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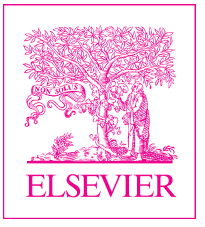

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Foreword

It is with especial pleasure that we welcome this new volume as an addition to the *Handbook of Clinical Neurology*. Professors Robert Griggs and Anthony Amato, the volume editors, have assembled an impressive group of internationally recognized authorities to provide clinicians and scientists with the latest information concerning the muscular dystrophies. Cogent summaries are provided about advances in molecular and cell biology, biochemistry, and other biological sciences, with particular regard to their application to this group of muscle disorders and to their clinical implications, thereby providing comprehensive new insights to a group of disorders that have profound effects on patients and their families. Comparison with earlier volumes of the *Handbook* that have covered related topics will indicate the remarkable changes that have occurred in our concepts of these disorders over the last two decades. Such advances clearly lay the foundation for future therapies. We hope that this volume will appeal to basic investigators by providing them with a greater understanding of the muscular dystrophies, and to clinicians by its emphasis on aspects that are of relevance to the care of diagnosis and management of patients with these disorders.

We are grateful to the two volume editors, who are both renowned clinicians and investigators, and to the numerous authors whom they assembled, for giving generously of their time and expertise to summarize developments in their field and thereby putting together this outstanding volume. As series editors, we each reviewed all of the chapters included in the volume, making suggestions for improvement as appropriate, but we were enormously impressed by their scholarly appraisal of developments in the field. In addition to the print form, the volume will be available electronically on Elsevier's Science Direct site. This will make it—and the other volumes of this third series of the *Handbook*—more accessible to readers and will also facilitate search for specific information.

It is a pleasure, as always, to thank the team at Elsevier—and, in particular, Michael Houston and Michael Parkinson in Edinburgh—for their unfailing and expert assistance in the development and production of this volume.

Michael J. Aminoff
François Boller
Dick F. Swaab

Preface

In recent years, new discoveries concerning the muscular dystrophies have occurred at breath-taking speed. It is only two decades since the first genetic cause of a muscular dystrophy was identified: dystrophin mutations as a cause of Duchenne muscular dystrophy. Since that time, close to 50 genes have been found mutated in a muscular dystrophy, and only a handful of disorders have not been categorized molecularly.

Unanticipated, however, has been the complexity of the pathogenesis for each genetically characterized disorder, as well as the persistence of unexplained phenotypic heterogeneity. Moreover, although we both remain optimistic that new therapeutic strategies will soon provide major improvement in one or more muscular dystrophies, the hurdles remain high and new obstacles continue to loom into view.

This book has been organized and its authors assembled to provide the latest information on both pathogenesis and the prospects for treatment—integrated with the clinical wisdom and perspective of highly experienced physicians who are the go- to experts in their sub-subspecialty. Each chapter is authoritative and complete, but with an emphasis on what matters clinically now and in the foreseeable future. We thank the contributors for doing a masterful job of summarizing the state of the art in their area.

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Muscular Dystrophies
A.A. Amato and R.C. Griggs, Editors

Chapter 1

Overview of the muscular dystrophies

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INTRODUCTION

Classification

Historically, the muscular dystrophies have been defined as progressive myopathies in which muscle biopsies demonstrate replacement of muscle fibers by adipose and connective tissue. The clinical onset of the dystrophy may be evident at birth, as in congenital muscular dystrophies, or may not develop until late adulthood. The dystrophies were felt to differ from congenital myopathies by the presence of specific ultrastructural abnormalities apparent in muscle biopsies in the latter (e.g., nemaline rods, cores, or minicores). However, with advances in molecular genetics the distinction between what constitutes a muscular dystrophy and a congenital myopathy has become blurred. For example, myofibrillar abnormalities and inclusions such as nemaline rods, cytoplasmic bodies, and reducing bodies that initially led to categorization as a congenital myopathy have been noted in disorders known to carry similar genetic defects that have been considered forms of dystrophy.

Dystrophies have been classified according to age of onset, mode of inheritance, and pattern of weakness (Table 1.1). For example, those that present at birth have been termed congenital muscular dystrophies (MDC). Dystrophies have also been named based on the patterns of muscle involvement, including limb-girdle muscular dystrophy (LGMD), facioscapulohumeral dystrophy (FSHD), oculopharyngeal muscular dystrophy (OPMD), distal myopathy/dystrophies, and scapuloperoneal dystrophy. Within the distal muscular dystrophies, subclassifications have been based on inheritance pattern, age of onset, and the specific muscle groups initially affected; for example, the Markesbery–Griggs, Udd, and Laing types of distal myopathy have preferential involvement of the anterior tibial muscles, Miyoshi myopathy the gastrocnemius, and Welander myopathy the extensor forearm muscles. Dystrophies associated with proximal greater than distal weakness are called limb-girdle dystrophies (LGMD). The LGMD, inherited in an autosomal dominant fashion, are termed LGMD type 1 (LGMD1), whereas autosomal recessive dystrophies are called LGMD2. Further subclassifications of the LGMDs are based on genotype differences (e.g., LGMD1A, LGMD1B).

There are problems with this traditional classification of muscular dystrophies. We now know that most of the genetic defects previously found to be associated with congenital muscular dystrophy can all be associated with milder, adult-onset dystrophy (see Table 1.1). In addition, clinical heterogeneity is sometimes evident within family members with specific mutations such that some may manifest with a limb-girdle pattern of weakness, whereas other members in the family have distal weakness (e.g., Miyoshi myopathy, anterior tibial myopathy, LGMD2B are all associated with dysferlin mutations). Thus, it may be more appropriate to classify the dystrophies by the genetic defect (e.g., dysferlinopathies, calpainopathy) and to understand the specific clinical phenotype, including age of onset and patterns of weakness that may be associated with the specific disorders. However, the classic terminology (e.g., LGMD) is so ingrained that it is likely to persist until a new generation of clinical investigators armed with the explanations for divergent phenotypes writes its textbooks.

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Table 1.1

Genetic classification of the muscular dystrophies

Disease	Inheritance	Chromosome	Affected protein
X-linked dystrophies			
Duchenne/Becker	XR	Xp21	Dystrophin
Emery–Dreifuss	XR	Xq28	Emerin
Scapuloperoneal/reducing body myopathy	XR	Xq26.3	Four and a half LIM domain protein 1 (FHL1)
Limb-girdle dystrophies (LGMD)			
LGMD1A	AD	5q22.3–31.3	Myotilin
LGMD1B	AD	1q11–21	Lamin A/C
LGMD1C	AD	3p25	Caveolin-3
LGMD1D	AD	6q23	?
LGMD1E	AD	7q	?
LGMD2A	AR	15q15.1–21.1	Calpain-3
LGMD2B*	AR	2p13	Dysferlin
LGMD2C	AR	13q12	γ-Sarcoglycan
LGMD2D	AR	17q12–21.3	α-Sarcoglycan
LGMD2E	AR	4q12	β-Sarcoglycan
LGMD2F	AR	5q33–34	δ-Sarcoglycan
LGMD2G	AR	17q11–12	Telethonin
LGMD2H	AR	9q31–33	E3 ubiquitin ligase (TRIM32)
LGMD2I	AR	19q13	*FKRP*
LGMD2J	AR	2q31	Titin
LGMD2K	AR	9q31	POMT1
LGMD2L	AR	11p14.3	Anoctamin-5
LGMD2M	AR	9q31–33	*Fukutin*
LGMD2N	AR	1p32	*POMGnT1*
LGMD2O	AR	14q24	POMT1
Congenital muscular dystrophies (MDC)			
MDC1A	AR	6q22–23	Laminin-α2 chain
α7 Integrin-related MDC	AR	12q13	α7 Integrin
MCC/LGMD2I	AR	19q13	*FKRP*
Fukuyama/LGMD2L	AR	9q31–33	*Fukutin*
WWS/LGMDK	AR	9q31	POMT1
MEB disease/LGMD2M	AR	1p32	*POMGnT1*
Rigid spine syndrome	AR	1p35–36	Selenoprotein N1
Ullrich/Bethlem	AR/AD	21q22.3 and 2q37	Collagens 6A1, 6A2, 6A3
Distal dystrophies/myopathies			
Welander	AD	2p13	?
Udd	AD	2q31	Titin
Markesbery–Griggs	AD	10q22.3–23.2	ZASP
Nonaka	AR	9p1–q1	GNE
Miyoshi 1	AR	2p13	Dysferlin
Miyoshi 2	AR	11p14.3	Anoctamin-5
Laing (MPD1)	AD	14q11	MyHC-7
Distal myopathy with vocal cord and pharyngeal weakness (VCPDM or MPD2)	AD	5q31	Matrin-3
Other dystrophies			
Facioscapulohumeral	AD	4q35	DUX4
Scapuloperoneal dystrophy	AD	2q35	Desmin
	AD	14q11	MyHC-7
	XR	Xq26.3	Four and a half LIM domain protein 1 (FHL1)
Emery–Dreifuss type 3	AD	6q24	Nesprin-1

Table 1.1

Continued

Disease	Inheritance	Chromosome	Affected protein
Emery–Dreifuss type 4	AD	14q23	Nesprin-2
Oculopharyngeal	AD	14q11.2–13	PABP2
Myotonic dystrophy 1	AD	19q13.3	DMPK
Myotonic dystrophy 2	AD	3q21	ZNF9
Myofibrillar myopathy	AD	5q22.3–31.3	Myotilin
	AD	10q22.3–23.2	ZASP
	AD	7q32.1	Filamin C
	AD	11q21–23	αβ-Crystallin
	AD/AR	2q35	Desmin
	AR	1p36	Selenoprotein N1
	AD	10q25–26	BAG3
Hereditary IBM			
AR hereditary IBM	AR		GNE
Hereditary IBM with FTD and Paget disease	AD		VCP
Hereditary IBM3	AD		MyHC-IIa

*LGMD2B and Miyoshi distal dystrophy are same condition.
AD, autosomal dominant; AR, autosomal recessive; BAG3, BCL2-associated AthanoGene 3; DMPK, myotonic dystrophy protein kinase; IBM, inclusion body myopathy; *FKRP*, *fukutin*-related protein; FTD, frontotemporal dementia; GNE, UDP-*N*-acetylglucosamine 2-epimerase/*N*-acetylmannosamine kinase; MCC, multiple congenital contractures; MEB, muscle–eye–brain; MPD, myophosphorylase deficiency; MyHC, myosin heavy chain; PABP, poly(A) binding protein; POMT1, protein O-mannosyltransferase gene; *POMGnT1*, protein O-mannose β-1,2-*N*-acetylglucosaminyltransferase; TRIM32, tripartite motif-containing protein 32; VCP, valosin-containing protein; VCPDM, vocal cord and pharyngeal weakness with distal myopathy; WSS, Walker–Warburg syndrome; XR, X-linked recessive; ZASP, Z-band alternately spliced PDZ motif-containing protein; ZNF, zinc finger protein 9.

MOLECULAR PATHOGENESIS FOR DYSTROPHIES

The muscular dystrophies can be caused by mutations that encode for sarcolemmal, basement membrane, sarcomeric, nuclear structural proteins, or enzymes (Figures 1.1 and 1.2, Table 1.2). Further, some disorders are caused by mutations that affect splicing of mRNA (e.g., the myotonic dystrophies) or by yet still unknown mechanisms (e.g., FSHD). The pathogenesis of the various forms of muscular dystrophy will be discussed in subsequent chapters.

CLINICAL FEATURES

Skeletal muscle weakness

The dystrophies are clinically heterogeneous. The most important aspect when evaluating a patient with a possible dystrophy – or any neuromuscular condition for that matter – is to try to define the pattern of muscle weakness. Most of the muscular dystrophies have a "limb-girdle" pattern of weakness with proximal leg and arm muscles being weaker than distal muscle groups. However, there is a group of disorders with more distal muscle involvement, as mentioned above, and others with peculiar patterns of involvement (e.g., oculopharyngeal, facioscapulohumeral, scapuloperoneal) that narrow down the differential diagnoses. It is important to look for facial weakness (FSHD), scapular winging (FSHD, LGMD2A), calf hypertrophy (dystrophinopathies, LGMD2C-F, LGMD2I), calf atrophy (LGMD2B/Miyoshi), significant asymmetries in strength (FSHD), and rippling muscles (LGMD1C). Muscles should be examined for myotonia that would lead to consideration of myotonic dystrophy 1 or 2, particularly if the patient has early cataracts, frontal balding, and temporal muscle wasting. Many patients with severe weakness due to dystrophies develop contractures. However, contractures in muscles groups that are not significantly weak should lead to consideration of Emery–Dreifuss muscular dystrophy or Ullrich/Bethlem myopathy. Axial muscle weakness can lead to progressive scoliosis, particularly once the individual is wheelchair bound.

Other muscle involvement

The dystrophies affect more than just skeletal muscles. Various dystrophies are associated with severe cardiomyopathy (Bushby et al., 2003; Norwood et al., 2007).

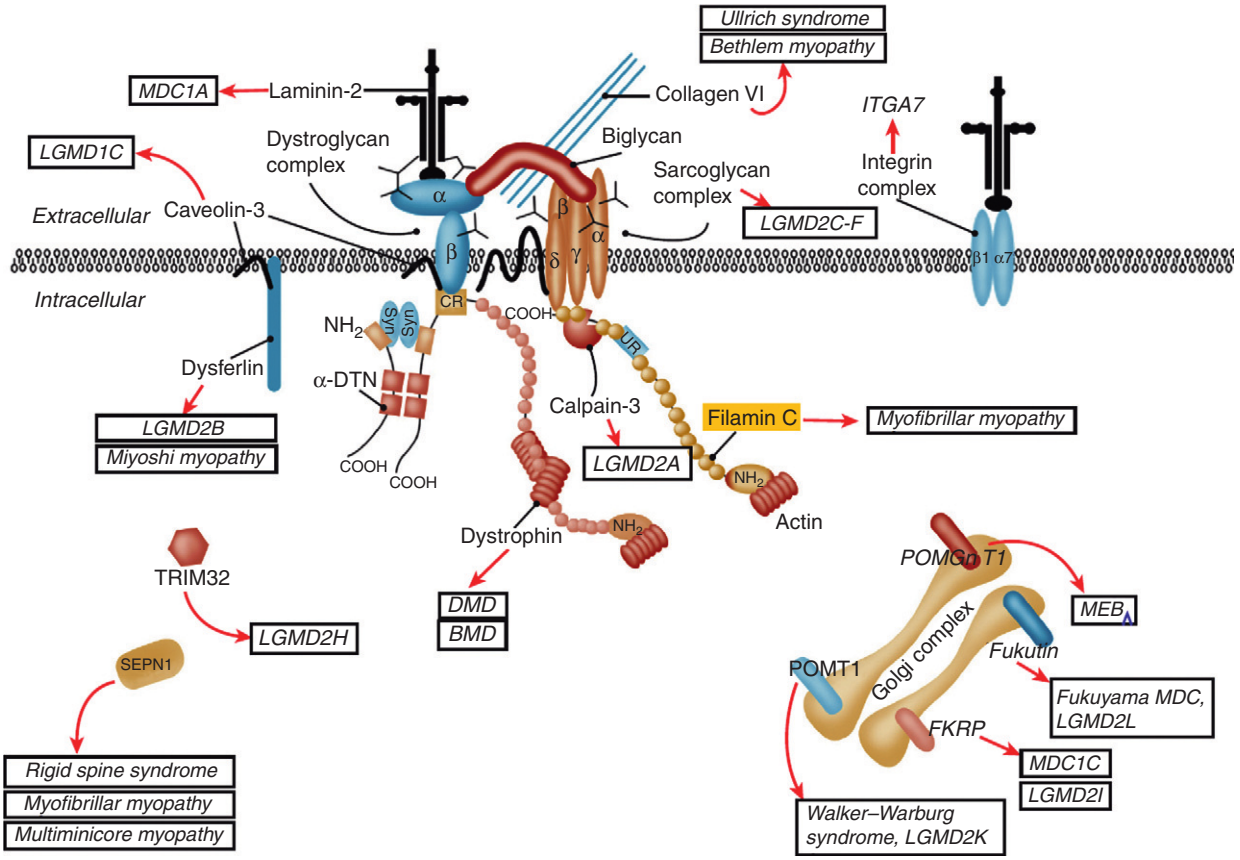

Figure 1.1. Sarcolemmal membrane and enzymatic proteins. This schematic shows the location of various sarcolemmal and enzymatic proteins associated with muscular dystrophies. The diseases caused by these molecules when mutated are shown in boxes. Dystrophin, via its interaction with the dystroglycan complex, connects the actin cytoskeleton to the extracellular matrix. Intracellularly, it interacts with dystrobrevin (α-DTN) and syntrophins (Syn) (shown in blue). Extracellularly, the sarcoglycan complex (orange) interacts with biglycan, which connects this complex to the dystroglycan complex and the extracellular matrix collagen. Intracellularly, δ- and γ-sarcoglycans interact with filamin C. The four proteins shown in the Golgi complex have been demonstrated to affect the glycosylation state of the α-dystroglycan and mediate its binding to the extracellular matrix. *Fukutin* and *fukutin*-related protein (*FKRP*) have been shown to localize to the medial Golgi. The localization of POMT1 (protein O-mannosyltransferase), *POMGnT1* (protein O-linked mannose β-1,2-*N*-acetylglucosaminyltransferase), and LARGE (another putative glycosyltransferase) is unknown but believed to be in the Golgi complex as these enzymes are involved in the glycosylation process. BMD, Becker muscular dystrophy; DMD, Duchenne muscular dystrophy; ITGA7, integrin-α7; LGMD, limb-girdle muscular dystrophy; MDC, congenital muscular dystrophy; MEB, muscle–eye–brain; SEPN, selenoprotein N; TRIM, E3 ubiquitin ligase. (From Amato AA, Russell J (2008) p. 530. Neuromuscular Disease. McGraw-Hill, New York, with permission.)

In some cases the cardiomyopathy may be the predominant feature of the illness, particularly in Emery–Dreifuss muscular dystrophy (EDMD), LGMD1B, sarcoglycanopathy, myotonic dystrophy, and the myofibrillar myopathies. The cardiomyopathy may manifest as rhythm disturbance or as congestive heart failure. Additionally, the ventilatory muscles are affected in many of the dystrophies, particularly LGMD2I and the myofibrillar myopathies. In such patients with early or prominent ventilatory muscle weakness, Pompe disease needs to be considered in the differential diagnosis. The primary cause of death in most patients with dystrophy are complications related to ventilatory failure (e.g., pneumonia) (Norwood et al., 2007). Smooth muscles may also be affected, leading to gastrointestinal hypomotility (gastroparesis and constipation). Decreased physical activity may compound the problem with constipation. Some patients develop difficulty swallowing as well, and may require a gastrostomy tube.

LABORATORY FEATURES

Muscle enzymes

Serum creatine kinase (CK) levels are usually increased (up to 20 times normal or greater) in most of the dystrophies, but in some of the more indolent disorders

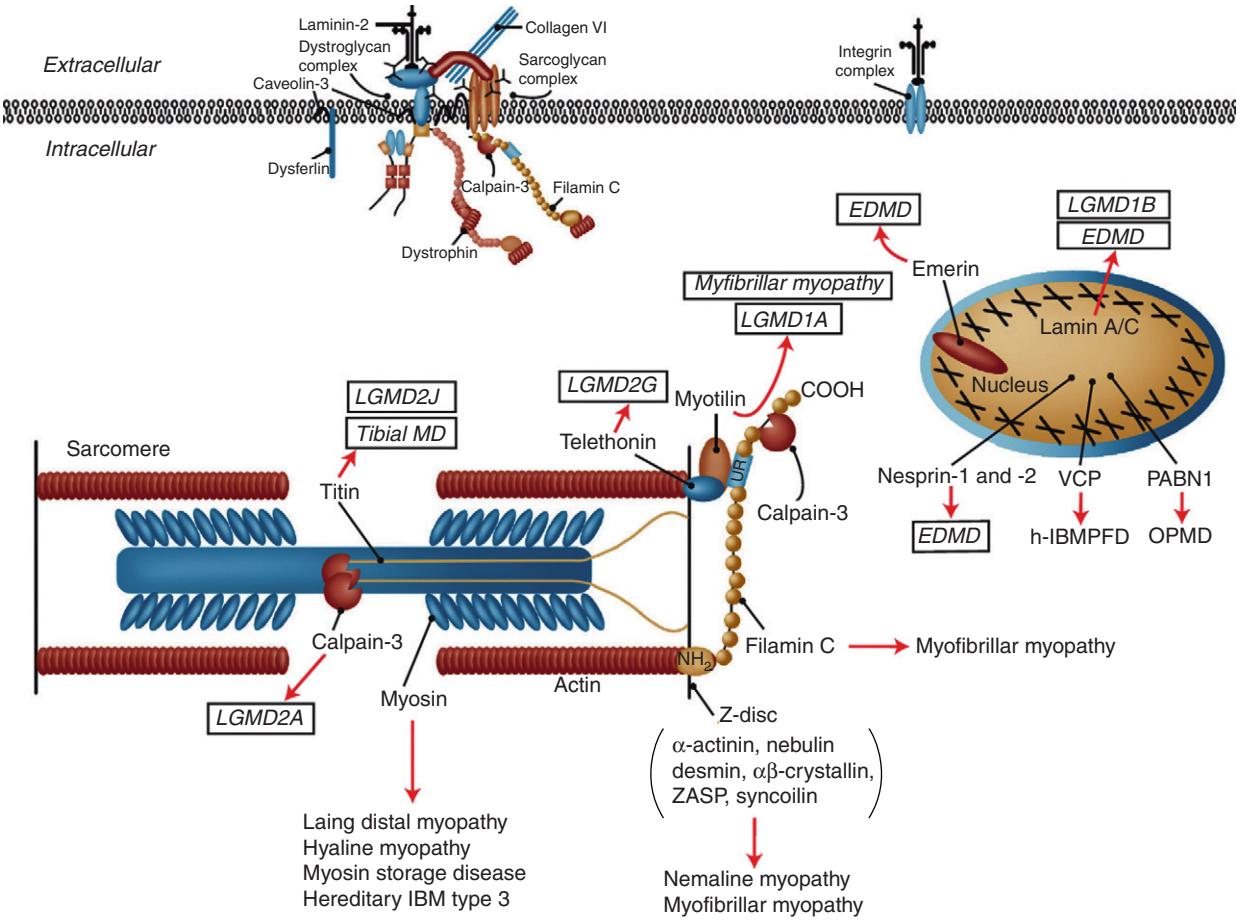

Figure 1.2. Sarcomeric and nuclear proteins involved in the muscular dystrophies. The schematic for the sarcomere and the nucleus showing the localization of the proteins involved in muscular dystrophies. The diseases they give rise to are shown in boxes. EDMD, Emery–Dreifuss muscular dystrophy; h-IBMPFD, hereditary inclusion body myopathy with Paget disease of bone; IBM, inclusion body myopathy; LGMD, limb-girdle muscular dystrophy; MD, muscular dystrophy; OPMD, oculopharyngeal muscular dystrophy; PABN, polyadenylate binding nuclear protein; VCP, valosin-containing protein; ZASP, Z-band alternately spliced PDZ motif-containing protein. (From Amato AA, Russell J (2008) p. 531. Neuromuscular Disease. McGraw-Hill, New York, with permission.)

(e.g., Ullrich/Bethlem myopathy, OPMD, and EDMD) the CK level may be normal or only slightly raised. Levels of other enzymes, including aldolase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH), may be raised as well, but the CK concentration is usually the most sensitive and specific marker for muscle destruction.

Neurophysiology

Electrodiagnostic testing in patients with suspected dystrophy is of limited value, particularly when there is a family history of the disorder. Electromyography (EMG) may be helpful in sporadic cases or in patients with normal or only slightly raised CK levels, in which case the differential diagnosis is broad and may include spinal muscular atrophy, neuromuscular junction disorders (congenital myasthenia), severe neuropathy (e.g., Charcot–Marie–Tooth disease type 3), or other myopathic conditions (e.g., congenital myopathies, metabolic myopathies, inflammatory myopathies). Needle EMG in affected muscle groups usually demonstrates increased insertional and spontaneous activity in the form of fibrillation potentials and positive sharp waves in those dystrophies with active necrosis. However, in more indolent myopathies there may be little abnormal insertional or spontaneous activity. Further, as muscle tissue is progressively replaced with both adipose cells and connective tissue, insertional activity diminishes. The presence of myotonic discharges on EMG is helpful in narrowing the differential diagnosis. Both short- and long-duration, polyphasic motor unit action potentials (MUAPs) may be apparent and reflect the chronicity of

Table 1.2

Molecular defects associated with muscular dystrophies

Mechanism	Protein or enzyme affected
Sarcolemmal	Dystrophin
	Sarcoglycans (α, β, γ, δ)
	Dysferlin
	Caveolin-3
Basement membrane	Merosin (α2 laminin)
	α7β1D integrin
	Collagen 6A1, 6A2, 6A3
Sarcomeric	Myotilin
	Titin
	Telethonin
	Z-band alternately spliced PDZ motif-containing protein (ZASP)
	Filamin C
	Desmin
	Myosin heavy chains
	FHL1 (four and a half LIM domain protein 1)
Nuclear	Emerin
	Lamin A/C
	Nesprin 1 and 2
	Poly(A) binding protein 2 (PABP2)
	Valosin-containing protein (VCP)
	Matrin-3
Enzymes	Calpain-3
	Tripartite motif-containing protein 32 (TRIM 32), also known as E3 ubiquitin ligase
	Fukutin
	Fukutin-related protein
	Protein O-mannose-β-1,2-N-acetylglucosaminyltransferase 1 (*POMGnT1*)
	Protein O-mannosyltransferase 1 (POMT1)
	LARGE
	UDP-*N*-acetylglucosamine 2-epimerase/*N*-acetylmannosamine kinase (GNE)
RNA splicing	Myotonic dystrophy type 1 and 2
Other or unclear mechanism	Facioscapulohumeral muscular dystrophy (FSHD)
	Selenoprotein N

the myopathic process. As opposed to neurogenic disorders in which there is a reduced number of MUAPs that fire at an excessive rate (decreased recruitment), in myopathic muscles one sees early recruitment pattern of MUAPs firing at normal rates even with low levels of force.

The condition in patients with lamin A/C mutations may present as a myopathy (LGMD1A or EDMD), but also as a hereditary neuropathy (dominant intermediate Charcot–Marie–Tooth disease type B) with mixed axonal and demyelinating features on the nerve conduction studies. Congenital muscular dystrophies associated with merosinopathy may also have slow conduction velocities on nerve conduction studies owing to the hypomyelination of nerves. Some of the myofibrillar myopathies are associated with concomitant peripheral neuropathy as well.

Radiological studies

Muscle imaging by means of computed tomography and, more recently, magnetic resonance imaging has been used to delineate the specific muscle groups that are affected, particularly early in the course of the disease. For example, in LGMD2A caused by calpain-3 mutations, imaging studies show early involvement of hip extensors and adductors as well as the hamstrings (Mercuri et al., 2005).

MUSCLE BIOPSIES

Histopathology

Muscle biopsies characteristically demonstrate scattered necrotic and regenerating muscle fibers with increased endomysial and perimysial connective tissue. Variability in fiber size with scattered hypertrophic and hypercontracted fibers in addition to small, rounded, regenerating fibers are typically found, as well as muscle fiber splitting and internalized nuclei. Endomysial inflammatory cell infiltrates also may be noted. In some dystrophies (e.g., dysferlinopathies, FSHD, calpainopathies), the infiltrate may be so prominent that the patient is misdiagnosed as having polymyositis. In biopsies of minimally affected muscles the histopathological findings may be subtle and nonspecific.

Immunohistochemistry

Immunohistochemistry is helpful in the evaluation of dystrophies. Immunostaining for dystrophin, sarcoglycan (α, β, γ, and δ), merosin, α-dystroglycan, and dysferlin are routinely employed for patients with limb-girdle syndrome. Emerin staining is done in suspected cases of EDMD. Immunostaining for lamin A/C and collagen-6 can also be performed, but these are often normal as the disorders associated with mutations affecting these proteins are usually autosomal dominant and thus there is sufficient protein to permit immunostaining. If routine light microscopy is suggestive of a myofibrillar myopathy then immunostaining for myotilin and desmin can be informative.

Immunoblot or western blot of muscle tissue is often performed to assess the quantity and size of the dystrophin, calpain-3, and dysferlin when the relevant disorders are suspected. Western blot can also be done on peripheral monocytes when dysferlinopathy is a consideration.

Electron microscopy

Electron microscopy (EM) can demonstrate abnormalities but usually is not helpful in distinguishing one type of dystrophy from another. The exception is the myofibrillar myopathies in which the structural changes seen are not typical of other forms of LGMD. Abnormalities evident only on EM are rarely helpful in diagnosis.

MOLECULAR ANALYSIS

Genetic testing

Usually, a strong suspicion of a specific muscular dystrophy based upon the clinical features or muscle biopsy findings leads to confirmation of the impression with genetic testing. Genetic testing may obviate the need for invasive muscle biopsy or EMG. Genetic testing is available for most, but not all, of the muscular dystrophies. For laboratories that perform genetic testing, visit www.genetests.com.

Recommended sequence of diagnostic genetic testing

Large genetic panels are seldom appropriate. The appropriate genetic test is usually suggested by the sex, ethnicity, inheritance pattern, and pattern of muscle involvement. In X-linked or sporadically affected boys or men with symmetrical limb-girdle weakness and large calf muscles, dystrophin mutations are likely. In males or females with limb-girdle weakness and/or calf atrophy associated with onset in the late teens or twenties and markedly raised CK levels, a dysferlinopathy is suggested. Scapular winging limb-girdle weakness in a patient from southern or eastern Europe or South America suggests a calpainopathy, whereas those from a UK or northern European background are more likely to have *fukutin*-related protein (*FKRP*) mutations.

TREATMENTS

Corticosteroids

Corticosteroids are the only medication shown to slow the rate of progression of weakness in randomized controlled trials of large numbers of patients (Manzur et al., 2008). Both prednisolone and deflazacort have been demonstrated to improve strength in children with Duchenne muscular dystrophy (Brooke et al., 1987; Mendell et al., 1989; Fenichel et al., 1991; Griggs et al., 1993; Moxley et al., 2005). There is insufficient evidence to comment on any possible benefit in patients with other forms of dystrophy. Personal experience suggests that many patients with various dystrophies (particularly dysferlinopathy, calpainopathy, and FSHD) who are misdiagnosed with polymyositis and treated with corticosteroids have not had apparent improvement. However, we do not know whether corticosteroids might slow the rate of progression of the disease. It seems reasonable, particularly with the childhood-onset sarcoglycanopathies and fukutinopathies, that corticosteroids may be beneficial.

Other medications

Other medications have been tried (e.g., creatine, oxandralone, albuterol) in small trials involving different types of dystrophy. Modest benefit has been reported in small trials of creatine in patients with DMD, BMD, and LGMD (Kley et al., 2007; Tarnopolsky, 2007) No functional benefit is seen with oxandralone in DMD (Fenichel et al., 2001). Lean body mass of

subjects with DMD and BMD was significantly higher following albuterol treatment compared with placebo (Skura et al., 2008). However, no differences were found in isometric knee strength or manual muscle tests. Although albuterol did not improve global strength or function in patients with FSHD, it did increase muscle mass and improve some measures of strength (Kissel et al., 2001).

Myostatin is an endogenous inhibitor of muscle growth. Mutations in the gene that encode for myostatin lead to muscle hypertrophy, suggesting that blockers of myostatin may be useful in treating muscle diseases. A recent trial of a neutralizing antibody to myostatin, MYO-029, in adult muscular dystrophies (Becker muscular dystrophy, FSHD, and LGMD) demonstrated that the drug was safe (Wagner et al., 2008). However, no improvement was observed in muscle strength or function, although the study was not powered to determine efficacy. Interestingly, the bioactivity of myostatin inhibition was supported by a trend in a limited number of subjects toward increased muscle size using dual-energy radiographic absorptiometry and muscle histology.

Gene therapy

Somatic gene therapy via myoblast has been disappointing, with several trials failing to demonstrate signs of efficacy (Mendell et al., 1995). Stem cell transplantation is still in the experimental stage in animals as modes to enhance delivery to the muscle need to be developed. A few small safety trials of direct gene replacement utilizing modified viral vectors have begun but the availability of gene therapy is still some way off (Muntoni and Wells, 2007). Studies are also ongoing of various compounds that have the ability to allow RNA transcriptase to read through stop codon mutations (Allamand et al., 2008) and antisense oligonucleotides in order to allow transcription of the missing protein (van Deutekom et al., 2007).

Supportive therapies

Patients are best managed using a multidisciplinary approach that involves neurologists, physiatrists, physical therapists, occupational therapists, speech therapists, respiratory therapists, dietitians, psychologists, and genetic counselors working together to meet the needs of individual patients. Physical therapy is a key component in the treatment of patients with muscular dystrophy. Because contractures develop early in the disease, particularly at the heal cords, iliotibial bands, and the hips, appropriate stretching exercises must be started early in the disease. Long leg braces may aid ambulation.

The cause of death in many of the dystrophies is secondary complications related to ventilatory failure (e.g., pneumonia) (Norwood et al., 2007). It is important to have patients vaccinated (e.g., influenza) for prophylactic management. In some conditions, such as LGMD2I, ventilatory muscle weakness can be an early manifestation of the disease. It is important to monitor ventilatory function and obtain baseline pulmonary function test results. In patients with dyspnea or with a forced vital capacity (FVC) of less than 80%, it is useful to assess FVC in the sitting and supine positions, as with diaphragmatic weakness the reduction in FVC is accentuated and may be apparent in mild disease only when the patient is supine. Reduction in FVC occurs prior to hypoxemia or hypercarbia on blood gas analysis. When the FVC falls below 60% or the patient has symptoms suggestive of nocturnal hypoventilation (e.g., morning headaches, frequent nocturnal arousals, excessive daytime sleepiness), overnight pulse oximetry should be performed. In patients who are dyspneic, low FVC ($< 50\%$), or who have hypoxemia on nocturnal oximetry, noninvasive positive pressure ventilation is initially indicated at night and later during the day. It is essential that, before patients develop severe respiratory failure, their wishes regarding tracheostomy and mechanical ventilation are discussed with them and family members, and documented in the records.

Because patients may develop a cardiomyopathy, baseline and periodic electrocardiography (every 2 years) is usually indicated. Some patients require a pacemaker or defibrillator. In those patients with dyspnea or with dystrophies associated with dilated cardiomyopathy, echocardiography is usually indicated. Patients with cardiac failure may benefit from afterload reduction (e.g., angiotensin-converting enzyme inhibitor). When the cardiomyopathy is severe but respiratory function and skeletal muscle impairment are mild, the patient may also be a candidate for heart transplantation.

Scoliosis is a common complication of many of the dystrophies. Spinal fusion in children with 35° scoliosis or greater can prevent pain (Cheuk et al., 2007). Ideally, FVC should be greater than 35% to minimize the risk of surgery. Quality of life may be enhanced following spinal stabilization, although scoliosis surgery may not improve ventilatory function.

SUMMARY

The muscular dystrophies are a clinically and genetically heterogeneous group of myopathies typically associated with progressive weakness. Weakness may be noted at birth or develop in late adult life. Some patients manifest with myalgias, rhabdomyolysis, or only raised serum CK levels without any symptoms

or signs of weakness. The muscular dystrophies can be inherited in an X-linked, autosomal recessive, or autosomal dominant fashion, and can result from mutations affecting structural proteins localizable to the sarcolemmal proteins, nuclear membrane, basement membrane, sarcomere, or nonstructural enzymatic proteins. Electrophysiological and routine histological examination of muscle biopsies are usually not helpful in distinguishing the different types of muscular dystrophy. Therefore, it is important for the clinician to understand the phenotypic differences that may be seen in the various types of dystrophy and the tests available to make accurate diagnoses, provide genetic counseling, and treatment for patients with dystrophies.

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Chapter 2

Dystrophinopathies

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INTRODUCTION

This chapter encompasses Duchenne muscular dystrophy (DMD), Becker muscular dystrophy (BMD) (inclusive of asymptomatic hyperCKemia, cramps and myalgia, quadriceps myopathy), X-linked dilated cardiomyopathy (XLDCM), and manifesting/nonmanifesting DMD/BMD carriers. All of these diseases are caused by mutations in the *DMD* (or dystrophin) gene at Xp21.2 which encodes the protein dystrophin. DMD is the most severe disorder and it will be described in detail, with related diseases discussed in comparison. The presentation of a young boy with progressive proximal weakness, calf hypertrophy, and raised creatine kinase (CK) levels almost always leads to the diagnosis of DMD. This fatal diagnosis affects 1 in 3500 boys (Emery, 1991), who will experience progressive loss of muscle strength and ambulation, develop deformities, and ultimately succumb to restrictive lung disease or cardiac death. Family, teachers, friends, and medical care providers will suffer with them through the diagnostic process, each functional loss, and early death. Corticosteroid treatment slows disease progression, but curative therapies are not yet available despite more than 20 years of research since the discovery of the *DMD* gene mutation. In this chapter, we will review natural history, a brief history of discovery, pathology, genetics, pathophysiology, pathogenesis, diagnosis, treatment, and emerging therapies in DMD and other dystrophinopathies.

