]. RYR1 is destabilized due to an aberrant binding with calstabin, with a subsequent opening of the channel and intracellular calcium leakage. In addition, the activity of sarco/endoplasmic reticulum calcium ATPase (SERCA), which normally functions to mediate calcium re-entry to SR, is reduced due to sarcolipin-induced down regulation, further contributing to increased intracellular calcium [30-32].

An additional crucial role of dystrophin is to anchor nNOS (neuronal nitric oxide synthase) to the sarcolemma, and thus muscle damage may be further aggravated by a functional ischemia caused by the mislocalization of nNOS in DMD, which is necessary for vasodilation during muscle contraction in order to normally supply exercising muscle with oxygen. [33, 34]. Muscle ischemia may in turn lead to activation of different parallel pathomechanisms, such as the release of inflammatory cytokines, calcium overload and an overproduction of ROS (reactive oxygen species) [35, 36], which may be in turn exacerbated by the microtubule-associated protein Rac1 activation of NADPH oxidase 2 (NOX2), with a subsequent severe free radical injury [37].

Recent data also support a possible mitochondrial dysfunction, implied by an aberrant mitochondrial morphology in dystrophic mice, which in fact precedes the onset of muscle fiber damage. Thus, a link between dystrophin and mitochondrial function is highly suspected but larger studies are needed to identify the underlying mechanisms [38-41].

In the early stages of the disease, muscle fibers have a greater regenerative capacity, which gradually decreases due to a progressive depletion of satellite cells [42]. Regenerative fibers often display a branched morphology that may further increase their susceptibility to damage. Moreover, muscle fiber branching may contribute to channel dysfunction and excessive calcium influx, creating a vicious cycle and maximizing muscle failure [26, 43].The progressive muscle fiber replacement with fat and fibrotic tissue further limits the ability of muscle regeneration.

CLINICAL CHARACTERISTICS

Although DMD and BMD are allelic disorders, they have also many differences as shown in Table 1. DMD is a continuum and although the diagnosis could be occasionally made in the first 2 years of life, the vast majority of patients are diagnosed at the age of 4-5 years. The disease is relentlessly progressive and initially leads to loss of the ability to run, to walk and then to ambulate, and DMD affected children finally end up wheelchair bound, approximately by the age of 10 years. The patients' autonomy is further limited by the concomitant loss of arm function. The early recognition of symptoms and signs of DMD becomes more necessary nowadays in the era of new evolving therapeutic approaches.

In a very early presymptomatic stage, there may be some indications of delayed developmental mile-

Table 1. Main differences between Duchenne and Becker muscular dystrophy

	Incidence	CPK levels	Onset (age)	Wheelchair dependency	Cardiomyopathy	Median survival	Muscle biopsy Immuno-histochemistry (dystrophin staining)	Muscle biopsy Western blot (dystrophin quantity)
DMD	15.9-19.5 per 100000 live male births	>10 normal	2-5 years	Before age 13	100% after age 18	27 yrs	Complete/ almost complete absence	0-5% dystrophin
BMD	1 per 6000 - 8000 live male births	>5 normal	Usually >7 years	After age 16 (if present)	30-70% of patients overall	Mid 40s	Normal appearing, or reduced/patchy intensity	

Abbreviations: DMD, Duchenne muscular dystrophy; BMD, Becker muscular dystrophy; CPK, Creatine phosphokinase

stones. Therefore, any difficulty in the acquisition of motor skills, such as a poor head control, the inability of a child to walk independently by the age of 18 months or to run by the age of 3 years, or a difficulty to jump, to climb stairs, or to get up easily from the floor should be considered as potential early indications of DMD [44, 45]. In addition, the presence of speech and language delay (no words spoken at the age of 18 months, unable to speak sentences by age 3), the detection of learning difficulties, the occurrence of behavioral issues or the recognition of an autistic spectrum disorder within the appropriate clinical context, may also raise the suspicion of an underlying dystrophinopathy [46-49]. An early ambulatory phase follows with affected children manifesting some signs of the disease, such as calf enlargement or pseudohypertrophy, which is usually asymmetric, due to adipose and connective tissue replacement, toe walking and difficulty standing up from a squatting position (Gower's sign). The patients usually also adopt a curved posture, to account for weaker chest and pelvic muscles. In a late ambulatory stage, the patients may exhibit a clumsy gait with frequent falls, and an increasing loss of walking ability. The patients can no longer climb stairs and an intermittent wheelchair use may be necessary. The early non-ambulatory stage is characterized by an absolute dependence on wheelchair and the development of scoliosis, while in the late non-ambulatory phase, the upper extremity function is severely impaired and there is also limited postural maintenance [12, 44, 50, 51].

It is also very important to emphasize that high levels of muscle enzymes, such as CK, LDH, ALT, AST and aldolase, may be incidentally detected at a presymptomatic stage and may be the first sign of the disease. There are also rare reports of a pseudometabolic phenotype associated with an underlying dystrophinopathy. These patients may present exertional myalgia and/or rhabdomyolysis, and usually run a more benign clinical course [52, 53].

The disease progression towards an increased need for ambulation support coincides with a rapid peak of fibrotic tissue approximately at the age of 7 years, with a concomitant loss of muscle tissue ability to regenerate, which should impact the decision of starting treatment [54]. Restrictive lung disease is also very common in DMD patients and pulmonary function progressively deteriorates due to respiratory muscles involvement, including the diaphragm [55-57]. Three distinct stages in the progression of respiratory function have been identified in DMD patients based on forced vital capacity (FVC) measurements: an initial annual rise in the ambulatory phase of the disease, a subsequent plateau during the early non-ambulatory stage, and finally a progressive decline during the late non-ambulatory period. An FVC reduction of less than 1 L is associated with a significantly higher mortality risk [56, 58, 59]. It has been shown that corticosteroids and particularly the use of respiratory support through mechanical ventilation resulted in a robust increase in the life expectancy of DMD patients, improving median survival from late teen years to 27.0 years of age [60-62].

Cardiac involvement in dystrophinopathies, although common, is not necessarily related to the severity of myopathy and in some cases of BMD, it may be predominant even with minimal muscular disease [63]. In DMD, at a preclinical stage of the disease, the heart manifestations are very subtle, with mild ECG abnormalities, some degree of diastolic dysfunction, or wall motion abnormalities. However, at a more advanced clinical stage, the progressively worsening dilatation of heart chambers and subendocardial fibrosis eventually lead to over 60% of DMD patients from adolescence onwards developing symptoms suggestive of heart failure and dilative cardiomyopathy with left ventricular ejection shortening (LVES) less than 28%, and left ventricular ejection fraction (LVEF) less than 45% [64-66]. Despite the severity of cardiac involvement, DMD patients are not considered good candidates for cardiac transplantation due to the shortage of donor availability and their poor prognosis [67-69].

Table 2. Clinical manifestations of female carriers

Signs/Symptoms	DMD mutations	BMD mutations
None	76%	81%
Muscle weakness	19%	14%
Myalgia/cramps	5%	5%
Left-ventricle dilation	19%	16%
Dilated cardiomyopathy	8%	0

Abbreviations: DMD, Duchenne muscular dystrophy; BMD, Becker muscular dystrophy
Adapted from Darras BT et al. [138].

