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Principles and Practice of the Muscular Dystrophies

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In loving memory of Dr. V. Narayanaswami, Mrs. Janaki Narayanaswami, and Ms. Fumiko Hamada.
We dedicate this book humbly and reverently to all who have suffered, are suffering, or will suffer from muscular dystrophy. We hope that the advances we describe in this book will result in cures for these disorders in the near future.

Foreword

The diagnostic evaluation and management of patients with muscular dystrophies can be quite daunting. I have always felt the most important and key first step is identifying the pattern of weakness on clinical examination. Increasingly, complementary testing such as imaging of muscle by magnetic resonance imaging and ultrasound have further advanced our understanding of patterns of muscle weakness and progression of muscle degeneration in these various dystrophies. The explosion of advances in genetics has led to the identification of many various types of muscular dystrophy that share similar clinical phenotypes and, likewise, the discovery of different clinical phenotypes associated with alterations in specific genes. With genetic testing becoming more commercially available, the use of invasive muscle biopsies has diminished. However, biopsies remain invaluable, particularly when, as in the majority of cases still at this time the diagnosis remains unclear even with extensive genetic testing.

I would like to congratulate the editors, Drs. Teerin Liewluck and Pushpa Narayanaswami, on this amazing new textbook, *Principles and Practice of the Muscular Dystrophies*. All the major forms of muscular dystrophy are covered by the leading experts in the field and are quite up to date. The need for a multidisciplinary team approach to care is emphasized. There is an important chapter developed to rehabilitation, often neglected in neuromuscular textbooks, which discusses the use of orthotics and assistive technologies to reduce disability and improve quality of life. The complexity and specific drawbacks of different forms of genetic testing are carefully explained, the utility of imaging and muscle biopsies to enhance diagnosis, and the importance of genetic counselling are described. With these advances in genetics has come a better understanding of the pathogenic bases of these different diseases which are well covered. This in turn has also led to new forms of gene therapy, including antisense oligonucleotides (ASO) that induce exon skipping, oligonucleotides that knock down mRNA expression through RNA interference (RNAi), gene replacement utilizing viral vectors, and more in development. The book even has a chapter devoted to clinical trial design.

The editors and all the authors should be commended on this excellent work, which will facilitate diagnosis and improve the care of patients with muscular dystrophy. It addresses the need for a comprehensive reference incorporating the advances in radiology, genetic testing, and genetic therapies. I believe that it will become an important resource and will be on the bookshelves of all clinicians who help manage these patients.

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Preface

"Technology has advanced more in the last thirty years than in the previous two thousand. The exponential increase in advancement will only continue," said Niels Bohr, the Danish physicist (1885–1962). This sentiment has never been truer than in the understanding of muscular dystrophies. From an initial descriptive period in the nineteenth century, to subsequent attempts at nosography of the disease, we arrived in the genetic era in 1987 when the gene for Duchenne muscular dystrophy was cloned and its protein product, dystrophin, identified by Hoffman, Kunkel, and colleagues. The rest, as they say, is indeed history. The classification of muscular dystrophies is now based on their genetic identity, and ongoing identification of the underlying abnormality associated with each genetic defect has provided a deeper understanding of not only the mechanisms underpinning abnormal muscle structure and function in these disorders, but also of normal muscle structure and function. The Holy Grail is an effective cure, and the last two decades have seen the beginnings of this glorious achievement.

In this book, we have attempted to provide a twenty-first century update on these disorders. The phenotypic approach to clinical diagnosis remains the basis of diagnosis, but the availability of next-generation sequencing techniques has revolutionized the diagnostic algorithm of the disease. Genetic testing has superseded muscle biopsy in the algorithm. Nevertheless, Duchenne's histologic harpoon is not ready to be laid to rest. The muscle biopsy remains relevant to confirm the effect of a genetic variant on myopathological changes and protein expression in muscle and to identify the pathological findings associated with novel genes. The phenotypic and genotypic heterogeneity of many muscular dystrophies is becoming increasingly apparent. Knowledge of the spectrum of extramuscular manifestations, particularly cardiovascular, informs judicious screening to improve outcomes. Muscle imaging has come of age as a diagnostic tool and is being investigated as a biomarker. Biomarkers in blood and tissue are being identified. Advances in rehabilitation interventions improve the quality of life at all stages of the disease. Finally, DNA- and RNA-based therapies and gene replacements have arrived. There is much to do yet, and we hope that this book will serve as a basic reference in this molecular era of muscular dystrophies.

This book would not have seen the light of day without Dr. Daniel Tarsy's encouragement. The authors who have so graciously provided their scientific, clinical, and literary expertise to the completion of this book are an absolutely stellar congregation of scientists and clinicians, and we cannot thank them enough. We extend our appreciation to Swathiga Karthikeyan, Gregory Sutorius, and Springer Nature Publishing for keeping us on task and making this book a reality. Finally, our grateful thanks to our families—Padma, Tom, Alamelu, Shruti, and Varun and to Eriko, Saya, Sota, Nikorn, and Supawadee for their unending support without which this work would not have been possible.

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An Introduction to the Muscular Dystrophies

1

Teerin Liewluck and Pushpa Narayanaswami

Introduction

The term muscular dystrophy is derived from the Latin, *musculus* (muscle), and the Greek, *dys* (bad, ill or difficult) and *troph* (nourishment). Muscular dystrophies encompass a large, clinically and genetically heterogenous group of primary progressive diseases of skeletal muscle leading to muscle wasting and weakness, with variable age of onset, ranging from in utero to late adulthood. Necrotic and regenerating myofibers and an increase in endomysial and perimysial fibrous and fatty connective tissue are the pathological hallmarks of muscular dystrophies, and these features are often referred to as "dystrophic changes" (Fig. 1.1) [1]. These disorders are often associated with extramuscular manifestations, most commonly cardiac and respiratory, but also with ophthalmological, dermatological, cognitive, and other manifestations. These extramuscular features can narrow the differential diagnosis of the type of dystrophy, and influence management. This chapter provides a broad overview of muscular dystrophies and their classification, pathogenesis, diagnosis, and management. Specific muscular dystrophies are discussed in their dedicated chapters.

In the pre-genetic era, the classification of muscular dystrophies was based on the pattern of weakness, age of onset, and mode of inheritance, if known. (Table 1.1). The first description of muscular dystrophies perhaps goes as far back as 1830, when Sir Charles Bell, famous for his description of facial paralysis, may have described a case of muscular dystrophy [2]. However, it was not until 1852, when Edward Meryon, an English neurologist, provided the first detailed clinicopathological description of a disorder of progressive muscle weakness affecting young boys. The first eponym associated with a muscular dystrophy, Duchenne muscular dystrophy (DMD), was that of Guillaume-Benjamin-Amand Duchenne, a French neurologist, who, in 1868, described all the cardinal clinical features of the disease, except for the hereditary component, calling it "progressive muscular atrophy with degeneration" [3]. By the late nineteenth century, another clinically distinct muscular dystrophy, currently known as facioscapulohumeral muscular dystrophy (FSHD), was recognized [4]. Patients with myotonic dystrophy and oculopharyngeal muscular dystrophy (OPMD) were first reported in 1909 and 1915, respectively [5, 6]. The term limb-girdle muscular dystrophy (LGMD) was coined in 1953 to describe a distinct type of autosomally inherited, proximal muscular dystrophy, which was clinically distinguishable from the hitherto recognized muscular dystrophies of that time [7]. In 1955, Dr. Peter Emil Becker, a German neurologist, described a new X-linked muscular dystrophy with later age of onset and milder phenotype compared to DMD, which was subsequently named Becker muscular dystrophy (BMD) [8]. Approximately a decade later, the first cases of Emery-Dreifuss muscular dystrophy (EDMD) were reported [9]. In 1977, Satoyoshi and Kinoshita described an autosomal dominant myopathy with preferential involvement of ocular, facial, bulbar and distal limb muscles, which is now known as oculopharyngodistal myopathy (OPDM) [10].

Owing to advances in molecular genetics, we now know that there are 2 genetically distinct subtypes of myotonic dystrophies [type 1 (DM1) and type 2 (DM2)] and FSHD [type 1 (FSHD1) and type 2 (FSHD2)]. LGMD and EDMD are not merely single entities, but, in fact, there are at least 30 genetically distinct subtypes of LGMD, and 6 genetic subtypes of EDMD. These further highlight the genetic heterogeneity of muscular dystrophies. Moreover, there is evidence that a mutation in a single gene can give rise to more than one clinical or histopathological phenotype (phenotypic heterogeneity), expanding the disease spectrum of individual

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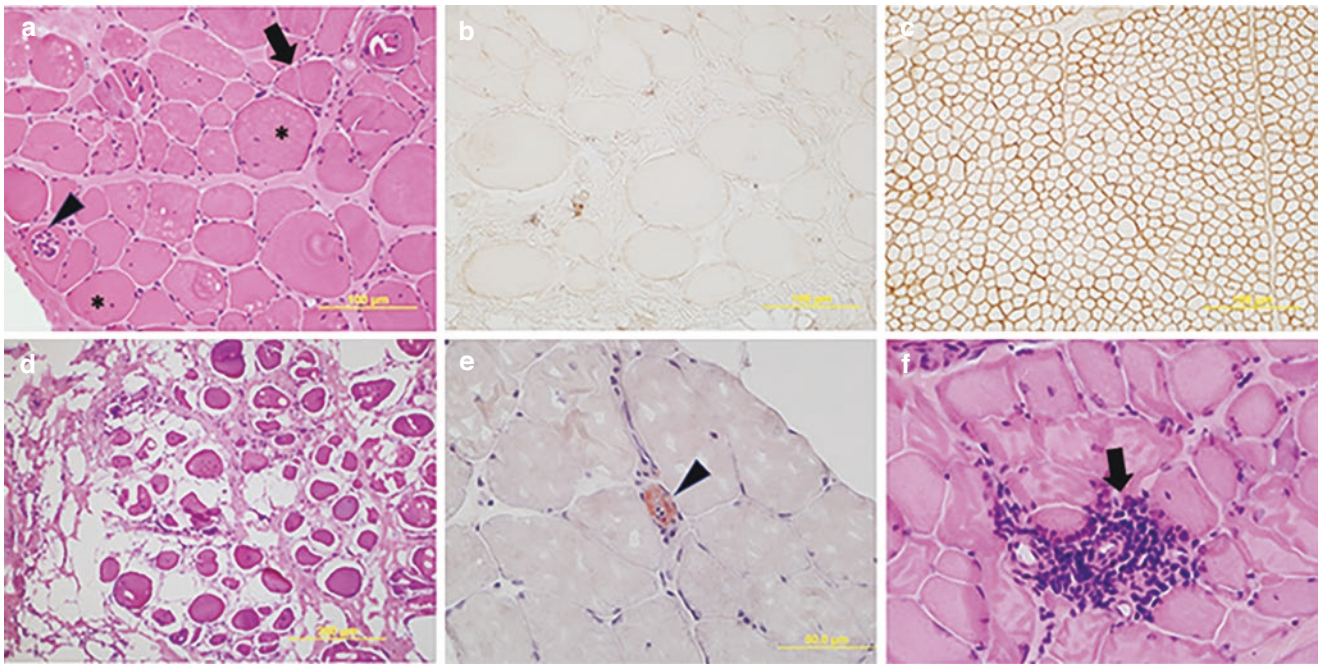