Brief history

In 1851, British physician Meryon described a family of four boys affected by progressive weakness, describing pathological changes of muscle with sarcolemmal involvement and anatomically normal brains. He recognized a male predominance (Emery, 1993; Meryon, 1851). French neurologist Duchenne du Bologne published his first description of DMD in 1861, with a more comprehensive account in 1868 (Duchenne, 1861, 1868). In 1879, British neurologist Gowers described and illustrated the characteristic physical features of calf muscle hypertrophy and proximal weakness, and the classic mode of coming to stand now known as Gowers' sign. A milder form of the disease with later onset was recognized by Becker in 1955 (Becker and Kiener, 1955). Marked increases in CK levels were discovered in affected males, and in carrier females to a lesser degree. Electrophysiological studies demonstrated myopathic discharges.

In 1986, the discovery of the *DMD* gene through the technique of positional cloning marked a new era in the field of neuromuscular medicine (Kunkel et al., 1986). Subsequent to gene discovery, the encoded protein was found and named dystrophin (Hoffman et al., 1987; Beggs and Kunkel, 1990), setting a precedent for naming of encoded proteins ending with –in (Hoffman and Kunkel, 1989). The spectrum of diseases related to mutations of this gene has broadened to include XLDCM, cramps and myalgia, some cases of quadriceps myopathy and asymptomatic hyperCKemia (Beggs and Kunkel, 1990; Sunohara et al., 1990; Saengpattrachai et al., 2006), and also includes carriers who may manifest musculoskeletal and cardiac abnormalities (Larsen and Juhl, 1988).

DYSTROPHINOPATHY SPECTRUM (TABLE 2.1)

Duchenne muscular dystrophy

DMD is the most severe dystrophinopathy. Boys are mildly delayed in motor and language development even before the first year of life (Cyrulnik et al.,

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Table 2.1

Dystrophinopathy spectrum

Disease	Age (range) of onset (years)	Clinical motor involvement (onset in years)	Other systems (onset in years)	Lifespan (years)	Common mutations (less common)
Males					
DMD	(3–7)	DD (1); hip and dorsiflexor weakness (3); calf hypertrophy (4); loss of walking (by age 13 years)	Dilated cardiomyopathy (6–8); tachyarrhythmias and bradyarrhythmias; restrictive lung disease; osteopenia; nonprogressive learning disabilities; autism	Without scoliosis surgery and NIV (20s); with (30s)	Deletion exons 45–51; large NH_2 terminus and rod domains in 60–70%
BMD	5–13 (7–20)	Toe walking, contractures, loss of walking (16 or over, mean 30)	Dilated cardiomyopathy (13–21); arrhythmias; learning disabilities; psychiatric disturbance	Variable	Large inframe NH_2 terminus and rod domains (85%), especially distal exons 45–48
Cramps and myalgia	Nonprogressive or slowly progressive	Normal		Normal	Del exons 45–52, 10–22, 13–18, 45–51, 45–48
XLDCM	(10–52)	Minimal or null	Raised CK levels	Sudden death after first decade	Exon 1 and promoter (muscle enhancer, Dp 427, dup exons 2–7, del 45–48)
Asymptomatic hyperCKemia	Oldest detected still asymptomatic (76)	Normal	Raised CK levels		Small partial duplication exon 34, del 50, 51, del 48–51, and 5′ and distal half central rod exons 44–53
Females					
Manifesting	Variable	DMD, limb-girdle phenotype, cramps, myalgia	Raised CK levels; muscle biopsy shows myopathic and inflammatory changes	Variable based on severity	All; other X-linked diseases
Nonmanifesting	Adult	Normal except decreased ability to perform steady-state work	CK levels raised or normal; ECG changes increase with age	Essentially normal	All

BMD, Becker muscular dystrophy; CK, creatine kinase; DD, developmental disability; del, deletion mutation; DMD, Duchenne muscular dystrophy; dup, duplication mutation; ECG, electrocardiogram; NIV, noninvasive ventilation; XLDCM, X-linked dilated cardiomyopathy.
Sources: Berko and Swift, 1987; Comi et al., 1992; Arbustini et al., 2000; Aartsma-Rus et al., 2006b.

2007). Most boys never run or jump well. Progressive weakness of ankle dorsiflexor, hip flexor and extensor muscle groups begins between 3 and 5 years of age (Dubowitz, 1989) followed by quadriceps, hip adductor and extensor, neck and abdominal flexor weakness, proximal upper extremity and respiratory muscle weakness. This pattern of specific weakness and preservation of strength in other muscle groups results in standing and walking on toes (Figure 2.1), wide-based stance and gait, and exaggerated lumbar lordosis, all of which worsen progressively until ambulation is no longer possible. Ultimately almost all muscle groups exhibit weakness and there is complete physical dependence for personal care and mobility.

Gowers' sign is a nonspecific manifestation of proximal lower extremity weakness, causing patients to rise from the floor with buttocks up first, using arms to climb up the legs while buttocks remain elevated (Figure 2.2A, B). Similar strategies using hands and fingers to crawl up the arms to reach the face and head are employed as weakness progresses in order to preserve independent activities of daily living such as hair brushing, donning eyeglasses, or eating.

Hypertrophy of calf muscles becomes obvious by 4–5 years of age and persists well beyond the onset of atrophy in other muscle groups (see Figure 2.1).

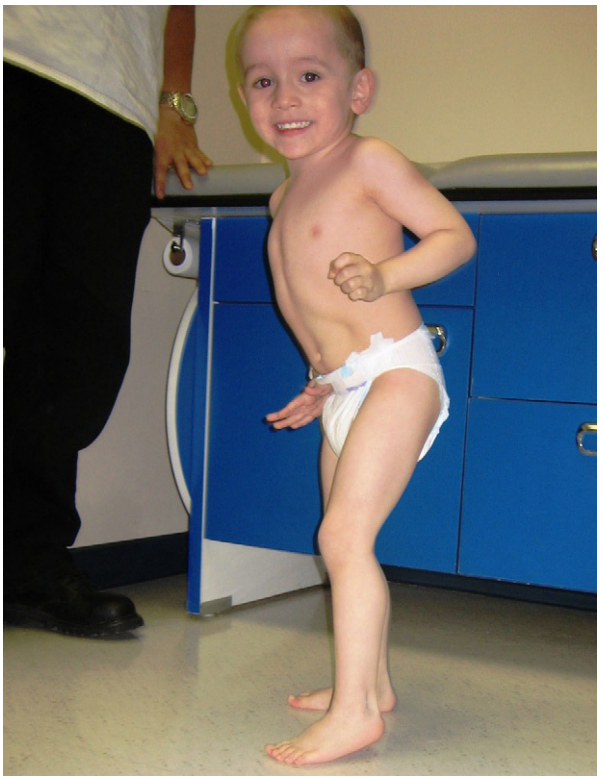

Figure 2.1. Four-year-old boy with stance illustrating plantar flexion and early calf muscle hypertrophy.

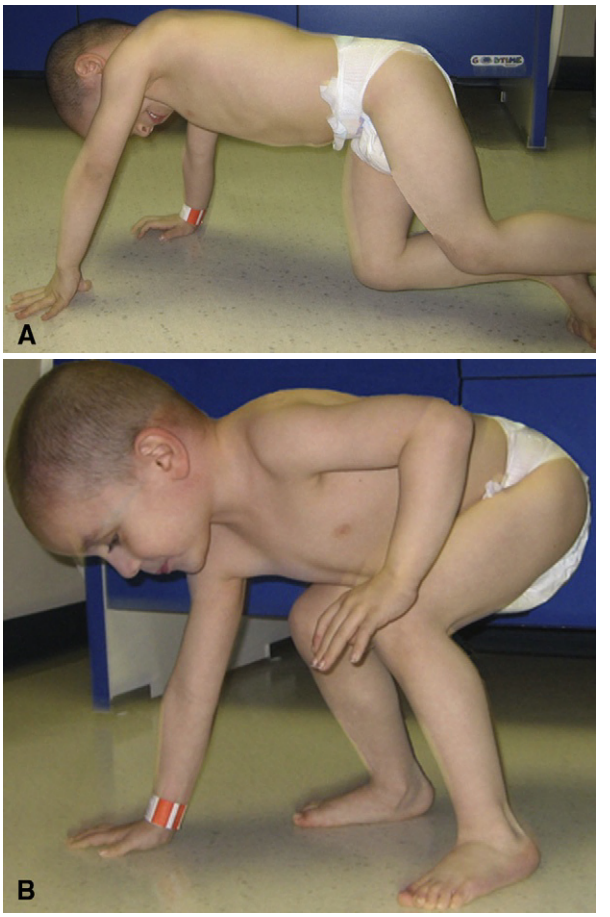

Figure 2.2. Four-year-old boy (**A**) arising from the floor using Gowers' sign and (**B**) completing stance.

Plantar flexor muscle strength is preserved and ankle jerk reflexes are retained even beyond the point of loss of ambulation in some cases. Hypertrophy may also be seen in vastus lateralis, infraspinatus (Pradhan, 1994; Pradhan and Mittal, 1995), deltoid, and tongue. Gracilis hypertrophy is noted on muscle imaging (Liu et al., 1993).

Loss of ambulation is an important clinical diagnostic milestone in the dystrophinopathy spectrum, best predicted by loss of strength in hip extension and ankle dorsiflexion (Bakker et al., 2002). DMD is clinically defined in noncorticosteroid-treated boys as those who cease ambulation before 13 years of age. An intermediate form exists where loss of ambulation occurs between 13 and 16 years of age. If still walking independently by 16 years, the clinical phenotype is known as Becker muscular dystrophy (BMD). These disorders appear as a clinical spectrum caused by *DMD* gene mutations, commonly grouped as DMD/BMD.

Contractures in ankle plantar flexion, hip flexion and abduction begin even before loss of ambulation. This is followed by knee flexion contractures which progress

rapidly after wheelchair confinement. In the upper extremities, elbow flexion contractures are followed by shoulder flexion, forearm pronation and finger flexion contractures, further limiting function and preventing comfortable positions in the wheelchair and bed.

Perceived pain is mild with less impact on function compared with other muscle diseases, but at least 40% of adult patients with DMD live with pain (Guy-Coichard et al., 2008). Pain may be caused by muscle cramping early in the disease. As deformity ensues, comfortable positioning becomes more challenging. Areas of bony prominence are subject to decubitus ulcers, which are often painful.

Systemic illness and rapid linear growth can accelerate disease progression. Periods of immobilization (postoperative, for fractures or illness) may precipitate loss of ambulation or rapid worsening of contractures.

Patients with DMD/BMD are susceptible to complications of anesthesia. In a retrospective series of 65 Canadian patients with DMD, complications included increased body temperature, abdominal pain,and dark-colored urine, and three had perioperative critical malignant hyperthermia-like complications associated with succinylcholine, and which occurred prior to the diagnosis of DMD in some cases (Larsen and Juhl, 1988).

Respiratory system

Sleep-related breathing disorders are prevalent in patients with DMD, 10-fold higher than in the general pediatric population (Suresh et al., 2005). Muscles of respiration continually weaken, particularly the diaphragm and intercostal muscles. Restrictive pulmonary disease may be subtle at onset and first noted in nocturnal hypoventilation. This is manifested by morning headaches, daytime somnolence and lethargy, nocturnal snoring, and sleep disturbance. Approximately one-third of patients are symptomatic at a mean age of 8 years (Suresh et al., 2005). This is followed by more frequent and prolonged infections due to ineffective cough from weakness in accessory muscles of respiration and decreased diaphragmatic excursion caused by fibrosis. As scoliosis progresses, there is increased chest deformity with decreased chest wall compliance. Without noninvasive ventilation (NIV), scoliosis surgery, or corticosteroid treatment, young men succumb to the disease by the early twenties, usually from respiratory causes (Toussaint et al., 2007b).

Cardiovascular system

Inappropriate tachycardia is common from an early age (Goodwin and Muntoni, 2005). Myocardial involvement is present by 6 years of age in a large proportion of boys with DMD. Some 30% of boys with DMD will have detectable cardiac abnormalities by the age of 14 years, half by 18 years, and virtually all survivors by age 24 years (Nigro et al., 1995). Many remain clinically asymptomatic because as they become more sedentary there is less demand placed on the compromised cardiac system (English and Gibbs, 2006). Electrocardiograms (ECGs) are abnormal in 90% of affected patients with tall right precordial R waves reflecting posterobasal fibrosis, and abnormal intra-atrial conduction defects with labile or persistent sinus tachycardia (Finsterer and Stollberger, 2003). Dilated cardiomyopathy and arrhythmias shorten lifespan and can result in sudden death (Chenard et al., 1993; Bianchi et al., 2003). Because survival has improved with NIV and mechanical ventilation by tracheostomy, more patients with DMD are living long enough to manifest cardiac disease (Wagner et al., 2007).

Skeletal

Osteopenia and osteoporosis are commonly seen by 10–12 years of age, even in noncorticosteroid-treated boys. Vitamin D levels may be deficient in boys with DMD. In a retrospective study, extremity fracture incidence was high (44%), typically resulting from falls (Larson and Henderson, 2000; Aparicio et al., 2002; Bianchi et al., 2003). In a survey of medical records and parent interviews in treated and untreated boys, the overall fracture incidence was somewhat lower at approximately 21%. Boys walking with knee–ankle–foot orthoses had more humeral fractures. Persistent and rapid functional loss after a fracture was not uncommon (McDonald et al., 2002).

Short stature is reported in DMD, but the cause is not readily identifiable, possibly related to muscle weakness or growth hormone or growth factor deficiencies. In boys with true short stature, the course may be milder. There are case reports in which the *SHOX* gene (pseudoautosomal region of Xp22) also has a mutation that causes short stature (Nagel et al., 1999).

Vertebral fractures are rare in noncorticosteroid-treated boys and they may be asymptomatic. Kyphoscoliosis occurs in more than 90% of untreated boys with DMD once they lose ambulation. Approximately 25% of patients have milder curves that do not require surgery, and 15% do not show progression (Oda et al., 1993). The natural lordotic position in standing and walking may forestall the development of lateral spine curvature, so boys who walk longer are less likely to acquire severe scoliosis. Curves begin in the thoracic region and involve many vertebral segments, starting as a long mild curve, but progressing to more severe and complex deformity. The rate of scoliosis progression is 2.1° per month (Smith et al., 1989). Hip asymmetry and forced vital capacity (FVC) at 11–12 years of age were

directly correlated with scoliosis severity. Progression of curves beyond 40° results in loss of function due to diminished sitting tolerance and use of upper extremities, pain, and diminished vital capacity (Hsu, 1983). An 8% decline in pulmonary function is predicted for boys with DMD and scoliosis (Galasko, 2001), and a 4% decline in pulmonary function is predicted for every 10° of curve (Kurz et al., 1983). Scoliosis is associated with cosmetic deformity and diminished height.

Contractures in the hip flexors and iliotibial bands sometimes precede loss of ambulation, and increase rapidly once patients are confined to a wheelchair. Flexion and ulnar deviation contractures were found in boys aged 8–14 years. Additional deformities of fingers were associated with pain on passive flexion of the proximal interphalangeal joint (Wagner et al., 1989). The combination of progressive weakness and deformity limits hand function necessary for personal care and use of electronic devices.

Gastrointestinal and nutrition

Gastrointestinal complaints are uncommon, but gastroparesis can occur and may be fatal (Barohn et al., 1988). Esophageal motility disorders were found in all of nine patients with DMD studied with manometry at a mean age of 8 years, and 77% of these boys had upper and lower gastrointestinal symptoms that did not correlate with manometric studies (Camelo et al., 1997). Constipation and obstipation occur in some boys, especially following surgery and with wheelchair confinement. Obesity is common after full-time wheelchair use, although some boys remain thin. Regardless of weight, there is progressive muscle atrophy and decreased muscle mass. In later stages, there is an ongoing concern for decubitus ulcers, especially in thin and poorly nourished sitters.

Brain and behavior

Duchenne recognized brain dysfunction in his first case reports, initially attributing muscle weakness to brain involvement. Brain function is clearly abnormal in DMD/BMD (Zellweger and Niedermeyer, 1965). Average IQ is 80–90, one standard deviation below the mean with verbal IQ more impaired than performance IQ. It is important to note that the IQ range is large with many bright patients counterbalancing those with more severe learning problems. Studies reveal a pattern of neuropsychological dysfunction with poor digit span, story recall and comprehension unrelated to physical disability (Hinton et al., 2000). Deficits in verbal span capacity are noted (Hinton et al., 2007b).

Most boys will require special assistance in school for this nonprogressive learning disorder. There are numerous examples of patients with more extreme brain dysfunction with autistic features (Komoto et al., 1984; Kumagai et al., 2001; Wu et al., 2005; Darke et al., 2006). A survey of parents in the Netherlands and the USA found rates of 11.7% for attention deficit/hyperactivity disorder (ADHD), 3.1% for autistic spectrum disorder, and 4.8% for obsessive–compulsive disorder in patients with DMD. There was no association between corticosteroid use and ADHD (Hendriksen and Vles, 2008). Boys with DMD had poorer facial recognition than their siblings, similar to the performance of autistic children (Hinton et al., 2007a). Eventually, chronic hypercapnia and hypoxemia may worsen brain dysfunction in more impaired patients but there are no reports of progressive decline in cognition in dystrophinopathies.

Eye

Extraocular muscles are relatively spared in DMD, inherently resistant to the lack of dystrophin possibly due to an intrinsic ability to maintain calcium homeostasis compared with other muscle groups (Kaminski et al., 1992; Khurana et al., 1995). Asymptomatic retinal abnormalities have been identified in some boys with DMD by electroretinography. About two-thirds of boys studied were found to have nonprogressive red–green colorblindness, compared with 10% of the normal male population. No genetic linkage to colorblindness was found in early studies (Emery, 1966; Zatz et al., 1974) but a later study found that no patients with DNA deletions upstream of exon 30 had colorblindness (Costa et al., 2007).

Other problems

Platelet dysfunction and fragility are manifested as a bleeding tendency during surgery. An increased risk of venous thrombosis may be related to factors associated with muscle necrosis (Porreca et al., 1999) or hypercoagulable factors related to cardiac dysfunction (Saito et al., 2005). Urinary control problems are reported, but are likely related to motor impairment (MacLeod et al., 2003).

Quality of life

Quality of life (QoL) was lower than normal peers in a group of younger patients with DMD, BMD, and sarcoglycanopathy (Grootenhuis et al., 2007). For most patients, QoL is good. This was measured by the Auto-questionnaire Qualité de Vie Enfant Imagé (AUQUEI) along with quantitative and qualitative interviews, and was concordant with the assessments of caregivers (Longo-Araujo de Melo and Moreno-Valdes, 2007).

The Short Form 36 (SF-36) health-related QoL measure was used in 35 Swiss patients treated or living in a specialized care facility. They had a surprisingly high QoL that did not correlate with the level of physical impairment or need for NIV (Kohler et al., 2005) or mechanical ventilation (Bach, 1992). Even so, patients may eventually decide to discontinue long-term ventilation for life prolongation (Hilton et al., 1993).

In a retrospective review of 25 adult patients with DMD (16 years and older) in the UK, 12 attended mainstream schools, 12 were in special residential schools, 16 received NIV, and 9 died during the period of observation. They reported problems with obtaining appropriate lifts and adequate personal care. Patients and parents were concerned that nonparental care providers would be inadequately trained, and help was sometimes rejected on this basis.

Patients did not usually wish to discuss end-of-life issues. Death often occurred unexpectedly, out of the hospital, and the cause was difficult to establish (Parker et al., 2005). Progressive carbon dioxide narcosis and overwhelming infection were found prior to death in some. QoL and end-of-life issues will be increasingly important as part of DMD management, especially for adult neuromuscular specialists. Care must be individualized and sensitive to the emotional (love life) and cultural needs of patients and their families (Rahbek et al., 2005).

Life expectancy

At the time of the initial description, life expectancy was limited to 16–18 years of age with death from pneumonia. Until the last decade, most reports referred to life expectancy in the late teens or early twenties owing to restrictive pulmonary disease, with 10–40% of deaths due to cardiac disease. With modern pulmonary management and surgeries, life expectancy has increased to 25 years on average, with some patients surviving into their thirties (Simonds et al., 1998; Eagle et al., 2002, 2007; Yasuma et al., 2004; Wagner et al., 2007).

Becker muscular dystrophy

BMD affects approximately 1 in 8000–10 000 males. Patients with BMD present later than those with DMD and they ambulate independently until at least 16 years of age, with a mean age of 30 years. Progression is slower and there is a longer life expectancy. Boys and young men may present with cramps and myalgia, or with an asymptomatic increase in levels of liver enzymes assayed for other reasons, sometimes accurately diagnosed only after extensive gastrointestintal workup, including liver biopsy. Pseudohypertrophy of calf muscles may be marked at the time of diagnosis. Electromyography (EMG) typically reveals fewer features of necrosis than DMD.

In BMD, full-scale cognitive profile is within the normal range, but learning disabilities in reading, spelling, and arithmetic are present, and there is a higher incidence of autistic spectrum disorders, behavioral and attentional problems than in unaffected peers despite normal IQ (Hinton et al., 2000, 2004; Cyrulnik et al., 2007; Della Coletta et al., 2007; Young et al., 2008). Psychiatric problems may be seen prior to the onset of weakness (North et al., 1996) with diagnosis discovered as a result of abnormal liver function test results, checked because of psychiatric medications in otherwise asymptomatic patients.

X-linked cramps and myalgia is the mildest form of dystrophinopathy with musculoskeletal involvement, with reports in many families (Doriguzzi et al., 1986; Gospe et al., 1989; Minetti et al., 1991; Ishigaki et al., 1996; Figarella-Branger et al., 1997; Kleinsteuber et al., 2000; Sanchez-Arjona et al., 2005). Myoglobinuria and fever with exercise are additional features found in males, with calf hypertrophy and cramps found in females of the same family (Sanchez-Arjona et al., 2005). Individual patients may remain nonprogressive (Gospe et al., 1989) but there is intrafamilial variation and most individuals had a slowly progressive course. Asymptomatic hyperCKemia is sometimes caused by mutations in the *DMD* gene (Liewluck, 2007).

Cardiac complications are rare in BMD before 13 years of age (Nigro et al., 1983, 1990; Towbin et al., 1993) and are not severe before age 21 years in most cases.

X-linked DCM

XLDCM is a rare, rapidly progressive, cardiomyopathy that presents in the second decade and can be rapidly fatal. Most cases are without detectable skeletal myopathy, but CK levels are usually raised (Towbin et al., 1993).

CARRIERS

Carriers and females with contiguous gene deletion syndromes may exhibit the entire spectrum of muscle weakness, from a full Duchenne phenotype to mild proximal muscle weakness, calf hypertrophy with mild pathological findings in muscle, to essentially normal muscle function. Manifesting women may be misdiagnosed as having polymyositis due to raised CK levels and inflammatory cells found on muscle biopsy. Treatment with corticosteroids does not produce the dramatic improvement expected with polymyositis. Manifesting carriers were first reported in 1974, with myopathic

features similar to autosomal recessive limb-girdle muscular dystrophy and increased CK concentrations (OMIM, 2008). In a group of 50 known DMD/BMD carriers, muscle biopsy specimen findings did not correlate with symptoms (Hoogerwaard et al., 2005). CK values in carriers are increased following aerobic exercise (Nicholson et al., 1986).

Carriers are divided into definite, probable, and possible categories. Definite or obligate carriers have an affected son and another affected maternal male relative or a confirmed mutation. Probable carriers have two affected sons, or one affected son and an affected grandson through her daughter but no other definite relatives. Nonmanifesting carriers have a normal lifespan unless cardiac complications are present. Although strength is normal, there is limited ability to perform steady-state work, and muscle spectroscopy reveals abnormal energy metabolism during isokinetic exercise. Muscle pathology is notable for abnormal dystrophin in a mosaic pattern, consistent with the lyonization (X-inactivation) of cells.

Carriers have normal cognitive status. Restrictive pulmonary disease, gastrointestinal and bone mineralization complications are not reported as features in nonmanifesting carriers, but carrier women have not been investigated thoroughly in these areas. Nearly half of carriers manifest changes in the ECG and 7–18% have cardiac dysfunction (Emery, 1969; Mirabella et al., 1993; Hoogerwaard et al., 1999a, b; Grain et al., 2001; Politano et al., 2003). Risk of cardiac involvement increases with advancing age. Risk of death from cardiomyopathy was low in a study of death certificates of female carriers in Scotland (Holloway et al., 2008).

NEUROPHYSIOLOGY AND LABORATORY FEATURES

The EMG shows nonspecific myopathic changes that include an increased number of small polyphasic motor unit potentials, increased recruitment, fibrillations, and positive sharp waves early in disease, followed by a decrease in insertional activity with disease progression. Patients with BMD have fewer features of necrosis on EMG than patients with DMD.

CK levels are increased in all forms of dystrophinopathy, beginning in the neonatal period, with the exception of some patients with XLDCM and some female carriers. CK values steadily decline by 8.7–20% per year after a peak in DMD at 3 years of age (Konagaya and Takayanagi, 1986; Sun et al., 2008). Approximately half of female carriers show increased CK levels, although a normal CK concentration in females cannot accurately exclude carrier status (Nicholson et al., 1979a, b, 1981). Fluctuations in CK levels occur with exercise and may also be influenced by cholesterol-lowering medications (Vandenhende et al., 2005). Serum alanine and aspartate transaminase levels are frequently raised (Urganci et al., 2006). Hypokalemia may be present as a result of vomiting, diarrhea, or diuretics (Soloway and Mudge, 1979).

PATHOLOGY

Pathological studies of muscle reveal progressive changes in muscle structure, including marked variation in fiber size due to fiber atrophy, hypertrophy of some muscle groups, fatty replacement, and connective tissue (Neuromuscular Disease Center, 2008). There are degenerating and regenerating fibers which appear as clusters of hypercontracted muscle fibers. The clinical distribution of muscle involvement reflects the distribution of more severe pathological findings. Necrotic fibers are surrounded by inflammatory cell macrophages and $CD4^+$ lymphocytes. Pathological changes are found first in large muscles are affected first with relative sparing of small muscles (Boland et al., 1995). In autopsy tissue from a 14-year-old boy, there were no pathological changes reported in extraocular muscles (Khurana et al., 1995).

The severity of dystrophin deficiency can be assessed using special markers of dystrophin on muscle biopsy specimens. Findings range from complete absence in DMD to partial absence in BMD, and a mosaic pattern in carrier females. Dystrophin is a subsarcolemmal membrane protein. Gradual destruction of this protein leads to segmental necrosis and calcium influx (Mokri and Engel, 1975; Carpenter and Karpati, 1979; Karpati and Carpenter, 1986).

In the brain, absence of dystrophin is associated with disordered central nervous system architecture, abnormalities in dendrites, and loss of neurons that normally express dystrophin (Anderson et al., 2002). Brain dystrophin is found in the cerebellum and hippocampus (Uchino et al., 1994a, b, 1996). There are seven promoter regions, all coding for different isoforms of dystrophin, with reports of Dp 140 associated with intellectual impairment (Bardoni et al., 2000; Felisari et al., 2000). In the heart, absence of dystrophin causes cardiomyocyte degeneration and fibrosis, beginning in the left ventricle and eventually causing hypokinesis and ventricular dilatation. Regional weakness can be detected prior to left ventricular dilatation. Late in the course, fibrosis leads to cardiac conduction defects. Dystrophin is found in gastric smooth muscle but its role is not known. Nonprogressive red–green colorblindness may be related to the specific dystrophin isoform Dp 260.

GENETICS, PATHOPHYSIOLOGY, AND PATHOGENESIS

Male predominance of DMD was recognized in the earliest published descriptions (Meryon, 1851). The X-linked inheritance pattern was recognized later (Walton and Nattrass, 1954). As molecular genetic technology advanced, DMD became an attractive target for gene localization, aided by the discovery of a female with phenotypic features of DMD and a large contiguous deletion on the X chromosome, Xp21.2 (OMIM 310 200). Ultimately, the dystrophin gene was discovered in 1986 (Kunkel et al., 1986). It remains the largest gene yet discovered, 90 times larger than any other known gene. It contains 79 exons, 8 promoters, and 2.2 million base pairs of genomic DNA. Three promoters produce isoforms that are active in skeletal muscle, smooth muscle, cardiac myocytes, or brain (Lapidos et al., 2004). In muscle, the 427-Da isoform links intracellular actin to extracellular matrix proteins.

Approximately one-third of mutations are new. Deletions of a single exon account for 55% of DMD and 65% of BMD cases, point mutations and small deletions or insertions for 30%, and duplications the remainder (Miller and Hoffman, 1994). There are "hotspots" for mutations in some regions, and premature stop codon mutations in approximately 10–15%, leading to truncated dystrophin production. Approximately 80% of deletions occur in the center domain, and 30% near the 5′ end of the gene, with the 200-kb region covering intron 44, exon 45, and intron 45, the major deletion breakpoint regions of the gene. The majority of large deletions initiate at the 5′ end of the gene (Prior and Bridgeman, 2005). Mutation size is not correlated with phenotype but deletions that produce premature truncation of dystrophin are more severe (Monaco et al., 1988).

Ninety percent of cases of DMD result from mutations that interrupt the messenger RNA open reading frame, either by frame shifting deletions or insertions, or premature stop codon mutations. BMD is usually seen when partially functioning dystrophin protein is formed. However, patients with BMD with out-of-frame deletions (e.g., exon 3–7) have been identified. These might be accounted for by alternate splicing or a new cryptic translational start site that produces a partially functional protein (Prior and Bridgeman, 2005) or other modifying genes, as in the case report of a reversal of a mutation by recombination (Hoop et al., 1994). The BMD phenotype has been observed in exon 45 deletion, which commonly causes DMD. This effect may be accounted for by instability of the protein by other factors such as modifier genes or damaging molecules, amount of regeneration, or cellular responses (Prior and Bridgeman, 2005).

Duplications or partial duplications are found in 5–10% of patients (Hu et al., 1990; Aartsma-Rus et al., 2006). Double deletions may confer a milder phenotype (El-Harouni et al., 2003).

In addition to the 70% of deletions and duplications, more than 124 small point mutations have been detected throughout the gene rather than in hotspots (Prior and Bridgeman, 2005; Deburgrave et al., 2007). These mutations typically result in protein truncation, lacking part or all of the C-terminus (Prior and Bridgeman, 2005). Pseudoexon mutations have been caused by deep intronic point mutations that alter splicing and allow intronic sequences to be included in the messenger RNA (Gurvich et al., 2008). Other mutations, such as deletions of exons 16 and 74, do not appear to cause disease (Schwartz et al., 2007; Kimura et al., 2009). Attempts to attribute specific disease severities to known mutations have been thwarted by numerous exceptions that defy explanation based on single dystrophin gene mutations alone, evidenced by clinical variability of features and severity even within affected families (Kesari et al., 2008).

X-linked dilated cardiomyopathy was reported in two boys with inframe deletion of exons 45–55, but another male with the same deletion had clinical BMD. The XLDCM mutations more typically occur in the dystrophin promoter region or exon 1, which results in phenotypic rescue of skeletal muscles by an alternative promoter in all tissues except the heart (Milasin et al., 1996; Muntoni et al., 2005). In patients with XLDCM, skeletal biopsy may show abnormal dystrophin-associated proteins in both skeletal and cardiac muscle.

Our understanding of dystrophinopathies has been aided greatly by the extensive study of animal models. The *mdx* mouse has a naturally occurring point mutation that results in a premature stop codon and in which no dystrophin is produced, although the phenotype is much less severe than in humans (Sicinski et al., 1989). The Golden Retriever canine model manifests progressive atrophy and disability more similar to that of DMD (Kornegay et al., 1988).

The dystrophin protein has four distinct domains. The amino-terminus associates with actin or an actin-like protein. The rod domain has long flexible rows of 24 spectrin-like α-helical repeats. There is a cysteine-rich region, and finally a unique carboxy-terminus. The dystrophin protein links the cytoskeleton to the basal lamina. It is tightly linked to a large oligomeric complex of sarcolemmal glycoproteins by the cysteine-rich domain and carboxy-terminus, with the amino-terminus interacting with actin or actin-like protein (Prior and

Bridgeman, 2005). It is one of the proteins in a large multiprotein complex. Mutations lead to breakdown of the entire critical complex, resulting in fragility of the sarcolemma. Intense muscle contraction intensifies this damage and leads to calcium influx and accelerated damage to muscle fibers. With their limited capacity to repair, these cells can be replaced by satellite cells, which reside between the basal lamina and the myofiber membrane and act as "stem-like" satellite cells that can grow muscle fibers and regenerate muscle fibers postnatally. Over time, the supply of satellite cells is exhausted and damage to muscle fibers progresses. Replacement by connective tissue and fat contributes in part to pseudohypertrophy in the calf and other muscle group (Cossu and Sampaolesi, 2007).

The function of dystrophin is still not known. Evidence shows that it supports the sarcolemma against mechanical stress (Koenig et al., 1988), helps regulate intracellular calcium and the cascade of calcium-related events (Franco-Obregon and Lansman, 2002), works in force and signal transduction, influences aggregation of neurotransmitter receptors (Kong and Anderson, 1999), and prevents excessive generation of reactive oxygen free radical species (Brown et al., 2005). Oxidative stress appears to hasten muscle degeneration, which may be influenced by exercise and physical therapy (Niebroj-Dobosz and Hausmanowa-Petrusewicz, 2005; Deconinck and Dan, 2007).

Immune responses are suspected to play a role in muscle destruction. A cDNA microarray analysis revealed increased expression of many genes including immune response (Noguchi et al., 2003), but how this translates into muscle destruction remains unknown. In the *mdx* mouse model, aquaporins (water channels) are linked to dystrophin, and a marked reduction of aquaporin-4 (AQP-4) expression has been identified in skeletal muscle (Frigeri et al., 1998; Wakayama et al., 2002). Human muscle biopsy tissues show increased AQP-1 transcript, and protein expression was significantly increased in DMD biopsies, localized to the sarcolemma and capillary endothelium (Au et al., 2008).

IMAGING

Muscle imaging provides a noninvasive method of evaluating muscle abnormalities in dystrophinopathies. Ultrasonography (Hughes et al., 2007), computed tomography (CT) (O'Doherty et al., 1977; Bulcke et al., 1979; Arai et al., 1995) and magnetic resonance imaging (MRI), including spectroscopy, reveal signal changes indicative of muscle atrophy and fat degeneration. In MRI there is myoedema in short TI inversion recovery (STIR) sequences and altered metabolism with spectroscopy (Pichiecchio et al., 2002). Progressive changes are noted on MRI of pelvis, thigh, and calf muscles in DMD that allow one to follow smaller incremental changes than possible with clinical Medical Research Council grading of muscle strength (Liu et al., 1993). ^{31}P-magnetic resonance spectroscopy (MRS) of muscle shows reduced total creatine and/or phosphocreatine concentration (Tarnopolsky and Parise, 1999). A study of nine boys with a Dixon three-point technique of fat quantification MRI of six upper leg muscles correlated better than manual muscle testing or dynamometer for disease severity as graded by the Brooke's scale (Wren et al., 2008).

CT reveals mild atrophy, and brain MRI shows occasional heterotopias. ^{31}P-MRS found higher inorganic phosphate ratios that did not correlate with intellectual ability (Tracey et al., 1995), increased choline-containing compounds in the cerebellum, and ratios of choline to *N*-acetyl aspartate that correlated with a measure of visuospatial cognitive ability (Rae et al., 1998). Brain imaging with fluorodeoxyglucose positron emission tomography shows hypometabolism in the cerebellum (Lee et al., 2002).

MRI has also been used to evaluate the heart in DMD/BMD, demonstrating abnormal T2 relaxation times in asymptomatic patients that were more prominent in older patients (Mavrogeni et al., 2005). Cardiac MRI detects strain in patients with DMD before clinical symptoms appear (Ashford et al., 2005). Echocardiography and multigated radionuclide ventriculography (MUGA; Shanmuga Sundaram et al., 2006) can be used to evaluate cardiac disease. Echocardiographic imaging quality may be limited by spinal deformity, but has the advantage of ability to be performed while patients are sitting in a wheelchair. MUGA is more difficult to perform as weakness and deformity limit positioning and comfort (Oguz et al., 1998). Cardiac MRI requires similar positioning and may not be feasible in older patients.