Finally, there is increasing data supporting the genetic predisposition for the outcome of both cardiac and respiratory function. More specifically, a better cardiac prognosis was observed in association with mutations in the dystrophin Dp116 coding region [70] and in patients carrying the polymorphisms rs28357094 in the SPP1 promoter, rs10880 and the VTTT/IAAM haplotype in LTBP4, which are also associated with age at loss of acquired motor skills [71]. Moreover, DMD patients amenable to skipping exon 44 seem to have a better respiratory function with higher FVC% and a slower rate of decline [72, 73].

Female carriers of *DMD* and *BMD* mutations may rarely have symptoms of myopathy or even cardiac involvement. Table 2 summarizes their main clinical characteristics

DIAGNOSTIC ALGORITHM

The typical myopathic presentation in a young boy combined with a significantly high CK are key features for coonsidering DMD. Though while DMD may be easily recognized in patients at an older age with the typical signs and symptoms of the disease, the diagnosis at an early stage is usually more difficult and requires a high suspicion index. A positive family history may be helpful, but as previously mentioned, there is a high proportion of patients carrying a *de novo* mutation. The high CK levels may be a very useful diagnostic clue, especially if randomly found at a preclinical stage. Although a CK increase is non specific and may be observed in various neuromuscular diseases and other conditions, the stable and very high levels can significantly narrow down the differential diagnosis [74].

In case that DMD is suspected, the initial diagnostic step is to perform genetic testing. Since deletions and duplications are the cause for the great majority of patients, it is considered cost-efficient to initially check for these mutations by using MLPA (multiplex ligation-dependent probe amplification) analysis or array comparative genome hybridization (array CGH) [75, 76]. In case of a positive result, the diagnosis is considered established, whereas if the mutation is not found, genetic testing must be completed with Sanger sequencing of the 79 exons of the *DMD* gene, in order to possibly detect a small causative mutation. However, this technique is laborious, time consuming and expensive, and not performed by all genetic laboratories [76]. If the results are still negative, but DMD remains highly suspected, there is also the rare possibility of deep intronic mutations that cannot be identified by the aforementioned techniques and may be picked up with more elaborate approaches, such as next generation sequencing (NGS) [76-78].

The need for muscle biopsy, which was historically the initial step for diagnosing DMD, has a limited role now. Although protein analysis through immunohistochemistry and western blot can provide further insights on the location, abundance and molecular size of dystrophin, the need for genetic testing is absolute, especially in the era of evolving specific genetic treatments, which require an accurate molecular diagnosis. Moreover, muscle biopsy is an invasive procedure and affected children at a young age have to undergo general anesthesia, which may pose an increased risk, given their cardiorespiratory status [76, 79, 80]. However, in case that a thorough genetic testing does not yield positive results, muscle biopsy should be considered to confirm or rule out the diagnosis [5, 74].

MANAGEMENT

Although there is currently no radical cure for DMD, there are many modern therapeutic approaches. In recent years, there has been a very large number of clinical trials investigating the safety and efficacy of multiple compounds with different mechanisms in DMD patients. They can be broadly divided into primary therapies, aiming to restore the missing or dysfunctional dystrophin, and secondary therapies,

targeting parallel pathophysiological processes due to the absence of dystrophin. An update on drug development for the treatment of DMD is provided on Table 3 and current information can be found at ClinicalTrials.gov.

However, the tremendous progress of genetic treatments and gene therapy in particular, should not downplay the importance of compliance to the standards of care, which have been updated in 2018 after their initial publication in 2010 by Bushby et al. [5, 44, 81-83]. The recent guidelines include more detailed recommendations for management of other aspects of the disease, such as endocrine abnormalities and bone health, and also emphasize the transition from childhood to adulthood care. Especially for the latter, an early transition planning is vital in order to assist DMD patients in better adjusting to the demands of the new setting. The participation of the individual in transition planning and decision making is also very important and ensures the maximum degree of independency a patient can achieve [84, 85].

The strict adherence to multidisciplinary management guidelines has decisively modified the natural course of the disease and can better control the symptoms of DMD patients, as they improve their quality of life and prolong their lifespan [4, 86]. It should be mentioned, however, that most guidelines are not evidence-based, due to the lack of large-scale randomized controlled studies for DMD, and are the result of expert opinions based on the available evidence rather than statistical approaches [44, 82, 83].

Respiratory complications

The strict application of respiratory guidelines with ventilatory support through non-invasive ventilation brought about improvements in the survival of DMD patients by approximately 10 years [60]. Respiratory assessment must be annually performed after the confirmation of the diagnosis. Many different methods are routinely used to assess lung function. Forced vital capacity % predicted (FVC%) is one of the most useful outcome measures of respiratory progression and when it is below 50%, there is an increased risk of sleep disordered breathing, while maximum expiratory and inspiratory pressure (MEP, MIP) are more specific for the evaluation of expiratory and inspiratory muscle function [55-57]. Especially in the early ambulatory stages of the disease, where the very young affected children cannot cooperate well in performing lung function tests, peak expiratory flow percentage predicted (PEF%) has proved a reliable and useful surrogate marker of respiratory progression [57, 87]. Sleep studies are also strongly recommended on suspicion of nocturnal hypoventilation and the use of mechanically assisted coughing and ventilation is highly advised when needed [57, 82].

Cardiac complications

The improvement of lifetime expectancy in DMD patients, mainly due to the best respiratory care, resulted in the emergence of cardiac complications and in an increase in cardiac-associated deaths owing to heart failure and conduction abnormalities. Current guidelines suggest starting cardiac monitoring with echocardiogram at the age of 6 years, which is later supplemented by cardiovascular MRI. It is also recommended to initiate angiotensin-converting enzyme (ACE) inhibitors or ACE blockers by the age of 10 years regardless of the presence of symptoms, which emphasizes the importance of a proactive approach [82, 88, 89].

Orthopaedic complications

Scoliosis, joint contractures, and a low bone mineral density due to impaired bone metabolism are commonly encountered in DMD patients. In ambulant patients, physiotherapy, occupational therapy and orthotics or other appropriate assistive devices are strongly encouraged to help them move and perform daily tasks. Especially the prevention of contractures developmt is of outmost importance for maintaining a patient's gait. In non-ambulant patients, the emphasis should be placed on the correct sitting position, to avoid worsening of scoliosis and to maintain as much as possible the upper limb function [82, 90]. Although the use of steroids has prevented the early development of severe scoliosis, it continues to be a common complication of the disease contributing to respiratory deterioration. In presence of scoliosis, radiological assessment should be performed at least annually, and any surgical intervention should be cautiously decided on a multidisciplinary basis [82, 91].

Other system complications

Gastrointestinal motor function disturbances due to visceral smooth muscle involvement, seem to be quite common in DMD patients, especially at an advanced age. Gastroparesis, constipation and gastroesophageal reflux disease (GERD) are the most prevalent manifestations [92]. Dietary guidelines and symptomatic treatment with the administration of osmotic and stimulant laxatives for bowel dysmotility or histamine 2 receptor antagonists and proton-pump inhibitors for GERD are highly recommended [44].

Urological management is also frequently required to address problems such as bladder hyperactivity, detrusor sphincter dyssynergia, and urinary tract infections. Pharmacological interventions may alleviate symptoms and improve quality of life. Special caution should also be paid to renal dysfunction, which may be observed in the late stages of the disease [93, 94].