Fig. 1.1 Histopathology of muscular dystrophy. (**a**, hematoxylin and eosin) Muscle biopsy of a patient with early stage Duchenne muscular dystrophy shows a marked variation in fiber size, a mild increase of fibers harboring internal nuclei (asterisk), scattered fiber splitting (arrow), occasional necrotic fibers (arrowhead) and increased endomysial connective tissue. Dystrophin C-terminal immunoreactivity is absent (**b**) compared to control (**c**). (**d**, hematoxylin and eosin) Muscle biopsy of a patient with advanced stage limb-girdle muscular dystrophy (LGMD) type R9 (LGMD-R9) displays marked increase of perimysial and endomysial fibrous and fatty connective tissue, consistent with near-endstage muscle. (**e**, Congo red) Muscle biopsy of an LGMD type R2 (LGMD-R2) patient reveals congophilic deposit in the blood vessel (arrowhead). (**f**, hematoxylin and eosin) There is a small perivascular collection of mononuclear cells in the perimysium (arrow)

Table 1.1 Classification of muscular dystrophies

Diseases	Inheritance	Gene(s) or underlying genetic defects	Typical age at onset	Typical pattern of weakness
Congenital muscular dystrophies	AR or AD	Several genes	In utero-infantile	Proximal predominant
Duchenne muscular dystrophy	XR	*DMD*	Early childhood	Proximal predominant
Becker muscular dystrophy	XR	*DMD*	Late childhood-adulthood	Proximal predominant
Myotonic dystrophies (DM)				
DM1	AD	CTG expansion in 3' UTR of *DMPK*	In utero-adulthood	Distal predominant and facial weakness
DM2	AD	CCTG expansion in intron 1 of *CNBP*	Adulthood	Proximal predominant with or without mild facial weakness
Facioscapulohumeral muscular dystrophies (FSHD)				
FSHD1	AD	Hypomethylation of contracted D4Z4 repeats on chromosome 4q35 and 4qA haplotype	Infantile to adulthood	Facial and scapuloperoneal weakness
FSHD2	Digenic	Hypomethylation of normal sized D4Z4 repeats and 4qA haplotype secondary to *SMCHD1, DNMT3B,* or *LRIF1* mutations	Infantile to adulthood	Facial and scapuloperoneal weakness
Limb-girdle muscular dystrophies (LGMD)				
LGMD-D	AD	Several genes	Childhood-adulthood	Proximal predominant
LGMD-R	AR	Several genes	Childhood-adulthood	Proximal predominant
Emery-Dreifuss muscular dystrophies	XR, AD or AR	Several genes	Childhood-adulthood	Scapuloperoneal weakness

Table 1.1 (continued)

Diseases	Inheritance	Gene(s) or underlying genetic defects	Typical age at onset	Typical pattern of weakness
Oculopharyngeal muscular dystrophy	AD > AR	GCN repeat expansion in exon 1 > point mutations in *PABPN1*; N represents any A, T, C or G nucleotide	Adulthood	Ocular, pharyngeal and proximal weakness
Oculopharyngodistal myopathy	AD	CGG expansion in 5'UTR of *LRP12, GIPC1, NOTCH2NLC* and *RILPL1*	Adulthood	Ocular, pharyngeal, facial and distal weakness
Myofibrillar myopathies (MFM)	AD, AR or XR	Several genes	Childhood-adulthood	Various patterns of weakness
Distal myopathies[a]	AD, AR or XR	Several genes	Childhood-adulthood	Distal predominant weakness

AD autosomal dominant, *AR* autosomal recessive, *UTR* untranslated region, *XR* X-linked recessive
[a] Not all subtypes of distal myopathies are considered muscular dystrophies

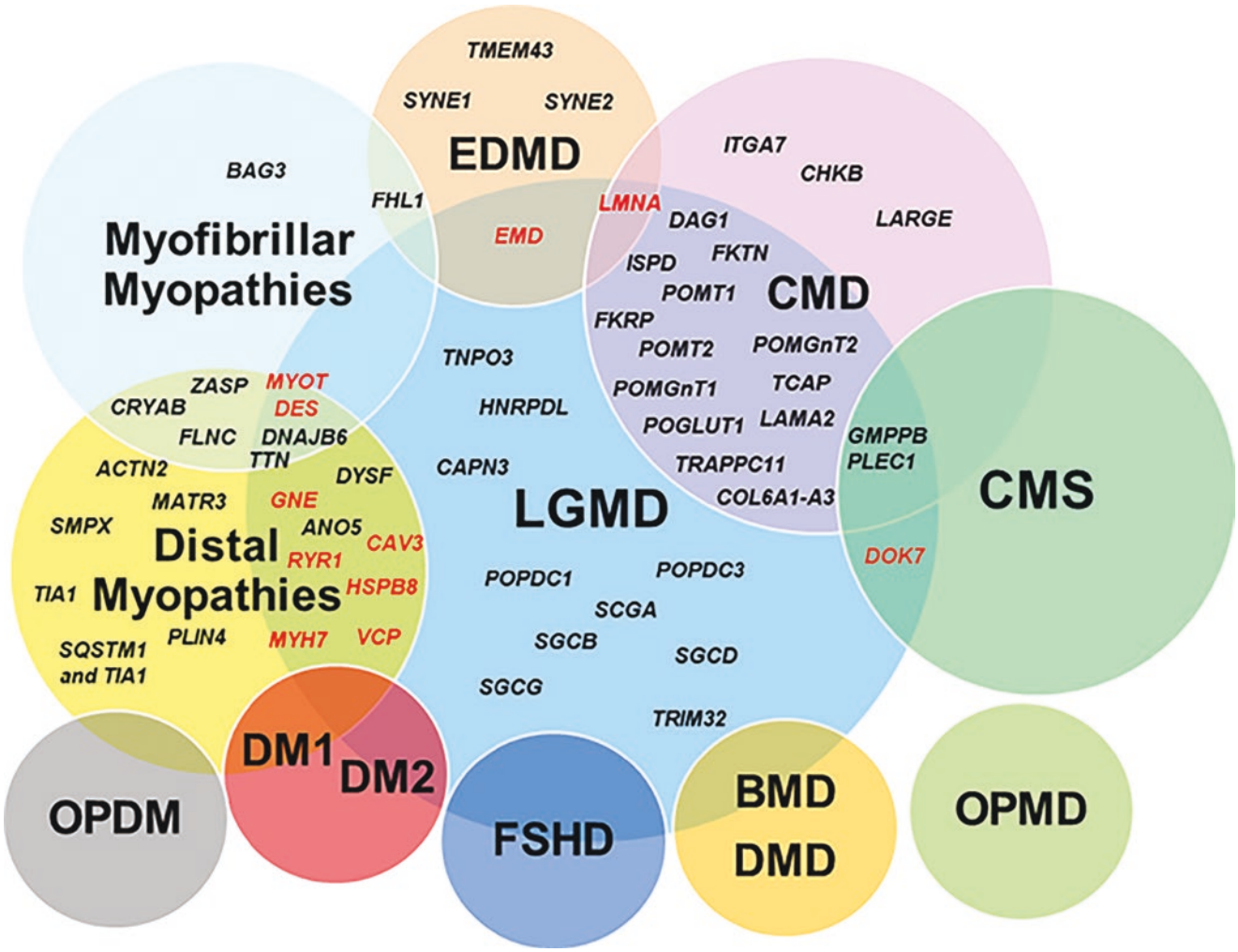

Fig. 1.2 Venn diagram displays overlapping clinical phenotypes between muscular dystrophy subtypes. In red font are the genes wherein mutations can cause a limb-girdle muscular dystrophy (LGMD)-like phenotype but are not classified as LGMD. It is important to note that Becker muscular dystrophy (BMD) can mimic LGMD. BMD is not classified as LGMD because of its X-linked inheritance. A few congenital myasthenic syndrome (CMS) genes are also shown

gene defects and blurring the boundaries of each muscular dystrophy subtype (Fig. 1.2). For example, mutations in the lamin A/C-encoding gene (*LMNA*) can cause EDMD, LGMD and congenital muscular dystrophy (CMD) [11], and defects in the dysferlin (*DYSF*)- and anoctamin 5 (*ANO5*)-encoding genes give rise to both autosomal recessive LGMD (LGMD-R2 and R12, respectively) and to the Miyoshi distal myopathy phenotype [12]. In fact, members of the same family bearing a mutation in these genes may present with either a LGMD or Miyoshi phenotype; these phenotypes tend to merge over time with disease progression. Mutations in the genes coding for merosin (*LAMA2*) and collagen VI (*COL6A1, COL6A2* and *COL6A3*) were first described in CMD patients and later in LGMD patients [1]. Some inherited diseases of skeletal muscle [e.g. myofibrillar myopathies (MFM) and certain subtypes of distal myopathies] are mislabeled as myopathies despite their progressive nature and the histopathological feature of a dystrophy [13, 14].

Pathogenesis

Defects in several components of muscle fibers, ranging from the extracellular matrix, basement membrane, sarcolemma, sarcomere, sarcoplasmic proteins, nuclear envelope and nuclear matrix can cause muscular dystrophies. The sarcolemma is subject to constant shear-stress due to contractile forces transmitted to it from the sarcomere. The resultant damage is repaired by proteins such as dysferlin and anoctamin-5. Mutations in genes encoding sarcolemmal proteins typically lead to destabilization of the sarcolemma and subsequent myofiber degeneration [15]. However, defects in some sarcolemmal proteins (dysferlin and anoctamin-5) primarily interfere with the repair machinery rather than the integrity of the plasma membrane [16].