DIAGNOSIS

Until the 1990s, diagnostic procedures included serum CK estimation followed by electrophysiological studies and muscle biopsy. Clinicians now screen patients with CK testing, and confirm the diagnosis by testing for DNA mutations in the dystrophin gene (Figure 2.3). Southern blot technique and multiplex polymerase chain reaction (PCR) are used to detect deletions, but confirm the diagnosis in only two-thirds of affected boys. Most deletions are found in a 3′ hotspot (exons 45 and 43). Duplications detected by Southern blot account for about 5–10% of mutations (Den Dunnen et al., 1989; Aartsma-Rus et al., 2006a). In BMD, a higher proportion of duplications, a different distribution of mutations, and a

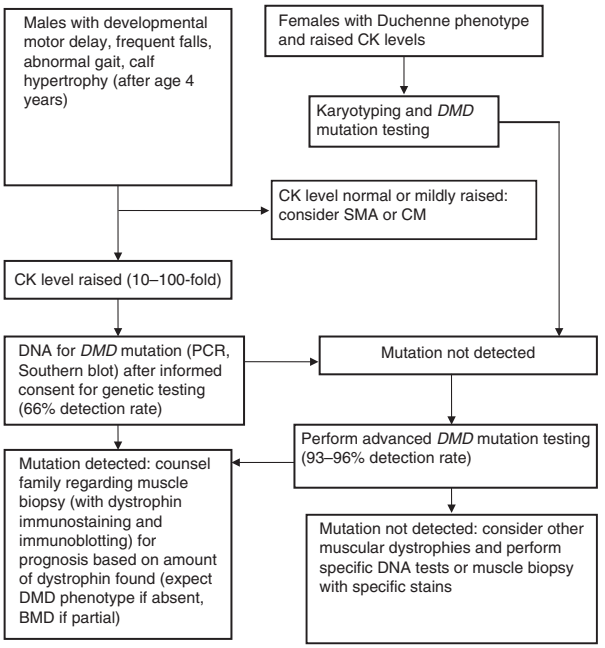

Figure 2.3. Diagnostic flow diagram. BMD, Becker muscular dystrophy; CK, creatine kinase; CM, congenital myopathy; DMD, Duchenne muscular dystrophy; PCR, polymerase chain reaction; SMA, spinal muscular atrophy.

higher exception to the reading frame rule are found (Beggs et al., 1990; Chamberlain et al., 1991; Freund et al., 2007; Kesari et al., 2008).

Point mutations were detected by a protein truncation test (Gardner et al., 1995; Flanigan et al., 2003). Currently, direct sequencing and dosage analysis with combination of techniques yields mutations in approximately 93–96% of patients (Beroud et al., 2004; Yan et al., 2004; Dent et al., 2005; Ashton et al., 2008) if the Southern blotting and PCR findings are negative.

Although DNA mutations confirm a dystrophinopathy, the phenotype cannot always be predicted reliably, leading some clinicians to perform muscle biopsies to confirm DMD or BMD based on the presence or complete absence of dystrophin with specific immunohistochemical dystrophin markers of muscle fibers. Muscle biopsy is still required for the best prognostic information regarding Duchenne or Becker phenotypes. It can be performed as an open procedure, or as a needle procedure, but both expose young patients to pain, anesthetic risk, and scarring.

With advances in DNA mutation detection rates, linkage analysis is rarely indicated but may be useful for identification of other affected family members or carriers when no mutation is identified in a child in whom the phenotype is suggestive of DMD/BMD. In some circumstances mutations can be detected only in mRNA, which requires muscle tissue. Immunoblotting may be used if dystrophin staining is unhelpful (Sanchez-Arjona et al., 2005).

GENOTYPE–PHENOTYPE CORRELATION STUDIES

Phenotypic variability is illustrated by intrafamilial variability among patients with identical mutations. This makes strict categorization and prognosis based on genotype difficult. However, a few statements can be made. XLDCM is reported to have higher incidence with deletion in exons in the N-terminus. Studies looking for correlation with brain function have not found deletions of the brain-specific promoter region, but distal deletions are more likely to be observed in boys with intellectual impairment (Prelle et al., 1992; Bushby et al., 1995). Mutations of Dp 140 were later found to be associated with intellectual impairment (Bardoni et al., 2000; Felisari et al., 2000). The expected association of autistic spectrum disorders with the promoter Dp 140 was not confirmed in 24 patients with BMD (Young et al., 2008). Red–green colorblindness is found only in DMD with deletions downstream of exon 30, possibly attributable to the Dp 260 isoform found in the outer plexiform layer of the retina (Costa et al., 2007).

GENETIC COUNSELING

Male offspring of female carriers have a 50% risk of inheriting the DMD/BMD mutation, and female offspring have a 50% risk of inheriting carrier status. Approximately one-third of dystrophinopathy mutations are *de novo* as predicted by the Haldane rule (Haldane, 2004). Some investigators believe that the rate of new mutation may be higher in males. The percentage of new mutations varies some around the world but most are close to the one-third estimate. Certain types of mutation may be more common in some populations (Vitiello et al., 1992).

CK testing is not considered adequate to rule out carrier status because levels may normalize in adult carrier females. DNA mutation testing is now the gold standard of evaluation. Carrier testing of females has been reserved for adult women or for those with clinical symptoms. However, there is a continuing debate about risks and benefits of knowing carrier status in childhood (Jarvinen et al., 1999, 2000). If mutations are not found in mothers of boys with detected mutations, a 10–15% risk of gonadal mosaicism remains (Wood and McGillivray, 1988). It is important to confirm whether or not female offspring have inherited carrier status to provide accurate genetic counseling in maternal relatives and sisters of affected boys

(Prior, 2003; Prior and Bridgeman, 2005). The location of the mutation can help determine the risk of a familial mutation (Passos-Bueno et al., 1992). Haplotype or linkage analysis can assist in identification of germline mutations where the mutation identified in the male offspring is not found in the mother (Ferreiro et al., 2004, 2005).

Prediction of severity is not always accurate based on DNA mutation testing even within families, owing to a variety of factors that influence outcome (see above). In a pair of monozygous twin females, one expressed a Duchenne phenotype and the other was clinically normal but had a son affected with DMD (Abbadi et al., 1994).

Prenatal testing in families with a known gene mutation is performed by amniotic sampling and chorionic villus sampling with multiplex PCR. These techniques can detect mutations but care must be taken to avoid misinterpretation based on maternal DNA. A gene dosage test determines whether there is no reduction or 50% reduction in the same bands deleted in the affected male relative. Preimplantation genetic diagnosis is possible for affected males or female carriers using the technique of multiple displacement amplification (Malcov et al., 2005; Ren et al., 2007, 2009). Risk of breech delivery is increased 5-fold, attributed to maternal rather than fetal factors (Geifman-Holtzman et al., 1997).

TREATMENT

Treatment begins with education about DMD/BMD spectrum disorders for the patient, family, and community. The diagnosis should be written down and the family provided with suggestions for reliable sources of information. The patient, family, and medical providers form a team for informed decision-making throughout the treatment course.

Medical therapies for neuromuscular weakness

Medical therapies for dystrophinopathies were ineffective until 1974. Thereafter, short-term improvement in muscle strength was demonstrated with corticosteroids (Drachman et al., 1974; Brooke et al., 1987; Fenichel et al., 1991; Emery, 1993). The seminal clinical trial by Griggs et al. (1993) showed increased strength in boys treated with prednisone at 0.75 mg/kg daily, but no greater benefit with a daily dosage of 1.50 mg/kg and a lesser effect with 0.375 mg/kg. Daily administration was more effective than treatment on alternate days (Fenichel et al., 1991). Deflazacort, a derivative of prednisone, at 0.9–1.2 mg/kg daily, is also effective but is not approved by the Federal Drug Administration in the USA. Prednisone and deflazacort improve muscle strength and function for 6 months to 2 years (Manzur et al., 2004, 2008; Moxley et al., 2005). Apart from strength function, these drugs prolong ambulatory function, reduce the risk of progressive scoliosis, and also stabilize pulmonary function variables (Biggar et al., 2006; King et al., 2007). Side-effect profiles show less weight gain with deflazacort but a greater risk of cataract formation (Alman et al., 2004). Both agents result in weight gain, risk of hypertension, increased osteopenia and fracture risk (King et al., 2007). Behavioral changes may be intolerable or require behavioral medication and therapy.

Most specialty clinics now routinely offer corticosteroids to all boys when they show clinical decline. The optimal age at which to begin corticosteroid treatment has not been established, although there is some evidence that treatment as young as 2–4 years of age results in an improved outcome (Merlini et al., 2003; Biggar, 2006; Houde et al., 2008). The recent Centers for Disease Control (CDC) care recommendations suggest that the timing of initiation of glucocorticoid therapy must be an individual decision, based on functional state, age, and pre-existing risk factors for adverse effects. They also suggest that treatment with glucocorticoid is not recommended for a child aged less than 2 years (Bushby et al., 2010).

The optimal schedule of delivery has not been established, although studies of alternate-day dosage of 1.25 and 2.5 mg/kg sustained benefit for only 3 months. A weekend-only protocol suggested benefit (Connolly et al., 2002). An alternating regimen of 10 days on, 10 days off corticosteroids may be used to minimize side-effects (Dubowitz, 2005). A daily dose of prednisone 0.3 mg/kg shows less robust but measurable improvement when compared with placebo (Mendell et al., 1989). A maximum dose of 40 mg prednisone per day is recommended (Ciafaloni and Moxley, 2008).

Duration of steroid use for maximal benefit is probably 18–36 months. Treatment may be discontinued owing to side-effects, or with wheelchair confinement. Continuation beyond loss of ambulation when tolerated is typically at a lower daily or alternate-day dose, at an average dose of 0.33–0.36 mg/kg prednisone, with some evidence that this slows the rate of cardiopulmonary complications (Wagner et al., 2007).

In BMD, corticosteroids have shown beneficial effects on muscle strength (Johnsen, 2001) but they are not recommended in most patients because of longer lifespan and potential for complications when used over many years. Manifesting carriers of DMD/BMD are not offered corticosteroid therapy because the risk may outweigh the potential benefit.

Corticosteroids appear to act directly on muscle strength rather than as anti-inflammatory agents (Kissel

et al., 1991, 1993). The mechanism of action remains elusive but it is known that steroids act on the regulation of signal transduction, with a direct nuclear effect (St-Pierre et al., 2004). They inhibit the immune response (Spuler and Engel, 1998), and inhibit myotube death during myogenesis (Sklar and Brown, 1991). There is evidence that steroids may enhance the proliferation of myogenic precursor stem cells (myoblasts) and increase muscle regeneration and growth (Rifai et al., 1995).

There is now increasing evidence that, in addition to the undisputed short-term benefits of corticosteroid therapy, there are long-term benefits (Angelini, 2007; King et al., 2007). Results of two pooled studies of pulmonary function with corticosteroid treatment demonstrated a mean improvement of 0.17 liters at 6 months (Brooke et al., 1987). Treatment with corticosteroids protects against cardiac ventricular dysfunction (Markham et al., 2008), and scoliosis is less severe with less spine surgery required. Treatment may improve QoL, but this has not been measured specifically (Silversides et al., 2003; Houde et al., 2008).

Management of adverse effects of corticosteroids (Table 2.2) includes supplementation of vitamin D and calcium with consideration of prescribing bisphosphonates (Hawker et al., 2005), nutrition counseling, education regarding risk of fractures, and management of behavior changes.

Some ineffective or marginally effective agents include leucine (Mendell et al., 1984), nifedipine (Moxley, 1985), flunarazine (Dick et al., 1986),

Table 2.2

Adverse effects of corticosteroid therapy*

Adverse effect	Therapeutic response or action
Weight gain	Prednisone 5.08 kg, deflazacort 2.17 kg in 12–24 months. Nutrition counseling: limit fat and simple carbohydrates, increase complex carbohydrate intake
Cushingoid appearance	Dosage adjustment (at least 0.3 mg/kg daily, max. 40 mg prednisone). If parents and siblings are also obese, start proactive dietary management for entire family
Asymptomatic cataracts	Occur in approximately one-third of patients receiving deflazacort. Regular ophthalmological care
Short stature	Endocrinology consult. Consider growth hormone cautiously
Acne	Dermatology consult; topical and oral treatment. Do not change glucocorticoid therapy unless emotionally distressed
Excessive hair growth	Consider psychosocial consequences. Assistance with personal grooming
Gastrointestinal symptoms	Avoid NSAIDs. Treat with H_2 blocker, antacid, or proton pump inhibitor. Seek gastroenterology consult
Behavioral changes	Consider change in dosage schedule. Counseling, psychotherapy; medication for depression, attention deficit disorder, psychosis
Osteopenia	Vitamin D and calcium supplementation. Consider bisphosphonates. Annual DEXA scan and 25-hydroxyvitamin D blood concentration
Fractures	Orthopedic consultation. Minimal immobilization; treat osteopenia
Glucose tolerance	Monitor for glycosuria and symptoms. If urine glucose is positive, assess fasting and postprandial levels – if elevated, seek endocrine consult
Hypertension	Routine monitoring. Restrict sodium, withdraw steroids. Treat in consultation with cardiologist
Immunosuppression and adrenal suppression	PPD placed prior to first dose of steroid. VZIG, varicella immunization. Annual flu shot; pneumovax every 5 years. Prompt treatment of bacterial infections. Careful tapering of steroids upon withdrawal
Myoglobinuria	Taper corticosteroids. Avoid excessive exercise. Evaluate other medications as possible contributors

*Prednisone 0.75 mg/kg daily; deflazacort 0.9 mg/kg daily.
DEXA, dual-energy X-ray absorptiometry; H_2, histamine type 2 receptor; NSAIDs, nonsteroidal anti-inflammatory drugs; PPD, purified protein derivative of tuberculin; VZIG, varicella zoster immunoglobulin;.
Sources: Bonifati et al., 2000; Talim et al., 2002; Soderpalm et al., 2008; Bushby et al., 2010.

azathioprine (Griggs et al., 1993), oral creatine alone (Felber et al., 2000), creatine monohydrate and glutamine (Escolar et al., 2005), and oxatomide (Buyse et al., 2007). Mazindol, a growth hormone inhibitor, appeared to show benefit when the more severe (and larger) of a twin pair received mazindol and the shorter twin received placebo (Zatz and Frota-Pessoa, 1981; Zatz et al., 1986), but a randomized trial did not show benefit for mazindol (Griggs et al., 1990).

Oxandrolone shows increased muscle strength but no functional benefit, and appears to be safe (Fenichel et al., 1997, 2001), although recent recommendations suggest that use of oxandrolone is not appropriate or necessary even with or without use of glucocorticoids (Bushby et al., 2010). Albuterol, a β_2-agonist used in reactive airway disease, increases lean body mass in ambulatory boys with DMD or BMD (Skura et al., 2008).

Rehabilitation and recreation

Until the first controlled trials of corticosteroids were published (Mendell et al., 1989; Fenichel et al., 1991), the mainstay of treatment for dystrophinopathies consisted of rehabilitation and surgical interventions. Rehabilitation strategies included physical therapy treatment with protocols for stretching, assistive devices for mobility and seating systems. The modern approach to rehabilitation is beginning to be evidence based. Multidisciplinary teams provide comprehensive care and are composed of physical, occupational and speech therapists, psychosocial interventions, psychiatrists, and neurologists in addition to primary care providers. Lightweight customized orthotics have largely replaced heavy, long leg, double upright, metal leg braces. Night splinting is typically started early but use of daytime ankle–foot orthotics remains controversial in ambulatory patients. Stretching programs, long the standard of care, are often abandoned as painful, impractical, and ineffective. Some forms of exercise result in more rapid deterioration of muscle function in animal models and humans (Petrof, 1998), necessitating professional guidance and supervision of exercise programs to avoid hastening the disease process (Grange and Call, 2007). Assistive devices including mobile arm supports, feeding devices, and wheelchair controls may be useful (Yasuda et al., 1986; Balaban et al., 2005).

Wheelchairs have improved substantially over the past two decades. Lightweight, durable materials, smaller batteries for power chairs, options for changing positioning throughout the day, and customization to prevent or accommodate deformities and improve comfort are now available. The use of a lumbar support to induce a lordotic position in sitting to delay scoliosis onset showed promise in a small study (Kerr et al., 2008). A variety of hand controls is available to optimize function at different disease stages (Pellegrini et al., 2004). A study comparing different electronic controls found that patients with severe disease preferred puff control, followed by sip, and key-pinch and push button systems (Pellegrini et al., 2007). Proper wheelchair fit with adequate foot support is essential (Liu et al., 2003). Power wheelchairs should include tilt-in-space and reclining functions to alleviate pressure points. Sit-to-stand function is now available, but use of this device may be limited by lower extremity contractures, limited trunk support, and cost. Devices for bathing, toileting, and transfers are now more widely available, but costly. Many insurance companies severely restrict the number of treatment sessions for physical, occupational, and speech therapies, leaving the school systems to provide those that are educationally relevant. Some children show marked deterioration over summer and vacation periods without therapy.

Sports are to be encouraged and modified as physical abilities decline. Muscular dystrophy camps offer one-on-one assistance for typical camping activities, and provide respite for families. Acquisition and development of computer skills should be encouraged soon after diagnosis.

Pulmonary

Routine pulmonary function testing and sleep studies were recommended for patients with DMD in a US consensus statement (Finder et al., 2004). In a group of patients with DMD and sleep-related breathing disorder (SRBD), tonsillectomy and adenoidectomy resulted in symptomatic improvement in all operated patients despite lack of measurable preoperative and postoperative changes. Nocturnal ventilation is effective for SRBD if surgery fails or is not performed, as measured by improvement of the apnea–hypopnea indices (Suresh et al., 2005). Recent CDC recommendations suggest an annual sitting FVC measurement for ambulatory patients aged 6 years or more. For all nonambulatory patients, oxygen saturation, sitting FVC, peak cough flow, and maximum expiratory and inspiratory pressure should be measured every 6 months. For patients with suspected hypoventilation, FVC of less than 50% and use of assisted ventilation and awake end-tidal carbon dioxide level by capnography should be measured annually (Bushby et al., 2010).

Manual and mechanical assisted cough techniques are indicated when:

- pulmonary infection is present or baseline peak cough flow is below 270 liters/min

- baseline peak cough flow is less than 160 liters/min or maximum expiratory pressure is below 40 cmH_2O
- baseline FVC is less than 40% that predicted or below 1.25 liters in an older teenager/adult.

Similarly, nocturnal ventilation is necessary when:

- signs or symptoms of hypoventilation (patients with FVC below 30% that predicted are at especially high risk)
- baseline Spo_2 is less than 95% and/or blood or end-tidal carbon dioxide is above 45 mmHg while awake
- the apnoea–hypopnoea index is greater than 10 per hour on polysomnography, or there are four or more episodes of Spo_2 below 92% or drops in Spo_2 of at least 4% per hour of sleep (Bushby et al., 2010).

NIV prolongs life in DMD (Raphael et al., 1994; Gomez-Merino and Bach, 2002; Eagle et al., 2007) and reduces hospital admissions and length of stay (Bach et al., 1997). Nocturnal NIV (Ward et al., 2005) and later addition of diurnal NIV alleviates breathlessness (Toussaint et al., 2006). These measures are thought to improve QoL and increase survival for 5–10 years (Toussaint et al., 2007b). Customized NIV systems are now compact and reliable, and can accommodate both nocturnal (nasal mask) and daytime (mouthpiece) requirements for greater independence. Frequent monitoring of mask fit and function is important to avoid skin breakdown around the mask. Pneumothorax has been reported in two patients on NIV (Vianello et al., 2004).

Steroids improve cough efficiency and respiratory muscle strength in patients with DMD as measured by peak cough flow and respiratory muscle strength (Daftary et al., 2007). Once weakness of cough is identified, use of an in-exsufflator provides benefit with excellent tolerance in children with DMD (Ward et al., 2005; Toussaint et al., 2007a; Fauroux et al., 2008). Additional pulmonary management includes prompt diagnosis and treatment of infection, chest percussion and drainage, and increased use of the in-exsufflator with infection. These strategies are critical for both prevention and acute management, sometimes preventing hospitalization. When adequate pressures can no longer be achieved by NIV and cough-assist is inadequate to clear secretions, discussions with the patient and family regarding tracheostomy and mechanical ventilation may be appropriate. Tracheobronchomalacia and tracheal hemorrhage are reported complications of long-term mechanical ventilation with uncuffed tracheostomies (Baydur and Kanel, 2003). QoL for patients with NIV or tracheostomy is reasonably good, but is underestimated by physicians and parents (Narayanaswami et al., 2000).

Cardiac

Screening at diagnosis, every 2 years through age 10 in DMD, at least every 5 years in BMD, and for carriers has been recommended by one group (Muntoni, 2003; Bushby and Griggs, 2007), whereas other clinicians recommend less frequent screening (English and Gibbs, 2006). There is little evidence that preventive treatment is effective for cardiomyopathy in dystrophinopathies. Treatment with perindropil in presymptomatic patients was well tolerated and the proportion of patients with left ventricular dysfunction was decreased compared with placebo (Duboc et al., 2005). Once symptomatic or abnormalities have been detected through screening, treatment with angiotensin-converting enzyme inhibitors (ACEIs) improves left ventricular function, unrelated to age at onset of left ventricular dysfunction or the specific gene mutation (Ramaciotti et al., 2006). Cough and angioedema are side-effects of ACEIs. β-Adrenergic blockers may also be helpful, and combination therapies may be more beneficial than beta-blockers or ACEIs alone (Kajimoto et al., 2006). For end-stage cardiac disease, continuous intravenous inotropes resulted in improvement for up to 30 months in three patients with DMD (Cripe et al., 2006).

In patients with BMD doing well from a neuromuscular perspective, the onset of cardiomyopathy may be an indication for cardiac transplantation (Quinlivan and Dubowitz, 1992). A female carrier (Melacini et al., 1998), a patient with DMD and a patient with BMD with end-stage cardiomyopathy underwent cardiac transplantation without complications (Rees et al., 1993). Pacemakers may be considered in patients with bradyarrhythmias.

Gastroenterology and nutrition

Patients with dystrophinopathy require monitoring and management of nutritional status to avoid obesity and further compromise of the neuromuscular system. Conversely, undernutrition diminishes the ability for muscles to undergo repair and predisposes patients to decubitus ulcers over bony prominences and deformities as muscle atrophy advances. The addition of corticosteroids increases the risk of obesity, decreases healing of wounds, increases risk of development of glucose intolerance, and results in more rapid progression of osteopenia. Supplemental calcium and vitamin D are recommended in the form of food and supplements. Nutritional counseling includes adequate protein, complex carbohydrates, hydration, and avoidance of foods likely to increase risk of obesity and diabetes. Some patients with dysphagia and gastroesophageal reflux

may require treatment. Gastrostomy tube placement may be indicated.

Orthopedic management

Scoliosis

Scoliosis screening should begin once ambulation is lost. Anteroposterior sitting radiographs are recommended every 6 months thereafter (Karol, 2007). Preventive rehabilitation strategies and corticosteroids may slow scoliosis progression, and the course may be milder. Although 90% of untreated boys develop scoliosis (Smith et al., 1989; Sussman, 2002), only 25% of boys on deflazacort require surgical intervention (Kinali et al., 2006). Approximately 15% of noncorticosteroid-treated boys in one series showed no progression in curvature over 4 years (Oda et al., 1993). Decreased scoliosis severity at 17 years of age correlated with longer walking and standing, and to some degree with corticosteroid treatment (Kinali et al., 2006). Neck extension contractures are not uncommon. Surgical lengthening of erector spinae muscles was successful in a case series of seven patients with an outcome of improved sitting balance and cosmesis (Giannini et al., 2006).

Nonsurgical treatment of scoliosis with spinal orthotics has been largely ineffective and may further compromise pulmonary function. Boys who are not surgical candidates for medical or philosophical reasons may derive some benefit from spinal orthotics (Heller et al., 1997).

Scoliosis surgery is performed to increase comfort and sitting tolerance, improve appearance, avoid the need for spinal orthoses, to ease care and for pain relief (Cheuk et al., 2007). Surgery is typically performed once the curvature exceeds 20–40°. Some surgeons feel that earlier operation with lesser degrees of curve may result in improved outcome due to reduction of risk factors (Miller et al., 1992; Heller et al., 2001; Cervellati et al., 2004). Preoperative assessment of pulmonary function is critical as many surgeons will not operate unless at least 30–35% of FVC is available. With careful management, successful surgery may be performed with smaller reserves (Harper et al., 2004).

The surgery is long and extensive, usually encompassing vertebral segments from T2 to the lumbar, pelvic, or sacral region. Titanium rods with wires are implanted and multiple laminectomies are performed. Surgical duration averages several hours. Life-threatening complications include anesthetic reactions, excessive blood loss, infections of the surgical site, pneumonia, and atelectasis. Less serious complications include pain and constipation. Increased sitting height may require modification of current transportation systems and feeding programs to avoid malnutrition (Iannaccone et al., 2003). Surgery improves cosmesis and possibly QoL (Bonnard et al., 1993). Benefit in pulmonary function has been more difficult to demonstrate (Kennedy et al., 1995). In a long-term retrospective series that compared boys with and without surgery, life expectancy increased up to a median age of 30 years when surgery was performed along with ventilation (Eagle et al., 2002, 2007).

Adverse events during and after surgery include ventilator-associated pneumonia, wound dehiscence, surgical wound infection, hemorrhage, loosening of fixation, pseudarthrosis, deteriorated respiratory function, and increased difficulty with hand-to-head access (Cheuk et al., 2007). Postoperative urinary dysfunction has been reported likely secondary to spinal cord injury (MacLeod et al., 2003).

The importance of prevention and management of complications is stressed. Newer techniques are now available to control bleeding (Shapiro et al., 2007). At least part of the bleeding tendency may be attributable to dysfunction of Dp 71 in the cytoskeleton of platelets, and may explain increased blood loss during scoliosis surgery in patients with DMD. Platelet transfusions may be beneficial in addition to packed red blood cells (Labarque et al., 2008). Controlled hypotensive therapies, preoperative use of NIV and early postoperative transition to NIV, and the use of in-exsufflators, postoperative chest physical therapy, and early mobility are recommended.

Orthopedic management of limb deformities

Stretching programs for prevention of limb contractures may be effective early in the course of DMD, especially when augmented by bracing. This includes active stretching, passive stretching, active-assisted stretching, and prolonged elongation using splinting, positioning, orthosis, and standing devices. Active, passive, and active-assisted stretching should be done for 4–6 days per week for any specific joint or muscle group to minimize contractures (Bushby et al., 2010). However, most patients continue to develop progressive contractures once wheelchair confined. Heel cord lengthening and iliotibial band release performed while there is still adequate strength for ambulation (Goertzen et al., 1995) may prolong independent ambulation. Other tenotomies may prolong the period of braced ambulation and standing, but function is not otherwise improved (Smith et al., 1993; Forst and Forst, 1995, 1999; Vondran et al., 1999).

Although tendon surgeries correct deformities, some patients deteriorate more rapidly postoperatively and

there is no benefit for strength. Walking may be extended to a small degree with the addition of tendon transfers to improve dorsiflexion (Manzur et al., 1992; Scher and Mubarak, 2002; Leitch et al., 2005). Serial casting to lengthen tendo-Achilles may be almost as effective as surgical lengthening of tendo-Achilles and iliotibial bands along with tendon transfer (Main et al., 2007). Single-level surgery is not indicated if there are knee flexion contractures of 10° or greater and quadriceps strength of grade 3/5 or less. Hamstring lengthening behind the knee is generally needed if there is a knee flexion contracture of more than 15° (Bushby et al., 2010). The use of botulinum toxin for reduction of a knee flexion contractures has been reported (von Wendt and Autti-Ramo, 1999), but long-term effects have not been studied adequately so this therapy cannot be recommended.

Treatment and prevention of fractures includes monitoring for decreases in bone mineral density (osteopenia and osteoporosis) by dual-energy X-ray absorptiometry (DEXA) scan (Soderpalm et al., 2008), and supplementation with calcium and vitamin D, particularly important in corticosteroid-treated patients. DEXA scans are recommended at baseline at age 3 years and at start of glucocorticoid therapy, followed by annual DEXA scans for those at risk with a DEXA score of less than −2 (Bushby et al., 2010). Vertebral fractures may be asymptomatic but detected by spine films or found incidentally on chest radiographs. The use of bisphosphonates is considered after fractures, or when the condition is severe or rapidly progressive, but these medications have not been studied thoroughly for long-term safety in children with DMD (Hawker et al., 2005).

Anesthetic and procedural sedation

As life expectancy improves in DMD, the need for patients to undergo surgery and procedures that require sedation has increased. Anesthetics can produce fatal malignant hyperthermia-like reactions with rhabdomyolysis or cardiotoxicity in patients with dystrophinopathies (Takagi and Nakase, 2008). General consideration should be given for all medications that might affect the musculoskeletal system, including the heart (Heiman-Patterson et al., 1986). An expert consensus panel published recommendations regarding anesthetic management in patients with DMD undergoing procedural sedation or general anesthesia. These include preoperative consultations, pulmonary testing, cardiology, nutrition, and discussion of resuscitation parameters with family including advances directives. Intubation may be complicated by macroglossia and jaw and cervical spine contractures. Succinylcholine is considered to be absolutely contraindicated. NIV and in-exsufflator devices should be available for rapid transition if assisted ventilation is required during the procedure, with monitoring of oxygen saturations and end-tidal carbon dioxide when possible, with cautious use of supplemental oxygen. Avoidance of constipation and obstipation is advised with attention to enteral nutrition as soon as possible after surgery (Birnkrant et al., 2007).

EMERGING THERAPIES

Curative treatments for dystrophinopathies are being investigated in animals and humans. Potential therapies can be divided into three major categories: drug, gene, and cell therapies. Discussion of emerging therapies will be confined to those already in or near human trials.

Drug therapy

The advent of large-scale screening programs in animals led to a series of human trials performed using a variety of agents already proven safe for children (Figure 2.4) (Khurana and Davies, 2003). Success has been greatest with strategies to attack premature stop codon mutations that affect approximately 15% of patients with DMD. A treatment trial with gentamicin did not produce improvement, but PTC124® (ataluren) shows promise (Welch et al., 2007). An open-label study of PTC124® in muscular dystrophy reported that 47% of study patients demonstrated visible improvements in dystrophin staining from muscle biopsies while receiving the study drug, and that during PTCI24® treatment serum CK levels were reduced. Results of a large prospective trial did not support a recommendation to use this drug in appropriate patients but further trials are being considered owing to group differences in response (PTC Therapeutics, 2009, 2010).

Oligodeoxynucleotide-mediated gene editing works in a similar fashion, but has the clear advantage that it results in true repair of DNA and does not require ongoing treatments (Lim and Rando, 2008). This technique has been studied in animals (*mdx* mouse, Golden Retriever) but is not yet in human trials. In another important discovery, the role of transforming growth factor β in stage-specific remodeling of human dystrophin-deficient muscle was demonstrated and led to the consideration of agents to reduce this factor (Chen et al., 2005). One of these drugs, losartan, normalized muscle architecture in a dysrophin-deficient mouse model of DMD and is currently under investigation in humans (Cohn et al., 2007; Kuehn, 2007). With high-throughput screening, an acceptable compound that upregulates utrophin was discovered, and human trials are scheduled for 2008.

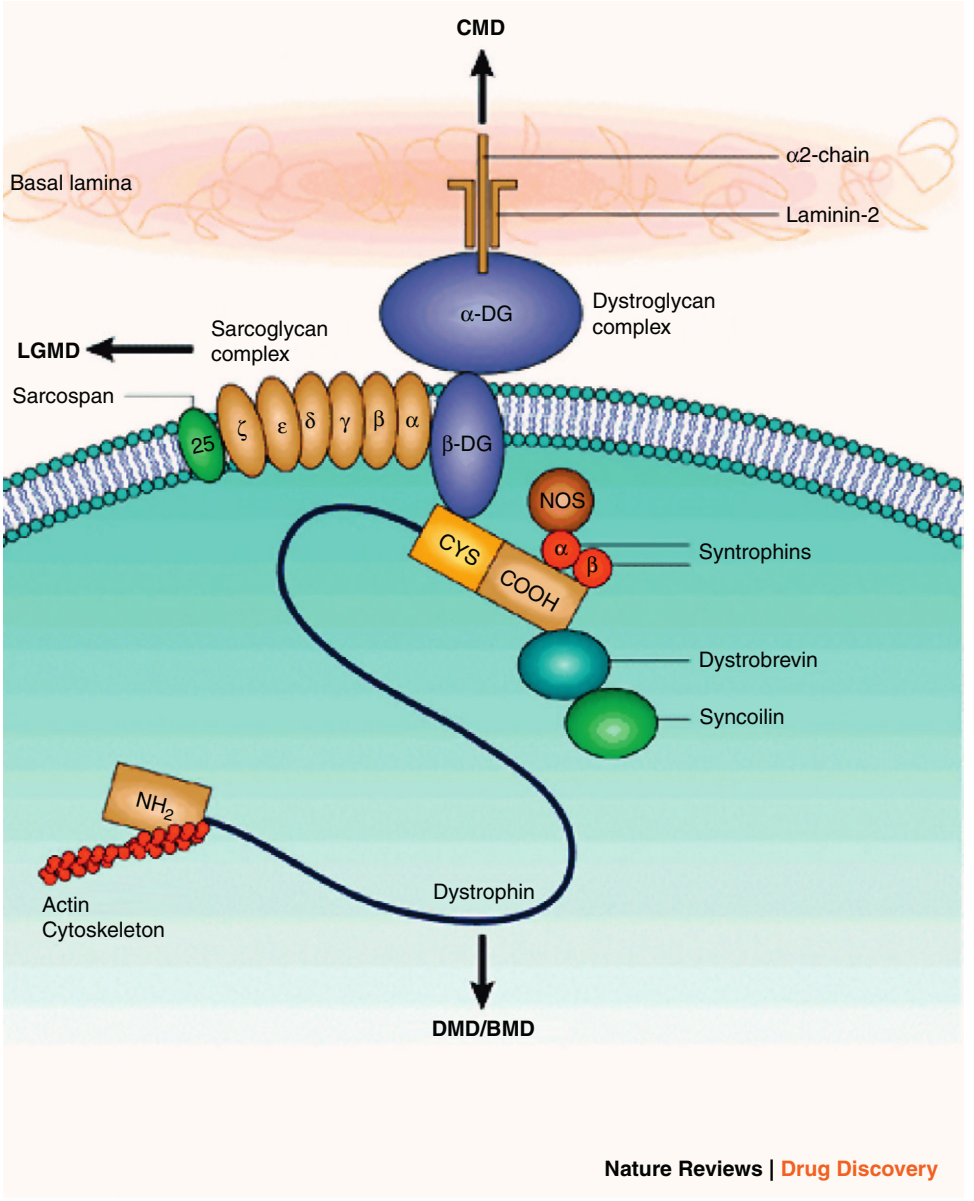

Figure 2.4. Diagram illustrating the dystrophin–glycoprotein complex, which is the site of pharmacotherapeutic intervention. α-DG, α-dystroglycan; β-DG, β-dystroglycan; CMD, congenital muscular dystrophies; CYS, cysteine-rich residue; DMD/BMD, Duchenne/Becker muscular dystrophy; COOH, carboxy-terminus; LGMD, limb-girdle muscular dystrophies; NH_2, amino-terminus; NOS, nitric oxide synthase. (From Khurana and Davies, 2003, with permission. © Nature.)

Strategies that focus on prevention or delay of muscle degeneration, reduction of inflammation, and promotion of muscle metabolism or regeneration are being explored. Myostatin negatively regulates muscle mass, and antibodies to block myostatin action showed functional improvement in dystrophic mice. The antibody MYO-019 increased the size of muscles and reversed pathological changes in the mouse model (Minetti et al., 2006), and showed good safety and tolerability in BMD and other types of muscular dystrophy in early safety trials (Wagner et al., 2008). Nitric oxide has a beneficial effect on muscle repair and has been delivered using a nonsteroidal anti-inflammatory drug in animals. The disease phenotype was ameliorated by reducing inflammation, preventing muscle damage, and preserving satellite cells. The compound also enhanced efficacy of donor stem cells through migration and then reconstitution of muscle fibers (Brunelli et al., 2007; Cossu and Sampaolesi, 2007).

Gene therapy

Gene therapy includes replacement of defective genes and strategies using other replacement genes to improve

cell function or to repair gene mutations (Rodino-Klapac et al., 2007a). Human trials were initially hampered by barriers that included immune reactions (Ferrer et al., 2000), inadequate methods of delivery, and later serious ethical concerns (Verma, 2000). There has been a transition from adenovirus to adeno-associated viral (AAV) vectors (Buchanan et al., 1981; Rodino-Klapac et al., 2007b). Highly functional mini- and micro-dystrophins are now available that prevent and reverse dystrophic pathology. Phase I trials using AAV vectors are now in progress in humans (Harper et al., 2002). Plasmids to carry the gene therapy instead of viruses are being explored.

Two strategies to repair the gene include antisense oligonucleotides or oligonucleotide-mediated gene editing (Bertoni, 2008; Lim and Rando, 2008). Exon skipping to restore the reading frame and produce a functional dystrophin with conversion to a Becker phenotype has been a promising strategy for specific mutations, with approximately 12 common mutations leading to 73.3% of deletions. Exon 51 alone accounts for approximately 20% of deletions and is a primary target in new clinical safety and efficacy trials. The first human oligonucleotide trial was done in 2007 with results showing that skipping of exon 51 corrects several mutations in the DMD deletion "hotspot", which amounts to about 13% of the mutations that cause DMD (van Deutekom et al., 2007). Another study in 2009 showed results similar to proof-of-concept study with intramuscular AVI-4658, a phosphorodiamidate morpholino oligomer that also targets exon 51 (Kinali et al., 2009). In the interim since gene discovery, innovative alternative approaches have made progress, but safety, sustained clinical benefits, expense, and more efficient production must be considered (Aartsma-Rus and van Ommen, 2007; Aartsma-Rus et al., 2007; Bertoni, 2008). Other approaches with antisense oligonucleotides (chemically synthesized 20–25-base single-stranded DNAs that are designed to hybridize with a complementary sequence in the target mRNA) to duplications and point mutations through double-exon and multi-exon skipping are being developed; these will increase the number of patients that can be treated (Aartsma-Rus et al., 2009). The first US trial of antisense oligonucleotide therapy (GSK2402968) opened in 2010 (see http://www.mdausa.org/).