Endocrinological monitoring for growth problems,

Table 3. Drug Development Pipeline for Duchenne Muscular Dystrophy

		Preclinical	Phase I	Phase I/II	Phase II	Phase III	Approved
PRIMARY THERAPIES (dystrophin restoration or replacement)							
Genetic treatments							
Non sense mutation readthrough	Ataluren (Translarna) *PTC Therapeutics*						EMA*
Exon Skipping	Golodirsen (exon 53) *Sarepta Therapeutics*						FDA*
	Eteplirsen/Exondys51 (exon 51) *Sarepta Therapeutics*						FDA*
	Viltepso (Viltolarsen/NS-065/NCNP-01 (exon 53)) *NS, Pharma, Inc.*						FDA/MHLW Japan*
	Casimersen (exon 45) *Sarepta Therapeutics*					√	
	SRP-5051 (exon 51) *Sarepta Therapeutics*				√		
	DS-5141b (exon 45) *Daiichi Sankyo*				√		
	NS-089/NCNP-02 (exon 44) *NS Pharma, Inc.*				√		
	scAAV9.U7.ACCA (exon 2) *Audentes Therapeutics*				√		
Gene Therapy	AAV9.microdystrophin (PF-06939926) *Pfizer*					√	
	rAAVrh74.MHCK7.micro-dystrophin (SRP-9001) *SareptaTherapeutics*				√		
	AAV9.microdystrophin (SGT-001) *Solid Biosciences*				√		
	GALGT2 genetherapy (rAAVrh74.MCK.GALGT2) *Nationwide Children's Hospital, Columbus, United States*				√		
Celltherapies	CAP-1002				√		
	Bone Marrow-derived autologous Stem Cells *Stem Cells Arabia*				√		
	Myoblasts *CHU de Quebec-Universite Laval, Canada*				√		
SECONDARY THERAPIES targeting...							
fibrosis	Pamrevlumab *FibroGen*					√	
inflammation	EMFLAZA (Deflazacort)-steroid *PTC Therapeutics*						FDA
	Vamorolone (VBP15) - steroid alternative *Santhera Pharmaceuticals*					√	
	Tamoxifen -SERM *University Hospital of Basel*					√	
	ATL1102 - antisense oligonucleotide *Antisense Therapeutics*				√		
	Canakinumab (ILARIS) - monoclonal antibody *Children's Research Institute*			√			
calciumhomeostasis	Rimeporide *EspeRare Foundation*			√			
musclegrowthand-protection	Givinostat Follastatin enhancement *ItalfarmacoSpA*					√	

Table 3. Continuity

		Preclinical	Phase I	Phase I/II	Phase II	Phase III	Approved
	Carmeseal-MD Membrane Sealant *PhrixusPharaceuticals*			√			
	EDG-5506 Muscle stabilizer *Edgewise herapeutics*		√				
	Spironolactone vs Prednisolone Aldosterone antagonist *Nationwide Children's Hospital*		√				
mitochondrial-function	EPM-01 mitochondrial biogenesis *Epirium Bio*		√				
	ASP0367 (MA-0211) Cellular function improvement Astellas Pharma Inc.		√				
cardiacfunction	Ifetroban Cardiomyocyte protection *Cumberland Pharmaceuticals*				√		
	Bisoprolol fumarate *Hoffmann-La Roche, Peking Union Medical College Hospital,* China					√	
	Nebivolol *Assistance Publique - Hopitaux de Paris, France*					√	

hypogonadism, delayed puberty and nutritional assessment should be regularly performed as well. Moreover, bone health and glucose metabolism should be given special attention, especially due to the long-term corticosteroid administration [44].

Moving on, neuropsychological status and neurodevelopmental progression should be carefully monitored in DMD patients, due to the high incidence of cognitive issues and psychiatric manifestations, such as anxiety, depression, autism, and attention deficit/hyperactivity disorder [95]. Regular neuropsychological and psychiatric evaluations and pharmacological treatment, when needed, should be provided. Moreover, specific educational programs could improve cognitive skills of patients, especially if applied early.

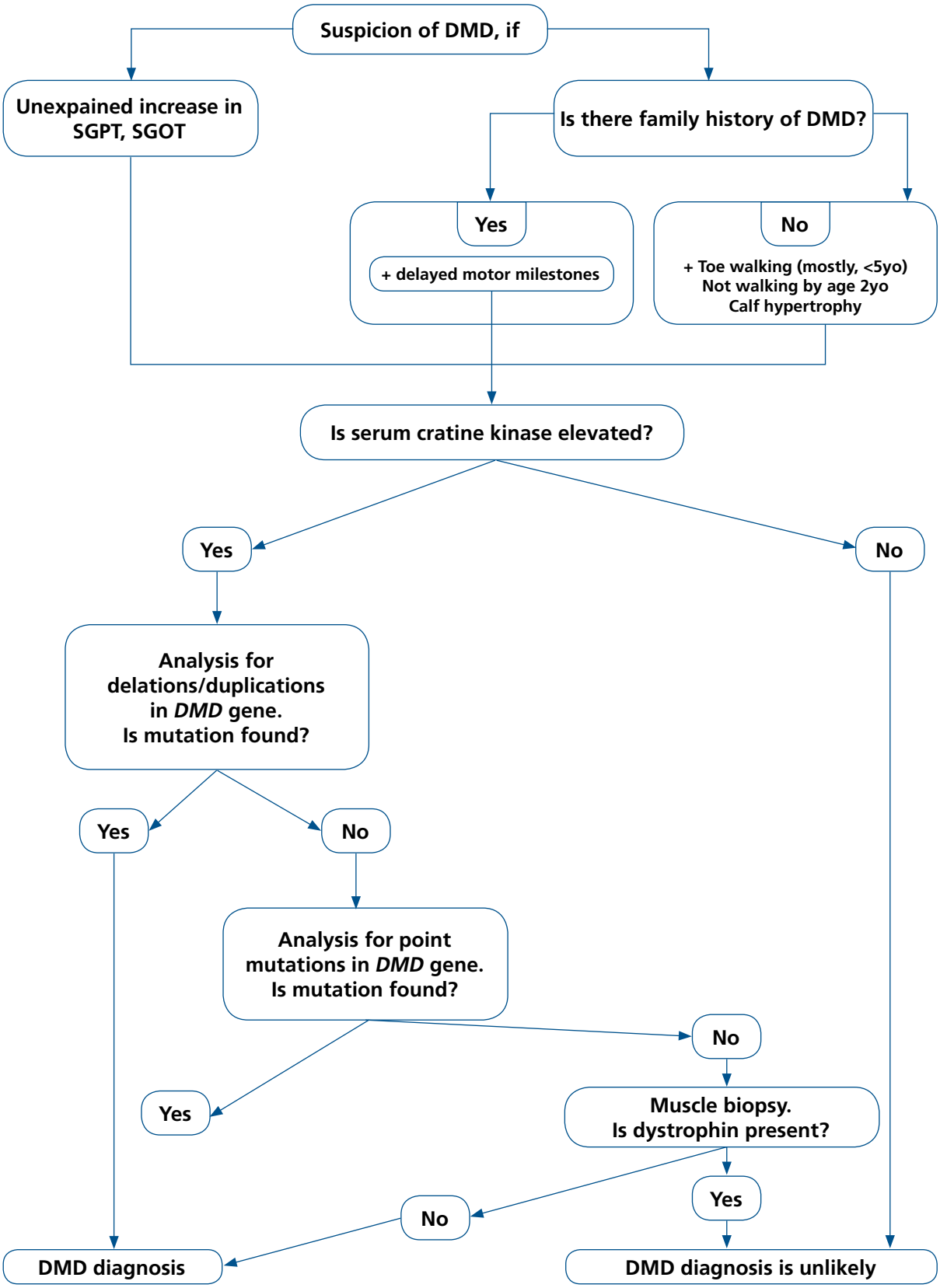
Steroids

Steroids have been shown to have a beneficial effect primarily on the respiratory function and in muscle strength maintenance. The early administration of steroids in children with DMD is included in the SOCs and aims at prolonging ambulation at least for 3 years, which is also very important for respiratory function, as it seems that there is a good correlation between loss of ambulation and respiratory function decline. Retaining ambulation may further delay spinal deformities, which is a major concern in DMD patients [44]. Despite the strict recommendations for steroid administration in paediatric DMD patients, there are no clear guidelines for the adult patients, and the treating physician must weigh the pros and cons of continuing treatment.