Generally, mutations in the extracellular matrix and basement membrane proteins (e.g., merosin and collagen VI) cause CMD, while mutations in sarcolemmal proteins cause DMD, BMD and LGMD. Defects in nuclear envelope proteins (nuclear envelopathies) typically give rise to EDMD [12, 17]. Defects in Z-disc-related proteins or chaperone-assisted selective autophagy (CASA) underlie MFM [13, 18]. However, the emerging phenotypic heterogeneity of mutant genes resists this over-simplification. For example, mutations in collagen VI- and merosin-encoding genes can also give rise to the LGMD phenotype [1]. Both CMD and LGMD phenotypes can also occur with mutations of several proteins involved in the glycosylation of alpha-dystroglycan, a heavily glycosylated sarcolemmal protein connecting the sarcolemma to the basement membrane [19].

In DM1, DM2 and FSHD, the primary genetic defects cause aberrant expression of toxic proteins or RNA, leading to myofiber degeneration and weakness [20, 21]. Finally, in some muscular dystrophies such as OPMD or OPDM, the pathomechanisms of the underlying genetic defect remains largely unknown.

Diagnosis of Muscular Dystrophies

Patients suspected to have muscular dystrophies require a comprehensive evaluation, combining clinical, serological, and electrophysiological studies to select an appropriate genetic test in order to achieve a definitive diagnosis. The place of the muscle biopsy in this algorithm has evolved in the era of next generation sequencing (NGS), and tends to be later in the diagnostic pathway, often after NGS testing.

Clinical Approach

An insidious onset of slowly progressive muscle weakness is characteristic of muscular dystrophies. History should focus on the age of onset and family history of similar illnesses. The age of onset may be difficult to identify in these insidious disorders. Information regarding the pregnancy, quickening, labor and delivery and neonatal abnormalities such as congenital hip dislocation should be obtained. Motor and mental milestones, childhood history of participation in sports and a history of learning disabilities should be evaluated. A family history of a muscular disorder may not be apparent, and indirect evidence of family members being unable to ambulate, requiring assistive devices or being wheelchair-bound should be sought. A family history of extramuscular manifestations is also important. A careful pedigree chart of the family should be constructed when a family history is present, to evaluate the probable mode of inheritance. Absence of family history does not preclude the diagnosis of muscular dystrophy.

A detailed neuromuscular examination to identify specific patterns of muscle weakness is an integral part of the evaluation of these patients. Weakness in the muscular dystrophies, like in most muscle diseases, is generally symmetric, but asymmetric weakness can be seen in some muscular dystrophies, e.g., FSHD and LGMD-R12 [4, 22]. Muscle pseudohypertrophy or atrophy may accompany the weakness. Scapular winging can be seen in FSHD and in other muscular dystrophies such as EDMD and LGMD-R1. Clinical myotonia (action or percussion-induced) is a key feature of both DM1 and DM2; these patients may report muscle stiffness or impaired relaxation of muscles in addition to weakness. Clinical myotonia tends to be less prominent in DM2 than in DM1[23]. Contractures are common in advanced stages of all muscular dystrophies when mobility is severely impaired; however, contractures occur in the early stages of certain muscular dystrophies (e.g., EDMD and col-

lagen VI-related muscular dystrophies) when weakness is not prominent and is a diagnostic feature of these disorders. These contractures often involve the elbow flexors and the Achilles tendon.

In addition to weakness, muscular dystrophy patients may develop myalgia and/or recurrent rhabdomyolysis. Myalgia may be persistent or episodic, precipitated by exercise or other factors such as infection. A history of episodes of myalgia associated with dark colored urine suggests rhabdomyolysis. Some DM2 patients may present with profound myalgia without significant muscle weakness [20]. Myalgias and recurrent rhabdomyolysis are classically considered indicative of metabolic myopathies, but may also be an initial presentation in certain subtypes of muscular dystrophies, e.g. dystrophinopathies and some subtypes of LGMD-R (e.g. R1, R2, R9 and R12) [24]. In these muscular dystrophies with recurrent rhabdomyolysis, the "pseudo-metabolic" phenotype, muscle weakness may not be evident between episodes of rhabdomyolysis, but serum creatine kinase (CK) levels generally remain elevated between episodes.

Although muscular dystrophies are primary diseases of skeletal muscle, extramuscular manifestations can occur in several muscular dystrophies. The most common are cardiac and respiratory involvement, which may vary from mild to severe and in some disorders, contribute significantly to quality of life and mortality. Other extramuscular systems involved include the central nervous system, eyes and skin. History should probe into extramuscular symptoms (dyspnea, chest pain, palpitations, developmental disabilities, cataracts, skeletal abnormalities, etc.). While there is no cure for muscular dystrophies, early recognition and prompt treatment of underlying cardiac and respiratory complications improves quality of life and prolongs life-expectancy. A history of early onset cataracts in the family raises the possibility of myotonic dystrophy. A positive family history of Paget disease of bone or frontotemporal dementia suggests multisystem proteinopathies. Extramuscular phenotypes of each muscular dystrophy are discussed with the individual disorders and are summarized in Chap. 17.

Laboratory Evaluation

Elevated serum CK levels are a well-known feature of primary disorders of muscle, including muscular dystrophies. However, there is no consensus regarding the degree of elevation. CK levels can be normal or mildly to markedly elevated, depending on the subtype of muscular dystrophy, and generally correlate with a number of necrotic fibers. Muscular dystrophies due to sarcolemmal defects (e.g. DMD, BMD and LGMD) typically have greater numbers of necrotic fibers and higher CK levels compared to muscular dystrophies due to defects of nuclear envelope (EDMD), myotonic dystrophies, FSHD, OPMD or collagen VI-related muscular dystrophies [15, 25]. There is wide overlap in the range of serum CK levels and they are usually not diagnostic of a specific subtype of muscular dystrophy. As the disease progresses, serum CK levels often fall and can be lower than normal, reflecting loss of muscle fibers and fibrofatty replacement ("end-stage muscle"). Some patients with muscular dystrophies (Calpainopathies [*CAPN3], ANO5*, Sarcoglycanopathies, and others) may present with asymptomatic/ pre-symptomatic or paucisymptomatic hyperCKemia [26, 27].

HyperCKemia can also occur in non-dystrophic myopathies, neuromuscular junction disorders and neurogenic disorders [e.g. spinal muscular atrophy (SMA), spinobulbar muscular atrophy (SBMA), and amyotrophic lateral sclerosis (ALS)] [28]. In motor neuron diseases, serum CK levels can be markedly elevated, similar to that observed in patients with muscular dystrophies featuring sarcolemmal defects [28].

Elevation of serum aspartase transaminase (AST) and alanine transaminase (ALT) is considered a diagnostic hallmark of liver disease. However, both enzymes are also expressed in skeletal muscle. Therefore, muscular dystrophy patients can have elevation of serum AST and ALT ("transaminitis" or "hypertransaminasemia") without underlying liver disease. It is not uncommon for patients to be detected to have transaminitis on routine laboratory testing, and then undergo extensive evaluation for underlying liver disease before being referred to a neurologist for consideration of a neuromuscular etiology. Gamma glutamyl transferase (GGT) is more specific to hepatocytes compared to AST and ALT. Hypertransaminasemia with normal GGT should prompt clinicians to measure serum CK levels [29].

Electrodiagnostic Evaluation

Nerve conduction studies are generally normal in muscular dystrophies, except for those disorders with a concomitant peripheral neuropathy (Chap. 17) or in the presence of severe distal weakness (Chap. 8). Coexistent disorders such as diabetes mellitus may cause an underling neuropathy. Low-frequency repetitive stimulation of motor nerves may elicit a decremental response in some muscular dystrophies that are associated with a defect of neuromuscular transmission, e.g. CMD or LGMD due to mutations in genes encoding GDP-mannose pyrophosphorylase B (*GMPPB*) and plectin (*PLEC*) (Chap. 17) [30]. Needle electromyography (EMG) generally shows an "irritable myopathy", characterized by increased insertional activity, fibrillation potentials or positive sharp waves, and short-duration, low-amplitude and complex motor unit potentials with early recruitment. The density of fibrillation potentials and positive sharp waves

correlates with the extent of necrotic fibers and fiber splitting in individual patients [31]. These abnormalities are usually seen in early disease when there is active muscle fiber necrosis. Decreased insertional activity and a mixed population of short-duration, low-amplitude and long-duration, large-amplitude motor unit potentials indicate chronicity and can be seen in advanced disease [32].

Myotonic discharges (electrical myotonia) are characteristic of DM1 and DM2, but these can also occur in non-dystrophic myotonias, some muscular dystrophies (e.g. LGMD-R12 and caveolin-3-associated muscular dystrophies) and other myopathies, especially acid-alpha glucosidase deficiency (Pompe disease), immune mediated necrotizing myopathy (IMNM) and MFM [32]. The presence of associated clinical myotonia should point to myotonic dystrophies, although clinical myotonia can be minimal or absent in DM2. Myotonic discharges in DM1 have a typical waxing and waning characteristic, while in DM2 they may appear as waning discharges or could be very subtle and hard to appreciate on the needle EMG [23, 33].

Rippling muscle diseases (RMD) refer to a group of muscle hyperexcitability disorders, clinically characterized by ripples that travel across the muscle and are typically electrically silent. RMD can be hereditary or immune-mediated [34]. Hereditary RMD is associated with mutations in genes coding for caveolin-3 and cavin-1. Antibodies to cavin-4 have been identified in patients with immune-mediated RMD [12, 34].

Genetic Diagnosis

In patients with a classical phenotype of repeat expansion disorders (DM1, DM2, and OPMD) and repeat contraction diseases (FSHD1 and FSHD2), genetic tests specific to these diseases should be performed as part of the initial evaluation; muscle biopsy is not necessary if the genetic test confirms the diagnosis [35]. OPDM is a repeat expansion disorder (Chap. 13), but a genetic test is not commercially available at this time. Muscle biopsy could serve as a diagnostic test for OPDM, although the findings are not entirely specific [36].