Cell therapy

Human myoblasts (10 million cells per procedure derived from father or brothers) were injected into affected muscles once per month in 12 boys, with sham treatment in the contralateral biceps brachii muscle group with biopsy 6 months later. There was poor cell survival and migration of myoblasts, and a possible immune response (Mouly et al., 2005). A phase I trial had encouraging results (Skuk et al., 2004, 2006), but a realistic system for delivery is not available.

A human trial of autologous stem cells (eight boys) revealed no adverse effects. The ratio of capillary to muscle fibers increased and there was with a switch from slow to fast myosin-positive muscle fibers (Torrente et al., 2007). This followed successful treatment in a dog model (Sampaolesi et al., 2006).

Clinical care recommendations are summarized in Table 2.3.

CLINICAL RESEARCH ISSUES

The formation of patient registries with specific mutations and clinical data would enhance the ability to locate patients if and when personalized therapies become available. The risk of loss of privacy and costs of tracking patients must be considered. Presymptomatic and newborn screening would increase the pool of presymptomatic patients, with the possibility of complete prevention of the disease if curative treatments became available. Outcome measures for clinical trials rely on timed functional tests, hand-held dynamometry (Beenakker et al., 2005), standardized and validated instruments such as the Hammersmith Functional Motor Scale (Parreira et al., 2007), EK scale (Steffensen et al., 2001), and quantitative muscle testing (Mayhew et al., 2007). Step activity monitors may also have some value (McDonald et al., 2005). The search continues for appropriate biomarkers that parallel clinical disease severity and could assess differences in treated and placebo groups, such as MRI (Wren et al., 2008). Biomarkers for osteoporosis have been identified (Soderpalm et al., 2008).

NEWBORN SCREENING

Newborn screening has been reserved for diseases for which early treatment is available, but this has been evolving with this and other diseases now being considered somewhat treatable and with advances and clinical trials in process. Just as with a registry of known affected patients, the pool of presymptomatic patients would allow the earliest utilization of approved treatments or participation in clinical trials. Early detection would give a family the presymptomatic diagnosis and the choice to avoid producing more affected offspring or to prepare for the birth of another affected child. Screening for DMD has not yet been proven to have clear financial benefit (Kemper and Wake, 2007), as in other diseases with neonatal screening such as hypothyroidism and phenylketonuria (Clague and Thomas, 2002). There is concern that giving parents a

Table 2.3

Clinical care recommendations

	Recommendations		
Disease stage	Testing	Education	Clinic visits
Diagnosis and early weakness (preschool)	Baseline cardiology; baseline PFT; neurocognitive evaluation; baseline/periodic chemistry; bone density; eye examination	Provide printed materials and reference websites. Home stretching program	Genetic counseling. Initiate rehabilitations services, night orthotics. Discuss and initiate corticosteroids by age 4 if possible. Supplement calcium and vitamin D with emphasis on dietary consumption and weight management. Encourage early participation in sports. Introduce early computer skills. Consider clinical trial participation
Progressive weakness (elementary school)	Continue periodic cardiology, pulmonary bone density evaluation (ophthalmology if on steroids)	Provide school support for mobility, learning issues, psychosocial issues. Treat fractures with minimal immobilization	Review corticosteroid side-effects, behavior, and school performance. Monitor rehabilitation measures. Monitor for falls and discuss fracture risk. Begin wheelchair discussions. Consider surgical interventions
Wheelchair confinement (middle, junior, and high school)	Scoliosis evaluation; minimize contractures through orthotics, proper fit and positioning; provide maximum independent mobility	Daily or periodic use of in-exsufflator; prompt treatment of infections; consider nebulizer treatments, percussion, or vest	Discuss discontinuation of corticosteroids. Scoliosis monitoring. Gastrointestinal history (reflux, constipation, diarrhea, choking). Manual or power wheelchair. Home and school modification and accommodation (before wheelchair), including transportation to school and for personal/family
Progressive quadriplegia	Home care providers, residential facilities	Noninvasive ventilation	End-of-life discussions; palliative care

PFT, pulmonary function tests.
Source: Ciafaloni & Moxley, 2008.

presymptomatic diagnosis for their child could result in informational harm to both parents and the child. With careful early education and giving parents control over knowledge, much of this could be avoided (Parsons et al., 1996, 2003, 2004).

CONCLUSIONS

Diagnostic testing with improved DNA analysis has made it possible to avoid muscle biopsy in most cases. Ongoing research into disease mechanisms, high-throughput drug screening programs, and animal studies have hastened progress towards human trials. Effective treatment is available for short-term and long-term improvement with corticosteroids, and management of corticosteroid side-effects has improved. Progress has been made in the symptomatic treatment of pulmonary and cardiac systems. The optimal age to start and optimal dosage schedules have not been determined, so well-designed large-scale longitudinal clinical trials for corticosteroids are still needed. Improvement in international collaborations and data sharing will speed discovery of definitive treatments. Early and limited curative treatment trials are under way in humans. Basic and translational research is moving forward towards a cure.

RESOURCES

Physician resources

Genetests; http://www.genetests.org

Online Mendelian Inheritance in Man® (OMIM®); http://www.ncbi.nlm.nih.gov/omim

Family resources

Muscular Dystrophy Association (MDA); http://www.mda.org

Parent Project Muscular Dystrophy (PPMD); http://www.parentprojectmd.org

TREAT-NMD Neuromuscular Network. DMD interim recommendations; http://www.treat-nmd.eu

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Chapter 3

Sarcoglycanopathies

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INTRODUCTION

Muscular dystrophies are rare and genetically very heterogeneous. As a clinical entity muscular dystrophy was first appreciated with the detailed clinical description of Duchenne muscular dystrophy in 1852 and thereafter (Meryon, 1852; Duchenne, 1868). Duchenne muscular dystrophy and its milder allelic form, Becker muscular dystrophy, account for about two-thirds of the muscular dystrophies.

About a century later it became obvious that there was another group of clinically similar progressive muscular dystrophies distinct from the X-linked Duchenne/Becker muscular dystrophy as it also affected females and showed autosomal recessive inheritance. This group of diseases was originally called Duchenne-like autosomal recessive muscular dystrophy or severe childhood autosomal recessive muscular dystrophy (Kloepfer and Talley, 1958). Following the identification of dystrophin mutations as the underlying genetic cause of Duchenne and Becker muscular dystrophies (Hoffman et al., 1987), biochemical analysis led to the description of the dystrophin-associated proteins (DAPs). Further delineation of the sarcoglycan (SG) complex as a member of the DAPs formed the basis for the characterization of the so-called sarcoglycanopathies as a group a four genetically closely related muscular dystrophies with a phenotype often similar to, but more variable than, the X-linked Duchenne muscular dystrophy. It has been demonstrated that pathological mutations of the α-SG (Roberds et al., 1994), β-SG (Bönnemann et al., 1995, 1996; Lim et al., 1995), γ-SG (Noguchi et al., 1995), and δ-SG (Nigro et al., 1996a) genes cause autosomal recessive muscular dystrophies.

With the introduction of the new purely genetic classification of limb-girdle muscular dystrophies (LGMD), the genetic entities of α-, β-, δ-, and γ-sarcoglycanopathies were assigned LGMD2D, LGMD2E, LGMD2F, and LGMD2C, respectively (Bushby and Beckmann, 1995).

EPIDEMIOLOGY

The prevalence of LGMD2C–F varies among different populations. Duggan et al. (1997) analyzed a group of 332 patients with normal dystrophin gene findings from northern Italy and the USA using immunohistochemistry and mutation analysis. The overall prevalence of sarcoglycanopathies was about 9%. Subgroup analysis revealed that the prevalence of SG gene mutations was highest among patients with severe (Duchenne-like) muscular dystrophy starting in childhood (18 of 83 patients, 22%), lower among patients with proximal (limb-girdle) muscular dystrophy with a later onset (11 of 180 patients, 6%), and absent in the group of congenital muscular dystrophies (69 patients).

The relative frequency of the individual subtypes of sarcoglycanopathy varies significantly between different populations. Moreira et al. (2003) reported the identification of SG mutations in 35 Brazilian families. Among these, α-SG was the most prevalent (14 families, 40%) followed by β- and γ-SG (8 families each, 23%), and δ-SG (5 families, 14%). Likewise, most patients with sarcoglycanopathy in Europe and North America carry a mutation in the α-SG gene (Duggan et al., 1997; Fanin et al., 1997; Angelini et al., 1999), and δ-SG mutations seem to be rare. In northern Africa γ-SG mutations are common and account for almost all cases of sarcoglycanopathy in this region (Ben Othmane et al., 1995).

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PATHOPHYSIOLOGY

The SG complex has four subunits and is part of the dystrophin-associated protein complex. More recently epsilon (ε)- and zeta (ζ)-SG have been identified as additional members of the SG family. However, these genes do not cause muscular dystrophies and their exact function is under investigation. Molecular details of all six sarcoglycans are shown in Table 3.1.

All SG proteins are glycosylated transmembrane proteins with small intracellular and large extracellular domains. The constituents of the SG complex differ between different tissues: α- and γ-SGs are not expressed in smooth muscles and ε-SG is not a component of skeletal muscle.

α/ε-Sarcoglycan (SGCA/SGCE) and γ/δ-SG (SGCG/SGCD) are closely related proteins which are similar in size and sequence. The sarcoglycans form a tetrameric complex of membrane proteins fixed to the dystrophin axis by lateral association with the dystroglycan complex. In contrast to dystroglycan, which is found in nearly all cell types, the SG complex is predominantly found in muscle. Initial studies in patients with LGMD suggested that mutations in any of the four SG genes would lead to instability of the whole complex (Bönnemann et al., 1995; Lim et al., 1995; Noguchi et al., 1995; Duggan et al., 1997). However, further studies in patients and animal models revealed that not all sarcoglycans were equally important for the stability of the complex. For example, homozygous missense mutations of β-SG lead to a complete absence of all sarcoglycans from the membrane (Bönnemann et al., 1996), whereas patients with mutations of γ-SG often retain expression of α-SG at the membrane (Vainzof et al., 1996). Sakamoto et al. (1997) examined the interactions among the SG molecules by an *in vitro* pull-down binding study and an *in vitro* overlay technique using the recombinant proteins corresponding to their extracellular domains. The strongest binding was seen between β- and δ-SG. Crosslinking studies from C2C12 cells later suggested that δ-SG directly interacts with β-dystroglycan, and confirmed its tight association with β-SG (Chan et al., 1998). Taken together, these findings suggest that β- and δ-SG might form a tight core complex, with α- and δ-SG loosely associated.

The sarcoglycans are closely related to sarcospan, leading to the term sarcoglycan–sarcospan complex. Assembly of the complete tetrameric SG complex seems to be a prerequisite for membrane targeting and correct localization of sarcospan (Crosbie et al., 2000). In spite of this close interaction, mutations in sarcospan have not been identified as a cause of muscular dystrophy.

In contrast to the dystrophin–dystroglycan basal lamina axis, the functional and molecular interactions of the SG complex are much less clear. It is poorly understood how the sarcoglycans link with other members of the dystrophin-associated protein complex and how they interact with the extracellular matrix. Therefore, it remains speculative to what extent sarcoglycans contribute to signaling pathways and/or to the biomechanical stability of the dystrophin-associated protein complex.

On the cytoplasmic surface, SGCG and SGCD bind a muscle-specific form of filamin, which was originally termed filamin 2 (FLN2) (Thompson et al., 2000) but is now officially called filamin C. Filamin C is an actin binding protein, and mutations in filamin C itself give rise to a subtype of myofibrillar myopathy (Vorgerd et al., 2005).

Table 3.1

Genetic and biochemical characteristics of sarcoglycans

Protein	Gene symbol	Gene locus	Disease name	Length (aa)	Calculated weight (on gel) (kDa)	Gene size (kb)	Exons
α-SG	SGCA	17q21	LGMD2D	387	43 (50)	17	10
β-SG	SGCB	4q12	LGMD2E	318	34 (43)	18	6
γ-SG	SGCG	13q12	LGMD2C	291	32 (35)	144	8
δ-SG	SGCD	5q33	LGMD2F	290	32 (35)	441	9
ε-SG	SGCE	7q21	Dystonia, myoclonic	437	50	71	11
ζ-SG	SGCZ	8p22	No disease association	312	34	1148	8

SG, sarcoglycan.

The only potential extracellular binding partner of sarcoglycans that has been identified recently is biglycan. Using blot overlay assays and co-immunoprecipitation assays, Rafii et al. (2006) showed that biglycan binds to α- and γ-SG, and regulates SG expression during development.

ANIMAL MODELS

In 1962, Homburger and colleagues established a strain of Syrian hamsters termed BIO 14.6 that presented myopathy and cardiomyopathy. These animals show dystrophic skeletal muscles and die prematurely from progressive myocardial necrosis and heart failure. In 1997, one year after discovering and cloning the human δ-SG gene (Nigro et al., 1996b), Nigro and co-workers identified a deletion of exon 1 of the hamster δ-SG gene and thereby established the BIO 14.6 hamster as the first animal model of sarcoglycanopathy (Nigro et al., 1997). In 1998, Holt et al. successfully used adenovirus-mediated transfer of recombinant δ-SG cDNA to treat the quadriceps femoris muscle of the BIO 14.6 hamster. After intramuscular injection δ-SG was synthesized and all the other SG proteins were restored.

In addition to the hamster as a spontaneous animal model, different types of knockout mice lacking α-, β-, γ, and δ-SG have been raised. All of them show muscle fiber degeneration from about 2 weeks after birth. Cell infiltration, fibrosis, and, in some cases, calcification are observed in the skeletal muscle. However, severe pathology recovers within about 20 weeks at the latest (Ozawa et al., 2005). In addition to the skeletal phenotype, β-, γ-, and δ-SG knockout mice showed myocardiac injury which was not present in the α-SG knockout mice.

Imamura et al. (2005) established several mouse lines overexpressing ε-SG in skeletal muscle. Overexpression of ε-SG in normal mice led to substitution of α-SG by ε-SG in the SG complex. Mice overexpressing ε-SG were crossed with α-SG-deficient mice, and α-SG-deficient mice overexpressing ε-SG exhibited no skeletal muscle cell membrane damage or abnormal contraction. Imamura et al. (2005) suggested that overexpression of ε-SG may represent a therapeutic strategy for treatment of LGMD2D.

Coral-Vazquez et al. (1999) analyzed genetically engineered mice deficient for either the *Sgca* or *Sgcd* gene. They found that only *Sgcd*-null mice developed cardiomyopathy, with focal areas of necrosis as the histological hallmark in cardiac and skeletal muscle. The authors speculated that disruption of the sarcoglycan–sarcospan in vascular smooth muscle might perturb vascular function and thereby cause cardiomyopathy and exacerbate muscular dystrophy.

However, later Durbeej et al. (2003) injected recombinant β- or δ-SG adenoviruses into skeletal muscle of corresponding null mice and found that the adenoviruses would not transduce vascular smooth muscle, but still prevented muscular dystrophy and protected against exercise-induced damage. They concluded that vascular dysfunction was not a primary cause of β- and δ-SG-deficient muscular dystrophy (Durbeej et al., 2003).

CLINICAL PRESENTATION

The four types of sarcoglycanopathy causing muscular dystrophy (α-, β-, δ-, and γ- sarcoglycanopathy or LGMD2C, D, E, and F, respectively) have to be included in the differential diagnosis of autosomal recessive limb-girdle muscular dystrophies. Like most entities in this group, they are characterized by a slowly progressive proximal muscle weakness. Typically, first symptoms are difficulties with climbing stairs and rising from the floor (positive Gowers' sign). Compared with other types of muscular dystrophy, sarcoglycanopathies are more frequently found among the more severe forms and the clinical course often resembles that of Duchenne muscular dystrophy with onset during childhood (Roberds et al., 1994; Bönnemann et al., 1995; Lim et al., 1995; Noguchi et al., 1995; Nigro et al., 1996a; Fanin et al., 1997; Ginjaar et al., 2000) However, the clinical spectrum is broader than that in Duchenne muscular dystrophy and onset can also be delayed into adulthood in some cases.

The clinical course of sarcoglycanopathies is invariably progressive, leading to loss of ambulation during adolescence in most patients. In patients with later onset, ambulation may also be preserved into adulthood. Contractures and scoliosis commonly develop in the course of the disease as a consequence of increasing weakness and fibrotic degeneration of skeletal muscles. With progression of the disease, muscle weakness will include respiratory muscles and often necessitates the initiation of respiratory support. In most patients respiratory insufficiency will not occur before loss of ambulation, but exceptions with early respiratory insufficiency have been reported (Walter et al., 2004). The incidence of cardiomyopathy in the different types of sarcoglycanopathy is not exactly known but in general it is less common than in Duchenne muscular dystrophy. Melacini et al. (1999) reported abnormal findings on electrocardiography or echocardiography in about 30% of patients with sarcoglycanopathy, indicating dilative cardiomyopathy. Patients with β- and δ-SG mutations may be at higher risk for cardiac manifestations that are clinically relevant. Heterozygous and

presumably dominant-negative mutations in δ-SG have been found to cause late-onset pure dilated cardiomyopathy in a few patients without clinical involvement of skeletal muscle (Tsubata et al., 2000).

Unlike Duchenne muscular dystrophy, there appears to be no cognitive involvement in patients with sarcoglycanopathies.

DIAGNOSTIC APPROACH

The diagnostic workup of patients presenting with proximal muscle weakness will always include serum levels of creatine kinase, which are invariably significantly increased in sarcoglycanopathies. If clinically indicated, electromyography might be used to differentiate muscular dystrophies from neurogenic disorders such as spinal muscular atrophy. Depending on the clinical presentation and availability, direct genetic testing for a specific type of muscular dystrophy such as Duchenne muscular dystrophy in an affected boy might be appropriate as the next step. If this is negative or the clinical presentation does not point to a specific genetic entity, a muscle biopsy is indicated. The quadriceps muscle is commonly the biopsy site in muscular dystrophies with proximal weakness. The muscle biopsy with appropriate workup in an experienced laboratory will help to confirm dystrophic changes in skeletal muscle and exclude other possible causes of proximal muscle weakness with high creatine kinase levels, such as myositis (Dubowitz and Sewry, 2006). Once routine histology has confirmed dystrophic changes in the muscle biopsy, immunohistochemistry and immunoblot technology should be used to look for specific protein deficiency.

Mutations in any single sarcoglycan gene will normally lead to the absence of this protein and a secondary deficiency of all other sarcoglycans at the sarcolemma. For example, patients with mutations in α-SG show marked deficiency or absence of α-, β-, γ-, and δ-SG on immunostaining of muscle. Using multiple antibodies against all four SG proteins may identify a SG deficiency more precisely; however, no immunostaining pattern is considered specific for any single SG gene.

Because patients with a primary dystrophinopathy often show a secondary deficiency of SG proteins in their muscle, and vice versa, it is important always to perform dystrophin and SG immunostaining on the same sample. The finding of normal dystrophin, and complete deficiency of any of the sarcoglycans, suggests that mutations of one of the SG genes may be causative. Immunostaining for SG proteins seems to be very sensitive in detecting a SG mutation as an underlying cause for a muscular dystrophy, but it is not specific as a secondary reduction of SG expression can be seen in other disorders.

Once muscle biopsy findings with absence or severe reduction of SG expression have led to the differential diagnosis of a sarcoglycanopathy, this should be confirmed by genetic analysis of the SG genes. The order in which the four SG genes are analyzed might be drawn from the expression levels in the biopsy. However, in most cases all four SG proteins will be absent or severely reduced, and it might be more appropriate to screen for the most frequent disorder first. In populations with low rates of consanguinity and endogamy, the relative frequency is highest for α-sarcoglycanopathies followed by β-, γ-, and δ-sarcoglycanopathies, respectively (Duggan et al., 1997; Fanin et al., 1997; Passos-Bueno et al., 1999; Vainzof et al., 1999). The Arg77Cys mutation is a recurring mutation in Europe, and accounts for about one-third of the mutated α-SG alleles (Carrie et al., 1997). In specific geographically or ethnically isolated populations, certain SG mutations can reach a high frequency. Founder mutations in β-SG have been observed in the Amish (Duclos et al., 1998). Specific mutations in γ-SG cause the large majority of LGMD cases in gypsies and Tunisians (Noguchi et al., 1995; Piccolo et al., 1996; Bönnemann et al., 1998; Lasa et al., 1998; Merlini et al., 2000). A specific mutation of δ-SG appears to be relatively common in Brazil (Moreira et al., 1998, 2003; Passos-Bueno et al., 1999). A database of published and unpublished mutations in the four SG genes can be found at http://www.dmd.nl.

TREATMENT

Although different research groups are working on causative treatment strategies for sarcoglycanopathies, such as vector-based gene transfer, currently none of these therapies has reached application in clinical practice (Sandonà and Betto, 2009). Therefore treatment is symptomatic and aims at amelioration of locomotor, respiratory, and cardiac manifestations of the disease. Owing to the paucity of affected patients, specific clinical trials for sarcoglycanopathies have not been published. As the clinical manifestation is often similar to that in Duchenne or Becker muscular dystrophy, comparable treatment strategies are used for patients with sarcoglycanopathies and will not be described in detail here. This includes physiotherapy, surgical intervention, such as correction of scoliosis and contractures, and initiation of respiratory support. The use of steroids has proven effective in Duchenne muscular dystrophy but there are only anecdotal reports about the use of steroids in patients with sarcoglycanopathies (Bönnemann et al., 1997; Angelini et al., 1998; Connolly et al., 1998), so that no general recommendations can be given.

Cardiomyopathy might develop in a subgroup of patients during the course of the disease and should be looked for by echocardiography and electrocardiography at least at yearly intervals. Comparable to common practice in Duchenne muscular dystrophy, treatment with angiotensin-converting enzyme inhibitors and possibly beta-blockers should be initiated once echocardiographic findings are abnormal.

Gene therapy may become an option for the treatment of patients with sarcoglycanopathies in the future. Compared with gene transfer for the more prevalent dystrophin deficiency (Duchenne muscular dystrophy), sarcoglycan deficiency (LGMD2C–F) may benefit from the smaller coding sequences for sarcoglycans, allowing packaging into a variety of viral vectors.

Gene therapy using vectors based on adeno-associated viruses has shown potential to transfer coding sequences of SG genes efficiently into skeletal muscle of the appropriate rodent models. However, these results cannot easily be transferred to humans because the immune system of humans is fundamentally different from that of rodents, conferring unexpected and adverse effects related to gene transfer. Well-designed phase I safety studies in humans will be necessary to explore these therapeutic avenues further.

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Chapter 4

Congenital muscular dystrophies

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CONGENITAL MUSCULAR DYSTROPHIES

Congenital muscular dystrophies (CMDs) are a heterogeneous group of disorders characterized by muscle weakness from birth and variable clinical manifestations of the eye and central nervous system (Figure 4.1). Some of these disorders are fatal in the first years of life, whereas others have a milder course, with survival into adulthood. The incidence of all forms of CMD has been estimated at 1 in 21500 with a prevalence of 1 in 125000 in north-eastern Italy (Mostacciuolo et al., 1996) and an incidence of 1 in 16000 in western Sweden (Darin et al., 2002). Since the first patient was described by Batten in 1903, the clinical and molecular classification of CMD has expanded considerably (Tables 4.1 and 4.2). The CMDs were initially classified by clinical features and country of origin; however, with new molecular techniques it is now possible to classify these patients better.

More than 10 genes that cause forms of CMD have been identified to date. These can be further subdivided into defects in structural proteins of skeletal muscle fibers, disorders of glycosylation, and proteins of the endoplasmic reticulum and nucleus (see Table 4.1). There are others that have been linked to a chromosomal location, but no gene has yet been identified, and additional forms that have yet to be mapped to a chromosomal location, let alone be identified molecularly. Even with current molecular diagnostic techniques, only approximately 25–50% of patients with CMD have an identifiable genetic mutation (Peat et al., 2008). In addition, some phenotypic classifications have been attempted. However, there is significant overlap between the phenotypic and molecular classifications, making diagnosis within this heterogeneous group of disorders difficult.

MUSCLE PROTEINS AND THE DYSTROPHIN–GLYCOPROTEIN COMPLEX

There is a strong connection between the intracellular actin cytoskeleton and the extracellular matrix. Structural proteins present in the extracellular matrix, including laminin α2 and collagen VI, connect to the intracellular actin–myosin complex within muscle fibers via the dystrophin–glycoprotein complex (DGC). These proteins are depicted in Figure 4.2.

Many of the proteins that are implicated in causing CMD attach to or alter the glycosylation of the DGC. This large multimeric complex connects the intracellular actin cytoskeleton through dystrophin and the transmembrane proteins to the extracellular matrix (see Figure 4.2) (Ervasti and Campbell, 1993). Enriched dystrophin preparations that were subjected to serial anion exchange chromatography and sucrose gradient centrifugation identified 10 proteins that were tightly associated with dystrophin (Ervasti et al., 1990). Four of these are glycosylated. Each of the genes encoding the core components of the DGC has been characterized and their interactions better defined over the last several years. The DGC functions to stabilize the sarcolemma against the repetitive stress incurred during muscle contraction (Petrof et al., 1993), has been implicated in cell signaling (Rando, 2001; Lapidos et al., 2004), and serves as a scaffold for membrane proteins (Judge et al., 2006).

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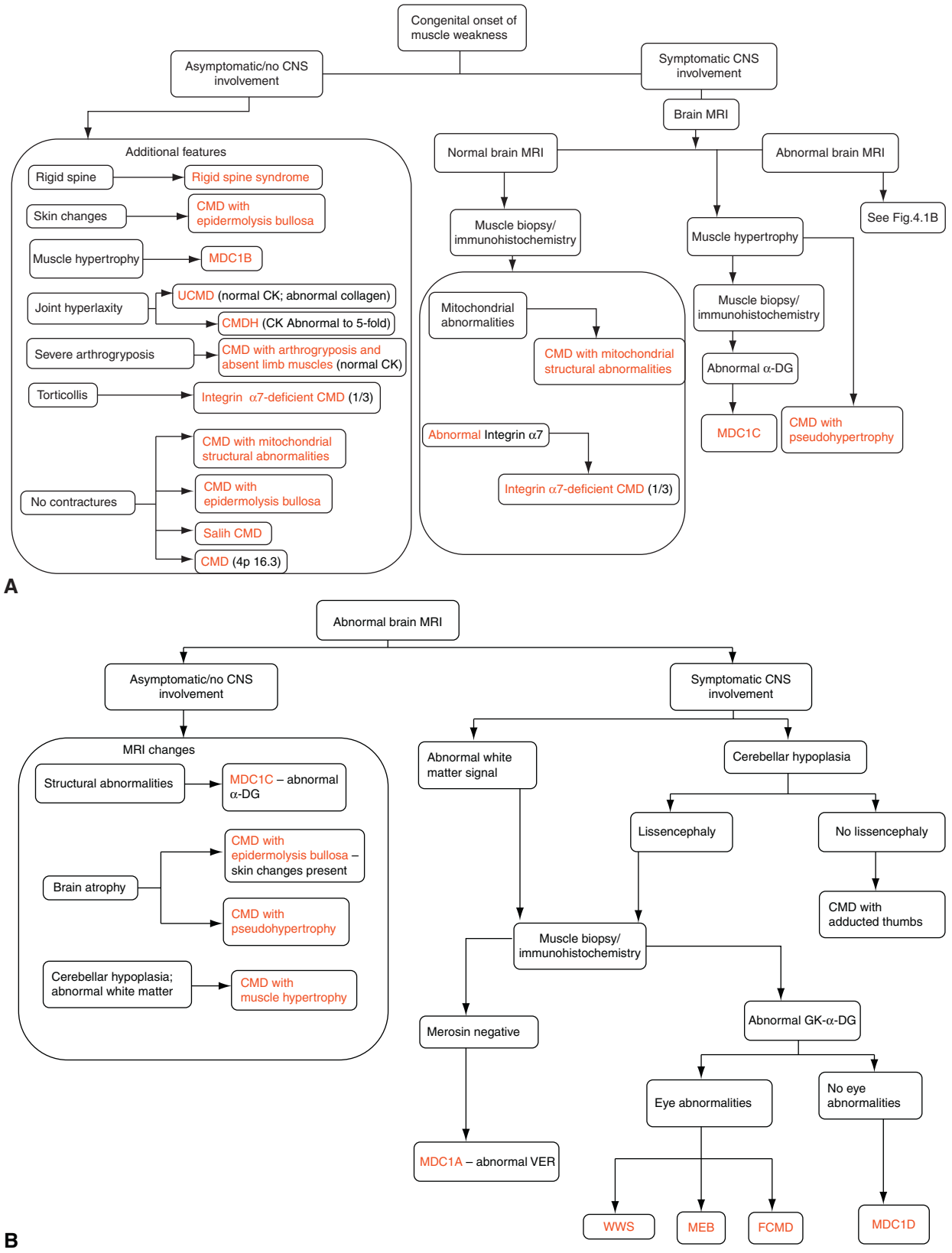

Figure 4.1. (**A, B**) Diagnostic algorithim for congenital muscular dystrophies, beginning with congenital onset of muscle weakness. Conditions are shown in red type. α-DG, α-dystrophin; CK, creatine kinase; CMD, congenital muscular dystrophy; CMDH, CMD with joint hyperlaxity; CNS, central nervous system; FCMD, Fukuyama CMD; MDC1A, CMD type 1A (merosin-deficient CMD); MDC1B, CMD type 1B (CMD with respiratory failure and muscle hypertrophy); MDC1C, CMD type 1C (CMD with muscle hypertrophy/*FKRP*-deficient CMD); MDC1D, CMD type 1D (CMD with severe intellectual impairment and abnormal glycosylation/*LARGE*-deficient CMD); MEB, muscle–eye–brain disease; MRI, magnetic resonance imaging; UCMD, Ullrich CMD; VER, visual evoked responses; WWS, Walker–Warburg syndrome.

Table 4.1

Congenital muscular dystrophies and associated defects

Disorder	Protein	Gene	Chromosome location
Defects of structural proteins			
Merosin-deficient CMD (MDC1A)	Laminin α2	*LAMA2*	6q22–q23
UCMD1	Collagen VI	*Col6A1*	21q22.3
UCMD2	Collagen VI	*Col6A2*	21q22.3
UDMD3	Collagen VI	*Col6A3*	2q37
Integrin α7-deficient CMD	Integrin α7	*ITGA7*	12q13
CMD with joint hyperlaxity (CMDH)	Integrin α9	*ITGA9*	3p23–21
CMD with epidermolysis bullosa	Plectin	*Plectin*	8q24.3
Defects of glycosylation			
Walker–Warburg syndrome (WWS)	Protein-*O*-mannosyltransferase 1	*POMT1*	9q34
	Protein-*O*-mannosyltransferase 2	*POMT2*	14q24
	Fukutin	*FCMD*	9q31–q33
	Fukutin-related protein	*FKRP*	19q13.3
	LARGE	*LARGE*	22q12.3–q13.1
Muscle–eye–brain disease (MEB)	*O*-linked mannose β1,2-*N*-acetylglucosaminyltransferase	*POMGnT1*	1p32–p34
Fukuyama CMD (FCMD)	*Fukutin*	*FCMD*	9q31–q33
CMD with muscle hypertrophy (MDC1C)	*Fukutin*-related protein	*FKRP*	19q13.3
CMD with severe intellectual impairment and abnormal glycosylation (MDC1D)	LARGE	*LARGE*	22q12.3–q13.1
Proteins of the ER and nucleus			
Rigid spine syndrome	Selenoprotein N 1	*SEPN1*	1p35–p36
LMNA-deficient CMD	Lamin A/C	*LMNA*	1q21.2
Other			
CMD with respiratory failure and muscle hypertrophy (MDC1B)			1q42
CMD			4p16.3

CMD, congenital muscular dystrophy; ER, endoplasmic reticulum; UCMD, Ullrich CMD.

The 156-kDa component of the DGC was identified as the heavily glycosylated α-dystroglycan (Ervasti and Campbell, 1991; Ibraghimov-Beskrovnaya et al., 1992). α-Dystroglycan is encoded along with β-dystroglycan by a single gene, *DAG1*. Post-translational cleavage gives rise to the two glycoproteins that are noncovalently associated (Michele and Campbell, 2003). Tissue-specific glycosylation of α-dystroglycan gives rise to products of different molecular weight (skeletal muscle is 156 kDa and brain and peripheral nerve is 120 kDa). α-Dystroglycan contains one potential *N*-linked glycosylation site, and β-dystroglycan contains three. However, it is the *O*-linked glycosylation located in the serine–threonine-rich mucin domain of α-dystroglycan that contributes most to the observed molecular weight (Endo and Toda, 2003).

β-Dystroglycan is a transmembrane protein that interacts with the C-terminal region of α-dystroglycan in the extracellular space and with dystrophin in the cytoplasm (Henry and Campbell, 1996). Complete knockout of *DAG1* is not compatible with life as a result of failure to form Reichert's membrane, the membrane that separates the maternal circulation from the embryo (Cohn et al., 2002). Conditional disruption of *DAG1* expression in the skeletal muscle results in loss of the DGC and muscular dystrophy in the mouse (Cohn et al., 2002). Similar brain-specific disruption affects the glia limitans, causes loss of cortical layering due to overmigration of the neurons in the subarachnoid space, and results in type II, or "cobblestone," lissencephaly, as is seen in some of the congenital muscular dystrophies (Michele et al., 2002).

O-linked glycosylation, in particular, *O*-mannosylation, of α-dystroglycan is important for the interaction with proteins in the extracellular matrix, including laminin, neurexin, agrin, and perlecan (Michele and Campbell, 2003). Defects in laminin cause congenital muscular dystrophy type 1A (MDC1A). Disruption of the glycosylation of α-dystroglycan is the underlying cause of several congenital muscular dystrophies, such as Walker–Warburg syndrome, muscle–eye–brain disease,

Table 4.2

Clinical features of congenital muscular dystrophies

Disorder	Age of onset	Weakness	Contractures	Joint hyperlaxity	Eye findings	Seizures	MR	MRI findings	CK level
Defects of structural proteins									
Merosin-deficient CMD (MDC1A)	Birth; partial deficiency can present at 1–12 years of age	Generalized	Multiple, but not severe	No	No; VER abnormal	Yes	6%	Abnormal white matter signal	> 1000 U/L
Ullrich syndrome (UCMD1)	Birth to 1 year	Generalized; distal > proximal	Hips, knees, fingers, elbows	Yes	No	No	No	None	Normal
Integrin α7-deficient CMD	Birth to 2 months	Proximal	Torticollis	No	No	No	1/3 pts	None	Mild increase
CMD with joint hyperlaxity (CMDH)	Birth	Generalized; progressive	Ankles, knees, shoulders	Yes	No	No	No	Normal	Normal to 5-fold
CMD with epidermolysis bullosa	Birth to 20 years	Generalized, including facial muscles	None described	No	No	No	No	Brain atrophy	> 1000 U/L
Defects of glycosylation									
Walker–Warburg syndrome (WWS)	Neonatal	Generalized; severe	Elbows	No	Micro-ophthalmia; coloboma; cataracts	Yes	Yes	Cobblestone lissencephaly	2–15-fold normal
Muscle–eye–brain disease (MEB)	Neonatal	Generalized	Elbows	No	Myopia; retinal dysplasia	Yes	Yes	Cobblestone lissencephaly	2–15-fold normal
Fukuyama CMD (FCMD)	Neonatal	Generalized	Hips, knees, ankles, and elbows possible	No	50% mild to severe	Yes	Yes	Migration defects	2–15-fold normal
CMD with muscle hypertrophy (MDC1C)	Birth to 6 months	Generalized; arms > legs	Elbows, knees, fingers	No	No	No	No	Normal to structural abnormality	20–75-fold normal
CMD with severe intellectual impairment and abnormal glycosylation (MDC1D)	Within the 1st year of life	Generalized	Ankles and elbows, mild	No	No	No	Yes	White matter abnormal; pachygyria; hypoplastic brainstem	3–25-fold normal

CMD with pseudohypertrophy, macroglossia, and respiratory insufficiency	Birth	Progressive; proximal then distal and facial	Elbows and lower extremities	No	No	No	?	Normal to mild atrophy	10–30-fold normal
Proteins of the ER/nucleus									
Rigid spine syndrome	Birth to 1 year	Mild to moderate, proximal	Rigid spine; elbows, fingers, hips, ankles	No	No	No	No		Normal or slightly raised
LMNA-deficient CMD	Birth	Generalized	Distal then proximal; lower extremity > upper extremity	No	No	No	No		Normal to 5-fold
Other									
CMD with respiratory failure and muscle hypertrophy (MDC1B)	Birth to 1 year	Proximal	Elbows, ankles, rigid spine	No	No	No	No	Normal	12–40-fold
CMD (4p16.3)	Birth	Trunk and shoulder girdle	No	Mild	No	No	No	Normal	2–4-fold
CMD and muscle hypertrophy	Birth	Generalized	Ankles, elbows	No	No	No	No	Cerebellar hypoplasia; abnormal white matter	15–40-fold
CMD with mitochondrial structural abnormalities	Neonatal	Proximal > distal	No	No	No	50%	Yes	Normal; mild brain atrophy	1.5–10-fold
CMD with arthrogryposis and absent limb muscles	Birth	Distal	Elbows, wrists, fingers, hips, knees, ankles	No	No	No	No	?	Normal
Salih CMD	Birth	Proximal	No	No	No	No	No	?	Slight increase
CMD with adducted thumbs	Birth	Generalized; distal > proximal	Congenital contractures of thumbs and toes	No	No	No	Yes	Cerebellar hypoplasia	3-fold

CK, creatine kinase; CMD, congenital muscular dystrophy; ER, endoplasmic reticulum; MR, mental retardation; MRI, magnetic resonance imaging; VER, visual evoked responses.