A significant body of evidence from recent clinical studies suggest that the early administration of steroids, before the age of 10 years, may increase the pulmonary function testing measures with a subsequent delay in the onset of decline, compared to naïve DMD patients. On the other hand, if given at a later stage, after the onset of respiratory deterioration, they do not seem to have any beneficial impact on the progression of the disease [72, 73, 96]. Previous studies have also shown that steroids slow the progression of scoliosis and delay the need for spinal surgery. Given the association of scoliosis and pulmonary function, it would be expected that the positive effect of steroids on spinal pathology may also indirectly impact the respiratory function [97, 98]. The role of steroids in the cardiac function of DMD patients is quite controversial. In a large retrospective study investigating the role of genetic modifiers in DMD, steroid treatment did not significantly affect the onset of dilated cardiomyopathy, which occurred at a mean age of 20 years [71]. Nevertheless, older studies suggest that steroids may delay progression of heart failure and can improve survival [99, 100].

Lastly, a further matter of interest is the potential different effect of frequently used corticosteroids and

Figure 1. Diagnostic algorithm for Duchenne muscular dystrophy



Abbreviations: DMD, Duchenne muscular dystrophy ; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, Serum glutamic pyruvic transaminase
Adapted from Birnkrant DJ et al. [44].

their various regimen schedules on the progression of cardiorespiratory function. According to a recent retrospective longitudinal study, steroids, either deflazacort or prednisone administered either daily or intermittently, had a significantly positive impact on both respiratory function and cardiomyopathy [73]. Notably, deflazacort is associated with less weight gain than prednisone and is the first glucocorticoid with a full FDA approval for DMD patients older than 5 years of age [44, 101]. A current ongoing trial is now comparing benefits and adverse effects between deflazacort and prednisone [102].

Genetic Treatments

Genetic therapies have attracted increasing attention and where indicated, are incorporated into the treatment plan. The approval of the first genetic drugs, ataluren by EMA in August 2014, and eteplirsen by FDA in September 2016, are considered important milestones in the treatment of the disease [44, 103].

➢ **Stop codon read through therapies**

In DMD, 11-30% of patients have a nonsense mutation in the *DMD* gene, resulting in a premature mRNA stop codon, which leads to termination of the translation before a full-length functional dystrophin is generated. Therefore, DMD patients carrying this type of mutation are eligible for ataluren, an orally administered small molecule, which promotes ribosomal read-through of mRNA with a premature stop codon, restoring the production of a full-length protein. Despite the failure to achieve the primary endpoints of improved walking distance in the 6-minute walk test (6MWT) after 48 weeks of treatment in two randomized, double-blind, placebo-controlled trials, there was a clear improvement in timed function tests and a significant 29-meter increase in 6MWT, which formed the basis of a conditional approval by EMA since 2014 [104-106]. On the other hand, ataluren has not gained approval from FDA yet, mainly because the 9% increase in dystrophin production induced by the drug was not considered statistically significant.

Finally, the efficacy and safety of ataluren has also been confirmed by the European Drug Registry (STRIDE), while another placebo-controlled study evaluating the effect of ataluren is underway [107].

➢ **Exon skipping therapies**

Exon skipping technology is being extensively used over the past few years in DMD. The aim is to restore the reading frame by converting an out-of-frame to an in-frame mutation, leading to a partially functional dystrophin and a milder BMD-like phenotype. Exon skipping is induced by the intravenous administration of antisense oligonucleotides (ASOs), which are short single-stranded nucleic acids that can bind to the pre-messenger RNA mutation preventing it from being included in the mature mRNA [108, 109].Obviously, the knowledge of the accurate genetic diagnosis is critical, as any frameshift mutation can be amenable to certain exon skipping therapy. Since deletions cluster in hotspots of the *DMD* gene, skipping of certain exons may be applied to a great majority of DMD patients [110]. More specifically, skipping of exon 51 is applicable to approximately 14% of patients, of exon 45 to 8%, of exon 53 to 8% and of exon 44 to 6%, respectively [111, 112]. Conditional approval has been already given by FDA to four exon skipping therapies: firstly eteplirsen (ExonDys51) in September 2016, to skip exon 51, golodirsen in December 2019 and vitolarsen in August 2020 to target exon 53 and more recently in March 2021, casimerson for skipping exon 45 [113-116]. Similarly, conditional approval has been granted to vitolarsen by the Japanese Ministry of Health, Welfare and Labour. Current studies are now assessing the long-term clinical effect of those compounds in order to obtain final approval.

➢ **Gene therapy**

Gene transfer therapy is an evolving therapeutic strategy for monogenic disorders, including DMD. The first double-blind placebo-controlled gene transfer therapy clinical trial for DMD patients (NCT03769116) started in 2018.

The aim of gene therapy is to prevent or slow the progression of the disease and relies on the use of viral vectors for efficient gene delivery. The vectors that are usually used for transferring functional genes are adenoviruses, adeno-associated viruses (AAVs), and lentiviruses, and are the most important determinants of safety and transduction efficiency. In DMD, the AAV9 and AAVrh74 vectors are suitable candidates for targeting both muscle and heart [117-119]. The AAV-induced immune response varies over time following administration. The first response is observed very early, hours to days after the injection and is mediated by innate immunity, while the adaptive immunity is activated later, weeks to months after drug delivery and may persist in the form of antigen-specific T and B cells [120, 121]. The most common adverse reactions of gene therapy may include an increase in transaminases, platelet reduction, nausea, vomiting, loss of appetite, diarrhea, increase of troponin and creatine kinase, fever and myalgia, while extremely rarely more serious side effects such as, liver, respiratory or heart failure, hemolytic uremic syndrome, intestinal bleeding, tumorgenicity, dorsal root ganglia toxicity, septicemia and death, have been reported [122-124]. An important concern with AAVs is that the delivery of gene therapy may be

prevented by neutralizing antibodies that block AAV entry into the cells. The pre-existing antibodies are mainly acquired through environmental exposure to wild-type AAVs and more rarely through AAV-based vaccination or AAV-based treatments [125-127].

A major issue of gene therapy for DMD is the huge size of *DMD* gene. As such, the dystrophin cDNA of 14kb far exceeds the 5kb packaging capacity of AAVs [128-130]. This problem was addressed with the discovery of microdystrophin, a shorter version of the *DMD* gene, which contains the important information for the production of a functional dystrophin protein, especially the coding region for binding to actin and to sarcoglycan complex. Therefore, it is expected that the expression of microdystrophin can keep patients at a stable state for a long period of time [131, 132]. Despite some initial promising results, an important question to be answered is the durability of gene transfer therapy. However, the treatment benefit will be potentially long term, since skeletal muscle cells are non-dividing and long-lived, while cardiomyocytes have a low turnover, with less than 50% of them being exchanged during a normal lifespan and the rate even decreases exponentially with age. A realistic goal would be an improvement of the disease trajectory of DMD patients compared to what could be expected from natural history studies [133-136]. However, further investigations are needed, particularly because in parallel with the effect of gene transfer therapy, there may be some extent of ongoing degeneration, which may lead to a clinical deterioration. Moreover, since AAV vectors are not integrating in the genome, the AAV-mediated dystrophin expression may decrease over time. There is currently no possibility of repeating gene transfer therapy, mainly due to the existence of neutralizing antibodies following the initial dose, which may affect subsequent administrations [137].