For other muscular dystrophies, previous diagnostic algorithms included clinical evaluation to identify distinguishing features such as ethnicity, clinical features, extramuscular manifestations etc. that may provide clues to narrow the differential diagnosis, followed by muscle biopsy, to identify histopathological and/or proteomic clues [37]. In the absence of specific distinguishing features, muscle biopsy would follow clinical evaluation; targeted genetic testing, often one candidate gene at a time, or small panels of genes, would be performed based on histopathologic features e.g., rimmed vacuoles, myofibrillar pathology, etc. [37]. The advent of NGS has revolutionized the diagnostic approach to inherited myopathies as it allows analysis of several genes simultaneously in a much shorter time and lower cost compared to Sanger sequencing. Therefore, NGS has become the diagnostic test of choice and bypasses the muscle biopsy in diagnosis of hereditary muscle diseases [35]. Chap. 14 outlines the genetic diagnosis of each type of muscular dystrophies and discusses pre-and post-test genetic counseling.

When a molecular diagnosis cannot be confirmed by NGS, muscle biopsy is the next step. Histopathological findings guide further evaluation or assist in interpretation of variants of uncertain significance (VUS) (see Section "Myopathological Diagnosis") [35]. VUS refers to a variation in a genetic sequence for which the association with disease is uncertain because although the variant has not yet been reported to be associated with a disease, it is also not reported in normal genetic libraries. Muscle imaging [Computerized Tomography (CT scan), Magnetic Resonance Imaging (MRI) and muscle ultrasound] (Chap. 15) is increasingly used in research and more recently, in clinical practice. Imaging provides specific patterns of muscle involvement in some hereditary myopathies, such as the Collagen VI disorders, which can be of diagnostic value. Additionally, imaging modalities provide both qualitative and quantitative estimates of adipose tissue deposition, which can be used as a biomarker of disease progression [38]. With emerging disease specific radiological patterns of muscle involvement, muscle imaging could be also useful in validating the pathogenicity of VUS [38]. If a diagnosis remains in doubt, further genetic tests, e.g. whole exome sequencing (WES), whole genome sequencing (WGS) or RNA sequencing, may provide the answer [35].

Myopathological Diagnosis

Muscular dystrophies result in a fairly uniform histopathological appearance, as described above, known collectively as dystrophic changes or dystrophic features. These non-specific dystrophic changes do not offer diagnostic clues to the underlying genetic defect or type of dystrophy. The severity of dystrophic findings varies with disease stage and type of dystrophy. Inflammatory infiltrates can occur in muscular dystrophies. In some types of muscular dystrophies (e.g., FSHD, LGMD-R1, LGMD-R2 and *LMNA*-CMD), the inflammatory reaction can be as prominent as that seen in inflammatory myopathies, and some patients may be misdiagnosed as having refractory myositis. Sarcolemmal expression of major histocompatibility complex-1 (MHC-1) is considered a pathological hallmark of inflammatory myopathies, but it has also been reported in the aforementioned muscular dystrophies featuring inflammatory infiltrates [39–42].

A new role of muscle biopsy in the genomic era is to validate the pathogenicity of VUS identified by NGS. This is

done by demonstrating the presence or absence of the expected functional consequences of the variant. For example, interstitial congophilic deposits without systemic amyloidosis have been reported in LGMD-R2 and LGMD-R12 [43]. Therefore, its presence could support the pathogenicity of a VUS in *DYSF* or *ANO5*. Pleomorphic hyaline materials observed on modified Gomori trichrome stained section and abnormal accumulation of Z-disc related proteins are the pathological hallmarks of MFM [13]. Its presence suggests that VUS in MFM-related genes could be pathogenic. Immunohistochemical or western blot studies of targeted proteins could also aid in validating the pathogenicity of VUS. For example, in patients with a VUS in fukutin-related protein-encoding gene (*FKRP*), abnormal alpha-dystroglycan immunoreactivity supports a diagnosis of LGMD-R9 due to *FKRP* mutations [19]. Chap. 16 elaborates the role of muscle biopsy in the genomic era.

Differential Diagnosis of Muscular Dystrophies

Phenotypic overlap between mutations in different genes is increasingly observed as more patients undergo genetic testing. It is important to consider other hereditary myopathies and congenital myasthenic syndromes (CMS) in the differential diagnosis. In cohorts of genetically uncharacterized LGMD, comprehensive genetic studies have identified pathogenic mutations in genes underlying hereditary non-dystrophic myopathies or CMS in a proportion of patients [44–46]. Therefore, a broader NGS panel, including not only muscular dystrophy-related genes, but also non-dystrophic hereditary myopathy and CMS-related genes, has a higher yield of achieving molecular diagnosis compared to an NGS panel limited to genes coding for the muscular dystrophies [35]. In patients without definite genetic diagnosis after undergoing a comprehensive genetic testing, one should consider the possibility of IMNM. Patients withIMNM typically present with subacute and rapidly progressive proximal weakness associated with marked elevation of CK levels; however, a rare chronic and slowly progressive form of IMNM, mimicking muscular dystrophies, has been recently recognized [47–49]. Atypical pathological findings e.g., myofibrillar pathology or mitochondrial abnormalities, have also been reported in IMNM [49]. Distal predominant weakness, resembling hereditary distal myopathies, can be a rare manifestation of IMNM [50]. Serologic testing for IMNM-associated antibodies [3-hydroxy-3-methylglutaryl CoA reductase (HMGCR) and signal recognition particle (SRP)] should be considered in these genetically uncharacterized muscular dystrophy or distal myopathy patients. The importance of recognizing IMNM cannot be overemphasized, because it is a treatable disorder.

Management of Muscular Dystrophies

Currently, the management of muscular dystrophies remains symptomatic and supportive. It requires a multidisciplinary team, consisting of neurologists, physiatrists, physical therapists, occupational therapists, speech therapists, respiratory therapists, nutritionists, geneticists or genetic counselors, cardiologists, pulmonologists, orthopedists, psychologists, and perhaps psychiatrists. Patients should be followed closely by a physical medicine and rehabilitation team (Chap. 19) because disabilities emerge and evolve as diseases progress. Early detection and prompt treatment of extramuscular manifestations (Chap. 17), especially cardiorespiratory complications, can improve quality of life and prolong life expectancy.

Although there is as yet no cure for muscular dystrophies, there are disease modifying therapies available for DMD, the most common type of muscular dystrophy. Glucocorticoids (prednisone, prednisolone, deflazacort and recently vamorolone) have been the cornerstone of pharmacotherapy for DMD for several years. In 2016, the United States Food and Drug Administration (FDA) approved the first drug, Eteplirsen, in a new class of genetic therapies, antisense oligonucleotides (ASOs), for a subset of DMD patients. (Chap. 18) [51]. Three more exon-skipping ASOs have been since approved. These agents restore the reading frame of dystrophin gene (*DMD*), converting the out of frame mutation to an in-frame one, and allowing the expression of a truncated dystrophin protein. This essentially converts the severe DMD phenotype to a milder phenotype, resembling BMD [51]. Very recently, the US FDA approved a recombinant gene therapy for DMD boys aged 4-5 years. This is designed to deliver a gene encoding micro-dystrophin, which is a truncated form of thedystrophin gene containing selected domains of the functional dystrophin protein. Other genetic therapies or pharmacotherapies have provided promising results in pre-clinical models of muscular dystrophies, but they failed to provide the same impact in human trials [52, 53]. A lack of knowledge of the underlying pathomechanisms, the natural history of each disorder or the appropriate outcome measures may, at least in part, be responsible for these failures. Clinical trial design and outcome measurement are discussed in Chap. 20.

Future Prospects

The treatment of muscular dystrophies continues to be an area of active research. DMD remains the major focus of this research. Improved exon-skipping strategies, microdystrophin gene replacement strategies and Cas-9/ CRISPR are some approaches being tested. Adeno- Associated Virus (AAV) based vector gene therapies are being investigated for some of the recessive LGMDs. Methods to block the forma-

tion of, or to effect degradation of, toxic mRNAs in FSHD and DM1 are being tested in pre-clinical studies. One challenge in the treatment of these disorders is the delivery of the drug effectively to target tissues. Nanomedicine is a fast-advancing field that develops and studies compounds that are between 1–100 nm (nanoparticles) to optimize drug delivery to target tissues. Nanoparticles have been used to deliver the Cas-9/CRISPR complex in mdx-mice which are deficient in dystrophin [54].

As potential therapeutic agents for these slowly progressive disorders are tested in clinical trials, the need for valid, reliable clinical outcome measures that are responsive to small changes becomes paramount. Refinement of surrogate outcomes such as imaging and identification of other biomarkers is critical. The rarity of these disorders will necessitate large multicenter collaborations, and registries will provide valuable observational information.

Conclusion

Since the first description of muscular dystrophies nearly 2 centuries ago, the advances of molecular genetics have clarified the heterogeneity of the muscular dystrophies, aided in their classification and transformed the diagnostic algorithm. The role of diagnostic muscle pathology has evolved and now plays a key role in demonstrating the functional consequences of VUS disclosed by NGS. Multidisciplinary care is a crucial part of patient management as the diseases are incurable at the present time. The advent of genetic therapies for a subset of DMD patients has spurred research into development of disease modifying therapies for other muscular dystrophies.

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Dystrophinopathies

2

Partha S. Ghosh and Basil T. Darras

Introduction

The dystrophinopathies are X-linked recessive disorders caused by mutations in the *DMD* gene leading to reduced or absent dystrophin, the protein product of the gene. Males are clinically affected, while females may be asymptomatic or manifesting carriers. There is a wide spectrum of clinical manifestations of dystrophinopathies: Duchenne muscular dystrophy (DMD), the most common form of muscular dystrophy due to absent or severely reduced amounts of dystrophin protein with a relentlessly progressive and fatal course; Becker muscular dystrophy (BMD), a milder phenotype due to reduced amounts of partially functional dystrophin protein; an intermediate phenotype and X-linked dilated cardiomyopathy (DCM). In this chapter, our primary focus will be on DMD and BMD. With advances in symptomatic and supportive management in the last 2 decades, the life expectancy of DMD patients has increased substantially. There have been several advancements in clinical and translational research that have paved the way for the development of new treatments to address the genetic defect.