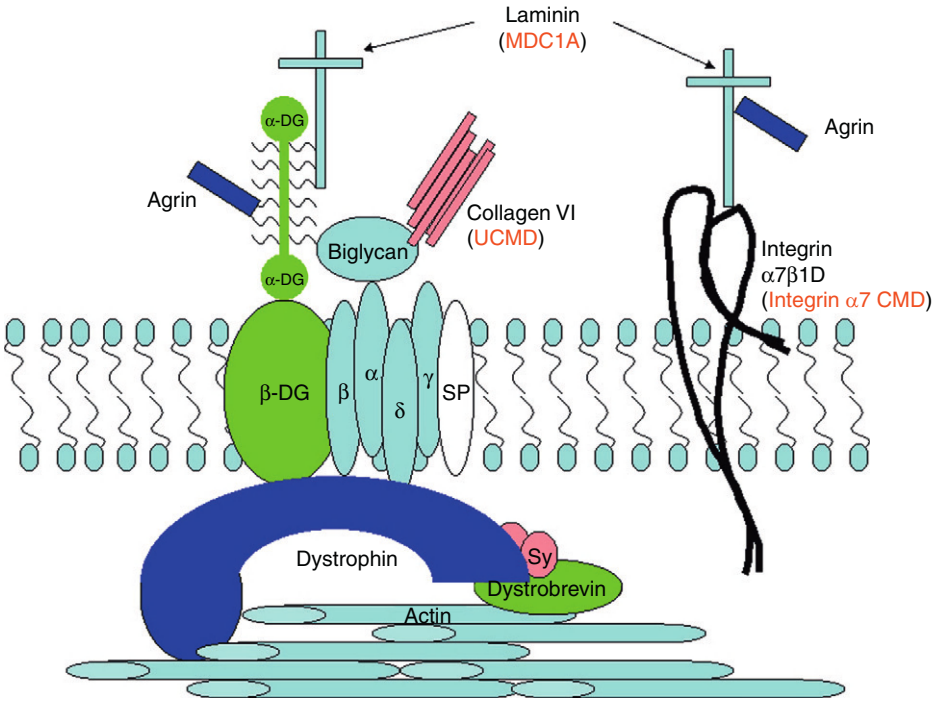

Figure 4.2. Dystrophin–glycoprotein complex and other structural proteins involved in congenital muscular dystrophy (CMD). α-DG, α-dystroglycan; β-DG, β-dystroglycan; α, β, γ, and δ in blue ovals are the sarcoglycans; MDC1A, CMD type 1A (merosin-deficient CMD); SP, sarcospan; Sy, syntrophin; UCMD, Ullrich CMD.

Fukuyama congenital muscular dystrophy, and congenital muscular dystrophy types lC and lD, as well as the milder phenotypes of limb-girdle muscular dystrophy (LGMD) types 2I, 2K, and 2L.

DEFECTS OF STRUCTURAL PROTEINS

Merosin-deficient congenital muscular dystrophy (MDC1A)

Clinical features

Merosin-deficient congenital muscular dystrophy (MDC1A) is the most common CMD in some countries, such as France and the UK, accounting for 30–40% of all cases (Fardeau et al., 1996). However, in other countries, such as Japan, Australia, and Italy, other forms are more common. MDC1A is characterized by onset of muscle weakness and hypotonia at birth or in the first few months of life (Tome et al., 1994). Proximal muscles are affected more than distal muscles, and axial muscles are severely affected as well. Calf hypertrophy can sometimes be observed, but more often the phenotype is one of atrophy. Contractures can occur, but severe arthrogryposis is not seen.

Motor milestones are invariably delayed. Maximal motor ability is typically only sitting unsupported. Often children with MDC1A can stand with support, but only about 25% can walk with support (Jones et al., 2001). The muscle weakness is stable over time, although progression has been documented. However, limitations are progressive with increased contractures noted over time. This is followed by rigidity and spinal deformities. limiting mobility even more.

Central nervous system (CNS) findings include typical white matter changes on MRI (see below). Cognition is typically normal unless there are extensive structural brain abnormalities or occipital agyria (Mercuri et al., 1999). Intellectual impairment has been described in 6% of patients (Jones et al., 2001). Some 8–30% of patients exhibit seizures, described as complex partial seizures with atypical absences (Hermann et al., 1996; Pegoraro et al., 1998; Philpot et al., 1999b; Jones et al., 2001).

Although visual function is normal, visual and somatosensory evoked responses are abnormal on electrophysiological studies (Mercuri et al., 1995). Children with MDC1A have a peripheral motor demyelinating neuropathy, but sensory nerve defects are observed only in older patients (Shorer et al., 1995). Extraocular muscles are spared in MDC1A (Kjellgren et al., 2004; Nystrom et al., 2006).

Typically, feeding difficulties and failure to thrive are present. The feeding difficulties can lead to aspiration pneumonia. Respiratory issues include a severe restrictive respiratory syndrome. From the age of 5 years

there can be nocturnal hypoventilation which responds to overnight noninvasive positive pressure ventilation (Philpot et al., 1999a). Death occurs between 15 and 30 years of age, secondary to respiratory failure. Cardiac involvement may be subclinical in 3–35% of cases (Jones et al., 2001), and cardiac failure is rare (Gilhuis et al., 2002); however, some patients with MDC1A have mild to moderate left ventricular hypokinesia (Spyrou et al., 1998).

Laboratory/radiology features

Laboratory studies include a raised serum level of creatine kinase (CK). In the neonatal period, serum CK levels are typically over 1000 units/l, but decrease over time. Later in the disease, CK levels may be in the normal range.

Muscle biopsy findings in infants include significant inflammation, which may confuse the diagnosis with infantile polymyositis (Pegoraro et al., 1996). During this neonatal period, there are only a few degenerating and regenerating muscle fibers. Over time, the inflammation resolves and is replaced by a more typical dystrophic picture with variable fiber size and increased endomysial connective tissue. Unlike other muscular dystrophies, in particular Duchenne muscular dystrophy (DMD), regeneration is less prominent. With advancing age, the picture is one of a static myopathy with extensive fibrofatty infiltrate (Pegoraro et al., 1998). In patients with MDC1A there is dissociation between the muscle pathology, which is severe, and the clinical picture, which is milder.

Brain magnetic resonance imaging (MRI) in all patients with MDC1A shows a characteristic hypomyelination and hypodensity of white matter (best visualized on T_2-weighted images), although the abnormality may not be present in the first 6 months of life. This abnormality is diffuse, but spares the internal capsule, corpus callosum, basal ganglia, thalami, and cerebellum. This distinctive pattern has been attributed to a dysfunction of the blood–brain barrier leading to abnormal water distribution in the white matter, and not due to decreased or abnormal myelination (Villanova et al., 1997; Taratuto et al., 1999; Alkan et al., 2007). Some patients may have MRI findings of leukoencephalopathy in the periventricular and subcortical white matter (Gilhuis et al., 2002). Neuronal migration defects are seen in approximately 4% of cases (Jones et al., 2001). Rarely, there may be structural brain abnormalities as well, including pontocerebellar hypoplasia (Sunada et al., 1995; Caro et al., 1999; Philpot et al., 1999b, Taratuto et al., 1999). Despite the abnormal white matter signal, patients generally demonstrate normal cognitive development. However, low IQ is seen in patients with more extensive structural abnormalities and occipital agyria (Mercuri et al., 1999). Leite et al. (2005a) evaluated the MRI findings of 25 Brazilian patients with MDC1A. The parietal, frontal, and temporal regions of the brain had more white matter involvement than the brainstem and internal and external capsules. The cerebellum was rarely affected. The extent of the white matter abnormality did not correlate with the clinical status and the degree of laminin deficiency (partial versus total). In those patients who underwent follow-up MRI, changes seen did not correlate with the clinical status of the patient.

Brain magnetic resonance spectroscopy (MRS) was performed on nine patients with MDC1A and compared with findings in 10 normal, aged-matched controls (Leite et al., 2005b). Abnormally high free-water concentrations were found in the white matter of patients with MDC1A, but *N*-acetylaspartate/creatine and choline/creatine ratios were normal. In another study, five patients with MDC1A were studied with short-echo time localized proton MRS to determine metabolite concentrations in gray and white matter (Brockmann et al., 2007). In the affected white matter there were reduced levels of *N*-acetylaspartate and *N*-acetylaspartylglutamate, creatine, and phosphocreatine. This is consistent with edema and increased free water, and decreased cellular density with a relative astrocytosis.

Electromyography (EMG) reveals nonspecific myopathic findings (more often in proximal muscles than in distal muscles), and nerve conduction studies show slowing of conduction velocity consistent with demyelinating polyneuropathy (Gilhuis et al., 2002; Di Muzio et al., 2003; Quijano-Roy et al., 2004). Quijano-Roy et al. (2004) reviewed EMGs and motor and sensory nerve conduction studies (NCS) in seven patients with MDC1A. All had abnormal EMGs with myopathic changes in at least two muscles. One newborn had a normal EMG, and became myopathic by 2 years of age. Abnormal NCS were seen in five of the seven patients. In the three patients in whom both motor and sensory nerves were tested, the conduction velocities were slow. In another study of 10 patients with MDC1A, all had reduced motor nerve conduction velocities (Shorer et al., 1995). Visual and somatosensory evoked responses are abnormal on electrophysiological studies, although visual function is typically normal (Mercuri et al., 1995).

Molecular pathogenesis/genetics

MDC1A is caused by a defect in *LAMA2* which encodes the heavy-chain α2 laminin. Laminins are a group of heterotrimeric glycoproteins composed of a heavy α chain and two light chains, β and γ each of

which is encoded by separate genes (Engvall, 1993). To date, five α chains (α1–α5), four β chains (β1–β3), and three γ chains (γ1–γ3) have been identified which combine to form 15 laminin isoforms, each with tissue and/or developmental stage-specific expression (Suzuki et al., 2005). In skeletal muscle, the predominant isoforms are laminin-2 (also known as merosin), which is composed of α2, β1, and γ1 chains, and laminin-4, composed of α2, β2, and γ1 (Gullberg et al., 1999). Even though MDC1A was initially considered merosin-deficient CMD, both laminin-2 and laminin-4 are affected, as merosin antibodies recognize the α2 laminin chain. Laminins are secreted into the extracellular matrix where they bind to neurexin, agrin, and collagen IV in the extracellular matrix, and to dystroglycan, integrins, and syndecans in the basement membrane (Muntoni and Voit, 2004). The biological functions of laminins are varied and include cell–cell recognition, growth, differentiation, cell shape, and migration (Vachon et al., 1996; Colognato et al., 1999; Suzuki et al., 2005).

The *LAMA2* gene is localized to chromosome 6q22–q23 (Vuolteenaho et al., 1994); it spans 260 kb and contains 64 exons (Zhang et al., 1996), producing a 400-kDa protein that is post-translationally cleaved into 300- and 80-kDa subunits which remain associated by disulfide bonds. Laminin-2 is expressed in the striated muscle basement membrane, as well as in the cerebral blood vessels including the capillaries that form the blood–brain barrier, the glia limitans, the developing axon tracts, and Schwann cells (Villanova et al., 1997; Buttery and Ffrench-Constant, 1999).

Absence of laminin α2 immunostaining on snap-frozen muscle biopsy tissue confirms the diagnosis of MDC1A. If there is residual laminin α2 staining, it is important to use multiple antibodies to different portions of laminin α2 (Sewry et al., 1997; Cohn et al., 1998). A marked reduction in α-dystroglycan, laminin β2, and integrin α7 can be observed in patients with complete laminin α2 deficiency. Overexpression of laminin heavy chains α4 and α5 can be seen, but the light chains, β1 and γ1, in addition to β-dystroglycan, are expressed normally (Sewry et al., 1995; Cohn et al., 1997, 1999). Laminin α2 staining and molecular testing (either with confirmed familial mutation or by analysis of markers compared with those of an affected sibling) can also be done on trophoblasts obtained by chorionic villus sampling for prenatal diagnosis (Naom et al., 1997; Yamamoto et al., 2004; Vainzof et al., 2005).

Loss-of-function mutations in *LAMA2* typically lead to severe MDC1A with complete laminin α2 deficiency. Mutations have been demonstrated throughout the gene and include nonsense mutations, deletions leading to frameshift mutations with premature stop codons downstream, and splice site mutations deleting an entire exon and leading to a premature stop codon (Helbling-Leclerc et al., 1995; He et al., 2001; Coral-Vazquez et al., 2003). However, with 64 exons and 9.5 kb of coding sequence, and no hotspots for mutations, routine mutation screening of *LAMA2* is impractical at best.

Clinical variant of MDC1A: partial laminin α2 deficiency

Patients with a partial deficiency of laminin α2 demonstrate a milder phenotype than complete deficiency, and typically have missense mutations in *LAMA2* (Hermann et al., 1996; Nissinen et al., 1996; Tan et al., 1997). The spectrum of partial laminin α2 deficiency ranges from mild CMD with independent ambulation to LGMD. Similar to patients with MDC1A, epilepsy is also seen in patients with partial laminin α2 deficiency, even though the skeletal muscle phenotype may be mild. CK levels are increased (3–30-fold) and nerve conduction velocities can be reduced in patients with this variant. However, one patient with partial laminin α2 deficiency had a severe course presenting with hypotonia and joint contractures at birth, delayed motor milestones, seizures, moderate intellectual impairment, and dilated ventricles, white matter abnormalities, and pachygyria on brain MRI (Pegoraro et al., 2000).

Management

The treatment for the congenital muscular dystrophies is currently symptomatic, as there are no definitive treatments. Management should be tailored to each individual. It should include physical therapy and stretching exercises to promote mobility and prevent contractures; use of assistive devises as needed to help with mobility and ambulation, including canes, walkers, orthotics, and wheelchairs; and orthopedic intervention for complications of foot deformities and scoliosis. Respiratory function should be monitored regularly and assisted ventilation devices should be implemented when needed. Early speech, language, and dietary input are necessary, because feeding difficulties along with failure to thrive are common. Gastrostomy should be considered in children with significant swallowing issues or severe failure to thrive (Philpot et al., 1999a).

In the authors' experience, prednisone at a dose used for DMD (0.75 mg/kg daily) can have a significant and longlasting effect in improving motor function and therefore should be offered under strict physician monitoring. There are no clinical studies published to this effect, but preclinical data in the α2-deficient mice

showed a beneficial effect of weekly prednisone treatment by prolonging survival and improving strength (Connolly et al., 2002).

Animal models

The *dy* mouse was initially described by Michelson et al. (1955). The homozygous mice demonstrated severe progressive muscular dystrophy, were smaller than their littermates, and died between 2 and 6 months of age from an unknown cause. The *dy* locus was mapped to chromosome 10, the same location as the mouse *LAMA2* gene, encoding merosin. Sunada et al. (1994) demonstrated deficient expression of merosin in the skeletal muscle, cardiac muscle, and peripheral nerve of *dy*/*dy* mice. The molecular defect in the *dy* mouse was identified as a splice site mutation in the laminin α2 gene leading to expression of multiple mRNAs (Xu et al., 1994). Since then, the dy^{2J}/dy^{2J} mouse has been identified (Meier and Southard, 1970), and mice with targeted deletions of *LAMA2* gene have been generated: dy^{W}/dy^{W} (Kuang et al., 1998) and dy^{3K}/dy^{3} (Miyagoe et al., 1997). These mouse models have varying severity and expression of laminin α2 (Guo et al., 2003). Comparison of these different mouse models has helped in the characterization of MDC1A and in treatment options (see Active Research, below). *LAMA2* deficiency has been described in Brittany–Springer Spaniel mixed breed dog and in several cat breeds, including Siamese, Maine Coon cat, and a domestic short-haired mixed breed (O'Brien et al., 2001; Shelton and Engvall, 2005).

Active research

With several mouse models of MDC1A available, it is possible to test different therapeutic approaches. Transgenic expression of the human laminin α2 gene in the skeletal muscle of dy^{W}/dy^{W} mice improves the muscle function (Kuang et al., 1998). Similarly, in dy^{3K}/dy^{3K} mice, overexpression of a cDNA encoding mouse laminin α1 under the chicken β-actin promoter shows significant improvement (Gawlik et al., 2004). The similarity between the laminins makes these results predictable. Improved muscle function and overall health has been described in laminin α2-deficient mice with overexpression of a mini-agrin construct, either transgenically or by somatic gene transfer (Moll et al., 2001; Bentzinger et al., 2005; Qiao et al., 2005). As agrin provides a link between laminin-8 and α-dystroglycan, it is hypothesized that overexpression of agrin may stabilize α-dystroglycan at the muscle membrane. In addition, the regenerative capacity of laminin α2-deficient muscle is improved after injury in mice overexpressing agrin; thus, agrin may also activate intracellular signals that allow successful regeneration (Bentzinger et al., 2005). Linker molecules such as a mini-agrin molecule and a chimeric protein containing the dystroglycan-binding domain of perlecan can also ameliorate the disease in *dy*/*dy* mice (Meinen et al., 2007).

Connolly et al. (2002) demonstrated that complement deficiency and prednisolone administration (0.01 mg/g per week divided in two oral doses) prolonged survival and improved muscle strength in *dy*/*dy* mice. Both the classical and alternate pathways of complement may be involved in the pathogenesis of muscular dystrophy, and prednisolone inhibition of the alternate pathway (Weiler and Packard, 1982) may be synergistic in this model.

Another mechanism that has been tested in mouse models of MDC1A is the inhibition of apoptosis. There are significant signs of skeletal muscle death by apoptosis in patients with MDC1A and in laminin α2-deficient mice (Miyagoe et al., 1997; Mukasa et al., 1999; Hayashi et al., 2001b). Therefore it is possible that apoptosis plays a role in the pathogenesis of MDC1A. Girgenrath et al. (2004) crossed $LAMA2^{-/-}$ mice with $Bax^{+/-}$ heterozygous mice or mice overexpressing the *pMyoD–hBcl-2* transgene to look at the effects of inhibition of apoptosis on the survival of the $LAMA2^{-/-}$ mice. Bax is a proapoptosis protein and Bcl-2 is an antiapoptosis protein. Both of these crosses prolonged the survival of the $LAMA2^{-/-}$ mice severalfold. In addition, the $LAMA2^{-/-}$ mice that had inactivation of Bax also had improved muscle histology and decreased fixed contractures. These researchers followed up these results by showing that Bcl under a myogenic regulatory factor (MRF) promoter produced the same results (Dominov et al., 2005). The MyoD promoter is active in both proliferating myoblasts and myofibers (Tapscott et al., 1992), whereas the MRF promoter is active only in myofibers (Pin et al., 1997). Therefore, therapeutic interventions that inhibit apoptosis may be of benefit in MDC1A.

Transgenic mice with overexpression of laminin α1 crossed with mice deficient in laminin α2 have shown that laminin α1 can substitute for laminin α2 in multiple tissues and correct the muscular dystrophy and peripheral neuropathy in the dy^{3K}/dy^{3K} mice (Gawlik et al., 2006). It is possible that treatment with laminin α1 may be beneficial in patients with MDC1A.

More recently, bone marrow transplantation (BMT) in the *dy* mouse has improved the outcome in this animal model of MDC1A (Hagiwara et al., 2006). BMT was shown to promote survival and growth of *dy* mice, with transplanted mice living twice as long as their littermate *dy* mice that did not receive a transplant. BMT also improved the muscle pathology and restored laminin α2 expression in the *dy* muscle. Muscle strength in

the *dy* mice who received BMT was significantly improved, as was their respiratory function.

Studies in the dyW mouse model have shown that overexpression of the cytotoxic T-cell carbohydrate blocks the dystrophic muscle phenotype in the mice. This was done by overexpressing the GlcNAc transferase (*N*-acetylgalactosaminyltransferase, Galgt2) in dyW homozygous mice (Xu et al., 2007). This suggests that increased glycosylation can help MDC1A.

Ullrich syndrome (see also Chapter 5)

Clinical features

Ullrich congenital muscular dystrophy (UCMD) was initially described in 1930 by Ullrich, who called it scleroatonic muscular dystrophy. Patients typically present in the neonatal period with hypotonia, congenital contractures of the proximal joints, torticollis, and kyphoscoliosis. There is hyperelasticity of the distal joints, with extended talipes and protruding calcaneus. Often there is hip dislocation. Hyperlaxity may be absent in severe cases. The kyphosis and proximal contractures can be transient, or at least improve with physical therapy. However, the contractures tend to recur and eventually affect the ankles, wrists, and fingers, which were previously lax. The finger flexion contractures can be particularly severe. Surgery for the kyphoscoliosis is often necessary.

Maximal motor function is very variable. Some patients never walk, whereas others achieve ambulation in time or with delay up to the fourth year. In a review of 15 patients with classical UCMD, 7 had severe motor disability, whereas 8 acquired ambulation or had only mild motor delay (Mercuri et al., 2002b). Increased contractures can lead to progressive functional difficulties and loss of ambulation after a period of independence. Patients have normal intelligence.

A characteristic facial appearance has been described in many patients with UCMD. The face is rounded with slight drooping of the lower eyelids and prominent ears (Mercuri et al., 2002b). The skin typically shows follicular hyperkeratosis. Additional more uncommon features are cheloid formation and a softer consistency of the skin on the palms and soles (Muntoni and Voit, 2004). Ultrastructural analysis of skin from patients with UCMD demonstrates abnormalities in the extracellular matrix including variable size of collagen fibrils, irregular shape of the fibrils (described as "cauliflower-like"), loosely packed collagen bundles, and infiltration of ground substance material, all reminiscent of Ehlers–Danlos syndromes (Kirschner et al., 2005). Like patients with Ehlers–Danlos syndromes, patients with UCMD may have excessive scar formation following trauma.

Respiratory complications with ventilatory insufficiency almost invariably develop in the first or second decade. Cardiac involvement has not been described (Lampe and Bushby, 2005). In addition to the typical phenotype, patients with milder disease who did not have neonatal contractures and showed normal motor milestones have now been reported. At the other end of the spectrum, there are patients in whom the distal laxity is absent.

Laboratory/radiological features

Serum CK levels are typically normal in patients with UCMD. They may be mildly increased, but rarely more than five times the upper limit of normal (Lampe and Bushby, 2005). Muscle MRI in patients with UCMD demonstrates diffuse involvement of the thigh muscles with relative sparing of the sartorius, gracilis, and adductor longus (Mercuri et al., 2005b). In one study, 9 of 10 patients with UCMD demonstrated concentric involvement of the vastus lateralis muscles, and 8 of these 9 patients had a central area of abnormal signal in the rectus femoris (Mercuri et al., 2005b). There was also diffuse involvement of the calf muscles.

EMG studies in three patients with UCMD demonstrated myopathic changes (Quijano-Roy et al., 2004). Low–borderline motor and sensory nerve conduction velocities were demonstrated in two patients at 2 years of age (Quijano-Roy et al., 2004).

Pathology of skeletal muscle from patients with UCMD ranges from mildly myopathic to completely dystrophic. The dystrophic muscle demonstrates increased variation in fiber size, some necrotic fibers, and prominent endomysial and perimysial fibrosis and adipose tissue substitution. Electron microscopy of muscle demonstrates a complete absence of microfibrils (typically seen associated with collagen fibrils in the interstitium), but an intact basal lamina and normal-appearing collagen fibrils in the interstitium (Ishikawa et al., 2002).

Reduced or absent collagen VI (Col6) labeling on immunohistochemistry suggests a diagnosis of UCMD. However, decreased collagen VI labeling has been described with no subsequent mutation in the *COL6* genes. Therefore, there may be secondary downregulation of this protein in some other conditions (Ishikawa et al., 2004). Some patients with UCMD have a partial or milder deficiency of collagen VI (Ishikawa et al., 2004). Perlecan, collagen IV, and laminin α2 expression is normal in UCMD (Muntoni and Voit, 2004).

Molecular pathogenesis/genetics

UCMD is typically an autosomal recessive condition caused by a defect in collagen VI. Collagen VI, like other collagens, is an extracellular matrix protein composed of three chains, α1, α2, and α3, that form a monomer made up of two globular domains connected by a triple helical

structure. The three chains assemble into antiparallel dimers via disulfide bonds which associate laterally into tetramers prior to secretion into the extracellular space (Zhang et al., 2002). The tetramers associate end to end to form a microfibrillar network that interacts with the fibronectin network, biglycan and collagen IV.

COL6A1, *COL6A2*, and *COL6A3* encode the three chains, α1, α2, and α3, respectively. *COL6A1* and *COL6A2* are located on chromosome 21q22.3, and *COL6A3* is located on chromosome 2p37. Mutations in the *COL6A2* gene leading to UCMD were first reported in 2001 (Camacho Vanegas et al., 2001), followed by identification of mutations in *COL6A1* and *COL6A3* genes (Demir et al., 2002; Pan et al., 2003; Giusti et al., 2005). In 79 patients with UCMD or Bethlem myopathy, Lampe et al. (2005) sequenced all three *COL6* genes from genomic DNA. Some 62% of the patients had a putative mutation in one of the *COL6* genes. Some patients showed changes in more than one of these genes, and some appeared to have dominant rather than recessive disease. Therefore, these results may explain why some of the cases of UCMD are unlinked to any of the *COL6* genes under a recessive model. Mutations in UCMD tend to result in premature termination codons, by either nonsense mutations or frameshift mutations caused by insertions, deletions, duplications, or splice site mutations. At least five patients with UCMD have been described with dominant mutations in one of the *COL6* genes (Pan et al., 2003; Baker et al., 2005).

Prenatal diagnosis of UCMD has been done by haplotype analysis and collagen VI immunocytochemistry on chorionic villus cells (Brockington et al., 2004).

Management

The management of UCMD is symptomatic, similar to MDC1A (see above). Active mobilization is key, and standing frames may be helpful in severely affected children (Lampe and Bushby, 2005). Nocturnal ventilatory support is often required (Wallgren-Pettersson et al., 2004). Prophylaxis with vaccination against influenza and pneumococcus, chest physiotherapy, and early and aggressive use of antibiotics may prevent further respiratory complications of UCMD (Wallgren-Pettersson et al., 2004; Lampe and Bushby, 2005). The combination of restrictive lung disease and progressive orthopedic deformity in severely affected children makes the timing of surgical interventions critical.

Animal model

Reduced contractile force and disturbed intracellular calcium homeostasis have been demonstrated in muscle fibers from a *Col6a1* knockout mouse model. More recently, Irwin et al. (2003) demonstrated ultrastructural alterations of the sarcoplasmic reticulum and mitochondria along with spontaneous apoptosis in $Col6a1^{-/-}$ muscles. This was secondary to abnormal activation of the mitochondrial permeability transition pore and could be rescued following administration of ciclosporin A (Irwin et al., 2003; Angelin et al., 2007). This provides a link between the defect in the extracellular matrix to mitochondrial dysfunction and apoptosis which can be inhibited by ciclosporin. Therefore, therapeutic trials with ciclosporin in UCMD are logical.

Active research

In patients with mutations that lead to nonsense-mediated mRNA decay, inhibition of the kinase and helicase central to this process has rescued the phenotype in fibroblasts from patients with UCMD (Usuki et al., 2006). This was done by using small interfering RNA (siRNA) that specifically inhibited either SMG-1 (a phosphoinositide 3-kinase-related protein kinase) or Upf1 (ATP-dependent mRNA helicase and a physiological substrate of SMG-1). The downregulation of SMG-1 and Upf1 led to the production of collagen VI with the formation of a partially functional extracellular matrix.

In a recent study, mitochondrial dysfunction was ameliorated with ciclosporin A and methylAla3ethyl-Val4 ciclosporin (Angelin et al., 2007). In particular, these ciclosporins were able to inhibit the apoptosis and ultastructural defects seen in cells from patients with UCMD.

Integrin α7-deficient congenital muscular dystrophy

Clinical features

Only three patients with a deficiency of integrin α7 have been described (Hayashi et al., 1998). They all demonstrated a mild congenital myopathy with delayed motor milestones, although independent ambulation was achieved. Torticollis and congenital dislocation of the hip was observed. These patients were not able to run, jump, or climb stairs unassisted. Intellectual impairment was described in one patient, but not in the others.

Laboratory/radiology features

Serum CK levels were mildly raise. MRI and electroencephalogram findings were normal. Muscle biopsies showed variation of fiber size and fatty replacement, and were more myopathic than dystrophic (Muntoni and Voit, 2004; Jimenez-Mallebrera et al., 2005).

Molecular pathogenesis/genetics

Integrins are heterodimeric transmembrane glycoproteins consisting of an α and a β chain. Integrin α7β1 is a major laminin α2 receptor in skeletal myotubes and mature myofibers. Integrin α7β1 expression and

localization is laminin α2 dependent (Vachon et al., 1997). Integrin α7 is encoded by *ITGA7*, which is located on chromosome 12q13. Primary deficiency of integrin α7 appears to be an exceptionally rare form of CMD. Of 117 patients with unclassified congenital myopathy and CMD, only three had integrin α7 deficiency on muscle immunohistochemistry, which was confirmed molecularly (Hayashi et al., 1998). The direct diagnosis of integrin α7 deficiency from immunostaining is hampered by the developmental regulation and interindividual variation seen especially in the first 2 years of life, where integrin α7 expression, as detected with the available antibodies, is frequently low. Pegoraro et al. (2002) also noted secondary deficiencies of integrin α7, which complicates diagnosis. Laminin α2 immunostaining is normal in integrin α7 deficiency (Hayashi et al., 1998).

Animal model

Mayer et al. (1997) generated an *ITGA7* knockout mouse. Mice homozygous for the null allele are viable and fertile. Histological analysis of skeletal muscle showed typical signs of progressive muscular dystrophy, starting soon after birth. There was variability in different muscle types due to impairment of the function of the myotendinous junctions.

Active research

Transgenic overexpression of integrin α7 in dystrophin/utropin-deficient mice attenuates the muscle pathology and extends the lifespan of these mice (Engvall and Wewer, 2003). It is possible that upregulation of integrin α7 in patients with DMD could be beneficial. Thus, the study of these rare disorders of muscular dystrophy has provided valuable insight into treatment for the more common muscular dystrophies, in particular DMD.

Congenital muscular dystrophy with joint hyperlaxity (CMDH)

Clinical features

Tetreault et al. (2006) presented 14 individuals from 11 different French-Canadian families who have a clinical phenotype similar to that of UCMD, but with no defect in any of the three collagen IV genes. All were hypotonic and presented with contractures at birth. The muscle weakness was generalized and slowly progressive. There were distal joint laxities mainly of the fingers, wrists, and toes, but some patients had elbow laxity as well. The contractures were proximal at the ankle, knee, and shoulder. There was no rigidity of the spine; instead, cervical spine hypermobility was frequently observed. All patients learned to walk between 14 months and 3 years of age. Scoliosis was seen in 64% of the patients and ranged from mild to severe. Two patients required surgical correction. Unlike UCMD, there was no high arched palate, torticollis, or protruded calcaneus seen in CMDH. Intelligence was normal or only mildly impaired.

Pulmonary vital capacity was normal or diminished on average by 21%, but seemed to be stable through decades. However, unlike in UCMD, respiratory complications are not seen in CMDH.

Laboratory/radiology features

CK levels are normal to mildly increased (range 17–959 units/l).

Molecular pathogenesis/genetics

Using 10 affected and unaffected participants belonging to two unrelated CMDH families, one of which was consanguineous, the CMDH locus was mapped to chromosome 3p23–21. Fine mapping and molecular analysis identified *ITGA9* as the causative gene (Tetreault et al., 2008).

Congenital muscular dystrophy with familial junctional epidermolysis bullosa

Clinical features

De Weerdt and Castelein (1972) first described a family with the combination of epidermolysis bullosa and muscular dystrophy. Since then, several other families have been described with infantile onset of blistering with continued predisposition to blistering, and progressive muscular dystrophy with onset ranging from birth to the third decade (Salih et al., 1985; Niemi et al., 1988; Doriguzzi et al., 1993; Abanmi et al., 1994; Patrizi et al., 1994; Gache et al., 1996; Pulkkinen et al., 1996). Several members of these families died in infancy from complications of the epidermolysis bullosa. Skin findings are described as severe blistering with atrophic scarring, nail dystrophy, scalp alopecia, and oral involvement (Fine et al., 1989). The muscle weakness is generalized, including facial muscle weakness. The degree and onset of weakness may correlate to residual plectin function. Other systemic symptoms include growth retardation and anemia. Infantile respiratory complications and laryngeal webs have been described (Shimizu et al., 1999). One patient had immature circulating white cells and bone marrow histology suggestive of a preleukemic state (Patrizi et al., 1994).

Laboratory/radiology features

CK levels are raised, typically over 1000 units/l. A myopathic pattern is seen on EMG. Brain MRI demonstrates brain atrophy and enlarged ventricles. Variable muscle

fiber size, internal nuclei, and increased connective tissue are seen on muscle histology. Histopathology of the blisters demonstrates dermal–epidermal blister formation and transmission electron microscopy reveals tissue separation at the level of the hemidesmosomal attachment plaque (Pulkkinen et al., 1996). Immunofluorescence reveals attenuated plectin expression.

Molecular pathogenesis/genetics

A defect in the *plectin* gene on chromosome 8q24 causes the autosomal recessive condition of congenital muscular dystrophy with familial junctional epidermolysis bullosa (Smith et al., 1996). In affected members of four families, a deficiency of plectin was demonstrated by immunohistochemistry.

Plectin is 32 kb in length and contains 32 exons, with genomic structure similar to that of bullous pemphigoid antigen 1 (Liu et al., 1996). It encodes a 500-kDa intermediate filament. The plectin protein has three domains, an N-terminal globular domain with homology to the actin-binding domain of the dystrophin family (Elliot et al., 1997), a central rod domain, and a C-terminal globular domain with homology to the intermediate filament-associated protein desmoplakin (McLean et al., 1996). Plectin is expressed in epithelia, muscle cells, and cells that constitute the blood–brain barrier (Wiche 1989; Errante et al., 1994). Plectin is located at the plasma membrane attachments of intermediate filaments and microfilaments such as hemidesmosomes (Wiche et al., 1984; Pulkkinen et al., 1996) and desmosomes (Eger et al., 1997), and at Z-line structures and dense plaques of smooth and skeletal muscle and intercalated discs of cardiac muscle (Wiche et al., 1983; Seifert et al., 1992) critical for binding of the intermediate keratin filament network to the hemidesmosomal complexes (Pulkkinen et al., 1996). Plectin associates with desmin, metavinculin, dystrophin, and actin (Hijikata et al., 2003). Thus, plectin may provide mechanical strength to cells and tissues by acting as a crosslinking element of the cytoskeleton.

Management

In addition to the supportive management that is necessary for all of the CMDs, specific management includes supportive care to protect the skin from blistering, appropriate dressings that will not further damage the skin and will promote healing, and prevention and treatment of secondary infection. Activities should minimize trauma to the skin. New blisters should be lanced and drained to prevent further spread from the pressure from the fluid. Appropriate dressings typically involve three layers. The first layer consists of a nonadherent dressing that will not strip the top layers of the epidermis. The second layer provides stability for the primary layer and adds padding to allow for more activity. The final layer has some elastic properties and ensures the integrity of the dressing. Surveillance for infection is important, and topical or systemic antibiotics may be necessary.

Animal model

Plectin knockout mice exhibit skin blistering and die within a few days after birth (Andra et al., 1997). The skin blisters showed degeneration of basal keratinocytes, but formation of hemidesmosomes and desmosomes was preserved. Necrotic muscle fibers were seen in the skeletal muscle, particularly those of the paravertebral and distal limb muscle groups.

DEFECTS OF GLYCOSYLATION

The defects of glycosylation are a group of disorders that affect the glycosylation of α-dystroglycan, an important component of the dystrophin–glycoprotein complex. All of these disorders affect the *O*-mannosylation of α-dystroglycan. It is this particular glycosylation that is important for binding the ligands in the extracellular matrix, such as laminin, perlecan, and neurexin (Ervasti and Campbell, 1991; Kanagawa et al., 2005). The oligosaccharide linkages are mannose linked to a serine or threonine in the mucin domain of α-dystroglycan, followed by *N*-acetylglucosamine (GlcNAc) linked via a β1,2 linkage to the *O*-linked mannose, followed by a galactose linked via β1,4 linkage to the GlcNAc, and finally capped by an α2,3-linked *N*-acetylneuraminic acid (Neu5Ac or NANA).

In addition to the common feature of muscular dystrophy with neonatal onset, disorders in this group also have CNS and eye involvement. The CNS pattern varies, but typically demonstrates abnormal migration, and at the severe end of the spectrum has type II, or "cobblestone," lissencephaly. Eye involvement ranges from myopia to micro-ophthalmia. At the milder end of the spectrum, LGMD may be the only manifestation, without brain or eye involvement. As a group these disorders are referred to as dystroglycanopathies.