CONCLUSION

DMD should be regarded as a continuum, with signs and symptoms that may manifest very early and go unnoticed if there is no high suspicion. Although DMD still remains an incurable condition, significant progress has been made, especially in the field of genetic therapies and as such, an early diagnosis becomes more important, since it allows patients to receive timely any available modifying treatment or to participate in clinical trials. The main therapeutic goal is firstly to delay the progression to each milestone, especially to prolong ambulation as much as possible, and to partially restore respiratory, cardiac and skeletal muscle function. Finally, adhering to the guidelines and the international standards of care for DMD in a multidisciplinary approach should be strongly encouraged.

REFERENCES

[1] Mercuri E, Bonnemann CG, Muntoni F. Muscular dystrophies. *Lancet.* 2019;394:2025-2038.

[2] Ryder S, Leadley RM, Armstrong N, et al. The burden, epidemiology, costs and treatment for Duchenne muscular dystrophy: an evidence review. *Orphanet J Rare Dis.* 2017;12:79.

[3] Aartsma-Rus A, Van Deutekom JC, Fokkema IF, et al. Entries in the Leiden Duchenne muscular dystrophy mutation database: an overview of mutation types and paradoxical cases that confirm the reading-frame rule. *Muscle Nerve.* 2006;34:135-144.

[4] Duan D, Goemans N, Takeda S, et al. Duchenne muscular dystrophy. *Nat Rev Dis Primers.* 2021;7:13.

[5] Bushby K, Finkel R, Birnkrant DJ, et al. Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and pharmacological and psychosocial management. *Lancet Neurol.* 2010;9:77-93.

[6] Zhong J, Xie Y, Bhandari V, et al. Clinical and genetic characteristics of female dystrophinopathy carriers. *Mol Med Rep.* 2019;19:3035-3044.

[7] Kamdar F, Garry DJ. Dystrophin-Deficient Cardiomyopathy. *J Am Coll Cardiol.* 2016;67:2533-2546.

[8] Soltanzadeh P, Friez MJ, Dunn D, et al. Clinical and genetic characterization of manifesting carriers of DMD mutations. *Neuromuscul Disord.* 2010;20:499-504.

[9] López-Hernández LB, Gómez-Díaz B, Luna-Angulo AB, et al. Comparison of Mutation Profiles in the Duchenne Muscular Dystrophy Gene among Populations: Implications for Potential Molecular Therapies. *International Journal of Molecular Sciences.* 2015;16.

[10] Garcia S, de Haro T, Zafra-Ceres M, et al. Identification of de novo Mutations of Duchénnè/Becker Muscular Dystrophies in Southern Spain. *International Journal of Medical Sciences.* 2014;11:988-993.

[11] Kesari A, Pirra LN, Bremadesam L, et al. Integrated DNA, cDNA, and protein studies in Becker muscular dystrophy show high exception to the reading frame rule. *Hum Mutat.* 2008;29:728-737.

[12] Chen WJ, Lin QF, Zhang QJ, et al. Molecular analysis of the dystrophin gene in 407 Chinese patients with Duchenne/Becker muscular dystrophy by the combination of multiplex ligation-dependent probe amplification and Sanger sequencing. *Clin Chim Acta.* 2013;423:35-38.

[13] Yu H, Chen YC, Liu GL, et al. A De novo Mutation in Dystrophin Causing Muscular Dystrophy in a Female Patient. *Chin Med J (Engl).* 2017;130:2273-2278.

[14] Caskey CT, Nussbaum RL, Cohan LC, et al. Sporadic occurrence of Duchenne muscular dystrophy: evidence for new mutation. *Clin Genet.* 1980;18:329-341.
[15] Helderman-van den Enden AT, de Jong R, den Dunnen JT, et al. Recurrence risk due to germ line mosaicism: Duchenne and Becker muscular dystrophy. *Clin Genet.* 2009;75:465-472.
[16] Gao QQ, McNally EM. The Dystrophin Complex: Structure, Function, and Implications for Therapy. *Compr Physiol.* 2015;5:1223-1239.
[17] Prins KW, Humston JL, Mehta A, et al. Dystrophin is a microtubule-associated protein. *J Cell Biol.* 2009;186:363-369.
[18] Stone MR, O'Neill A, Catino D, et al. Specific interaction of the actin-binding domain of dystrophin with intermediate filaments containing keratin 19. *Mol Biol Cell.* 2005;16:4280-4293.
[19] Moser H. Duchenne muscular dystrophy: pathogenetic aspects and genetic prevention. *Hum Genet.* 1984;66:17-40.
[20] Hoffman EP, Brown RH, Jr., Kunkel LM. Dystrophin: the protein product of the Duchenne muscular dystrophy locus. *Cell.* 1987;51:919-928.
[21] Allen DG, Whitehead NP, Froehner SC. Absence of Dystrophin Disrupts Skeletal Muscle Signaling: Roles of Ca2+, Reactive Oxygen Species, and Nitric Oxide in the Development of Muscular Dystrophy. *Physiol Rev.* 2016;96:253-305.
[22] Aartsma-Rus A, van Putten M. Assessing functional performance in the mdx mouse model. *J Vis Exp.* 2014.
[23] Mokri B, Engel AG. Duchenne dystrophy: electron microscopic findings pointing to a basic or early abnormality in the plasma membrane of the muscle fiber. *Neurology.* 1975;25:1111-1120.
[24] Turner PR, Westwood T, Regen CM, et al. Increased protein degradation results from elevated free calcium levels found in muscle from mdx mice. *Nature.* 1988;335:735-738.
[25] Reeve JL, McArdle A, Jackson MJ. Age-related changes in muscle calcium content in dystrophin-deficient mdx mice. *Muscle Nerve.* 1997;20:357-360.
[26] Mareedu S, Million ED, Duan D, et al. Abnormal Calcium Handling in Duchenne Muscular Dystrophy: Mechanisms and Potential Therapies. *Front Physiol.* 2021;12:647010.
[27] Vallejo-Illarramendi A, Toral-Ojeda I, Aldanondo G, et al. Dysregulation of calcium homeostasis in muscular dystrophies. *Expert Rev Mol Med.* 2014;16:e16.
[28] Vandebrouck C, Martin D, Colson-Van Schoor M, et al. Involvement of TRPC in the abnormal calcium influx observed in dystrophic (mdx) mouse skeletal muscle fibers. *J Cell Biol.* 2002;158:1089-1096.
[29] Constantin B. Dystrophin complex functions as a scaffold for signalling proteins. *Biochim Biophys Acta.* 2014;1838:635-642.
[30] Bellinger AM, Reiken S, Carlson C, et al. Hypernitrosylated ryanodine receptor calcium release channels are leaky in dystrophic muscle. *Nat Med.* 2009;15:325-330.
[31] Kushnir A, Wajsberg B, Marks AR. Ryanodine receptor dysfunction in human disorders. *Biochim Biophys Acta Mol Cell Res.* 2018;1865:1687-1697.
[32] Voit A, Patel V, Pachon R, et al. Reducing sarcolipin expression mitigates Duchenne muscular dystrophy and associated cardiomyopathy in mice. *Nat Commun.* 2017;8:1068.
[33] Lai Y, Thomas GD, Yue Y, et al. Dystrophins carrying spectrin-like repeats 16 and 17 anchor nNOS to the sarcolemma and enhance exercise performance in a mouse model of muscular dystrophy. *J Clin Invest.* 2009;119:624-635.
[34] Sander M, Chavoshan B, Harris SA, et al. Functional muscle ischemia in neuronal nitric oxide synthase-deficient skeletal muscle of children with Duchenne muscular dystrophy. *Proc Natl Acad Sci U S A.* 2000;97:13818-13823.
[35] Kalogeris T, Baines CP, Krenz M, et al. Ischemia/Reperfusion. *Compr Physiol.* 2016;7:113-170.
[36] Kuroda Y, Togashi H, Uchida T, et al. Oxidative stress evaluation of skeletal muscle in ischemia-reperfusion injury using enhanced magnetic resonance imaging. *Scientific Reports.* 2020;10:10863.
[37] Khairallah RJ, Shi G, Sbrana F, et al. Microtubules underlie dysfunction in duchenne muscular dystrophy. *Sci Signal.* 2012;5:ra56.
[38] Moore TM, Lin AJ, Strumwasser AR, et al. Mitochondrial Dysfunction Is an Early Consequence of Partial or Complete Dystrophin Loss in mdx Mice. *Front Physiol.* 2020;11:690.
[39] Nghiem PP, Bello L, Stoughton WB, et al. Changes in Muscle Metabolism are Associated with Phenotypic Variability in Golden Retriever Muscular Dystrophy. *Yale J Biol Med.* 2017;90:351-360.
[40] Vila MC, Rayavarapu S, Hogarth MW, et al. Mitochondria mediate cell membrane repair and contribute to Duchenne muscular dystrophy. *Cell Death Differ.* 2017;24:330-342.
[41] Barker RG, Wyckelsma VL, Xu H, et al. Mitochondrial content is preserved throughout disease progression in the mdx mouse model of Duchenne muscular dystrophy, regardless of taurine supplementation. *Am J Physiol Cell Physiol.* 2018;314:C483-C491.
[42] Dumont NA, Rudnicki MA. Targeting muscle