History

DMD is named after the French neurologist Duchenne [1, 2]. He first described this entity in 1861 under the term "hypertrophic paraplegia of infancy of cerebral origin" [3]. In 1865, he devised an instrument ("histologic harpoon") for muscle biopsy and provided a detailed analysis of 13 of his own cases [4–7]. In 1868, Duchenne revised the term to "pseudohypertrophic muscular paralysis" to emphasize the fact that the weakness was of muscular rather than of cerebral origin [5, 6]. Before this description by Duchenne, isolated cases were reported in the first half of the nineteenth century by other European physicians [8, 9]. In 1955, Becker, Kiener and Walton first proposed the milder form of X-linked muscular dystrophy which was subsequently named as Becker muscular dystrophy (BMD) [10]. However, at that time, it was not clear that DMD and BMD were allelic disorders. The mapping of the gene responsible for DMD at chromosome Xp21 was made possible with advances in genetics in the early 1980s [11, 12]. In 1987–1988, Kunkel and colleagues cloned and sequenced the complete complementary DNA (cDNA) of the *DMD* gene and the protein product was named dystrophin [13–15]. Dystrophin was localized within the sarcolemma and was noted to be absent in DMD and decreased in BMD [16–18]. The journey of DMD thus evolved from the description of the clinical entity in the nineteenth century to understanding the genetic basis of the disease in 1980s and to the first US Food and Drug Administration (FDA) approved dystrophin restorative therapy in the form of exon skipping using antisense oligonucleotide technology in 2016.

Epidemiology

DMD is the most common form of muscular dystrophy with an estimated incidence of about 1 in 5000 live male births [19]. The incidence of BMD is about one-third of DMD and varies from 1 in 18,000 to 1 in 31,000 male births [20–22]. Population studies in northern England report an incidence of 1 in 5618 live male births for DMD [22], whereas in Nova Scotia, Canada, the incidence of DMD was 1 in 4500 live male births from 1968–2008 [23].

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Etiopathogenesis

DMD is the largest known human gene with a 14 kilobase (Kb) transcript and 79 consecutive exons spanning 2.2 megabases (Mb) on the short arm of the X chromosome [14–16, 24].

Dystrophin Isoforms

There are several tissue specific isoforms of dystrophin, driven by a specific promoter which facilitates transcription from their first exon [25–28]. (Fig. 2.1). The three main promoters of the *DMD* are the Brain (B), the Muscle (M) and the Purkinje (P), which drive the production of the full-length dystrophin protein of 427 kilo Dalton (KDa) designated as B/Dp427, M/Dp427, and P/Dp427, respectively [28]. The muscle isoform is expressed in skeletal, smooth and cardiac muscles. It is first detected at 9 weeks of gestation and its expression increases as myoblasts continue to mature [12]. The brain isoform is highly expressed in the neocortex and hippocampus while the Purkinje isoform is expressed in the cerebellum [12]. There are several short isoforms of dystrophin which are transcribed by at least four first exons situated adjacent to the promoters and localized within introns 29 in the DMD gene (Retinal isoform or Dp260, R), 44 (Brain specific isoform or Dp140, B3), 55 (Schwann cell isoform or Dp116, S) and 62 (General isoform or Dp71, G) [24–28].

Dystrophin Protein (Fig. 2.2a, b)

Dystrophin has four functional domains: amino (N)-terminal, rod, cysteine-rich and carboxy (C)-terminal. The N-terminal domain is encoded by exons 1–8 and binds to actin through three high-affinity actin-binding sites. This domain shares homology with other actin-binding proteins (e.g. α-actinin and β-spectrin) and interacts with the cytoskeleton [28]. Studies have shown that deletion of these actin binding sites do not cause a significant reduction of in-vitro actin-binding affinity [29]. In addition, deletions of these regions are seen in some BMD patients [30, 31]. These observations suggest that dystrophin may contain other actin-binding domains or is able to associate with additional cytoskeletal proteins [28]. The large central rod domain is encoded by exons 9–63, comprising of 24 homologous "spectrin-like" repeats forming an α-helical structure [32]. Each repeat is encoded by two exons and repeats are interrupted by two non-helical regions known as "hinges" which confer flexibility to the rod domain during muscle contraction [32]. The cysteine-rich domain is encoded by exons 64–69, located near the C-terminal region and stabilizes the binding between β-dystroglycan and dystrophin on the sarcolemmal membrane [33]. The C-terminal domain encoded by exons 70–79 plays an important role in binding to various adjacent proteins. The C-terminal and cysteine-rich domains act as a bridge that link the cytoskeleton with sarcolemmal proteins that in turn bind with extracellular matrix proteins. These membrane proteins are collectively called dystrophin associated protein complex (DAP), comprising of 3 main groups: dystroglycans (α- and β-dystroglycan); sarcoglycans (α, β, γ, and δ –sarcoglycan); syntrophin/dystrobrevin group (α-syntrophin, β1-syntrophin, β2-syntrophin, α-dystrobrevin, and β-dystrobrevin) [12, 28]. Specifically, the region encoded by exons 71–74 binds to α – and β syntrophin in-vitro and modulates the functional interaction between dystrophin and syntrophin [34–36]. This region also links with nitric oxide synthase (nNOS) via dystro-

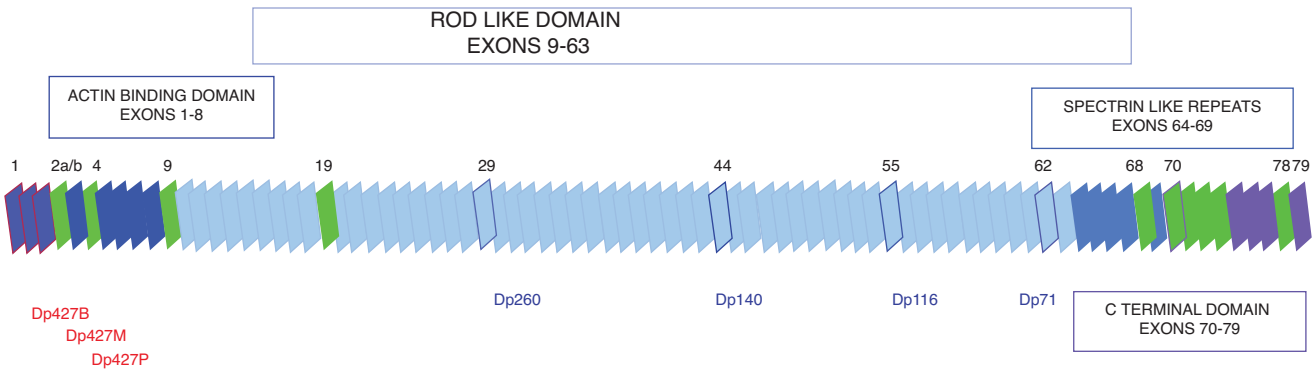

Fig. 2.1 Dystrophin gene structure and protein domains. Schematic representation of 79 exons of dystrophin gene with isoforms and protein domains. Lines in red represent the 5′ full length promoters and their first exon (isoforms Dp427B-M-P). Lines in blue represent the 3′ promoters and their first exons of isoforms: Dp260 (retinal), Dp140 (brain 3), Dp116 (Schwann cells), Dp71 (general). In green are represented exon alternatively spliced or skipped. Boxes' different blue/violet colors explain the protein domains corresponding to the different exonic regions. Reproduced with permission from Elsevier from Ferlini A. et al. The medical genetics of dystrophinopathies: Molecular genetic diagnosis and its impact on clinical practice. Neuromuscular Disorders 2013;23 (1):4–14 (Fig. 1)

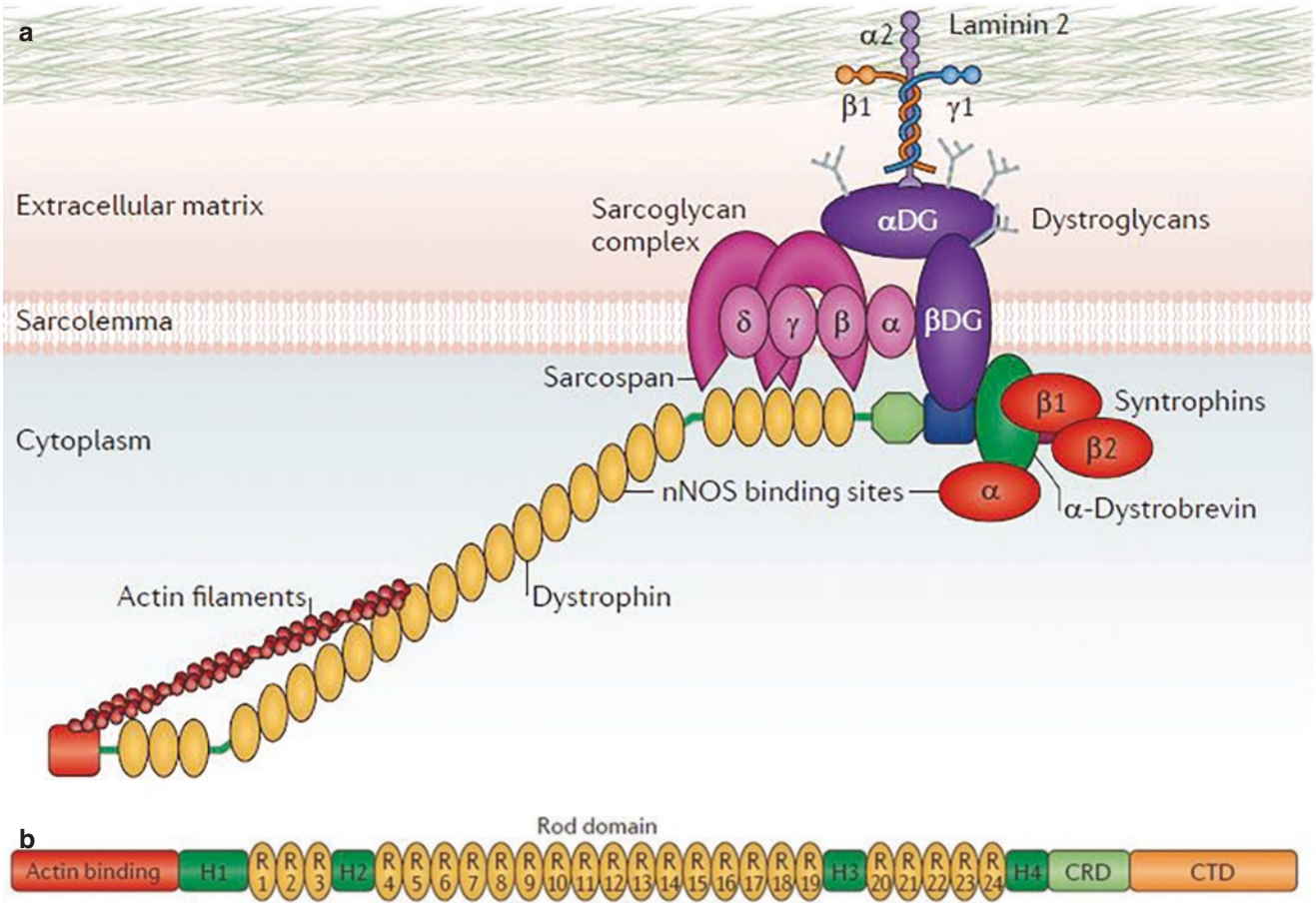

Fig. 2.2 (**a**) The dystrophin-associated protein complex. Dystrophin acts as an important link between the internal cytoskeleton and the extracellular matrix. Neuronal nitric oxide synthase (nNOS) binds to α-syntrophin but also has a binding site in repeat 17 of the rod domain of dystrophin. αDG, α-dystroglycan; βDG, β-dystroglycan. Reproduced with permission from Springer Nature from Fairclough RJ et al. Therapy for Duchenne muscular dystrophy: renewed optimism from genetic approaches. Nature Reviews Genetics 2013;14:373–378 (Fig. 1). (**b**): Wild-type dystrophin. Full-length dystrophin comprises an aminoterminal actin-binding domain, four hinge domains (H1–H4) and a rod domain consisting of 24 spectrin-like repeats (R1–R24), within which lie a second actin-binding domain, a cysteine-rich domain (CRD) and a carboxy-terminal domain (CTD). Reproduced with permission from Springer Nature from Fairclough RJ et al. Therapy for Duchenne muscular dystrophy: renewed optimism from genetic approaches. Nature Reviews Genetics 2013;14:373–378 (Figure 2a)

brevin. Absence of dystrophin in DMD downregulates nNOS which plays a critical role in reduced tissue perfusion and muscle damage [37].