CLINICAL DYSTROGLYCANOPATHY SYNDROMES

Walker–Warburg syndrome (WWS)

Walker–Warburg syndrome (WWS) was initially described by Walker in 1942, and further characterized by Warburg in 1978. It is an extremely severe condition, with a life expectancy of less than 3 years. Signs of WWS are already present at birth, and imaging techniques can detect features such as encephaloceles and

severe hydrocephalus prenatally. There is profound weakness and generalized hypotonia at birth. Muscle bulk is reduced and contractures develop soon after birth, although they may take a few months to develop.

Brain abnormalities include migration defects with type II lissencephaly/agyria ("cobblestone type"), combined with pontocerebellar hypoplasia (Dobyns et al., 1989). Obstructive hydrocephalus may complicate the clinical picture. White matter shows hypomyelination. Additional structural abnormalities include hypoplasia/agenesis of the corpus callosum, encephalocele, and Dandy–Walker malformations (Vajsar and Schachter, 2006). Seizures have been described.

Eye abnormalities can include microcornea and/or micro-ophthalmia, either unilateral or bilateral, hypoplastic or absent optic nerves, and colobomas, which may involve the retina. Retinal dysplasia or detachment may be present. Other anterior chamber malformations include cataracts, iris hypoplasia or malformation, and abnormal or shallow anterior chamber angle, which can result in glaucoma.

Additional features include male genital anomalies such as small penis and undescended testes and severe feeding difficulties that likely require tub or gastrostomy feeding. Rarely there can be mild facial dysmorphisms such as low-set or prominent ears and cleft lip or palate (Vajsar and Schachter, 2006). Mild renal dysplasia and imperforate anus have been reported (Dobyns et al., 1989).

Laboratory/radiology features

CK levels are raised. Brain MRI demonstrates the "cobblestone type" of lissencephaly, often with ventriculomegaly. There are hindbrain malformations with atrophy of the cerebellum vermis and hemispheres, and a flattened aspect of the pons and brainstem. Arachnoid cysts are common, especially in the posterior fossa. Muscle pathology demonstrates myopathic or dystrophic changes, and immunohistochemistry of muscle and peripheral nerve with the glycosylated form of α-dystroglycan is absent or severely reduced (Beltran-Valero de Bernabe et al., 2002; Muntoni et al., 2003). Laminin α2 concentration may be reduced, but perlecan and collagen VI levels are normal (Jimenez-Mallebrera et al., 2003).

Molecular pathogenesis/genetics

There is genetic heterogeneity in WWS. Mutations have been demonstrated in the *O*-mannosyltransferase 1 (*POMT1*) gene (Beltran-Valero de Bernabe et al., 2002; Kim et al., 2004; van Reeuwijk et al., 2006), the *O*-mannosyltransferase 2 (*POMT2*) gene (van Reeuwijk et al., 2005), the *fukutin* gene (Beltran-Valero de Bernabe et al., 2003), the *fukutin*-related protein (*FKRP*) gene (Beltran-Valero de Bernabe et al., 2004), and most recently in *LARGE* (van Reeuwijk et al., 2007). These genes are discussed in detail below. Abnormal α-dystroglycan expression has been documented in patients with WWS, with up to 90% of cases having no molecular diagnosis (Jimenez-Mallebrera et al., 2003; Vajsar and Schachter, 2006).

Muscle–eye–brain disease (MEB)

Muscle–eye–brain disease (MEB) was initially described in 1977 by Santavuori et al., with the combination of congenital muscular dystrophy, intellectual impairment, and eye and structural brain involvement. There is an increased prevalence of MEB in Finland, but the condition has been demonstrated in persons from all ethnic backgrounds (Santavuori et al., 1989; Taniguchi et al., 2003). The severity of MEB varies from a typical neonatal presentation to a milder presentation with seizures and autism (Haliloglu et al., 2004). The typical presentation of MEB is neonatal onset of profound muscle hypotonia and poor visual alertness. The muscle weakness is generalized and includes the facial and neck muscles. Patients at this severe end never achieve sitting and may die during the first years of life. Moderately affected patients usually show high myopia, but have some preserved vision enabling them to establish contact. Their maximum motor ability is to sit unsupported, and occasionally to speak a few words. Muscle enlargement may be present. Patients at the milder end of the spectrum may acquire ambulation for a number of years. Often their functional abilities are more impaired by the coexistence of spasticity and ataxia than by muscle weakness.

Typical eye features include high myopia, optic disc pallor, retinal dysplasia, persistent hyperplastic primary vitreous, glaucoma, and cataracts. Later, the progressive high myopia may lead to retinal detachment. Visual evoked potentials demonstrate high amplitudes and delay. Electroretinography shows abnormalities and progression (Pihko et al., 1995).

CNS findings include structural brain abnormalities on brain MRI including neuronal migration defects and type II "cobblestone" lissencephaly similar to that of WWS (Haltia et al., 1997). Other features include partial absence of the corpus callosum, hypoplasia of the pyramidal tracts, and obstructive hydrocephalus requiring a shunt. Patients with milder MEB may have only flattening of the brainstem, hypoplasia of the cerebellar vermis, and cerebellar cysts (Muntoni and Voit, 2004). Intellectual impairment is present in the typical and moderated patients with MEB. Seizures and myoclonic jerks may also be present.

Laboratory/radiology features

There is a consistent increase in CK levels. EMG shows myopathic potentials, although nerve conduction velocities are normal. Muscle biopsies demonstrate signs of dystrophic muscle. There is abnormal glycosylation of α-dystroglycan on immunohistochemistry of muscle from patients with MEB (Kano et al., 2002). Laminin α2 binding is also lost in MEB muscle, but expression of the core α-dystroglycan is preserved (Michele et al., 2002).

Molecular pathogenesis/genetics

Linkage analysis was used to localize the gene responsible for MEB to chromosome 1p32–34 (Cormand et al., 1999) and subsequently mutations in the glycosyltransferase *O*-mannose β-1,2-*N*-acetylglucosaminyltransferase (*POMGnT1*) were shown to cause MEB (Yoshida et al., 2001). See below for more detail on *POMGnT1*.

Animal model

A genetic model of MEB was created in mice by loss of *POMGnT1* through gene trapping (Liu et al., 2006). These mice are viable, but smaller than their littermates, in the first few days of life. Most of the mutant mice survive to adulthood (2 months) and several have survived to more than 20 months prior to being killed. The mice exhibit muscle weakness, CNS abnormalities (small cerebellum and thinner cerebral cortex, and migration defects), and eye findings (abnormal findings on electroretinography, small optic nerves, and reduced density of ganglion cells in the retina), all similar to patients with MEB. Glycosylation of α-dystroglycan was disrupted as determined by western blot, laminin overlay studies, and immunohistochemistry in skeletal muscle. Further studies with these mice have demonstrated that the defects in cortical lamination are the result of disappearance of the basement membrane and the glia limitans at the cerebral cortical surface during development (Hu et al., 2007).

Fukuyama congenital muscular dystrophy (FCMD)

Fukuyama congenital muscular dystrophy (FCMD) was initially described in Japan in 1960 (Fukuyama et al., 1960). It is the second most common form of muscular dystrophy in Japan after DMD. Clinically, there is a spectrum of severity ranging from severe, to typical presentation, to mild. In a group of 56 Japanese families with FCMD, 35 families had the typical presentation, 12 had a mild presentation, and 9 a severe presentation (Saito et al., 2000).

Classically, patients with FCMD present with neonatal onset of generalized muscle weakness, severe brain involvement, frequent seizures, intellectual impairment, and abnormal eye function. Symptoms may begin *in utero* with poor fetal movement. Asphyxia at birth is not uncommon. There may be functional improvement, and most patients achieve standing with support and occasional ambulation with support. Muscle enlargement is common. There is a myopathic facial appearance. Involvement of respiratory muscles is progressive, and respiratory failure in the middle–late teens is an invariable complication. Life expectancy averages about 15 years, although survival into the mid 20s is possible (Osawa et al., 1997). There are progressive contractures of the hips, ankles, and knees. There is loss of independent sitting after 9 years of age, and scoliosis commonly develops. At the mild end of the spectrum patients can walk or stand with support, and at the severe end of the spectrum head control and ability to sit without support are never achieved (Saito et al., 2000).

Brain abnormalities are similar to those of WWS and MEB, with migration defects, cobblestone lissencephaly, and hindbrain malformations. Cognitive ability ranges from profound intellectual impairment to mild–moderate, where the patients learn to speak in short sentences and may even be able to read and write a few characters. Most patients develop seizures before 3 years of age. Neurofibrillary tangles have been reported in the locus ceruleus, basal nucleus of Meynert (Takada et al., 1986), and the hippocampus of patients with FCMD (Oka et al., 1999). In addition, a unique, 50-kDa form of tau was demonstrated in the brains of patients with FCMD, in addition to the common forms of tau ranging from 60 to 68 kDa, as is seen in Alzheimer disease (Saito et al., 2005).

Cardiac involvement is almost invariable and typically develops in the second decade of life. Of 34 patients with FCMD, echocardiography revealed increased left ventricular end-diastolic dimension in 2 patients and left ventricular fractional shortening in 16. This shortening was not observed in patients less than 10 years old, but was seen in most patients over 15 years of age. No patient demonstrated abnormalities on electrocardiography. One patient had tetralogy of Fallot. Five patients in the study died from heart failure or respiratory problems, and myocardial fibrosis was noted on pathological examination (Nakanishi et al., 2006).

About 50% of classical FCMD cases show signs of ocular involvement, ranging from abnormal eye movements, poor visual pursuit, and strabismus to severe myopia, hyperopia, or cataracts. At the more severe end of the spectrum, however, there can be retinal detachment and micro-ophthalmos (Osawa et al., 1997).

Laboratory/radiology features

Serum CK levels are raised. They are 10–60 times higher than normal in children under 6 years of age, and 5–20 times higher than normal in older children. The levels may be normal in bedridden individuals. Brain MRI demonstrates pachygyria in the cerebral cortex and increased white matter signal on T_2-weighted images, due to delayed myelination (Fukuyama et al., 1981; Osawa et al., 1997). There may be hypoplasia of the pons, and cerebellar cysts (Aida et al., 1994). Muscle histology demonstrates a dystrophic process. Immunohistochemistry of skeletal muscle (Hayashi et al., 2001a) and neurons (Saito et al., 2006) from patients with FCMD shows deficiency of the glyosylated form of α-dystroglycan.

Molecular pathogenesis/genetics

FCMD is caused by mutations of the *fukutin* gene on chromosome 9q31 (Toda et al., 1993, 1996; Kobayashi et al., 1998).

Animal model

There is no reported, naturally occurring mouse model of *fukutin* deficiency (Toda et al., 2003). Targeted homozygous deletion of *fukutin* is embryonic lethal at day 6.5–7.5 (Horie et al., 2002). Chimeric mice have been generated using embryonic stem cells. Those mice that have a high number of *fukutin*-deficient cells develop a severe muscular dystrophy, neuronal migration defects, and abnormalities of the eye (Takeda et al., 2003). Further studies using these mice have demonstrated cortical defects and distortion of the laminar organization as early as embryonic day 14 (Chiyonobu et al., 2005).

Congenital muscular dystrophy with muscle hypertrophy (MDC1C)

The typical form of MDC1C presents as a combination of weakness and maximal functional achievements similar to those observed in MDC1A (Mercuri et al., 2000). MDC1C is characterized by secondary laminin α2 chain deficiency, but brain imaging and intelligence are normal. Weakness and hypotonia are noted at birth, or in the first few months of life without arthrogryposis, followed by marked delay of motor milestones. The maximum motor achievement is to sit or to take a few steps with support in the first decade of life. Progressive respiratory muscle weakness leads to ventilatory insufficiency in the first or second decade of life. Other characteristic features include marked enlargement of the leg muscles, sometimes followed by striking tongue hypertrophy. Wasting and weakness of the shoulder muscles and facial weakness are common. Cardiac involvement is present in the form of a dilated cardiomyopathy.

The spectrum of MDC1C has expanded to include structural brain involvement. This includes patients with mild intellectual impairment and structural changes of the cerebellum with cerebellar cysts but normal brainstem and eye examinations (Topaloglu et al., 2003). A number of similar cases have also been described from Tunisia and Algeria (Driss et al., 2003; Louhichi et al., 2004).

Laboratory/radiology features

CK levels are greatly increased, 20–75-fold (1000–10 000 IU/l). Routine histological analysis of muscle biopsies does not differentiate these patients from other patients with CMD. Brain MRI findings in MDC1C range from normal, to isolated cerebellar cysts, to cerebellar cysts associated with structural brain changes. These changes involved the posterior fossa and the cortex. In one patient there was focal nodular heterotopia and in another cerebellar dysplasia and pons hypoplasia (Mercuri et al., 2006). Signs of cortical or subcortical atrophy and focal and diffuse white matter changes have been observed in some patients with MDC1C (Quijano-Roy et al., 2006).

Brief, repetitive discharges provoked by electrical nerve stimulation were observed in the muscle from one patient with MDC1C, but not in three others tested (Quijano-Roy et al., 2004). Nerve conduction studies were normal in these four patients, even in examinations performed up to 12 years of age.

Molecular pathogenesis/genetics

MDC1C is caused by mutations in the *fukutin-related protein*, or *FKRP*, gene (see below).

Congenital muscular dystrophy with severe mental retardation and abnormal glycosylation (MDC1D)

To date, only a single patient with MDC1D has been reported. She was a 17-year-old girl who presented at 5 months of age with congenital onset of muscular dystrophy, profound intellectual impairment, white matter changes, and subtle structural abnormalities on brain MRI. Her maximal motor ability was walking 200 yards and jumping at 9 years of age, but thereafter a gradual decline in function was observed, and at 17 years she was able to walk only a few steps independently. There was muscle hypertrophy of the quadriceps, calf, and arm muscles. Contractures at the ankles and elbows were mild. Hearing and vision were normal, and there was no cardiac involvement (Longman et al., 2003).

Laboratory/radiology features

Serum CK levels were increased, ranging between 467 and 4500 IU/l. Peripheral motor and sensory nerve conduction studies were normal and the EMG was myopathic. A skeletal muscle biopsy showed reduced immunolabeling of glycosylated α-dystroglycan, and immunoblotting demonstrated a reduced molecular weight form of α-dystroglycan. The laminin binding activity was retained (Longman et al., 2003).

Brain MRI at 14 years of age demonstrated abnormal white matter changes, mostly in the periventricular region to the arcuate fibers, particularly anteriorly and in the temporal regions. The brainstem was hypoplastic. There was mild pachygyria with the cortex in the frontal lobes being moderately thick and dysplastic, and in the posterior frontal, temporal, and parietal regions the gyri were mildly simplified with shallow sulci (Longman et al., 2003).

Molecular pathogenesis/genetics

MDC1D is caused by a mutation in *LARGE* (see below; Longman et al., 2003).

Animal model

The myodystrophy (*myd*, now renamed *Large*^{myd}) mouse carries a loss of function mutation in the *LARGE* gene (Grewal et al., 2001). Homozygous *Large*^{myd} mice display a severe, progressive muscular dystrophy (Lane et al., 1976; Mathews et al., 1995) and a mild cardiomyopathy in addition to ocular, peripheral, and central nervous system involvement (Holzfeind et al., 2002). Abnormalities in neuronal migration are observed in the brain, particularly the cortex and cerebellum (Qu and Smith, 2005). A profound loss of muscle α-dystroglycan has also been observed (Grewal et al., 2001; Michele et al., 2002). The muscular dystrophy in *Large*^{myd} mice is caused by changes in the interactions of α-dystroglycan and the distribution of proteins in the sarcolemma (Reed et al., 2004). In addition, it is the interaction of α-dystroglycan and its ligands in the pontine nuclei that is required for correct neuronal migration (Qu et al., 2006). Another mouse model with a mutation in *LARGE* has been described: *Large*^{vls} (Lee et al., 2005). In addition to muscular dystrophy, these mice also have retinal defects.

Management

As with all of the CMDs, treatment for the dystroglycanopathies is mainly supportive (see MDC1A). Anticonvulsants are used if seizures are present. Neurosurgical management of the hydrocephalus with shunting or encephaloceles may be required.

Interestingly, the three patients with LGMD2L all responded to oral corticosteroids (prednisolone or prednisone) with increased endurance, increased strength, and decreased frequency of falls. However, when the steroids were discontinued there was rapid decline in muscle strength and endurance, which again responded with improvement when rechallenged (Godfrey et al., 2006).

GENES INVOLVED IN THE DYSTROGLYCANOPATHIES (TABLE 4.3)

POMT1 and *POMT2*

POMT1 catalyzes the first step in *O*-mannosyl glycan synthesis, with the attachment of a mannose via an *O*-glycosyl linkage to the Ser/Thr of the protein (Willer et al., 2003). *POMT2* is a second *O*-mannosyltransferase, which complexes with *POMT1* for the *O*-mannosyltransferase activity (Manya et al., 2004; Akasaka-Manya et al., 2006). Mutations in *POMT1* and *POMT2* cause WWS. Mutations in *POMT1* have also been described in patients with a milder phenotype consisting of congenital muscular dystrophy, calf

Table 4.3

Molecular etiology of dystroglycanopathies

Gene	Protein	Phenotype
POMT1	Protein-*O*-mannosyltransferase 1	WWS, LGMD2K
POMT2	Protein-*O*-mannosyltransferase 2	WWS
POMGnT1	*O*-linked mannose β1,2-*N*-acetylglucosaminyltransferase	MEB
Fukutin	*Fukutin*	WWS, FCMD, LGMD2L
FKRP	*Fukutin*-related protein	MDC1C, LGMD2I
LARGE	Large	WWS, MDC1D

FCMD, Fukuyama congenital muscular dystrophy (CMD); LGMD, limb-girdle muscular dystrophy; MDC1C, CMD type 1C (CMD with muscle hypertrophy/*FKRP*-deficient CMD); MDC1D, CMD type 1D (CMD with severe intellectual impairment and abnormal glycosylation/*LARGE*-deficient CMD); MEB, muscle–eye–brain disease; WWS, Walker–Warburg syndrome.

hypertrophy, microcephaly, and intellectual impairment (Van Reeuwijk et al., 2006), and in LGMD2K (Balci et al., 2005). Mutations in *POMT2* have been shown to cause a milder phenotype, more consistent with MEB, as well as in patients with CMD and microcephaly, severe intellectual impairment, with or without ocular involvement (Yanagisawa et al., 2007).

It has been shown that *POMT1* gene expression is highest in the muscle, eye, and brain in the developing embryo, and later in development expression is maintained in these tissues. In the brain, the strongest expression is seen in those regions that are most affected in WWS (Prados et al., 2007).

POMGnT1

Linkage analysis was used to localize the gene responsible for MEB to chromosome 1p32–34 (Cormand et al., 1999) and subsequently mutations in the glycosyltransferase *O*-mannose β-1,2-*N*-acetylglucosaminyltransferase (*POMGnT1*) were shown to cause MEB (Yoshida et al., 2001). *POMGnT1* has 22 exons, with the coding region beginning in exon 2 (Yoshida et al., 2001). Mutations are located throughout the gene and include a combination of missense, nonsense, and frameshift mutations (Yoshida et al., 2001; Taniguchi et al., 2003; Vervoort et al., 2004). There has been some genotype–phenotype correlation, with a more severe CNS phenotype of the patients carrying mutations towards the 5′ end of the gene compared to those located towards the 3′ end (Taniguchi et al., 2003). However, in one family with two siblings carrying the same mutation, one had typical features of MEB but her sibling was much more severe, with some features suggestive of WWS (Teber et al., 2008). The *POMGnT1* gene encodes a 660-amino-acid type II transmembrane protein. It is expressed constitutively, although the spinal cord, lymph node, and trachea have an additional transcript that is produced either by an alternative transcriptional start site or alternative splicing in these tissues (Yoshida et al., 2001). *POMGnT1* catalyzes the transfer of *N*-acetylglucosamine to the *O*-linked mannose of glycoproteins including dystroglycan. Reduction of *POMGnT1* activity in skeletal muscle of patients with MEB has been demonstrated (Yoshida et al., 2001; Manya et al., 2003; Zhang et al., 2003).

Fukutin

FCMD is caused by mutations of the *fukutin* gene on chromosome 9q31 (Toda et al., 1993, 1996; Kobayashi et al., 1998). The *fukutin* gene spans more than 100 kb of genomic DNA and is composed of 10 exons (Kobayashi et al., 2001). Its protein product, *fukutin*, is a 461-amino-acid, 53.7-kDa transmembrane protein with sequence homology to a bacterial glycosyltransferase, but its precise function is unknown (Toda et al., 2003). A recent report has shown there is colocalization and molecular interaction of *fukutin* and *POMGnT1*, suggesting that *fukutin* may form a complex with *POMGnT1* and modulate its enzymatic activity (Xiong et al., 2006).

A retrotransposal insertion into the 3′ untranslated region of *fukutin* mRNA accounts for 87% of FCMD chromosomes and is considered to be a relatively mild mutation as it only partially reduces the stability of the full-length mRNA. This mutation can be identified using a polymerase chain reaction-based method (Kato et al., 2004). Carrier frequency in Japan is 1 in 90 (Toda et al., 2000). Combined heterozygotes between this mutation and deletions or nonsense mutations have a more severe phenotype than individuals homozygous for the retrotransposal insertion (Saito et al., 2000). Although targeted inactivating mutations of both alleles in the mouse are not compatible with life, two patients with functional null mutations in a homozygous state were identified recently. Interestingly, they both had a more severe WWS-like phenotype, indicating that complete loss of *fukutin* function is compatible with life in the human (Beltran-Valero de Bernabe et al., 2003; Silan et al., 2003). *Fukutin* mutations have also been reported in patients with LGMD phenotype LGMD2L (Godfrey et al., 2006). Some of these patients presented in the first months of life with a CMD phenotype. Two of these reported patients (Figure 4.3) are siblings and have been followed by one of the authors (D.M.E.) for 10 years. They have benefited significantly from prednisone treatment, started at 12 and 18 months. The younger sibling diagnosed at 3 months became ambulatory; both patients remain steroid dependent.

A study in Japan noted that patients who were heterozygous for the founder mutation had seizures earlier than those who were homozygous for the founder mutation. In addition, some heterozygotes demonstrated intractable seizures (Yoshioka et al., 2008).

FKRP

MDC1C is caused by mutations in the *fukutin-related protein*, or *FKRP*, gene. The *FKRP* gene consists of four exons, one of which encodes a 495-amino-acid protein that, like *fukutin*, is targeted to the medial Golgi apparatus (Torelli et al., 2005). Sequence homology suggests *FKRP* is a member of the glycosyltransferase family (Brockington et al., 2001). Mutational analysis in patients with MDC1C revealed either two missense mutations or a missense mutation combined with a null mutation (Poppe et al., 2003). To date, no patient has been reported with two null *FKRP* alleles and perhaps this would not be compatible with life.

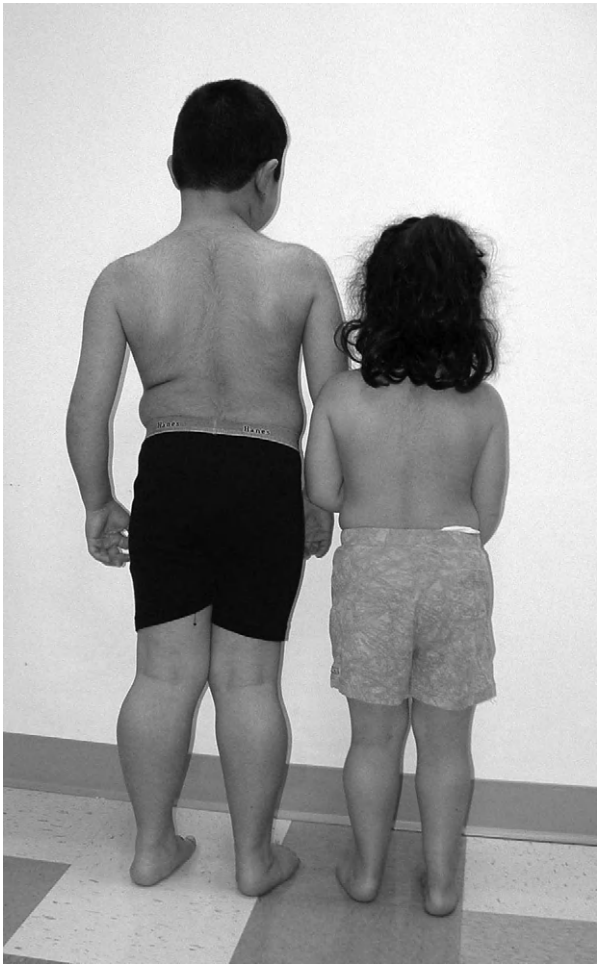

Figure 4.3. Siblings with limb-girdle muscular dystrophy type 2L, presenting in the first year of life, treated with steroids. Note calf hypertrophy in both children.

Mutations in *FKRP* also cause the milder allelic condition of LGMD2I. The most common mutation in *FKRP* is the 826C>A (Leu276Ile) mutation which is associated with LGMD2I and has not been seen in MDC1C (Poppe et al., 2003). This common mutation has been calculated to occur at a heterozygous frequency of 1 in 400 in the UK (Poppe et al., 2003). The second allelic mutation in patients with the Leu276Ile change determines the severity of the LGMD2I phenotype, although the degree of intrafamilial clinical variability suggests that additional factors may also play a significant role (Mercuri et al., 2003). Patients with LGMD2I may present in childhood, adolescence, or adult life and can often maintain the ability to walk for life. Intelligence and brain MRI are normal. Patients with LGMD2I may be divided into a DMD-like group with early onset and loss of independent ambulation in the teens, and a milder group with later onset and preserved ambulation after the second decade (Mercuri et al., 2003). Dilated cardiomyopathy has been seen in more than half of patients with LGMD2I (Poppe et al., 2003), and respiratory failure after the age of 16 years has been described in a third of the patients with a DMD-like phenotype (Muntoni and Voit, 2004).

From a biochemical point of view, α-dystroglycan glycosylation is abnormal in all patients with *FKRP* mutations. There is typically a clear correlation between the residual expression of glycosylated α-dystroglycan and the phenotype. Patients with MDC1C display a profound depletion of glycosylated α-dystroglycan. Patients with LGMD2I and a DMD-like phenotype have a moderate reduction in glycosylated α-dystroglycan, whereas individuals with the milder form of LGMD2I show a variable but subtle alteration in glycosylated α-dystroglycan immunolabeling (Brown et al., 2004). In addition, patients with mutations in *FKRP* also demonstrate decreased immunolabeling in muscle with β-dystroglycan and α-, β-, and γ-sarcoglycan antibodies, suggesting that *FKRP* mutations can alter membrane-associated proteins beyond α-dystroglycan (MacLeod et al., 2007).

One patient with MEB has been described with a mutation in the *FKRP* gene (described below; Beltran-Valero de Bernabe et al., 2004). This patient had CMD, anterior chamber abnormalities, and characteristic brain abnormalities including cobblestone lissencephaly.

There are two surprising features associated with *FKRP* gene alterations. The first is that *FKRP* mutations are very common, especially among Caucasians, with a heterozygous frequency of 1 in 400 (Poppe et al., 2003). The second is the large range of severity of phenotype associated with mutations in a single gene, *FKRP*. This ranges from an *in utero* onset of muscular dystrophy with a WWS phenotype to mild LGMD variants with muscular dystrophy onset in adulthood. *FKRP* localizes to the Golgi apparatus. Mutations in *FKRP* lead to retention of the altered protein in the endoplasmic reticulum and decreased localization in the Golgi. Therefore, individual mutations can confer at least two independent effects on the protein, including reduction or loss of enzyme activity directly and mislocalization that could further alter the function of the protein. This complexity of the effect of individual *FKRP* mutations may partly explain the wide variation of the *FKRP*-related myopathies (Keramaris-Vrantsis et al., 2007).

LARGE and *LARGE2*

The *LARGE* gene is the fifth largest gene in the human genome, spanning 664 kb of genomic DNA on chromosome 22q12.3–q13.1, and has homology to the glycosyltransferase gene family (Peyrard et al., 1999). The predicted protein contains an N-terminal cytoplasmic domain, a transmembrane region, and two putative

catalytic domains (Grewal et al., 2001). The proximal catalytic domain has close homology to Waaj, a bacterial family GT8 glycosyltransferase (Coutinho et al., 2003) involved in lipo-oligosaccharide synthesis (Heinrichs et al., 1998). The distal catalytic domain demonstrates homology with the human UDP-GlcNAc:Galβ1,3-*N*-acetylglucosaminyltransferase (Coutinho et al., 2003). There is a second gene, *LARGE2*, with close homology to *LARGE* (Brockington et al., 2005; Fujimura et al., 2005). Mutations in *LARGE* were originally described in MDC1D. More recently, a large intragenic deletion was demonstrated in the *LARGE* gene in a patient with severe CMD, more consistent with WWS (van Reeuwijk et al., 2007).

Active research

Overexpression of *LARGE* in cell cultures derived from patients with FCMD, MEB, and WWS can correct the glycosylation defect of α-dystroglycan and restore its interaction with laminin, agrin, and neurexin (Barresi et al., 2004). Therefore, modulation of *LARGE* expression *in vivo* may be a therapeutic option in patients with the defects in glycosylation of α-dystroglycan.

OTHER DYSTROGLYCANOPATHIES

CMD with pseudohypertrophy, macroglossia, and respiratory insufficiency

Clinical features

Quijano-Roy et al. (2002) described four unrelated patients with progressive CMD. Two of the patients had a milder phenotype initially and were able to walk for a few years before the disorder progressed, but the other two had a severe condition from infancy and were never ambulatory. In the advanced stages of the condition, all four patients were tetraplegic, mechanically ventilated, had significant macroglossia, and wasting of the distal musculature of the hands and feet. Weakness affected axial and proximal muscles first, followed by facial and distal musculature. Distal contractures developed in all patients. There was calf hypertrophy in all four patients, with one patient demonstrating a generalized pseudohypertrophy. Due to the hypotonia, all four patients developed spinal deformities requiring bracing.

Developmental milestones were delayed in all four patients. In the two patients with milder disease, walking was obtained at 17 and 36 months. Sitting was the maximal motor milestone achieved in the two patients with severe disease, acquired at 12 months and 5 years, and was lost later, within 3–6 years.

Cardiac function was abnormal in two patients. All patients had involvement of diaphragmatic muscles along with respiratory insufficiency. Tracheotomy and mechanical ventilation was required in the second decade in three patients. The parents of the fourth patient refused tracheotomy and the patient died at 10 years of age, secondary to respiratory complications. There were no seizures in any of the patients.

Laboratory/radiology features

Serum CK levels were increased by 10–30-fold early in the disease, but normalized over time. Brain MRI was normal in one patient and showed mild atrophy in the other three. There was no evidence of migration or structural defects.

Muscle biopsies of all four patients demonstrated increased variation in fiber size, mildly increased frequency of centralized nuclei, significant increase in interstitial connective tissue, and a predominance of type 1 fibers. Regenerating and necrotic fibers were present. The two patients who had later muscle biopsies demonstrated progression with complete replacement of the muscle with fibrofatty tissue.

Immunolabeling with dystrophin, α-, β-, and γ-sarcoglycans, β-dystroglycan, and laminins β1 and γ1 were normal in all four patients. Immunolabeling of the 80-kDa portion of laminin α2 was reduced in all four patients, whereas laminin α5 was upregulated. In two of the patients, immunolabeling of the 300-kDa portion of laminin α2 was performed; in one patient it was significantly reduced and in the other it was absent. In the two patients where α-dystroglycan immunohistochemistry was done, there was no expression. This was likely the result of a glycosylation defect that has not yet been determined.

Molecular pathogenesis/genetics

Polymorphic markers for the *LAMA2*, *FCMD*, *MEB*, and *MDC1B* loci were evaluated in all four patients. These four loci were excluded in the only consanguineous family based on the absence of homozygosity. In fact, the patient shared identical haplotypes at the *FCMD* and *MEB* loci as his unaffected sibling. In one patient, the entire *fukutin* gene was sequenced and no mutations were found.

PROTEINS OF THE ENDOPLASMIC RETICULUM AND NUCLEUS

Rigid spine syndrome

Clinical features

The clinical features of rigid spine syndrome (RSS) were first described in 1973 by Dubowitz. RSS most commonly presents in the first year of life with axial

hypotonia and weakness, but there is only mild delay in achieving motor milestones and no significant contractures. Motor difficulties may occur because of a combination of mild/moderate proximal muscle weakness, mild Achilles tendon tightness, and rigidity of the spine. However, it is rare that patients do not achieve independent ambulation. Ambulation is usually preserved into adulthood unless a severe progressive scoliosis that cannot be treated surgically develops. The overall muscle bulk is reduced, especially the medial aspects of the thighs. The most prominent clinical feature is spinal rigidity and scoliosis due to contractures of the spine extensor muscles which typically develops between 3 and 12 years of age. In typical cases this will be in the form of a lumbar lordoscoliosis, with a pelvic tilt and cervical spine stiffness. If present, stabilization by thoracolumbar orthosis and early surgical fixation of the spine may be helpful. Contractures usually are mild and affect the ankles, and only occasionally the temporomandibular joint, with limitation of mouth opening, or also the finger extensors. An additional feature of these patients is palatal weakness, which manifests as nasal speech.

There is respiratory compromise in most individuals with RSS. Vital capacity is low due to stiffness of the rib cage, and decreases over time. This is aggravated by diaphragmatic weakness which leads to respiratory failure (Morita et al., 1990). There may be a discrepancy between the overall functional abilities of affected patients and the compromise of lung function. Many patients require ventilatory assistance already in the first decade, some as early as at 2 years of age. On the other hand, if noninvasive ventilation is instituted early enough, an active life with good quality and reasonable muscle function can be maintained over many years (Yang et al., 2002).

Cardiac function may be compromised secondary to respiratory involvement (Mercuri et al., 2002a). Other cardiac manifestations including bradycardia, dilated cardiomegaly, and complete conduction block have been described in a 32-year-old man with RSS (Takase et al., 1990), and incomplete left bundle branch block was described in a 12-year-old boy with RSS (Niamane et al., 1999). Because of these cardiac manifestations, RSS can be confused with Emery–Dreifuss muscular dystrophy (EDMD) in adults. RSS was reported to present as cor pulmonale in one patient (Venance et al., 2005).

The differential diagnosis of RSS includes nemalin myopathy, EDMD, and pituitary dwarfism III. Nemalin myopathy involves bulbar, neck lexor, and respiratory muscles. The face is myopathic with a high arched palate. Chest deformities are common, and hyperlordotic spine with rigidity is seen. Nemalin myopathy is caused by mutations in the *nebulin* gene. It differs from RSS in that the muscle biopsy demonstrates nemaline bodies and type 1 predominance and myopathic changes without signs of dystrophy or inflammation (Wallgren-Pettersson et al., 1999). X-linked EDMD is caused by mutations in the *emerin* gene and is characterized by muscle weakness beginning at the age of 4–5 years, contractures, and cardiac involvement, and may also include rigidity of the spine (Dubowitz, 1973; Wettstein et al., 1983). Pituitary dwarfism III is caused by mutations in the *LHX3* gene and demonstrates a combination of pituitary hormone deficiency and a rigid cervical spine (Netchine et al., 2000).

Laboratory/radiology features

CK levels are normal or mildly raised. EMG demonstrates a myopathic pattern. A specific pattern of skeletal muscle involvement on muscle imaging (computed tomography or MRI) has been described. This includes selective involvement, with adductors, sartorius, and biceps femoris more markedly involved and rectus femoris and gracilis relatively spared (Flanigan et al., 2000; Mercuri et al., 2002a). This pattern of involvement helps distinguish RSS from other muscular dystrophies such as UCMD, EDMD, and central core myopathy (Mercuri et al., 2002a).

Muscle specimens show evidence of myopathy with increased variation of fiber size and some increase in endomysial fibrosis. Early on there is usually no or very little necrosis and also little regeneration. Fiber type disproportion has been reported (Unal et al., 1999). Biopsies of severely affected patients or from paraspinal muscles may show overt dystrophic changes with a strong increase of endomysial and perimysial fibrosis, and some increase of internal nuclei, but frank necrosis and regeneration are still not prominent features (Ferreiro et al., 2002). Staining for laminin $\alpha 2$ and collagen VI is normal.

Molecular pathogenesis/genetics

Using consanguineous families from Morocco, Iran, and Turkey, one locus for RSS was linked to chromosome 1p35–36, and termed *RSMD1* (Moghadaszadeh et al., 1998). Further studies identified the selenoprotein N gene, *SEPN1*, as the cause of RSMD1 (Moghadaszadeh et al., 2001). *SEPN1* contains 13 exons spanning 18.5 kb of genomic sequence encoding a 590-amino-acid protein (Moghadaszadeh et al., 2001). Selenoprotein N is a 70-kDa endoplasmic reticulum glycoprotein containing a single selenocysteine residue (Petit et al., 2003). Frameshift, nonsense, and missense mutations in *SEPN1* have been described (Moghadaszadeh et al., 2001; Okamoto et al., 2006). In a study of nine families with RSS, there

was linkage to chromosome 1p35–36 in only one family; thus there is genetic heterogeneity and other genes may be involved in RSS (Moghadaszadeh et al., 1999).