stem cell intrinsic defects to treat Duchenne muscular dystrophy. *npj Regenerative Medicine.* 2016;1:16006.
[43] Burr AR, Molkentin JD. Genetic evidence in the mouse solidifies the calcium hypothesis of myofiber death in muscular dystrophy. *Cell Death Differ.* 2015;22:1402-1412.
[44] Birnkrant DJ, Bushby K, Bann CM, et al. Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and neuromuscular, rehabilitation, endocrine, and gastrointestinal and nutritional management. *Lancet Neurol.* 2018;17:251-267.
[45] van Dommelen P, van Dijk O, de Wilde JA, et al. Early developmental milestones in Duchenne muscular dystrophy. *Dev Med Child Neurol.* 2020;62:1198-1204.
[46] Cyrulnik SE, Fee RJ, De Vivo DC, et al. Delayed developmental language milestones in children with Duchenne's muscular dystrophy. *J Pediatr.* 2007;150:474-478.
[47] Parsons EP, Clarke AJ, Bradley DM. Developmental progress in Duchenne muscular dystrophy: lessons for earlier detection. *Eur J Paediatr Neurol.* 2004;8:145-153.
[48] Fujino H, Saito T, Matsumura T, et al. Autism spectrum disorders are prevalent among patients with dystrophinopathies. *Neurol Sci.* 2018;39:1279-1282.
[49] Hendriksen JG, Vles JS. Neuropsychiatric disorders in males with duchenne muscular dystrophy: frequency rate of attention-deficit hyperactivity disorder (ADHD), autism spectrum disorder, and obsessive--compulsive disorder. *J Child Neurol.* 2008;23:477-481.
[50] Emery AE. The muscular dystrophies. *Lancet.* 2002;359:687-695.
[51] Chen YW, Nagaraju K, Bakay M, et al. Early onset of inflammation and later involvement of TGFbeta in Duchenne muscular dystrophy. *Neurology.* 2005;65:826-834.
[52] Allen NM, Ewer A, Nakou V, et al. Unusual Presentations of Dystrophinopathies in Childhood. *Pediatrics.* 2018;141:S510-S514.
[53] Serratrice J, Chabrol B, Attarrian S, et al. Dystrophinopathie pseudométabolique sans anomalie immunohistochimique. *Revue neurologique (Paris).* 2000;156:175-178.
[54] Peverelli L, Testolin S, Villa L, et al. Histologic muscular history in steroid-treated and untreated patients with Duchenne dystrophy. *Neurology.* 2015;85:1886-1893.
[55] Khirani S, Ramirez A, Aubertin G, et al. Respiratory muscle decline in Duchenne muscular dystrophy. *Pediatr Pulmonol.* 2014;49:473-481.
[56] Mayer OH, Finkel RS, Rummey C, et al. Characterization of pulmonary function in Duchenne Muscular Dystrophy. *Pediatr Pulmonol.* 2015;50:487-494.
[57] Finder J, Mayer OH, Sheehan D, et al. Pulmonary Endpoints in Duchenne Muscular Dystrophy. A Workshop Summary. *Am J Respir Crit Care Med.* 2017;196:512-519.
[58] LoMauro A, Romei M, Gandossini S, et al. Evolution of respiratory function in Duchenne muscular dystrophy from childhood to adulthood. *Eur Respir J.* 2018;51.
[59] Sheehan DW, Birnkrant DJ, Benditt JO, et al. Respiratory Management of the Patient With Duchenne Muscular Dystrophy. *Pediatrics.* 2018;142:S62-S71.
[60] Passamano L, Taglia A, Palladino A, et al. Improvement of survival in Duchenne Muscular Dystrophy: retrospective analysis of 835 patients. *Acta Myol.* 2012;31:121-125.
[61] Rall S, Grimm T. Survival in Duchenne muscular dystrophy. *Acta Myol.* 2012;31:117-120.
[62] Abresch R, McDonald C, Henricson E, et al. P.11.11 Pulmonary function characteristics of boys with Duchenne Muscular Dystrophy by age groups, ambulatory status and steroid use. *Neuromuscular Disorders.* 2013;23:801-802.
[63] Mavrogeni SI, Markousis-Mavrogenis G, Papavasiliou A, et al. Cardiac Involvement in Duchenne Muscular Dystrophy and Related Dystrophinopathies. *Methods Mol Biol.* 2018;1687:31-42.
[64] D'Amario D, Amodeo A, Adorisio R, et al. A current approach to heart failure in Duchenne muscular dystrophy. *Heart.* 2017;103:1770-1779.
[65] D'Amario D, Gowran A, Canonico F, et al. Dystrophin Cardiomyopathies: Clinical Management, Molecular Pathogenesis and Evolution towards Precision Medicine. *J Clin Med.* 2018;7.
[66] Adorisio R, Mencarelli E, Cantarutti N, et al. Duchenne Dilated Cardiomyopathy: Cardiac Management from Prevention to Advanced Cardiovascular Therapies. *J Clin Med.* 2020;9.
[67] Papa AA, D'Ambrosio P, Petillo R, et al. Heart transplantation in patients with dystrophinopathic cardiomyopathy: Review of the literature and personal series. *Intractable Rare Dis Res.* 2017;6:95-101.
[68] Perri G, Filippelli S, Adorisio R, et al. Left ventricular assist device as destination therapy in cardiac end-stage dystrophinopathies: Midterm results. *J Thorac Cardiovasc Surg.* 2017;153:669-674.
[69] Ryan TD, Jefferies JL, Sawnani H, et al. Implantation of the HeartMate II and HeartWare left ventricular assist devices in patients with duchenne muscular dystrophy: lessons learned from the first applications. *ASAIO J.* 2014;60:246-248.