Pathogenesis

The primary role of dystrophin in skeletal muscle is mechanical stabilization of the sarcolemma, as evidenced by increased susceptibility to contraction-induced sarcolemmal rupture in the *mdx* mouse model [38]. Secondary loss of DAP due to dystrophin deficiency contributes to further destabilization of the muscle cell membrane from contractile forces, resulting in focal tears during contractile activity [39]. This in turn leads to muscle fiber necrosis from activation of proteolytic enzymes like calpains due to an influx of extracellular calcium [40]. There is progressive degeneration of larger muscle fibers while smaller fibers like those in extraocular muscles are relatively spared because the mechanical stress per unit of the muscle membrane surface is much less in the smaller fibers [41].

Mutations in the Dystrophin Gene (Table 2.1)

The mutation rate is relatively high in the dystrophin gene. One third of the cases are due to *de novo* mutations; this presents a challenge to reduce population disease burden as new dystrophinopathy cases cannot be prevented even with good prenatal genetic counseling [42]. This high mutation

Table 2.1 Type and Frequency of Mutations Held within the TREAT-NMD DMD Global Database

Total	7149	Percentage of total mutations
Large mutations	5682	79
Large deletions (≥ 1 exon)	4849	68
Large duplications (≥ 1 exon)	784	11
Small mutations	1445	20
Small deletions (< 1 exon)	358	5
Small insertions (< 1 exon)	132	2
Splice sites (<10 bp from exon)	199	3
Point mutations	756	11
Nonsense	726	10
Missense	30	0.4
Mid-intronic mutations	22	0.3

rate is largely attributed to the unusually large intron sizes of *DMD* (for example intron 44) [28]. The most common mutations are deletions (approximately 65%) and duplications (roughly 10%) of one of more exons in the *DMD* with two mutational hot spots concentrated between exons 44–53 and exons 3–7 [24, 28, 42, 43]. The genomic breakpoints of the 3′ hot spots usually lie within intron 44, while the 5′ end hot spots lie within introns 2 and 7, which are evolutionary conserved and contain regulatory regions [28]. The remaining 25% of the mutations are small mutations which include point mutations (nonsense and missense), frameshift mutations, insertion-deletion mutations (indels), and other rare types (small inversions, complex small rearrangements) [28]. Point mutations can lead to premature stop codons (nonsense mutations), accounting for about 10%–13% of the cases [42]. Point or small mutations can disrupt splice sites (either donor or acceptor sites) resulting in the exon not being recognized by the splicing machinery. Splice-site mutations generally cause a single-exon deletion at the mRNA level, which can be in-frame or out-of-frame [42]. Missense mutations are rare in *DMD*; they are usually located in the cysteine-rich domain of dystrophin and prevent its binding to β-dystroglycan, thus disrupting the link between dystrophin and the extracellular matrix [44, 45]. Pseudo-exons are deep intronic mutations wherein an intronic region is recognized as an exon by the splicing machinery leading to its inclusion in the mRNA and thus disrupting the reading frame or creating premature stop codons. These mutations account for about 1% of *DMD* mutations [28, 32]. Finally, autosomal translocation and non-random inactivation of the X chromosome can involve *DMD,* causing a dystrophinopathy phenotype in females [46, 47].

Reading Frame Rule

The functional consequences of *DMD* mutations are mainly related to the ability to maintain an open reading frame which allows transcription/ translation of the dystrophin protein, the so called "reading frame rule" [48]. The open reading frame is the sequence of three consecutive, non-overlapping nucleotides, the triplet codon, each coding for an amino acid, with a start codon that initiates transcription/ translation to a stop codon that terminates transcription/ translation [48]. In general, when the mutations (deletions or duplications) maintain the reading frame ("in-frame") there is production of abnormal (reduced amount or truncated size), albeit partially functional protein resulting in a milder phenotype of BMD [28, 42]. On the contrary, in the more severe DMD phenotype, mutations cause disruption of the reading frame ("out-of-frame") resulting in unstable mRNA that results in virtually undetectable levels of non-functional truncated dystrophin protein [28]. This phenomenon is called nonsense-mediated mRNA decay, which depletes the major part of the dystrophin mRNA [28]. The reading frame hypothesis is accurate in about 90% of cases and is commonly used to predict the phenotype of dystrophinopathy [44, 49]. In case of large deletions or duplications involving one or more exons, if the number of nucleotides that are deleted or duplicated is divisible by 3, the reading frame will be intact since the critical N- and C- terminal domains of *DMD* are maintained [42]. If the nucleotides are not divisible by 3, reading frame is disrupted. It is important to note that although the reading frame rule is generally applicable to duplications, there are some limitations as most of the commonly used genetic techniques used to detect duplications may not determine if a duplication is arranged in a head-to-tail orientation [50].

Exceptions to the reading frame hypothesis involve BMD patients with "out-of-frame" mutations (frame-shift deletions/duplications) or DMD patients with "in-frame" deletions/duplications [42].

The following mutations are examples that do not follow the reading frame rule. Frame-shift or nonsense mutations proximal to exon 8 may result in a BMD phenotype due to the activation of alternative translation initiation sites in exon 6 or 8 (deletion of exon 2 can activate translation initiation in exon 6) [51]. Some patients with out-of-frame deletion of exons 3–7 may have a variable phenotype depending on whether an alternative translation initiation site in exon 8 is activated [52]. Patients with nonsense mutations can present with BMD phenotype due to exon skipping, which bypasses the nonsense mutation and maintains the reading frame [53]. DMD patients with out-of-frame mutations flanking exon 44 typically show a milder phenotype which is predicted to be

due to low level spontaneous exon skipping [54]. This idea is supported by the fact that these patients have higher than normal amounts of dystrophin on muscle biopsy compared to other out-of-frame mutations [55].

For in-frame mutations, the location and size of mutations can influence disease severity and these patients may present with a severe phenotype than would be otherwise predicted by the reading frame rule [35, 49]. In-frame mutations affecting critical points of the dystrophin molecule such as the cysteine-rich and C-terminal domains (encoded by exons 64–70) which are involved in DAP protein complex assembly or affect all three actin binding domains (encoded by exons 2–10 and exons 32–45) result in DMD phenotype [42]. In-frame deletions affecting the first 10 exons delete the first two actin binding domains while sparing the third one, encoded by exons 32–45, typically result in a 'severe BMD' phenotype rather than the DMD phenotype [42]. Deletions in the hotspot region (exons 45–55) are generally associated with a milder disease presentation. Deletions between exons 10 and 40 are mild and may present with cramps and myalgia or are found in asymptomatic individuals [56]. As long as the N- and C-terminal domains are intact, removal of large portions of the rod domain typically results in BMD [57]. The Leiden Duchenne Muscular Dystrophy database (http://www.dmd.nl/) is an excellent resource for various phenotypes observed in patients with deletions. These variations, which do not follow the reading frame rule, pose considerable challenges in predicting the phenotype, BMD or DMD, particularly in young children without a family history [28]. Muscle biopsies are no longer routinely performed in the diagnostic work up of dystrophinopathies due to widespread availability of genetic testing. In cases where genetic testing does not provide definitive information about the phenotype, muscle biopsy can provide essential information by immunohistochemical (IHC) staining or quantification of the dystrophin protein by Western blot (WB), which supplements clinical and genetic data to assist phenotyping.

Clinical Phenotypes (Table 2.2)

DMD

Skeletal Muscle Involvement

One of the early manifestations of DMD is gross motor delay/impairment in a boy in the first 2 years of life. By 3 years, most patients have evidence of proximal leg weakness resulting in frequent falls, difficulty in climbing stairs, jumping, running and getting up from a sitting position [12, 58]. Some parents notice enlargement of the calf muscles. Children may complain of intermittent pain in their leg muscles associated with physical activities. The mean age at diagnosis with negative family history is approximately 4 years 10 months (range 16 months-8 years) [59–61]. Often DMD is diagnosed following work-up of patients with elevated hepatic transaminases [11, 12].