Management

Like other CMDs, the mainstay of management is primarily supportive. This includes early intervention with noninvasive nocturnal ventilatory support. Treatment of the scoliosis includes physical therapy and bracing. Surgical correction may be indicated (Arkader et al., 2005). However, owing to the neck contractures which can make intubation difficult, and the cardiac and respiratory complications of RSS, anesthesia risks are not trivial (Jorgensen et al., 1999). Botulinum toxin therapy has been tried in the neck extensors, particularly the trapezius, sternocleidomastoid, and paracervical muscles, to help with the imbalance between the neck flexor and extensor muscles that leads to fixed neck extension, with success in one patient (Sastre-Garriga et al., 2001).

LMNA-related myopathy

Clinical features

Clinically, patients show progressive hypotonia and weakness predominantly both proximally in the upper limbs and distally in the lower limbs. There is thoracic spine rigidity with cervical and dorsal lordoscoliosis. Initially there are contractures of the distal joints, with lower limbs (ankles) affected more than upper limbs (fingers and wrists). This is followed by development of severe contractures of the more proximal joints, also first in lower limbs (knees, hips). Elbow contractures, typical of EDMD, are not observed, or are seen only very late in the course of the disease. The disease follows a progressive course and the restrictive respiratory insufficiency is a major complication, observed earlier in patients with absence of milestones, who need mechanical ventilation from the first 2 years of life (Quijano-Roy et al., 2008). In the course of the disease, some of the clinical findings resemble EDMD, with scapulohumeral and peroneal distribution of amyotrophy and weakness, and spinal stiffness (Emery, 1989), but the absence of elbow contractures, the severe axial hypotonia, and the progressive course with severe respiratory insufficiency are distinctive and characterize this clinically recognizable CMD subtype.

Laboratory/radiology features

CK levels are increased, but usually not more than 5-fold over normal values. Muscle may show dystrophic or nonspecific myopathic features (Mercuri et al., 2004, 2005a; D'Amico et al., 2005; Quijano-Roy et al., 2008). Markedly atrophic fibers are common, most often type 1, and a few patients showed positive inflammatory markers (Quijano-Roy et al., 2008).

Molecular pathogenesis/genetics

LMNA encodes the nuclear envelope proteins lamin A and lamin C. Mutations in *LMNA* are responsible for a wide variety of clinical disorders, including the autosomal form of EDMD, LGMD type 1B, cardiomyopathy, inherited lipodystrophies, and Hutchinson–Gilford progeria (see Broers et al., 2006, for a review). Mutations in the *LMNA* gene have been identified in a group of children with CMD either with a very severe neonatal hypotonia without any motor acquisition (Mercuri et al., 2004, 2005a) or with acquisition of sitting and even walking ability and ultimate loss of head control leading to a dropped-head syndrome (D'Amico et al., 2005; Quijano-Roy et al., 2008).

Management

Like other CMDs, the mainstay of management is primarily supportive. Progressive respiratory compromise results in the greatest morbidity and mortality (Quijano-Roy et al., 2008).

OTHER CONGENITAL MUSCULAR DYSTROPHIES WITH CHROMOSOMAL LINKAGE

Congenital muscular dystrophy with respiratory failure and muscle hypertrophy (MDC1B)

Clinical features

MDC1B was initially described in four affected individuals from a consanguineous family from the United Arab Emirates (Muntoni et al., 1998). MDC1B is characterized by congenital onset of proximal muscle weakness including facial weakness, generalized muscle hypertrophy, and early respiratory failure. There was delay of early motor milestones, but all were able to walk but not run. There is rigidity of the spine and ankle contractures. The respiratory failure was secondary to severe diaphragmatic involvement, and occurred in one individual at the age of 4 years. Intellect was normal.

Laboratory/radiology features

Serum CK levels are increased (2270–7650 IU/l). Brain imaging is normal. Muscle histology demonstrates signs of dystrophy. Muscle immunohistochemistry demonstrates a secondary deficiency of laminin α2 (Muntoni et al., 1998).

Molecular pathogenesis/genetics

Using the original family, MDC1B has been linked to chromosome lq42. A second German family has been described with similar clinical features and linkage to the same locus (Brockington et al., 2000). The gene for MDC1B has not been identified.

Management

Nocturnal ventilatory support is necessary in MDC1B.

Congenital muscular dystrophy with linkage to chromosome 4p16.3

Clinical features

Individuals with CMD linked to 4p16.3 have been described in a large consanguineous Palestinian family (Sellick et al., 2005). Affected individuals have congenital onset of muscular dystrophy with poor muscle build, with a stable course. Muscle involvement included severe weakness of the trunk and shoulder girdle muscles, and mild to moderate involvement of facial, neck, and proximal limb muscles. There was no involvement of the extraocular or bulbar muscles, and no muscle hypertrophy. The individuals who survived were able to walk, and all but one maintained this ability into adulthood. There were no early feeding or respiratory issues. There was mild joint laxity in adulthood. Intelligence was normal. Death occurred between 7 months and 36 years in four of the seven affected individuals.

Laboratory/radiology features

Serum CK levels were normal to mildly increased (2–4-fold). Brain computed tomography performed on two individuals was normal. Echocardiograms were normal. Muscle biopsies from two individuals demonstrated dystrophic changes and in two others there were myopathic changes with increased fiber size variation and a mild increase in connective tissue, but no signs of degeneration or regeneration. Immunohistochemical analysis revealed normal staining with laminins (α2, α5, β1, γ2), dystrophin, sarcoglycans (α, β, γ), and β-dystroglycan.

Molecular pathogenesis/genetics

Using this family, linkage to chromosome 4p16.3 was determined. Two candidate genes expressed in skeletal muscle in the linked region were screened for mutations, without success. One, *SPON2*, is predicted to encode an extracellular matrix protein (Manda et al., 1999), and the other, *MYL5*, codes for a regulatory light chain of myosin (Collins et al., 1992).

OTHER CONGENITAL MUSCULAR DYSTROPHIES

CMD and muscle hypertrophy

Clinical features

Four patients with congenital muscular dystrophy, calf hypertrophy, microcephaly, and severe intellectual impairment were described in three unrelated Italian families (De Stefano et al., 1996; Villanova et al., 2000). The hypotonia was severe and generalized, and included facial muscles. These patients also had joint contractures, mainly at the ankles and elbows, but also at the knees, hips, and feet in one patient. The muscle hypertrophy was noted mainly at the calf, but was also present in the quadriceps and minimally in the biceps and triceps. Macroglossia was noted in the two cousins. There was muscle wasting of the sternocleidomastoid, pectoralis muscles, and in muscles of the scapular region.

Developmental milestones were significantly delayed. Head control and the ability to sit without support was achieved in two of the patients by 3 years of age. One patient was able to sit at 9 months, and the other was not able to sit unassisted. None of the patients was ambulatory. Two patients had their first words at 4 years of age, but did not pronounce any words clearly. One patient began to vocalize at 3 years of age, but not clearly, and the other patient did not speak at the last evaluation at 3 years of age.

Respiratory infections were frequent in all the patients, many of whom required hospitalization. Two of the patients had fractures secondary to severe osteoporosis.

Laboratory/radiology features

Serum CK levels were increased in all of these patients (15–40-fold). Brain MRI in three patients showed enlarged cisterna magna, cerebellar hypoplasia (vermis more affected than the hemispheres), and an abnormal periventricular white matter signal, with no migration defects. The fourth patient demonstrated a thin corpus callosum, a Dandy–Walker variant with cerebellar vermis agenesis, and disturbance of myelination in the periventricular white matter. Motor and nerve conduction velocities were normal. EMG demonstrated small and polyphasic motor unit potentials in the two patients tested.

Muscle biopsies showed a marked variation in fiber size and increased interstitial connective tissue. However, degeneration and centralized nuclei were not a prominent feature. Immunofluorescence demonstrated reduced expression of laminin α2 and an upregulation of laminin α5. Dystrophin, α-sarcoglycan, β-dystroglycan, laminin β1n, and laminin γ1 were expressed normally.

Molecular pathogenesis/genetics

In the two consanguineous families, linkage to the *LAMA2*, *MEB*, and *FCMD* loci was excluded.

CMD with mitochondrial structural abnormalities

Clinical features

Four patients from three unrelated families were described with neonatal onset of generalized hypotonia and developmental delay (Nishino et al., 1998). Muscle weakness was more proximal than distal. Facial muscles were mildly affected.

Development was delayed, with head support obtained between 5 and 7 months, sitting unsupported between 9 months and 1 year, and walking independently between 21 months and 33 months. Gower's sign was present in all patients. Only one patient spoke any meaningful words, at 5 years of age, but she became mute at 6 years. IQ ranged from 39 to 44.

One patient developed dilated cardiomyopathy and died at age 13 years, secondary to cardiac failure. Two patients had generalized seizures and were treated with valproic acid. One patient had precocious pubertal development.

Laboratory/radiology features

Serum CK levels were mildly raised (1.5–10-fold). Brain computed tomography was normal in two patients, and demonstrated cavum septi pellucidi in one patient and mild brain atrophy in another. Muscle biopsies demonstrated moderate variation in fiber size and minimal-to-mild endomysial fibrosis. There was evidence of both necrosis and regeneration. However, the most striking feature was the presence of peripherally located mitochondria. None of the patients had deletion or duplication of mtDNA, and respiratory chain enzyme activities were normal in the one patient tested. Immunolabeling with dystrophin, α-sarcoglycan, β-dystroglycan, merosin, and desmin was normal.

CMD with arthrogryposis and absent limb muscles

Clinical features

Philpot et al. (2001) presented a case of CMD in a patient with arthrogryposis present at birth. Joint contractures were noted at the elbows, wrist, fingers, hips, knees, and ankles. Facial muscles were not affected. Motor development was delayed, but cognition was normal. The patient sat independently at 15 months, but never was able to ambulate.

Laboratory/radiology features

Serum CK levels were normal. Muscle biopsy demonstrated signs of dystrophy with fiber size variability, fibrosis, and hypercontracted fibers. Merosin staining on a skin biopsy (subsequent repeat muscle biopsy retrieved only fat) was normal. MRI of the muscles demonstrated significant involvement of the limbs with fat replacing the muscle, but sparing of the neck and trunk muscles.

Salih CMD

Clinical features

Two siblings with congenital muscular dystrophy, delayed motor development with normal cognition, and cardiomyopathy were described (Salih et al., 1998). There was proximal muscle weakness. Development was delayed, but both brothers were ambulatory.

Laboratory/radiology features

Serum CK was slightly increased. Muscle histology demonstrated minimal-change myopathy at 4 years of age, but repeat biopsy at 10 years demonstrated advanced dystrophic changes. Immunolabeling with dystrophin, sarcoglycan, and merosin was normal. Electrocardiographic findings were abnormal in both brothers. Echocardiography and multigated acquisition (MUGA) scans were normal in the younger brother at 15 years, but in the older brother demonstrated severe left ventricular dysfunction at 16 years.

CMD with adducted thumbs

Clinical features

Two siblings born to consanguineous parents from Sicily were described with CMD with adducted thumbs (Voit et al., 2002). Generalized hypotonia and adducted thumbs along with contractures of the toes were noted at birth. Muscle weakness was more evident distally, with wasting of the thenar, hypothenar, and interosseus muscles of the hands. Their facies were myopathic and ptosis was evident. One patient developed scoliosis, which required bracing at the age of 7 years.

Motor development was delayed, with walking at 2–3 years. Language development was normal. However, cognition was delayed, with IQ in the range of 65–70.

Laboratory/radiology features

Serum CK was slightly raised (up to 3-fold), but normalized with age. Electrocardiography and echocardiography were normal. Visual evoked potentials were normal in both children. Motor and sensory nerve

conductions were normal in the median, peroneal, and sural nerves. Somatosensory evoked potentials in the median and tibial nerves were delayed. MRI of the brain demonstrated cerebellar hypoplasia of both the vermis and the hemispheres. There were no white matter changes and gyration was normal. MRI of the pelvic and thigh muscles showed advanced fatty atrophy of all the muscles with some preservation of the adductor longus, the semimembranosus, and semitendinosus muscles bilaterally. Muscle histology demonstrated dystrophic muscle with increased variation of fiber size, increased centralized nuclei, and increased endomysial fibrosis. There were interspersed atrophic fibers and some whirled and target fibers present. Immunolabeling with laminins α2, α5, β1, γ1, β2, α-dystroglycan, β-dystroglycan, α-, β-, γ-, and δ-sarcoglycan, dystrophin, caveolin-3, and collagen VI was normal in all cases. Emerin and lamin A/C were expressed normally at the nuclear membrane as well.

Molecular pathogenesis/genetics

Haplotype analysis of both children were performed at the *LAMA2*, *FCMD*, *MEB*, and *MDC1B* loci. The haplotypes for *MEB* and *MDC1B* loci were different in the two children. At the *LAMA2* and *FCMD* loci, the haplotypes were the same, but not homozygous. Therefore, linkage to any of these loci is unlikely.

CMD with external ophthalmoplegia, bulbar syndrome, and white matter changes on MRI

Clinical features

Vondracek et al. (2007) reported a case of CMD in a 13-year-old girl with severe contractures, bulbar dysfunction, progressive external ophthalmoplegia, and white matter changes on brain MRI. Cognition was normal. There was profound proximal weakness and hypotonia. The patient had severe ankle contractures limiting mobility, and she became wheelchair bound at the age of 7 years. In addition, there was subcutaneous adipose deposition in the lower extremities. There was no rigid spine or joint hyperlaxity. In addition to the external ophthalmoplegia, there was mild bilateral ptosis, but no retinopathy. There were no respiratory issues, and no seizures.

Laboratory/radiology features

Serum CK levels were normal. EMG showed a typical myopathic pattern. Motor and sensory nerve conduction studies were normal. T_2-weighted images of the brain MRI showed increased signal intensities in the white matter which were spotty and nonconfluent, mostly affecting the left frontal white matter. Muscle biopsy demonstrated fiber necrosis and regeneration, significant fiber size variation with type 2 fibers predominating, endomysial fibrosis, and fibers with central nuclei. Additionally, there was a prominent multifocal endomysial lymphocytic infiltrate with B cells and T cells ($CD4^+$ and $CD8^+$). Immunostaining showed normal expression of merosin and α- and β-dystroglycan. Dysferlin was partially deficient, suggestive of a secondary defect.

Molecular pathogenesis/genetics

No mutations were found in *calpain3*, *dysferlin*, or *SEPN1*.

CONCLUSIONS

Molecular classification of the congenital muscular dystrophies will aid in better diagnosis and hopefully identify new and novel treatments for these disorders. The use of animal models will facilitate this goal. It is likely that there are still more genes to be identified as the underlying cause of some forms of CMD. Understanding of the molecular pathogenesis of these rare disorders gives insight into muscle physiology in general. In particular, treatments developed for the CMDs may be applicable to broader classes of muscular dystrophies, including the much more common DMD.

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Chapter 5

The collagen VI-related myopathies: Ullrich congenital muscular dystrophy and Bethlem myopathy

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INTRODUCTION

Collagen type VI is a microfibrillar collagen found in many extracellular matrices including those of muscle, skin, tendon, and vessels. It is composed of three α chains encoded by three independent genes on chromosomes 2 and 21 (Table 5.1). Mutations in these three genes have been found to underlie a group of muscle disorders that are now referred to as collagen VI-related myopathies. The collagen VI-related myopathies encompass a spectrum of disorders ranging from the more severe Ullrich congenital muscular dystrophy (UCMD) through phenotypes of transitional severity to the milder Bethlem myopathy (BM). An additional, mostly contractural, phenotype, referred to as myosclerosis, has also been delineated and associated with mutations in *COL6A2*.

UCMD was initially described in a series of papers in the 1930s by Otto Ullrich (Ullrich, 1930a, b), who referred to the condition as "atonic–sclerotic muscular dystrophy" because of the characteristic occurrence of weakness and striking joint hypermobility (together imposing an "atony" in the original description) in conjunction with significant and evolving contractures (the "sclerotic" part of the initial description). Ullrich's disease was maintained as a distinct diagnostic entity mainly in the Japanese and European literature (Furukawa and Toyokura, 1977; Nonaka et al., 1981; Voit, 1998). After collagen VI mutations had been identified in BM it was the realization by Enrico Bertini and Mimma Pepe in 2001 in Italy that some of the clinical features in UCMD were reminiscent of BM that led to the first discovery of collagen VI mutations in this condition as well (Camacho Vanegas et al., 2001).

BM was initially reported in 1976 by Bethlem and van Wijngaarden in the Netherlands as an autosomal dominant early-onset but relatively benign myopathy associated with the development of characteristic contractures. Several others then reported similar cases, including a report of a large French-Canadian kindred, the authors of which then suggested the name Bethlem myopathy (Mohire et al., 1988). Linkage to the collagen VI genes was first established in larger families in Holland and the USA, leading to the identification of collagen VI mutations in these original families in 1996 (Jöbsis et al., 1996) and 1998 (Pan et al., 1998). Genetic analysis in many patients with clinically convincing UCMD and BM has now clarified that the majority of patients with these phenotypes have underlying dominant or recessive mutations in the three known collagen VI genes *COL6A1*, *COL6A2*, and *COL6A3* (Lampe and Bushby, 2005). This has been true in particular for the clinically more distinct phenotype of UCMD. There is, however, a definite number of patients with convincing clinical features who have no detectable mutations in these three collagen VI genes (Tetreault et al., 2006; Petrini et al., 2007), indicating that there is some degree of genetic heterogeneity underlying an otherwise fairly typical phenotype. This number may indeed be larger in the sometimes less distinct clinical phenotype of Bethlem (K.M.D. Bushby, personal communication).

Prevalence numbers are slowly emerging. In the population followed by the Muscle Centre in Newcastle

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Table 5.1

The three collagen VI genes and α chains

α chains	Size (kDa)	Corresponding gene	Gene position
α1(VI)	140	*COL6A1*	21q22.3
α2(VI)	140	*COL6A2*	21q22.3
α3(VI)	260–330*	*COL6A3*	2q37

*Size variable depending on state of glycosylation.

upon Tyne, UK, the prevalence of UCMD has been calculated as 0.13 per 100 000 and that of BM as 0.77 per 100 000 (Norwood et al., 2009). In other populations it is now emerging that the collagen VI-related muscle disorders are one of the most common entities subsumed under the category of congenital muscular dystrophy (Okada et al., 2007; Peat et al., 2008).

CLINICAL FEATURES

As introduced above, there are two classical clinical phenotypes associated with mutations in the collagen VI genes: the severe Ullrich congenital muscular dystrophy and the milder Bethlem myopathy (outlined separately below), which were thought to be distinct entities but which are now connected by phenotypes showing clinical features of intermediate severity between the two. Not surprisingly, certain aspects of the clinical phenotype of the collagen VI-related myopathies are shared to a greater or lesser degree by all the disorders in this spectrum, in particular as far as the contractural and joint hypermobility aspects of the phenotypes are concerned. The typical severe congenital presentation in this group of disorders includes congenital weakness and hypotonia, associated with striking joint laxity particularly of the distal joints, whereas more proximal joints such as hips, knees, elbows, and spine may be affected by congenital contractures (Bertini and Pepe, 2002). In the milder presentation of Bethlem myopathy the laxity is less conspicuous but still is often present during the early phases of the disease in childhood. In contrast, later the clinical picture in the typical case of Bethlem myopathy is much more dominated by the prominent contractures that develop over time (Merlini et al., 1994). The relative contribution of laxity and contractures can vary widely in an individual patient, so that patients with a clinical presentation of predominant joint hypermobility as well as patients with a presentation almost entirely governed by contractures ("myosclerosis") may be seen. Meticulous attention to such connective tissue-related aspects of the phenotype probably provides the best clues for a clinical diagnosis in this group of conditions.

Ullrich congenital muscular dystrophy

Ullrich disease or congenital muscular dystrophy type Ullrich (UCMD; MIM #254090) (Ullrich, 1930a, b) presents with clinical manifestations that are typically readily apparent at birth or during the first year of life. Frequently, however, the diagnosis of a neuromuscular disorder is not readily made at birth but only later when the acquisition of motor milestones is delayed. During pregnancy there may be a history of perceived reduced prenatal movements, but polyhydramnion, indicative of decreased prenatal swallowing as sometimes seen in other congenital disorders of muscle, is rarely reported in Ulllrich CMD (Ullrich, 1930a, b; Nonaka et al., 1981; Voit, 1998; Bertini and Pepe, 2002; Lampe and Bushby, 2005).

Signs and symptoms at birth include hypotonia and weakness associated with extreme distal joint laxity while at the same time contractures and skeletal deformities can be seen in more proximal joints in about 50% of the patients (Figure 5.1). Hands, fingers, and feet are

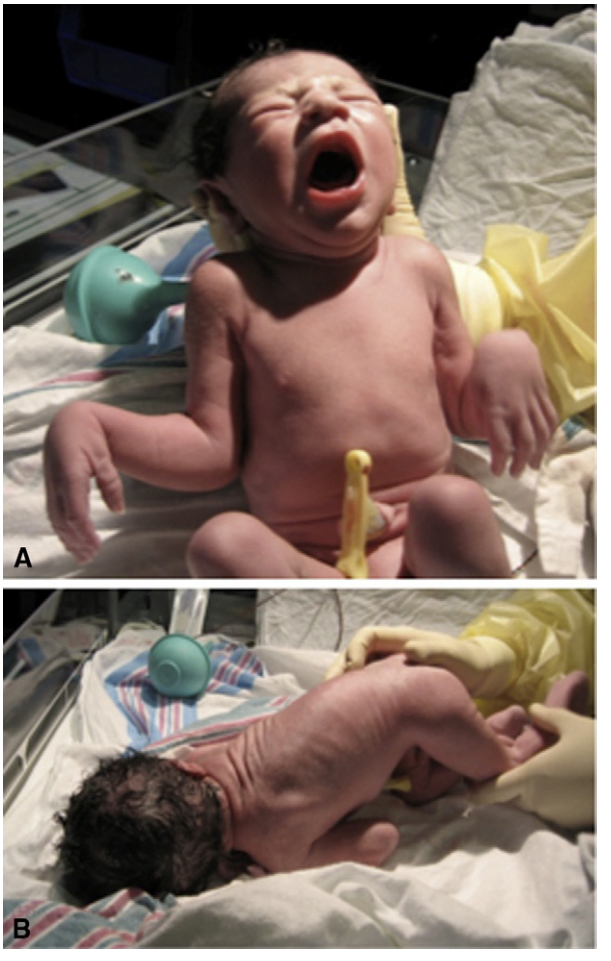

Figure 5.1. Neonatal presentation of Ullrich congenital muscular dystrophy. Note elbow contracture and flaccid-appearing hands in (**A**) and kyphosis in (**B**).

extremely hyperlax, allowing for the fingers to bend back onto the dorsum of the hands with ease, whereas the hand may drop down at the wrist, clinically reminiscent of Ehlers–Danlos syndrome (EDS). Hip dislocation/dysplasia is seen in about 50% of the patients. There may be congenital pes adductus, but, more commonly, the feet may be seen dorsiflexed against the shin because of initial hyperlaxity at the ankle. A prominent calcaneus is often evident at birth and remains a conspicuous feature in a patient with typical UCMD. It is, however, not a specific sign as it can also be seen in other congenital disorders associated with significant hypotonia.

Additional clinical features at birth may include dislocated hips, torticollis, and kyphoscoliosis, as well as contractures of the hips, knees, and elbows. Even though contractures may be present at birth, a clinical picture corresponding to a distally predominant arthrogryposis multiplex congenita (Hall, 1997) is not usually seen. Overall, at birth the presence of hyperlaxity is seen more consistently in UCMD than the presence of contractures. Frequently, the more severe contractures found immediately at birth have a tendency to improve over the first several months of life; however, new and eventually progressive contractures will set in later in most, but not all, patients.

In the most severe cases walking is never achieved, although some patients manage to crawl. More commonly, though, children with UCMD will achieve the ability to walk, often with some delay and sometimes only with the use of assistive devices (Nonaka et al., 1981; Voit, 1998). Walking then is typically lost again during childhood with a median age around 10 years (but as early as 3.5 years of age and as late as early adulthood) (Nadeau et al., 2009), mostly as a result of an increase in weakness and an even more noticeable increase in contractures, in particular in the knees and hips, with the contractures sometime progressing faster than the weakness (Figure 5.2). Thus, some children will have a period in which they prefer to ambulate on their knees as the contractures do not allow for the knees to be straight and yet there remains sufficient strength in the hip muscles to enable this mode of ambulation.

Even as the contractures progress to involve the spine, pectoralis, elbows, hips, knees, and long finger flexors, the hyperlaxity of the distal joints often persists to very late stages of the disease. This distal hyperlaxity typically involves all interphalangeal joints, including the most distal ones, while at the same time there will be increasing evidence of evolving contractures of the long finger flexors that first become evident upon extension of the fingers in the metacarpophalangeal joint. Scoliosis is often a serious and progressive problem, requiring surgical intervention in many patients. The scoliosis may evolve out of kyphoscoliosis present at birth, or start to develop independently before ambulation is lost (Nadeau et al., 2009), and even more significantly in the nonambulant patient and during the teenage years. Surgical intervention for scoliosis may be necessary before the loss of ambulation, so that changes in functional abilities after surgery will have to be considered as well. We have the impression that prominent rigidity of the spine may sometimes help delay excessive scoliosis. Osteopenia has been observed in a number of patients as early as in Ullrich's original description (Ullrich, 1930a, b). Weakness tends to be diffuse and can be variable in its relative distribution. Antigravity strength in arms and legs seems initially preserved, even in severely affected infants.

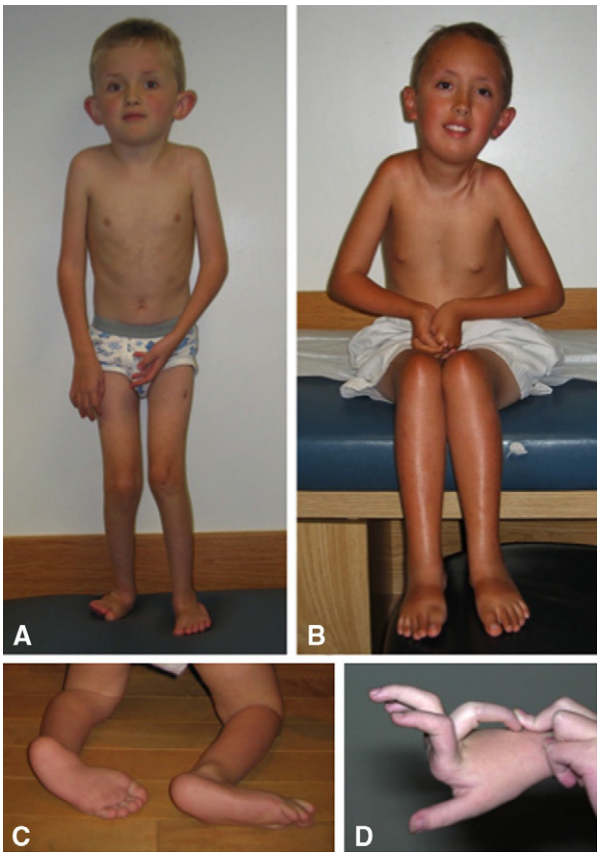

Figure 5.2. Ullrich congenital muscular dystrophy (UCMD). The patient in (**A**) is able to walk, but note knee and elbow contractures. This patient lost the ability to walk at 9 years of age. The patient in (**B**) shows typical contractures in the nonambulatory patient, affecting the pectoralis, elbows, hips, knees, and Achilles tendons. The feet in (**C**) show the typical prominent calcaneus and the soft plantar skin, whereas (**D**) demonstrates the striking distal hyperlaxity seen in the fingers in a patient with UCMD. (D: Courtesy of Marjo van der Knaap, Amsterdam, Netherlands)

The nature of the contractures, which can progress independently from the weakness, and the simultaneously persistent significant distal hyperlaxity already point to the nature of this condition as a disorder

affecting both skeletal muscle and connective tissue. Skin is another significantly affected tissue in collagen VI-related disorders, leading to a number of notable dermatological findings (Kirschner et al., 2005). Excessive scar formation including the formation of large keloids can be a significant problem in some but not all patients, and has to be taken into account when surgical interventions are considered. Keratosis pilaris often is seen prominently on extensor surfaces of the limbs. This dermatological finding is not specific to collagen VI-related disorders, but is quite consistent in the more severely affected patients, so that it is diagnostically useful in the overall context of the clinical presentation. In contrast to the roughness of the keratosis pilaris, the skin in the palms of the hands and feet is notably soft and velvety, akin to the skin texture in EDS. A general tendency for hyperhidrosis that some of the children show was commented upon by Ullrich (1930a, b).

Respiratory involvement manifesting as progressive respiratory insufficiency due to restrictive lung disease occurs in the majority of severely affected patients during the first 15 years of life and is due to a combination of weakness of the diaphragm and accessory muscles as well as stiffness of the chest wall. Early clinical signs are mostly related to night-time hypoventilation, so that sleep studies are the most effective way to monitor for early respiratory insufficiency. Regular pulmonary function testing should also be performed in all patients in order to monitor the decline in predicted forced vital capacity. In most patients there will be a clear decline in forced vital capacity toward the end of the first decade of life and into the beginning of the second decade, when the decline will be less marked on average (Nadeau et al., 2009). In those patients who need it, night-time noninvasive ventilatory support will be necessary during the second decade on average, but may be as early as 3 years of age or delayed into adulthood, reflecting the significant variability in clinical severity seen in these patients. In contrast to Duchenne muscular dystrophy, in some patients with UCMD night-time ventilatory support may become necessary before the ability to walk is lost (Nadeau et al., 2009), although in the majority ambulation is lost before onset of respiratory support.

After the initiation of noninvasive ventilation the respiratory situation tends to be quite stable over many years. Failure of initiation of noninvasive respiratory support in patients with signs of respiratory failure will lead to death in a relatively short amount of time (Nonaka et al., 1981; Nadeau et al., 2009). Cardiac involvement in UCMD usually is not seen, although there has been a rare observation of cardiac arrhythmia in UCMD (F. Muntoni, personal communication) and it is not clear whether this was coincidental or is more common.

Feeding difficulties and, at times, prominent gastroesophageal reflux have been observed in more severely affected infants. Some infants have required transient nasogastric tube feeding, whereas a minority have received G-tube feeding later in life to avert the risk of nutritional deficiency and dehydration with intercurrent illnesses (Nadeau et al., 2009). Swallowing difficulties have been reported and may need to be monitored (Nadeau et al., 2009).

Much of the natural history and the incidence of late complications in patients with severe collagen VI deficiency remain to be fully explored but will probably become clearer as patients now regularly survive well into adulthood with the institution of well-managed ventilatory support.

Intermediate phenotypes

Both UCMD and BM had been described initially as distinct clinical syndromes, but the subsequent discovery of collagen VI mutations in both of these conditions and the availability of better diagnostic tools leads to the realization that there are clinical presentations of transitional severity that are difficult to classify as clearly Ullrich or Bethlem (Demir et al., 2002, 2004; Mercuri et al., 2002). Included in this group are patients with clinical presentations that are more severe than classical Bethlem but milder compared with classical Ullrich, indicating that there is a phenotypic spectrum bridging the two classical presentations. Patients with intermediate phenotypes between Ullrich and Bethlem present with significant weakness in early childhood and often show features typical of both presentations, including the Ullrich-like distal laxity of the most distal interphalangeal joints, while at the same time showing early Bethlem-like contractures of the long finger flexors. Ambulation in these patients is achieved, although weakness can be considerable. Ambulation may be maintained into adulthood – beyond the age at which walking is usually lost in patients with typical UCMD. Walking may become difficult for these patients, however, necessitating aids such as crutches or walkers, and ambulation may be lost as early as late teenage and early adult years. Progressive respiratory impairment is an important feature in the intermediate patients also.

Bethlem myopathy

In the milder Bethlem myopathy (BM, MIM #158810) (Bethlem and van Wijnaarden, 1976) the onset of the disease may also be congenital, but children are affected to a much milder degree (Mohire et al., 1988; Merlini et al., 1994; Jöbsis et al., 1999; Bertini

and Pepe, 2002; Lampe and Bushby, 2005). Equinovarus deformity or, more commonly, foot dorsiflexion contractures and torticollis (50%) have been noted at birth in infants born with Bethlem myopathy (Jöbsis et al., 1999). Hypotonia at birth may not have been noted or is remembered only in hindsight. Although surgical release of the torticollis has been performed successfully in a number of cases (Jöbsis et al., 1999), the early contractures, when present, tend to resolve. Children may be found to have only mild weakness during childhood, frequently associated with some degree of notable distal joint laxity.

New contractures then set in, typically towards the end of the first decade of life, affecting Achilles tendons, elbows, pectorales muscles, long finger flexors, and the interphalangeal joints of digits 2 to 5 in particular, as well as in other muscle groups (Jöbsis et al., 1999) (Figure 5.3). Once established, contractures often are stable, or they may progress and become disabling in their own right, including restricted hand function due to the flexion contractures. Compared with the Ullrich phenotype, spinal rigidity is moderate in Bethlem myopathy. The distribution of the muscle weakness often shows a proximal predominance, but there can be distal weakness as well. The weakness is stable or may even improve somewhat in time with the normal increase in strength in puberty. There is, however, a slowly progressive increase in weakness starting in the third to fourth decades of life, so that on average two-thirds of patients over the age of 60 years need assistance with ambulation (Jöbsis et al., 1999). In other patients the onset of symptoms may be delayed into childhood or young adulthood, or the patient may have been noted to be a toe-walker in childhood before the onset of any perceptible weakness. Some family members may not even be aware of the presence of mild contractures or mild weakness (Merlini et al., 1994), so it is important to examine family members closely in order to establish inheritance within a family.

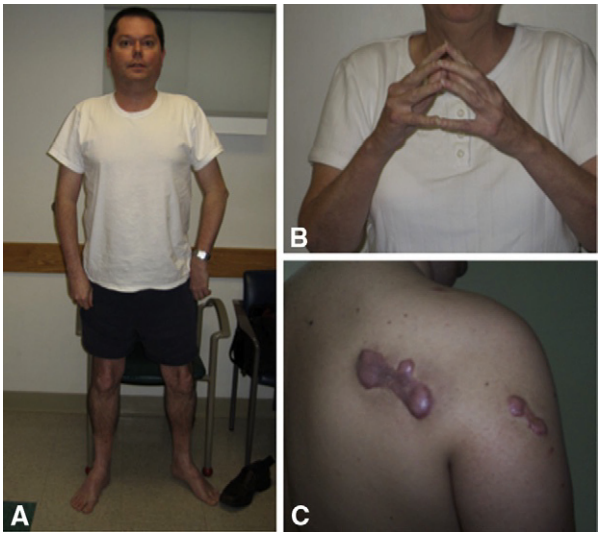

Figure 5.3. (**A**) Adult patient with Bethlem myopathy (BM). Note typical elbow and Achilles tendon contractures. (**B**) demonstrates the typical finger flexor contractures in BM that make it impossible to put the fingers close together with the elbows extended. (**C**) demonstrates excessive keloid formation in a patient with BM. (C: Courtesy of Claudia Castiglione, Santiago, Chile.)

A potential complication associated with progression of Bethlem myopathy is the development of respiratory insufficiency resulting from restrictive pulmonary disease on the basis of a combination of stiffness of the rib cage together with respiratory/diaphragmatic muscle weakness (Haq et al., 1999). Most of the more typically affected patients have milder respiratory symptoms, but should be monitored by regular sleep studies. Cardiac involvement has not been reported so far in patients with Bethlem myopathy, except for one patient in whom asymmetrical septal hypertrophy was seen, probably a coincidental finding (de Visser et al., 1992).

Involvement of the skin is similar to that seen in UCMD, although soft and velvety skin is less typical whereas the development of keloidal scars can be substantial (Nadeau and Muntoni, 2008).

There is wide clinical variability associated with the Bethlem phenotype. Some patients may present with predominantly proximal weakness and very few contractures, thus presenting more akin to a limb-girdle muscular dystrophy (LGMD) (Scacheri et al., 2002), whereas others may show a more contractural picture with relatively mild weakness, a phenotype historically referred to as myosclerosis (Bradley et al., 1973); see below. Later onset with rather minimal clinical manifestation has also been seen. Even within the same family there may be striking degrees of variability in the degree of the contractures and of the weakness.

LGMD presentation of Bethlem myopathy

Some patients have been reported as presenting with mostly proximal weakness, without any significant contractures over the course of the disease or showing development of contractures much later, thus carrying a diagnosis of LGMD on clinical grounds (Scacheri et al., 2002). Dominantly acting collagen VI missense mutations found in this context were located in the *COL6A1* and *COL6A2* genes (COL6A1: K121R; COL6A2: D630N) in two such families (Scacheri et al., 2002), indicating that collagen VI-related myopathies have to be considered within the differential diagnosis of a patient

with proximal muscle weakness and a nonspecifically myopathic muscle biopsy. However, the same mutation seen in one of the families with the LGMD presentation (COL6A2: D630N) was also seen by us in a family with typical Bethlem myopathy in some family members. The LGMD-like presentations as well as contractural presentations may even coexist in the same family. Thus, limb-girdle weakness without significant contractures is best viewed as one possible clinical presentation along the phenotypic spectrum of collagen VI-associated myopathies and not as a separate clinical entity. Nonetheless, it is important to be aware of this presentation as there may be few typical clinical clues pointing towards a collagen VI-associated myopathy.