[70] Yamamoto T, Awano H, Zhang Z, et al. Cardiac Dysfunction in Duchenne Muscular Dystrophy Is Less Frequent in Patients With Mutations in the Dystrophin Dp116 Coding Region Than in Other Regions. *Circ Genom Precis Med.* 2018;11:e001782.
[71] Barp A, Bello L, Politano L, et al. Genetic Modifiers of Duchenne Muscular Dystrophy and Dilated Cardiomyopathy. *PLoS One.* 2015;10:e0141240.
[72] Bello L, D'Angelo G, Villa M, et al. Genetic modifiers of respiratory function in Duchenne muscular dystrophy. *Ann Clin Transl Neurol.* 2020;7:786-798.
[73] Trucco F, Domingos JP, Tay CG, et al. Cardiorespiratory Progression Over 5 Years and Role of Corticosteroids in Duchenne Muscular Dystrophy: A Single-Site Retrospective Longitudinal Study. *Chest.* 2020;158:1606-1616.
[74] van Ruiten HJ, Straub V, Bushby K, et al. Improving recognition of Duchenne muscular dystrophy: a retrospective case note review. *Arch Dis Child.* 2014;99:1074-1077.
[75] Lalic T, Vossen RH, Coffa J, et al. Deletion and duplication screening in the DMD gene using MLPA. *Eur J Hum Genet.* 2005;13:1231-1234.
[76] Aartsma-Rus A, Ginjaar IB, Bushby K. The importance of genetic diagnosis for Duchenne muscular dystrophy. *J Med Genet.* 2016;53:145-151.
[77] Okubo M, Minami N, Goto K, et al. Genetic diagnosis of Duchenne/Becker muscular dystrophy using next-generation sequencing: validation analysis of DMD mutations. *J Hum Genet.* 2016;61:483-489.
[78] Schussler SC, Gerhalter T, Abicht A, et al. Rare intronic mutation between Exon 62 and 63 (c.9225-285A>G) of the dystrophin gene associated with atypical BMD phenotype. *Neuromuscul Disord.* 2020;30:680-684.
[79] Anderson LV, Davison K. Multiplex Western blotting system for the analysis of muscular dystrophy proteins. *Am J Pathol.* 1999;154:1017-1022.
[80] Anthony K, Arechavala-Gomeza V, Taylor LE, et al. Dystrophin quantification: Biological and translational research implications. *Neurology.* 2014;83:2062-2069.
[81] Bushby K, Finkel R, Birnkrant DJ, et al. Diagnosis and management of Duchenne muscular dystrophy, part 2: implementation of multidisciplinary care. *Lancet Neurol.* 2010;9:177-189.
[82] Birnkrant DJ, Bushby K, Bann CM, et al. Diagnosis and management of Duchenne muscular dystrophy, part 2: respiratory, cardiac, bone health, and orthopaedic management. *Lancet Neurol.* 2018;17:347-361.
[83] Birnkrant DJ, Bushby K, Bann CM, et al. Diagnosis and management of Duchenne muscular dystrophy, part 3: primary care, emergency management, psychosocial care, and transitions of care across the lifespan. *Lancet Neurol.* 2018;17:445-455.
[84] Hamdani Y, Mistry B, Gibson BE. Transitioning to adulthood with a progressive condition: best practice assumptions and individual experiences of young men with Duchenne muscular dystrophy. *Disabil Rehabil.* 2015;37:1144-1151.
[85] Andrews JG, Conway K, Westfield C, et al. Implementation of Duchenne Muscular Dystrophy Care Considerations. *Pediatrics.* 2018;142.
[86] Saito T, Kawai M, Kimura E, et al. Study of Duchenne muscular dystrophy long-term survivors aged 40 years and older living in specialized institutions in Japan. *Neuromuscul Disord.* 2017;27:107-114.
[87] Ricotti V, Selby V, Ridout D, et al. Respiratory and upper limb function as outcome measures in ambulant and non-ambulant subjects with Duchenne muscular dystrophy: A prospective multicentre study. *Neuromuscul Disord.* 2019;29:261-268.
[88] McNally EM, Kaltman JR, Benson DW, et al. Contemporary cardiac issues in Duchenne muscular dystrophy. Working Group of the National Heart, Lung, and Blood Institute in collaboration with Parent Project Muscular Dystrophy. *Circulation.* 2015;131:1590-1598.
[89] Duboc D, Meune C, Pierre B, et al. Perindopril preventive treatment on mortality in Duchenne muscular dystrophy: 10 years' follow-up. *Am Heart J.* 2007;154:596-602.
[90] Suthar R, Reddy BVC, Malviya M, et al. Bone density and bone health alteration in boys with Duchenne Muscular Dystrophy: a prospective observational study. *J Pediatr Endocrinol Metab.* 2021;34:573-581.
[91] Yilmaz O, Karaduman A, Topaloglu H. Prednisolone therapy in Duchenne muscular dystrophy prolongs ambulation and prevents scoliosis. *Eur J Neurol.* 2004;11:541-544.
[92] Lo Cascio CM, Goetze O, Latshang TD, et al. Gastrointestinal Dysfunction in Patients with Duchenne Muscular Dystrophy. *PLoS One.* 2016;11:e0163779.
[93] Bertrand LA, Askeland EJ, Mathews KD, et al. Prevalence and bother of patient-reported lower urinary tract symptoms in the muscular dystrophies. *J Pediatr Urol.* 2016;12:398 e391-398 e394.
[94] Matsumura T, Saito T, Fujimura H, et al. [Renal dysfunction is a frequent complication in patients with advanced stage of Duchenne muscular dystrophy]. *Rinsho Shinkeigaku.* 2012;52:211-217.