Examination shows weakness of the proximal muscles. The lower limbs are more affected than the upper limbs in the early stages. The following muscles are preferentially affected: hip extensors (compensatory exaggerated lumbar

Table 2.2 Genetic, clinical and pathological features of the dystrophinopathies

Type	Gene location	Protein	Inheritance	Clinical features	Pathology
Duchenne	Xp21	Dystrophin	XR	Onset: 2–5 year. Pseudohypertrophy Diminished I.Q. Cardiac involvement Rapid decline Wheelchair confinement: 11–13 year. or earlier Death: 15–30 year	Severe dystrophic changes Complete/almost total absence of dystrophin by immunohisto-chemistry[a] Dystrophin 0–5% of normal quantity[b] by Western blot[a]
Intermediate "outliers"	Xp21	Dystrophin	XR	Intermediate severity Wheelchair confinement: 13–16 year	Dystrophin 5–20% of normal quantity by Western blot of muscle protein
Becker	Xp21	Dystrophin	XR	Onset: 5–20 year. or later More benign course Wheelchair confinement: After 16 year	Less marked changes Normal appearing or reduced intensity ± patchy dystrophin staining by immunohisto-chemistry Normal or abnormal molecular weight[c] dystrophin, quantity >20% by Western blot

XR X-linked recessive, *I.Q.* intelligence quotient. Reproduced with permission from Elsevier from Darras BT, Menache-Starobinski CC, Hinton V, Kunkel LM. Dystrophinopathies. Chap. 30. In: Neuromuscular Disorders of Infancy, Childhood, and Adolescence: A Clinician's Approach. 2nd edition. San Diego: Academic Press, 2015. pp. 551–92 (Table 30.1)

[a] Uses monoclonal antibodies to the carboxy-terminus, amino-terminus, and mid-rod domain (6–10 antibody) of dystrophin

[b] The quantity of dystrophin is expressed as a percentage of control values (standardized versus myosin post transfer with Coommasie stain)

[c] Normal molecular weight is 427 kDa

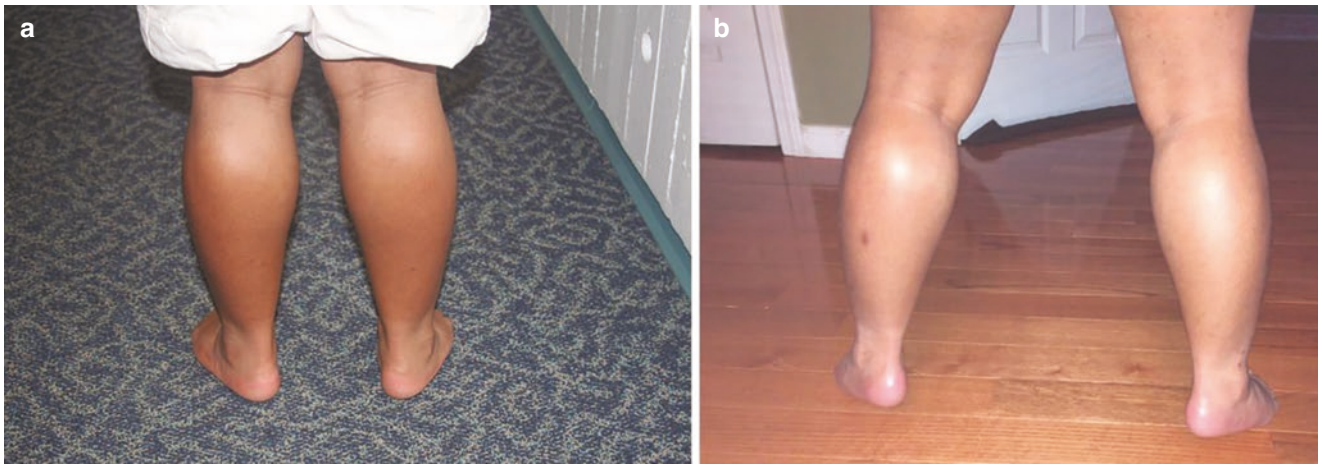

Fig. 2.3 A boy with Duchenne muscular dystrophy, at the ages of 8 years (**a**) and 11.5 years (**b**). Note enlargement of gastrocnemii muscles bilaterally, known as "pseudohypertrophy." Also note the progression in foot position from plantigrade (**a**) to mild equinovarus (**b**). Reproduced with permission from Elsevier from Darras BT, Menache-Starobinski CC, Hinton V, Kunkel LM. Dystrophinopathies. Chap. 30. In: In: Darras BT, Jones HR Jr., Ryan MM, De Vivo DC (editors). Neuromuscular Disorders of Infancy, Childhood, and Adolescence: A Clinician's Approach. second edition. San Diego: Academic Press, 2015. pp. 551–92 (Fig. 30.5)

lordosis), knee extensors more than flexors, elbow flexors and extensors more than deltoids. Gowers' sign which is a manifestation of proximal lower limb muscle weakness is a useful bedside test where affected patients turn their face to the floor when arising from a supine position, then spread their legs and use their hands to climb up their thighs to an upright position [12]. Early involvement of the neck flexors as evidenced by the inability to lift the head against gravity in the supine position is common in DMD [12]. Hypertrophy of the calf muscles (Fig. 2.3) is a useful sign. In the early phase, there is true hypertrophy of the muscle fibers which are then replaced by fibrous and fatty tissue in the late stages of the disease (pseudohypertrophy) [12]. However, several other muscles can be hypertrophied, such as quadriceps, gluteal muscles, deltoid, infraspinatus, tongue and rarely masseter muscles [12]. DMD is a relentlessly progressive disease with gradually evolving weakness of the lower limb muscles from 7 years onwards to non-ambulatory status by 12–13 years historically in steroid naïve patients. This is followed by weakness of the upper limb muscles and development of scoliosis from paraspinal muscle weakness and atrophy. However, the rate of progression of weakness varies substantially among DMD patients and even within siblings of the same family, suggesting the presence of genetic modifiers in disease severity [42]. Baseline 6-minute walk distance (6MWD) which is an important functional measure in neuromuscular disorders and age (≥7 years) are strong predictors of loss of ambulation (LOA) in DMD patients; baseline 6MWD <350 meters is predictive of greater functional decline [12].

Tendon reflexes are typically preserved in early stage of the disease and help to differentiate myopathies from other conditions which present with proximal weakness, such as spinal muscular atrophy. By 10 years, triceps, biceps, and knee reflexes are difficult to elicit in 50% of the patients while ankle reflexes are preserved in one-third of the cases even in the late phase of the disease [62].

Preferential involvement of the ankle dorsiflexors and evertors with preservation of plantar flexors and invertors lead to heel cord contractures and toe walking [12]. The majority of patients develop contractures of the heel cords, iliotibial bands, and hip flexors, causing toe walking and limitation of hip flexion by 6–10 years [63]. This is followed by contractures at the knees, elbows and wrists. Progressive weakness of the respiratory muscles (intercostal and diaphragm) commences in the later part of the first decade and is an important cause of morbidity and mortality in DMD patients [64]. Nocturnal hypoventilation and carbon dioxide retention can cause early-morning headaches and significantly impact the quality of life [65]. Swallowing difficulty can result from involvement of the skeletal muscle fibers in the upper third of the esophagus. Dysarthria and hypophonia from involvement of laryngeal muscles may precede the decline of pulmonary function [65]. In the past, the majority of patients died in their late teens to late twenties from respiratory failure and/or cardiac failure secondary to progressive cardiomyopathy [66]. However, life expectancy of DMD patients now extends into the late thirties and early forties [67].

DMD (Extra-Muscular Involvement)

Cardiac Involvement

DMD-associated cardiac involvement manifests as a dilated cardiomyopathy (DMD-CM), and or cardiac arrhythmia [68]. The incidence of cardiac involvement increases after

the first decade; one-third of patients are affected by 14 years of age and almost all patients after age 18 years [69]. There is limited data regarding the correlation of the severity of the cardiac phenotype and the genotype in DMD [68]. Patients typically do not experience classic symptoms of heart failure in the early stages due to diminished physical activity and non-ambulatory status and often report nonspecific symptoms like fatigue, weight loss, vomiting and sleep disturbances [68]. Resting sinus tachycardia is an early and consistent finding; other findings include tall R waves in V1–V3, increased amplitude Q waves in the left precordial leads, right axis deviation and right or left bundle branch block [68]. Transthoracic echocardiographic (TTE) findings include reduced systolic function as measured by left ventricular ejection fraction (LVEF) of <55% or fractional shortening (FS) < 28% [70]. Right ventricular function, however, is relatively preserved [71]. Echocardiographic values are now increasingly described as z-scores in children because of wide variation in normative data due to age and body habitus [68]. Echocardiograms can be challenging to interpret due to poor acoustic windows from severe scoliosis and obesity [72]. Recently, Cardiac MRI (CMRI) has become the non-invasive technique of choice in investigating cardiac structure and function because it provides accurate 3-dimensional analysis of global and regional functioning with better reliability and reproducibility than TTE [72]. Unlike echocardiography, it is not restricted by body habitus [73] and apical regions of the ventricles are better visualized by CMRI. Late gadolinium enhancement (LGE) or myocardial delayed enhancement (MDE) is an early sensitive marker of fibrosis in DMD patients typically affecting the basal infero-lateral wall before there is global cardiac dysfunction [74]. The disadvantages of CMRI include claustrophobia, patient discomfort and cost of the procedure compared to TTE [68]. CMRI is considerably underutilized in the DMD population currently but hopefully will be increasingly used in the coming years for improved detection and timely management of cardiomyopathy.

Brain

About 50% of DMD patients lack the full length dystrophin isoform in the brain (Dp427), while the other half lack both Dp427 and Dp140 isoforms and a small fraction lack Dp71/Dp40 [75]. Dystrophin is predominantly expressed in the cortex (temporal and frontal cortex> parietal and occipital cortex), hippocampus, amygdala and cerebellum. The Dp427 network plays an important role in transmembrane transporter activity and synaptic transmission by anchoring GABA-A receptors to the post-synaptic membrane of GABAergic neurons [76–79]. Much less is known about functions of the Dp71/Dp40 network; they may be associated with vascular development and cell motility [80, 81]. A shift of one standard-deviation in full scale intelligence quotient (IQ) compared to the general population is consistently reported in DMD patients [82–84]. Learning problems (particularly information processing, verbal working memory, reading) and behavioral disturbances are common in DMD even in patients with normal IQ, and can be detected early in development [85–87]. Associated neurobehavioral comorbidities include autism spectrum disorder (ASD) in 3–15%, attention deficit hyper activity disorder (ADHD) in 11–32%, obsessive compulsive disorder (OCD) in 5–60%, and anxiety in 27% patients [86, 88, 89]. The prevalence of epilepsy in DMD patients is about 6% [90]. However, the incidence of neurodevelopmental abnormalities is likely under represented as DMD patients are not universally screened for these conditions and there is no standardized diagnostic test battery for DMD patients [75]. Although there is no strong correlation between genotype and neurocognitive patterns, a recent study found a relationship between lower normalized forward digit span scores in nonsense *DMD* mutations downstream of exon 30, exon 45, and exon 63 [91].

Smooth Muscle

Smooth muscles of the gastrointestinal tract undergo degeneration in DMD leading to several dysmotility syndromes [12]. Acute gastroparesis may cause acute abdominal pain, vomiting and gastric dilatation. Chronic abdominal pain and constipation due to intestinal hypomotility may be seen, and can lead to intestinal pseudo-obstruction.