[95] Banihani R, Smile S, Yoon G, et al. Cognitive and Neurobehavioral Profile in Boys With Duchenne Muscular Dystrophy. *J Child Neurol.* 2015;30:1472-1482.
[96] McDonald CM, Gordish-Dressman H, Henricson EK, et al. Longitudinal pulmonary function testing outcome measures in Duchenne muscular dystrophy: Long-term natural history with and without glucocorticoids. *Neuromuscul Disord.* 2018;28:897-909.
[97] Alman BA, Raza SN, Biggar WD. Steroid treatment and the development of scoliosis in males with duchenne muscular dystrophy. *J Bone Joint Surg Am.* 2004;86:519-524.
[98] Kurz LT, Mubarak SJ, Schultz P, et al. Correlation of scoliosis and pulmonary function in Duchenne muscular dystrophy. *J Pediatr Orthop.* 1983;3:347-353.
[99] Barber BJ, Andrews JG, Lu Z, et al. Oral corticosteroids and onset of cardiomyopathy in Duchenne muscular dystrophy. *J Pediatr.* 2013;163:1080-1084 e1081.
[100] Markham LW, Kinnett K, Wong BL, et al. Corticosteroid treatment retards development of ventricular dysfunction in Duchenne muscular dystrophy. *Neuromuscul Disord.* 2008;18:365-370.
[101] Griggs RC, Miller JP, Greenberg CR, et al. Efficacy and safety of deflazacort vs prednisone and placebo for Duchenne muscular dystrophy. *Neurology.* 2016;87:2123-2131.
[102] Guglieri M, Bushby K, McDermott MP, et al. Developing standardized corticosteroid treatment for Duchenne muscular dystrophy. *Contemp Clin Trials.* 2017;58:34-39.
[103] Stephenson AA, Flanigan KM. Gene editing and modulation for Duchenne muscular dystrophy. *Prog Mol Biol Transl Sci.* 2021;182:225-255.
[104] Bushby K, Finkel R, Wong B, et al. Ataluren treatment of patients with nonsense mutation dystrophinopathy. *Muscle Nerve.* 2014;50:477-487.
[105] Haas M, Vlcek V, Balabanov P, et al. European Medicines Agency review of ataluren for the treatment of ambulant patients aged 5 years and older with Duchenne muscular dystrophy resulting from a nonsense mutation in the dystrophin gene. *Neuromuscul Disord.* 2015;25:5-13.
[106] McDonald CM, Campbell C, Torricelli RE, et al. Ataluren in patients with nonsense mutation Duchenne muscular dystrophy (ACT DMD): a multicentre, randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet.* 2017;390:1489-1498.
[107] Mercuri E, Muntoni F, Osorio AN, et al. Safety and effectiveness of ataluren: comparison of results from the STRIDE Registry and CINRG DMD Natural History Study. *J Comp Eff Res.* 2020;9:341-360.
[108] Harding PL, Fall AM, Honeyman K, et al. The influence of antisense oligonucleotide length on dystrophin exon skipping. *Mol Ther.* 2007;15:157-166.
[109] Niks EH, Aartsma-Rus A. Exon skipping: a first in class strategy for Duchenne muscular dystrophy. *Expert Opin Biol Ther.* 2017;17:225-236.
[110] Aartsma-Rus A, Fokkema I, Verschuuren J, et al. Theoretic applicability of antisense-mediated exon skipping for Duchenne muscular dystrophy mutations. *Hum Mutat.* 2009;30:293-299.
[111] Bladen CL, Rafferty K, Straub V, et al. The TREAT-NMD Duchenne muscular dystrophy registries: conception, design, and utilization by industry and academia. *Hum Mutat.* 2013;34:1449-1457.
[112] Wang RT, Barthelemy F, Martin AS, et al. DMD genotype correlations from the Duchenne Registry: Endogenous exon skipping is a factor in prolonged ambulation for individuals with a defined mutation subtype. *Hum Mutat.* 2018;39:1193-1202.
[113] Alfano LN, Charleston JS, Connolly AM, et al. Long-term treatment with eteplirsen in nonambulatory patients with Duchenne muscular dystrophy. *Medicine (Baltimore).* 2019;98:e15858.
[114] Mendell JR, Goemans N, Lowes LP, et al. Longitudinal effect of eteplirsen versus historical control on ambulation in Duchenne muscular dystrophy. *Ann Neurol.* 2016;79:257-271.
[115] Drug U. FDA grants accelerated approval to first targeted treatment for rare Duchenne muscular dystrophy mutation. 2019. 2019.
[116] Casimersen (Amondys 45) for Duchenne muscular dystrophy. *Med Lett Drugs Ther.* 2021;63:e104-e105.
[117] Colella P, Ronzitti G, Mingozzi F. Emerging Issues in AAV-Mediated In Vivo Gene Therapy. *Mol Ther Methods Clin Dev.* 2018;8:87-104.
[118] Bulcha JT, Wang Y, Ma H, et al. Viral vector platforms within the gene therapy landscape. *Signal Transduct Target Ther.* 2021;6:53.
[119] Lau CH, Suh Y. In vivo genome editing in animals using AAV-CRISPR system: applications to translational research of human disease. *F1000Res.* 2017;6:2153.
[120] Mingozzi F, High KA. Immune responses to AAV vectors: overcoming barriers to successful gene therapy. *Blood.* 2013;122:23-36.
[121] Capasso C, Garofalo M, Hirvinen M, et al.

The evolution of adenoviral vectors through genetic and chemical surface modifications. *Viruses.* 2014;6:832-855.
[122] Bolt MW, Brady JT, Whiteley LO, et al. Development challenges associated with rAAV-based gene therapies. *J Toxicol Sci.* 2021;46:57-68.
[123] Wang D, Tai PWL, Gao G. Adeno-associated virus vector as a platform for gene therapy delivery. *Nat Rev Drug Discov.* 2019;18:358-378.
[124] Buning H, Schmidt M. Adeno-associated Vector Toxicity-To Be or Not to Be? *Mol Ther.* 2015;23:1673-1675.
[125] Long BR, Sandza K, Holcomb J, et al. The Impact of Pre-existing Immunity on the Nonclinical Pharmacodynamics of AAV5-Based Gene Therapy. *Mol Ther Methods Clin Dev.* 2019;13:440-452.
[126] Calcedo R, Wilson JM. Humoral Immune Response to AAV. *Front Immunol.* 2013;4:341.
[127] Boisgerault F, Mingozzi F. The Skeletal Muscle Environment and Its Role in Immunity and Tolerance to AAV Vector-Mediated Gene Transfer. *Curr Gene Ther.* 2015;15:381-394.
[128] Naso MF, Tomkowicz B, Perry WL, 3rd, et al. Adeno-Associated Virus (AAV) as a Vector for Gene Therapy. *BioDrugs.* 2017;31:317-334.
[129] Ferrer A, Foster H, Wells KE, et al. Long-term expression of full-length human dystrophin in transgenic mdx mice expressing internally deleted human dystrophins. *Gene Ther.* 2004;11:884-893.
[130] Lostal W, Kodippili K, Yue Y, et al. Full-length dystrophin reconstitution with adeno-associated viral vectors. *Hum Gene Ther.* 2014;25:552-562.
[131] Zhao J, Kodippili K, Yue Y, et al. Dystrophin contains multiple independent membrane-binding domains. *Hum Mol Genet.* 2016;25:3647-3653.
[132] Mendell JR, Sahenk Z, Lehman K, et al. Assessment of Systemic Delivery of rAAVrh74.MHCK7.micro-dystrophin in Children With Duchenne Muscular Dystrophy: A Nonrandomized Controlled Trial. *JAMA Neurol.* 2020;77:1122-1131.
[133] Asher DR, Thapa K, Dharia SD, et al. Clinical development on the frontier: gene therapy for duchenne muscular dystrophy. *Expert Opin Biol Ther.* 2020;20:263-274.
[134] Partridge TA. Cells that participate in regeneration of skeletal muscle. *Gene Ther.* 2002;9:752-753.
[135] Spalding KL, Bhardwaj RD, Buchholz BA, et al. Retrospective birth dating of cells in humans. *Cell.* 2005;122:133-143.
[136] Yin H, Price F, Rudnicki MA. Satellite cells and the muscle stem cell niche. *Physiol Rev.* 2013;93:23-67.
[137] Ilyinskii PO, Michaud AM, Roy CJ, et al. Enhancement of liver-directed transgene expression at initial and repeat doses of AAV vectors admixed with ImmTOR nanoparticles. *Sci Adv.* 2021;7.
[138] Darras BT, Urion DK, Ghosh PS. Dystrophinopathies. In: Adam MP, Ardinger HH, Pagon RA, et al., eds. *GeneReviews((R)).* Seattle (WA), 1993.