BMD

Skeletal Muscle Involvement

There is considerable phenotypic heterogeneity in BMD. Usually patients present between ages 5 and 20 years with the mean age of onset about 12 years [92–95]. There are patients on the milder end of the clinical spectrum with symptom onset after 40 years who remain ambulatory past their 60 s [96–98]. Exertional myalgias and cramps involving the calves are common presenting symptoms in BMD; rhabdomyolysis and myoglobinuria occurs infrequently [99, 100]. The degree of muscle weakness is milder than DMD but follows a similar pattern starting with proximal weakness of the lower limbs and calf hypertrophy [12]. Calf, distal upper extremity and neck flexors remain strong until the late stage of the disease [12]. Joint contractures are also less frequent than DMD. Patients remain ambulatory beyond 16 years, the mean age of LOA is in the fourth decade [92–94, 101]. Deletion of >60% of the rod domain (deletion of exons 17–48) in one BMD patient resulted in a very mild disease and forms the basis of microdystrophin constructs for DMD gene transfer therapy [102]. Survival is typically beyond the

third decade, and patients usually die from respiratory failure or cardiomyopathy in their fourth to sixth decades of life [60, 99].

Extra-Muscular Involvement

On occasion, cardiac involvement in BMD may be more severe than the skeletal muscle involvement and can precede muscle weakness by several years [103–106]. Patients with deletions affecting N-terminal domain are more likely to experience early-onset cardiomyopathy [107]. Similarly, cognitive and behavioral problems are less severe in BMD patients, although mean IQ scores are slightly lower than the general population [12].

Other Dystrophinopathy Phenotypes

Intermediate Phenotype

These patients are so called "mild DMD or severe BMD" as they are in between the two classic phenotypes. In a natural history study, these patients usually remain ambulatory before age 13 but become wheelchair dependent before age 16 [12]. An important clinical clue is preservation of their ability to flex their neck against gravity which differentiates them from the classic DMD phenotype [12]. This phenotypic variability can be partly explained by the genetic modifiers that influence ambulatory status, steroid responsiveness, and cardiomyopathy [65]. Some of these genetic modifiers have negative or positive effect on the phenotype. Osteopontin, known as secreted phosphoprotein 1 (SPP1), is an acidic glycoprotein that plays important role in bone-remodeling, immune function, and muscle repair; it's promoter is activated by transforming growth factor β (TGFβ) family members [65]. A single nucleotide polymorphism (SNP) in the promoter of *SPP1* is associated with early LOA in DMD patients [108]. It is also noted that patients with certain SPP1 variants respond poorly to steroids [109]. Latent TGFβ binding protein 4 (LTBP4) is a member of the fibrillin superfamily that binds to TGFβ in the extracellular matrix and regulates TGFβ activity [110]. Certain LTBP4 genotypes have a protective effect with delayed LOA, glucocorticoid responsiveness as well as late onset of cardiomyopathy [110–112]. In two sets of brothers with DMD who were discordant for their LTBP4 haplotypes, the brothers with the protective allele had delayed LOA compared to the brothers without that allele [110].

DMD-Associated Dilated Cardiomyopathy

Several members of a large multi-generation family were described in 1987 to have dilated cardiomyopathy without skeletal myopathy and linkage analysis identified the locus to Xp21 of *DMD* [113, 114]. DMD-CM typically presents in males in the second or third decade with rapidly progressive course; associated ventricular arrhythmias are common [114, 115]. Female carriers develop mild cardiomyopathy in the fourth or fifth decade and exhibit slow progression [12]. Elevated CK is an important finding in this condition [12] and should alert the cardiologist to suspect DMD-CM. Patients with severe cardiomyopathy do not produce dystrophin in their cardiac muscle while their skeletal muscle is unaffected [116].

Female Carriers

As dystrophinopathies are X-linked recessive disorders, women carry and transmit the affected gene on one X chromosome but usually do not manifest the disease due to the presence of a normal X chromosome. Women carriers can infrequently develop clinical manifestations, the so called "manifesting carriers" (MC). Several mechanisms have been proposed to explain MC [117–121]. The most frequently described mechanism is non-random or skewed X-chromosome inactivation (XCI) wherein expression of the X chromosome with the mutated allele is favored [120]. It is generally thought that more severe skewing of XCI (ratio > 90:10) is associated with more severe symptoms in MC, however, this association is not definitive [121]. The phenotype in monozygotic female twins with DMD gene mutations are often discordant due to differential XCI in the early embryonic stage [122, 123]. Other mechanisms for MC status are balanced X-autosome translocations with breakpoints at Xp21 (most common) [124], Turner syndrome [125], X chromosome uniparental disomy [126], and male pseudohermaphroditism due to a mutation in the androgen receptor gene [127].

With regards to the clinical features of MC, one study found that 5% of the carriers had myalgias/cramps without muscle weakness, 17% experienced mild-to-moderate muscle weakness, and 8% had DCM [128]. Another descriptive study of clinical and genetic features of 15 MC (excluding those with only myalgias/cramps) among 860 patients in the United Dystrophinopathy Project (UPD) found that symptom onset ranged from 2 to 47 years. The phenotype varied from DMD-like progressive disease to very mild-BMD like presentation. Eight patients had male relatives with DMD [129]. Manifesting carriers can pose diagnostic challenges in the absence of a family history of dystrophinopathy, as 7 out of 15 MC in this study had negative family history [129]. About 10% of women with elevated CK (typically >1000 U/L) and myopathic histology were found to be MC [130]. CK was elevated (2–10 times the upper limit of normal, mean 306 U/L) in 30–50% of dystrophinopathy carriers in one study; 22% were MC in this study [128]. In this study there was no significant difference of CK level between asymptomatic and MC [128].

Differential Diagnosis of Muscular phenotype of Dystrophinopathies

Limb-girdle muscular dystrophy (LGMD) are a diverse group of disorders that can be of autosomal dominant or recessive inheritance. Among LGMD types, sarcoglycanopathies (LGMD-R3–5) and LGMD-R9 resemble dystrophinopathies (proximal weakness, high CK and calf hypertrophy) and may have cardiomyopathy (see Chap. 6) [11, 12]. Clinically, these conditions are difficult to differentiate from dystrophinopathies in boys without a family history. Emery-Dreifuss muscular dystrophy (EDMD) is characterized by the clinical triad of early onset proximal joint contractures, progressive muscle weakness typically starting in a scapulo-peroneal distribution, and cardiac involvement (arrhythmias and cardiomyopathy) (see Chap. 10). Proximal joint contractures and scapulo-peroneal pattern of weakness help to differentiate EDMD from dystrophinopathies. EDMD can be X linked [Emerin (*EMD)* and four and a half LIM domain 1 (*FHL1)*], autosomal dominant [Lamin A/C (*LMNA),* Nesprin-1 *(SYNE1),* Nesprin-2 *(SYNE2)*, Transmembrane Protein 43 (TMEM43)] and autosomal recessive [*LMNA*, SUN domain containing protein-1 (*SUN1*), Titin (TTN].

Congenital muscular dystrophies are a phenotypically and genotypically diverse group of disorders (see Chap. 11). These patients present with early onset weakness, hypotonia, high CK and sometimes central nervous system manifestations (seizures, cortical malformations, and white matter changes) [11, 12]. Most congenital muscular dystrophies present with muscle weakness before 2 years of age, which is uncommon in DMD patients.

Spinal muscular atrophy (SMA) is an autosomal recessive disorder due to homozygous deletions of *SMN1* (5q-SMA) in >95% of cases. SMA type 3 can present with progressive proximal limb weakness after 18 months of age and rarely can have calf hypertrophy. Early loss of tendon reflexes, normal or mild elevation of CK, and neurogenic changes on electromyography (EMG) help to differentiate SMA from muscular dystrophies [11, 12].

Investigations

Creatine Kinase

Among several serum muscle enzymes used to detect myopathies, CK is the most sensitive and cost-effective screening test in clinical neuromuscular practice [131]. CK levels are invariably elevated in patients with dystrophinopathy and continue to increase with age, reaching a peak by 2–3 years of age [132]. CK levels then progressively decline with age at a rate of about 20% per year due to replacement of the muscle with fibrous tissue [133, 134]. As a general rule, CK levels are much higher in DMD compared to BMD; by age 5 CK levels are about 50–200 times the upper limit of normal in DMD and 20–200 times the upper limit in BMD [132, 134]. However, it is not always possible to reliably differentiate DMD from BMD based on CK levels alone because of the overlap in the range of levels. One study found that CK levels were 2–10 times the upper limit of normal in 30–50% of the female carriers of DMD or BMD; 22% of carriers were MC. Mean CK level was not different in MC and asymptomatic carriers [128]. Another study found that daughters of obligate carriers have a disproportionate decline in CK and pyruvate kinase (PK) with age as compared to non-carrier females, suggesting that the rate of carrier detection will be higher in the first two decades [135].

Electromyography

Electromyography (EMG) in DMD reveals increased insertional activity, abnormal spontaneous activity (fibrillation potentials and positive sharp waves) and brief, small amplitude motor unit potentials with early recruitment. The irritability is attributed to muscle fiber necrosis and is less apparent in BMD. In end stage disease, when muscle fibers are replaced by fibro-fatty tissue, insertional activity is reduced, spontaneous activity is no longer seen and both short and long duration polyphasic motor units are seen, reflecting chronic disease. However, because the findings are nonspecific [12], and the procedure is associated with some discomfort, EMG is of limited utility in the diagnosis of DMD especially when a family history of the disorder is present. In sporadic cases and in BMD, because the differential diagnosis is broader, EMG may be useful in confirming a myopathic process and directing further testing.

Magnetic Resonance Imaging (MRI)

Muscle MRI (mMRI) is a noninvasive imaging modality to asses morphologic dystrophic abnormalities in DMD [136]. (see Chap. 14). Qualitative measures (signal intensity changes on T1 and T2 W images) assess muscle edema, fat infiltration and muscle volume; quantitative techniques (T1map, T2map, diffusion-weighted imaging [DWI], and Dixon) can precisely measure "fat fraction" (FF) of the muscle. During the early phase of DMD, muscle edema is observed (suggesting inflammation, seen as hyperintense signals on short tau inversion recovery, water T2-Dixon, or fat-suppressed T2-weighted sequences) while fatty infiltration and atrophy of the muscles (seen as hyperintense signals on T1-weighted images) occur later [137–141]. There is a