Myosclerosis

A phenotype that is related to Bethlem myopathy but that also shows some significant differences is known as myosclerosis. The term "myosclerosis" was used by Guillaume Duchenne when he observed that muscle fibers in the biopsy from patients were completely encased by connective tissue (sclerotic) (Duchenne, 1868). A phenotype of myosclerosis was then more described clearly by Lowenthal in 1954, when the term was used to refer to the development of contractures without significant weakness, as well as a certain "woody" feeling upon palpation of the muscles (Bradley et al., 1973). It has been pointed out in the latter report that there was evidence for etiological heterogeneity in patients considered to have this diagnosis on clinical grounds (Bradley et al., 1973). In 2008, however, Merlini described a pair of siblings fulfilling the clinical diagnosis of myosclerosis, who were homozygous for a truncating mutation at the end of the C1 domain of *COL6A2* (Merlini et al., 2008b), situated just before the beginning of the alternate splice events that lead to the three C2 domain isoforms recognized in the *COL6A2* gene. This mutation has not been seen outside of this phenotype. The patients presented with moderate weakness and significant and progressive contractures of multiple joints, including masseter muscles, neck, shoulders, elbows, fingers, knees, and Achilles tendons, without significant joint hyperlaxity at any point. Muscles had a firm "woody" feel. Biopsies were severely fibrotic with partial collagen VI deficiency in the basement membrane and complete collagen VI deficiency around intramuscular capillaries, which also had a thickened basement membrane. Myosclerosis, with its predominance of contractures and comparatively mild weakness, could be understood as lying at the opposite end of a weakness/contracture spectrum to the LGMD-like presentation, with almost no contractures but significant weakness. Whether myosclerosis will maintain a separate phenotypic and genetic position within the collagen VI-related myopathies remains to be seen as more cases are identified and correlated with collagen VI mutations.

COLLAGEN VI AND MOLECULAR PATHOGENESIS

Collagen VI synthesis and interactions

Collagen VI belongs to the nonfibrillar collagens forming a network of beaded microfibrils in the extracellular matrix (Chu et al., 1990a; Timpl and Chu, 1994). The major three α chains, α1(VI), α2(VI), and α3(VI), are encoded by three genes: *COL6A1* and *COL6A2* on chromosome 21q22 and *COL6A3* on chromosome 2q37 (Weil et al., 1988; Heiskanen et al., 1995) (Figure 5.4). These three chains form the heterotrimeric monomer that is the basic building block of most of collagen VI. All three chains have relatively short triple helical collagenous domains of 335–336 amino acids containing single

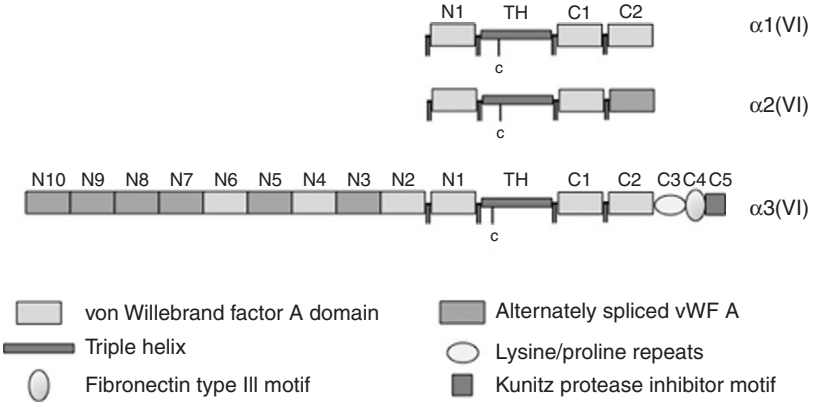

Figure 5.4. Schematic depiction of the three α chains, α1(VI), α2(VI), and α3(VI), highlighting the domain structure and the triple helical domains with the cysteine residues that are involved in higher-order assembly.

cysteine residues in the N-terminal part of the triple helical domains that are important for later intracellular assembly of the dimer and tetramer before secretion into the extracellular matrix (Chu et al., 1988).

The α1(VI) and α2(VI) chains are related and likely arose by gene duplication on chromosome 21q22 where they are oriented head to tail (Heiskanen et al., 1995). They both have two C-terminal and one N-terminal globular von Willebrand factor A domain (Chu et al., 1990b). The α3(VI) chain on chromosome 2q37 has a larger and extensively spliced N-terminal domain that is again rich in von Willebrand factor A domains (Chu et al., 1990a). The regulation and physiological function of these splice isoforms is not known, but when a stop mutation occurs in one of the alternately spliced exons its effect seems to be lessened compared with that of the nonspliced exons (Demir et al., 2002). The C-terminal domains of the α2(VI) and α3(VI) domain undergo further splicing and post-translational processing respectively.

The *COL6A2* gene generates two splice versions of the C2 domain in addition to the full-length version, which is predominant. The C1 domain alone is sufficient for assembly of the monomer, yet lack of the C2 domain or mutations in the C2 domain cause significant disease (Baker et al., 2005; Petrini et al., 2007; Merlini et al., 2008b). The most C-terminal domain of α3(VI) protein undergoes proteolytic processing in the extracellular matrix after secretion. More recently two additional *COL6A3*-related collagen VI chain genes have been identified: *COL6A5* and *COL6A6* in human and *col6a4, 5, 6* in the mouse (*COL6A4* in the human is interrupted no longer functional) (Fitzgerald et al., 2008; Gara et al., 2008).

The expression pattern of these novel chains differs from that of the traditional chains, but there is evidence now that they are able to combine into a heterotrimer with the α1(VI) and α2(VI) chains (Fitzgerald et al., 2008; Gara et al., 2008). Yet, it seems that there is no clear evidence that this heterotrimer has the ability to ameliorate the phenotype, as the disease in patients with *COL6A3* mutations is not obviously milder than that in patients with mutations in the *COL6A1* and *COL6A2* genes, as would be expected if the *COL6A5* or *COL6A6* chains were able to compensate for mutations in the *COL6A3* gene. It remains to be seen whether mutations in these additional genes will be found to cause disease or whether variation in expression of these genes is capable of modifying the clinical phenotype of patients with mutations in the classical three genes.

Collagen VI undergoes a complex assembly process inside and outside of the cell (Engvall et al., 1986; Timpl and Chu, 1994) (Figure 5.5). All three primary α chains have to combine to form a heterotrimeric monomer, the basic building block of collagen VI (Engvall et al., 1986). The monomer will thus contain the α1(VI), α2(VI), and α3(VI) chains in equal stoichiometry. Assembly proceeds from the C1 domain along the triple helical domains to the N-terminal domain. Similar to other collagens, the collagenous Gly-X-Y motifs at the C-terminal ends of the individual triple helical domains are crucial for the initiation of the triple helix formation, which successively proceeds from the C-terminal end towards the N-terminal end of the triple helical domains. Two of these triple helical monomers then associate in an antiparallel arrangement mediated by a single cysteine residue located in the N-terminal part of the α1(VI) or α2(VI) collagen chain triple helical domain, interacting with a cysteine residue in one of the C-globular domains (Furthmayr et al., 1983; Chu et al., 1988; Colombatti et al., 1995). Two dimers then associate in a parallel orientation (Engvall et al., 1986), mediated by a similar triple helical cysteine in the α3(VI) chain to form a tetramer (Furthmayr et al., 1983; Chu et al., 1988; Bonaldo et al., 1990). The tetramers are then secreted into the extracellular space, where they associate end-to-end to form the beaded microfibrillar network that is characteristic of collagen VI (Furthmayr et al., 1983; Engvall et al., 1986; Lamande et al., 1998).

The assembled extracellular collagen VI microfibrils have a diameter of 4.5 nm and a periodicity of 100–105 nm (Furthmayr et al., 1983; Chu et al., 1989; Baldock et al., 2003). Collagen VI has a widespread distribution, and is found in most matrices and tissues, including muscle, vessels, skin, intervertebral discs, cartilage, eye, and others. It shows a distinctly pericellular distribution in particular around tendon cells (Senga et al., 1995; Ritty et al., 2003) and also displays a particular affinity for basement membranes (Keene et al., 1988; Kuo et al., 1997). Thus, on immunohistochemical examination, collagen VI immunofluorescence is found to overlap with markers of basement membranes such as perlecan, laminin-γ1 and collagen type IV around endothelium, nerve, and muscle (Pan et al., 2003). Collagen VI interactions that have been suggested include collagen types II (Bidanset et al., 1992), IV (Kuo et al., 1997), and possibly type I, fibulin-2 (Sasaki et al., 1995), fibronectin and perlecan (Tillet et al., 1994), microfibril-associated glycoprotein 1 (Finnis and Gibson, 1997), heparin and hyaluran (Specks et al., 1992), decorin and biglycan (Bidanset et al., 1992; Wiberg et al., 2001), collagen XIV (Brown et al., 1993) and, amongst cell surface receptors, NG2 (Burg et al., 1996), integrin (Aumailley et al., 1991; Pfaff et al., 1993), and CD44. Through decorin and biglycan, collagen VI also interacts indirectly with chondroadherin and matrilin (Wiberg et al., 2003) and possibly also with the sarcoglycan and

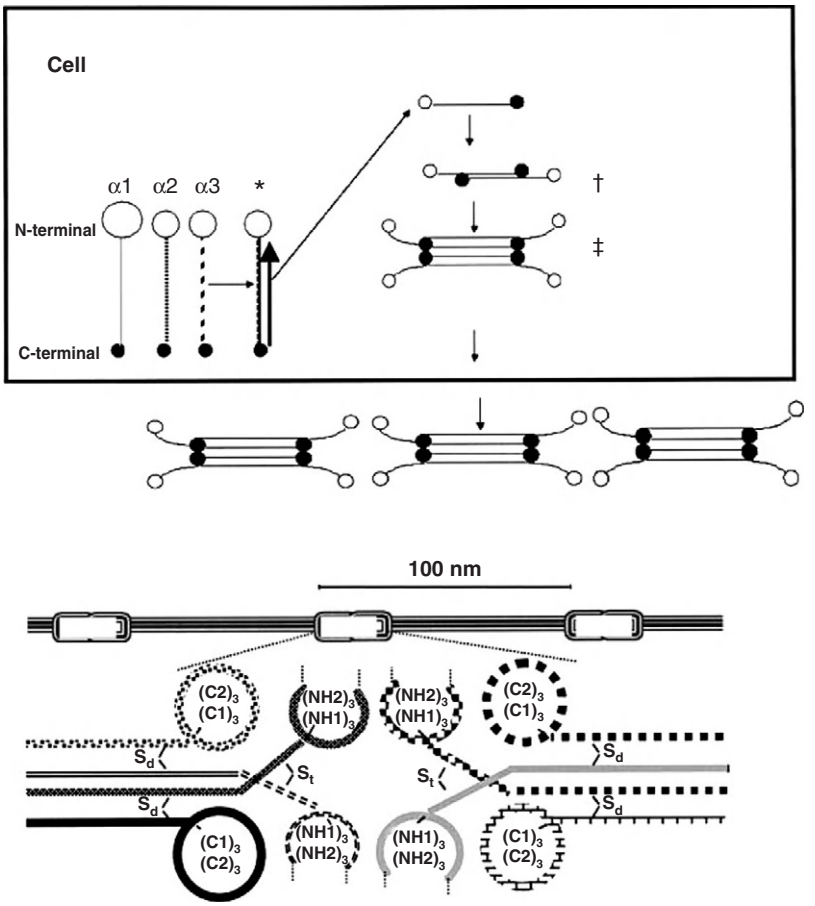

Figure 5.5. Schematic of the collagen VI assembly process. Upper panel depicts intracellular assembly from the heterotrimeric monomer composed of all three α chains (*), via antiparallel dimer (†) to tetramer (‡) formation. The lower panel depicts the formation of the beaded microfilaments with 100-nm periodicity, formed by close interaction of the C- and N-terminal globular domains. Also note the interchain disulfite bridges. (Reproduced with permission from Bertini and Pepe (2002), incorporating a modified schematic from Timpl and Chu (1994).)

dystroglycan complexes (Bowe et al., 2000; Rafii et al., 2006). Collagen VI is able to bind to the collagen-binding integrins α1β1 and α2β1 via its native triple helical domain, whereas unfolded collagen VI is able to bind to α5β1 and αvβ3 via a cryptic Arg-Gly-Asp (RGD) motif (Aumailley et al., 1991; Pfaff et al., 1993). The membrane-associated chondroitin sulfate proteoglycan NG2 is the other known cell surface receptor for collagen VI (Stallcup et al., 1990; Nishiyama and Stallcup, 1993; Burg et al., 1996). The precise receptor or receptors for collagen VI in skeletal muscle are currently unknown.

Collagen VI functions, molecular pathogenesis

Functions suggested for collagen VI have included roles in cell adhesion (Aumailley et al., 1991; Klein et al., 1995), proliferation, stimulation of DNA synthesis of mesenchymal cells (Atkinson et al., 1996), and neural crest cell migration (Perris et al., 1993). Work in patient biopsies and cell culture has suggested that there is a loss of connection between the basement membrane and the interstitial matrix (Ishikawa et al., 2002, 2004; Pan et al., 2003), resulting in reduced anchorage to the matrix (Kawahara et al., 2007). Abnormal regulation of the putative collagen VI receptor NG2 has been seen (Petrini et al., 2005), although it is currently not clear whether there is a direct link from the muscle via NG2 and collagen VI to the rest of the matrix. In several cellular systems collagen VI seemed to infer resistance to apoptosis through as yet poorly understood pathways (Ruhl et al., 1999).

Further analysis of a mouse model of collagen VI deficiency generated by homologous inactivation of the *col6a1* locus (Bonaldo et al., 1998) revealed apoptosis in the muscle mediated by mitochondrial changes (Irwin et al., 2003). In this mouse model there was evidence for easy breakdown of mitochondrial potential because of dysfunction of the mitochondrial permeability transition

pore (PTP) in mitochondria prechallenged with oligomycin. There also were morphological differences in the mitochondria, which looked abnormally ballooned, as well as abnormalities of the sarcoplasmic reticulum. This inappropriate opening of the PTP is mediated by cyclophilin D and was correctable by the addition of wild-type collagen VI and of the cyclophilin D inhibitor ciclosporin A (Irwin et al., 2003) and the ciclosporin analog Debio25 (Tiepolo et al., 2009). Crossing the collagen VI null mouse with a cyclophilin D knockout mouse to create a collagen VI cyclophilin D double-knockout situation also prevented the inappropriate opening of the PTP and significantly decreased apoptosis in the muscle, reaffirming the central role of cyclophilin D in this part of the disease process (Palma et al., 2009). The same phenomenon of increased apoptosis because of inappropriate opening of the PTP has also been described in human cell culture and in biopsies obtained from human patients with collagen VI mutations (Angelin et al., 2007; Merlini et al., 2008a). Thus, an apoptotic mechanism, rather than dystrophy due to an unstable plasma membrane, appears to be a major contributor to myofiber loss in this condition, although this mechanism involving cyclophilin D is not specific to collagen VI deficiency and was also observed in a merosin-deficient CMD mouse model (Millay et al., 2008). The pathways mediating between the presence of collagen VI in the matrix and the mechanisms controlling apoptosis are less clear and remain to be worked out in detail.

MUTATIONAL SPECTRUM/GENOTYPE–PHENOTYPE CORRELATIONS

Bethlem myopathy

Many mutations have now been described in the three collagen VI genes both in patients with UCMD and in those with BM, so that a number of observations about genotype–phenotype correlations are beginning to emerge. Many of the mutations have been summarized by Lampe and colleagues (Lampe and Bushby, 2005) in great detail. Thus far, mutations in BM have mostly been dominant, i.e., acting from one allele either as an inherited change in families with Bethlem myopathy or as *de novo* mutations. Typical mutation types seen in BM are missense mutations of glycine residues of the collagenous Gly-X-Y motif at the N-terminal end of the triple helical domain (Jöbsis et al., 1996; Pepe et al., 1999a; Scacheri et al., 2002; Lampe et al., 2005; Lucioli et al., 2005). This mutation introduces a kink into the triple helical domain of the assembled tetramer, thus exerting a dominant negative effect on the structure of the tetramer (Lamande et al., 2001). The effect of these N-terminal triple helical glycine mutations is variable, correlating with the variable clinical phenotype associated with this type of mutation (Pace et al., 2008). If the effect on assembly is more profound, the phenotype will be more severe and fall into the intermediate or even Ullrich range of severity (Okada et al., 2007; Pace et al., 2008). The other recurrent type of mutation in BM is an inframe deletion of exon 14 in the α1(VI) chain (Pepe et al., 1999b, 2006; Vanegas et al., 2002; Pan et al., 2003; Lampe et al., 2005; Lucioli et al., 2005). This mutation removes one of the cysteines necessary for dimer assembly, blunting its dominant negative effect on the assembly (Pan et al., 2003; Baker et al., 2007). As outlined below, cysteine-sparing mutations in this location are otherwise associated with the more severe phenotype of UCMD because of the more pronounced dominant negative effect. It should be noted, however, that similar to the effect of dominant glycine missense mutations at the N-terminal end of the triple helical domain skipping of exon 14 in the α1(VI) chain can also result in phenotypic severities in the interim range between Ullrich and Bethlem. A range of other mutations has been described in BM, including missense mutations that do not affect the triple helical glycine residues (Scacheri et al., 2002; Lucioli et al., 2005), including in the families with a LGMD-like presentation.

Not all of the hitherto reported mutations associated with BM have been fully analyzed as to their pathogenic effect on collagen VI. Particular caution is necessary as there are many polymorphisms in the collagen VI genes that have not yet been fully catalogued, so that it may be hard to determine whether a newly encountered sequence change in a patient is in fact pathogenic or a polymorphism. Most recently, recessive mutations in the *COL6A2* gene have been seen in patients with typical Bethlem myopathy (Foley et al., 2009; Gualandi et al., 2009). These patients (from three independent families to date) were carrying a null mutation on one allele and a missense mutation on the other, and all these mutations have so far occurred in the *COL6A2* gene. The mutations were asymptomatic in the carrier state. Homozygosity for the null allele would, of course, give rise to the more severe phenotype of UCMD, so that the missense mutation on the other allele appears to ameliorate the phenotype into the Bethlem range. Thus, both clinical and genetic data clearly support the notion of a clinical and molecular spectrum of the collagen VI-associated myopathies.

Ullrich congenital muscular dystrophy

In UCMD the first mutations that were detected were recessive null mutations, leading to an absence of

collagen VI in muscle biopsy sections and in culture of dermal fibroblasts (Camacho Vanegas et al., 2001; Higuchi et al., 2001). A larger variety of recessively acting mutations, mostly leading to premature termination codons, has subsequently been described, including some with milder manifestations because of their localization in alternatively spliced exons (Demir et al., 2002; Giusti et al., 2005; Lampe et al., 2005; Okada et al., 2007). Splice-site mutations may lead to out-of-frame exon skipping, thus acting as recessive null mutations (Camacho Vanegas et al., 2001; Ishikawa et al., 2002; Lucarini et al., 2005). Haploinsufficiency for one of the collagen VI chains in general does not lead to a clinical phenotype, so that carriers of these null mutations are not significantly affected clinically (Camacho Vanegas et al., 2001; Higuchi et al., 2001; Peat et al., 2007; Foley et al., 2009). Subsequently, it has become evident that *de novo* dominant mutations in all three collagen VI genes are responsible for a substantial proportion of patients presenting with sporadic UCMD (Pan et al., 2003; Baker et al., 2005; Lampe et al., 2005, 2008; Okada et al., 2007). These mutations are typically inframe exon-skipping mutations (on the basis of splice-site mutations or genomic deletions; Pepe et al., 2006) of exons coding for the N-terminal part of the triple helical domain, but sparing the cysteine residues responsible for higher-order assembly of the basic heterotrimer into the dimer and tetramer state (Pan et al., 2003; Lampe et al., 2008). As the skipped exon is located at the N-terminal end of the triple helical domain, the deleted chain is effectively incorporated into the heterotrimeric monomer. Owing to the fact that the deleted chain preserves the cysteine, the following higher-order assembly will include the mutant containing monomers, so that 15 of 16 secreted tetramers will then include at least one mutant chain as the basis of the strong dominant negative effect that these mutations exert (Pan et al., 2003; Baker et al., 2005; Lampe et al., 2008). In contrast, inframe exon skipping mutations that occur more towards the C-terminal end of the triple helical domain will be excluded from assembly into even the basic heterotrimeric monomer and, therefore, act in a recessive way (Demir et al., 2002; Ishikawa et al., 2004; Baker et al., 2005; Lampe et al., 2005, 2008). These dominant negative mutations in collagen VI are associated with a phenotypic range approaching the severity of recessive null mutations; however, patients with dominant negative mutations may be more likely to at least achieve some limited time of ambulation, compared with those with a complete null situation. In the dominant negative mutations, immunohistochemical analysis of the muscle biopsy will show presence of collagen VI in the extracellular matrix; however, careful labeling with markers of the basement membrane shows that the normally tight connection between collagen VI and basement membrane has been lost (Pan et al., 2003; Ishikawa et al., 2004). This double-labeling technique has emerged as the most sensitive immunohistochemical technique to suggest the presence of a collagen VI disorder (see below).

DIAGNOSIS, DIFFERENTIAL DIAGNOSIS, GENETIC COUNSELING, AND TREATMENT

Clinical diagnosis and differential diagnosis

The first step in the diagnosis of a collagen VI-related condition is the recognition of the salient clinical features that raise the index of suspicion for the presence of UCMD, BM, or a related disorder of intermediate severity. In a juvenile or adult patient with the findings of a prominent contractural phenotype associated with weakness, a collagen VI-related myopathy of intermediate severity, Bethlem myopathy, and myosclerosis are definite diagnostic considerations.

The most important differential diagnosis to consider in this scenario are the Emery–Dreifuss muscular dystrophies (EDMDs), caused by mutations in *emerin* for the X-linked form and *lamin A/C* for the autosomal form. There can be considerable overlap in the pattern of contractures, making a reliable distinction based on that feature alone difficult (Bonne et al., 2000), although, in our experience, contractures of the long finger flexors, in particular, are seen more commonly in the collagen VI-related disorders compared with the Emery–Dreifuss group. There is no Emery–Dreifuss typical cardiac involvement in the collagen VI disorders, whereas clinically relevant involvement of the skin seen in the collagen VI-related disorders does not occur in the Emery–Dreifuss group of disorders. For the UCMD group, in the younger child with EDS-like hyperlaxity together with contractures and weakness, this differential diagnosis of EDMD disorders is less relevant, although a CMD presentation of *lamin A/C* mutations is now being recognized and can be the basis of some confusion (Quijano-Roy et al., 2008). Usually the hyperlaxity of the distal joints is sufficiently striking to provide a high enough index of suspicion for the presence of a collagen VI disorder; however, hyperlaxity can also be a feature of other neuromuscular disorders (Voermans et al., 2009). For example, core disorders such as multi-minicore disease or more severe neonatal central core disease can also lead to a high degree of joint laxity in some patients and may have to be considered in the differential diagnosis (Ferreiro et al., 2002). The presence of additional and purely dermatological findings, such as striking keloid formation and

extensive keratosis pilaris, is a strong indicator of the presence of a collagen VI-related disorder (Kirschner et al., 2005).

In the child, adolescent, or young adult with a predominantly rigid spine presentation, the differential diagnosis to consider in addition to the collagen VI-related disorders includes again the EDMDs caused by *emerin* and *lamin A/C* mutations, *FHL1*-related myopathies (reducing body myopathy and an EDMD-like presentation of *FHL1* mutations), partial merosin deficiency, the contractural phenotype of LGMD2A (*calpain* mutations), and early-onset myofibrillar myopathies.

Muscle imaging

Muscle imaging can be helpful in the clinical differentiation of these disorders as there are suggestive patterns of muscle involvement associated with each of the conditions (Deconinck et al., 2010; Mercuri et al., 2010). Imaging in the collagen VI disorders will reveal a picture with characteristic fatty and connective tissue replacement of muscle starting around the fascias surrounding or traversing the muscle, as seen on muscle magnetic resonance imaging (Mercuri et al., 2005). The rectus femoris and vastus lateralis muscles show this pattern most consistently. A similar appearance can be appreciated on muscle ultrasonography, where the degeneration around the central fascia in the rectus femoris generates the appearance of a "central cloud" (Bönnemann et al., 2003). This peculiar "outside-in" picture of degeneration seen on muscle imaging is helpful when present, although it is not seen in all patients or may no longer be discernible in advanced cases, such as in late BM, or in more severely involved UCMD (Mercuri et al., 2005).

Muscle biopsy and dermal fibroblast analysis

Muscle biopsy findings in the collagen VI-related disorders can be quite variable and range from close to normal or mildly myopathic-appearing muscle with atrophic fibers and some degree of fiber-type disproportion (Schessl et al., 2008), to more dramatically myopathic pictures with variability of fiber diameter including the appearance of sometimes extremely atrophic fibers and build-up of extracellular connective and fat tissue. These histological abnormalities usually become more evident with increasing age of the patient. Evidence for myofiber degeneration also becomes more evident later in the disease, although it is never a strikingly prominent aspect of the histological picture. Core-like abnormalities in the myofibers may also be seen on occasion and can be source of confusion with the true core myopathies (personal observations).

Collagen VI immunhistochemistry on muscle biopsy sections can be performed and may be helpful, particularly in recessive cases of UCMD where staining is absent or severely reduced. In the case of dominant mutations in UCMD there will be strong labeling for collagen VI immunoreactivity in the matrix, but the proper overlap of collagen VI with the basement membrane will be lost, indicating that the mutant collagen VI protein is being secreted but is not capable of proper function (Pan et al., 2003; Ishikawa et al., 2004) (Figure 5.6). Careful double labeling of the basement membrane with antibodies to collagen IV, laminin γ1 or perlecan is thus important in order to assess collagen VI localization in the tissue properly. In the milder BM, this lack of connection between collagen VI and the basement membrane may only be partial and sometimes may not be apparent at all (Pan et al., 2003), thus the muscle immunohistochemical analysis can appear to be normal. Analysis of collagen VI production in dermal fibroblast cultures can also be helpful in implicating collagen VI as being involved, ranging from completely absent or severely reduced with intracellular retention in UCMD to more subtle abnormalities in BM (Jimenez-Mallebrera et al., 2006; Hicks et al., 2008).

Molecular genetic analysis

Mutational analysis can now be achieved on genomic DNA by sequencing all exons for all three collagen VI chains (Lampe et al., 2005). As alluded to before, it is important to point out that this analysis will not infrequently generate sequence changes that may be unknown in their significance, i.e., a given change may not yet have been seen as a disease-associated mutation or as a polymorphism. In this situation, it is important to trace the mutation in the family to see

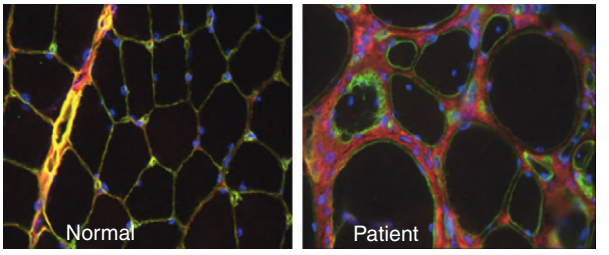

Figure 5.6. Immunolocalization of collagen VI in the muscle of a patient with a dominant negative mutation in collagen VI. In the normal biopsy, collagen VI (red) overlaps with the basement membrane (green), resulting in a yellow color. In the patient's biopsy there is a considerable amount of collagen VI immunoreactivity in the matrix; however, the colocalization with the basement membrane is lost, resulting in the green color of the basement membrane. Nuclear counterstain in blue.

whether it follows the expected pattern of inheritance for a disease causing change in a given family.

In patients in whom the clinical suspicion for the presence of a collagen VI-related myopathy is strong, but, in using genomic sequencing, no mutations in the collagen VI genes are found or a change of uncertain significance is uncovered, it may also be useful to analyze a dermal fibroblast culture for collagen VI production and deposition as well as for possible effects on splicing, by performing reverse transcription–polymerase chain reaction analysis on RNA isolated from the dermal fibroblasts. This latter analysis may be important, as a change of unknown significance may influence splicing or there may be a deep intronic mutation interfering with splicing that is not detected on exon-based genomic sequencing (Lucarini et al., 2005; Martoni et al., 2009).

Lastly, it has become apparent recently that larger genomic deletions can occur, particularly in the *COL6A1* and *COL6A2* loci, that also will not be detected on exon-based genomic sequencing (which is dosage insensitive); therefore, these deletions require dosage-sensitive techniques such as multiplex ligation-dependent probe amplification or genomic single nucleotide polymorphism arrays for detection (Foley et al. in press 2010). Latest-generation molecular tools including chip-based sequencing arrays will be able to detect large genomic deletions as well as deep intronic sequence changes.

Genetic counseling

Genetic counseling in BM usually assumes the presence of an autosomal dominant condition with a risk of 50% for an affected individual of passing on the mutant allele. Although this assumption is holding up for the majority of patients, a potential caveat is the possibility of recessively acting mutations underlying Bethlem myopathy (Foley et al., 2009; Gualandi et al., 2009), which obviously carry a very different recurrence risk for the offspring of an affected patient. Thus, sporadic patients with BM likely carry a *de novo* dominant mutation, but they may rarely carry recessive mutations. The identification of the actual collagen VI mutation will help greatly in understanding the inheritance pattern so mutation analysis should always be attempted. In such a patient, in whim one missense mutation is detected that is inherited from an asymptomatic parent, the presence of a larger genomic deletion of the other allele should be suspected and pursued appropriately (see above). In the sporadic patient with UCMD both recessive mutations as well as a *de novo* dominant negative mutations can be expected with about equal likelihood, depending on the patient population of origin. Patients from populations with a degree of consanguinity or common ancestry are more likely to present with recessive UCMD, whereas patients from populations with mixed heritage are more likely to present with *de novo* dominant mutations, although either mutation type is possible in both settings.

Recessive versus *de novo* dominant mutations are obviously associated with greatly different recurrence risk estimations for future pregnancies of a couple with a single (sporadic) affected child and a negative family history. The risk of recurrence would be 25% for the recessive scenario, whereas for the a *de novo* dominant mutation only the theoretical risk of germline mosaicism has to be assumed. Only by the definitive identification of the causative mutations in the collagen VI genes can this situation be clarified.

In general, counseling for the possibility of a certain degree of individual clinical variability is important in families with collagen VI-related disorders, even if the mutation found is a known disease-associated mutation with a given phenotype. Prenatal diagnosis is possible by haplotype analysis and collagen VI staining of a chorionic villus biopsy (Brockington et al., 2004), but is much more straightforward and reliable once the disease-causing mutation(s) are known in the family, allowing for direct testing of the pregnancy.

Management and therapeutic interventions

Therapeutic intervention in the collagen VI disorders currently consists mainly of careful clinical and preventive management of the various clinical aspects of these conditions. Contractures are usually addressed initially by an aggressive stretching program in combination with dynamic splinting. Such a program is helpful in delaying worsening of the contractures; however, rarely can the progression of the contractures be entirely stopped. Surgical release of the contractures can be helpful, in particular in the Achilles tendons to preserve normal walking in patients with Bethlem myopathy, although the contractures have a tendency to recur after surgery. Very importantly, surgical release of other joint contractures, such as flexion contractures in the knees, has less clear efficacy and there is much less underlying clinical experience.

Management of early and progressive scoliosis can be challenging. Bracing may have a temporizing effect but never stops the eventual progression of the scoliosis. Experience with newer scoliosis surgical techniques such as the VEPTER (vertical expandable prosthetic titanium rib) is limited; however, it will likely be explored more in the future and will likely have a place in the surgical management of young children with UCMD and early progressive scoliosis. Careful respiratory monitoring by pulmonary function testing (upright and supine to assess for diaphragmatic involvement) as well as by sleep studies with timely institution of

respiratory support is of prime importance and usually consists of noninvasive ventilatory support such as bilevel positive airway pressure. Respiratory insufficiency is clearly progressive in the collagen VI-related myopathies (Wallgren-Pettersson et al., 2004, Nadeau 2009); however, once ventilatory support is instituted, there will be a long period of stability in respiratory status. Adequate nutritional support is of great importance as many of the more severely affected children will have inadequate oral intake of food and fluids, even though there is no frank dysphagia. It is important to monitor bone density and consider calcium and vitamin D supplementation when appropriate. In selected children with collagen VI-related myopathies, a temporary percutaneous gastrostomy tube has become necessary.

Myofiber apoptosis has already been identified as a potential therapeutic target in the animal model of collagen VI deficiency. Pharmacological agents that may counteract the apoptosis that is part of the downstream effect of the collagen VI dysfunction are thus under investigation. A recent uncontrolled study of five patients with collagen VI mutations treated for 1 month with ciclosporin A (acting as a inhibitor of the mitochondrial permeability transition pore) demonstrated decreased apoptosis and increased stability of the mitochondrial permeability transition pore, although strength improvement was not recorded (Merlini et al., 2008a). Antiapoptotic agents with less long-term toxicity are now under active clinical investigation and may enter clinical trials in these patients.

A particular challenge lies in the combined predominance of dominant mutations in all of the collagen VI-related disease. In this situation, gene replacement approaches obviously will not work, and other strategies such as inactivation of the dominant negative allele will have to be devised. Stem cell therapy will have to take into account that the collagen VI cell of origin in muscle is predominantly the muscle interstitial fibroblast (Zou et al. 2008). As mentioned above, there are a minority of patients with UCMD for whom mutations in the three collagen VI genes have been ruled out, indicating that there should be additional genes causing their phenotype that still await discovery. Very recently reduced autophagocytic flow has also been implicated in the pathogenesis of collagen VI deficiency (Grumati P, Coletto L, Sabatelli P et al. (2010). Autophagy is defective in collagen VI muscular dystrophies, and its reactivation rescues myofiber degeneration. Nat Med 16: 1313–1320.)

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Chapter 6

Limb-girdle muscular dystrophy 2A

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INTRODUCTION

This chapter focuses on the clinical and laboratory features of limb-girdle muscular dystrophy 2A (LGMD2A, MIM #253600) and the possible pathogenic basis of the disorder. Before the cloning of *CAPN3* or *calpain-3* (Richard et al., 1995), the gene causing LGMD2A, all the genes described responsible for limb-girdle weakness corresponded to structural proteins expressed in the sarcolemma. This family of proteins, the dystrophin-sarcoglycan complex, provides a link between the sarcoplasm and the extracellular matrix stabilizing muscle membrane during contraction. However, calpain-3 is a nonlysosomal Ca^{2+} protease and this has posed the question of what its function might be in skeletal muscle. To date more than 300 different pathogenic mutations have been reported worldwide, distributed along the entire length of the gene. It has been reported that patients with a missense mutation develop a milder phenotype than those bearing a null mutation. However, the amount of protein does not correlate with the onset of the disease (Groen et al., 2007).

CLINICAL FEATURES

Classical clinical presentation

In most cases, the clinical characteristics of LGMD2A fit in with those reported previously by Fardeau et al. (1996a), van der Kooi et al. (1996), and Urtasun et al. (1998) (Table 6.1). Muscle weakness usually begins in the pelvic girdle, with problems running, climbing stairs or getting up from a chair. The lower limb girdle muscles are the most severely affected, even in the few patients in whom the disease started in the shoulder girdle or both simultaneously. Hip adductors and gluteus maximus are the earliest clinically affected muscles, and to a lesser degree the hip flexors and hamstring muscles are also involved (Figure 6.1) (Fardeau et al., 1996b). Hip abductors are relatively better preserved (Pollitt et al., 2001), as well as distal muscles in the lower limbs. However, some distal muscles are characteristically involved early, as shown by skeletal muscle magnetic resonance imaging (MRI). Clinical complaints about shoulder girdle and upper limb weakness appear later on in the course. However, the neurological examination discloses early muscle involvement in the scapular girdle muscles, including predominantly the latissimus dorsi, rhomboids, serratus magnus, and pectoralis, causing scapular winging (see Figure 6.1). To a lesser degree the trapezius, deltoid, biceps brachii, brachialis, and brachioradialis are also involved (Fardeau et al., 1996b). As the disease progresses, weakness and atrophy also involve the quadriceps and the tibialis anterior in the lower extremities, the triceps brachii, and the forearm muscles (Fardeau et al., 1996b). Abdominal muscles are also weak. Facial muscles are spared, helping to distinguish LGMD2A from facioscapulohumeral dystrophy. Extraocular and pharyngeal muscles are not affected. A similar pattern of muscle involvement is evident in most patients with LGMD2A of Turkish or Basque origin (Dincer et al., 1997; Topaloglu et al., 1997; Beckmann and Fardeau 1998; Urtasun et al., 1998).

Muscle hypertrophy is unusual (Urtasun et al., 1998; Passos-Bueno et al., 1999), although this trait has been observed in different series and was found in 75% of patients in the Brazilian population (de Paula et al., 2002). Several patients have significant contractures that are limited to calf muscles in the early stages of the diseases but may include the elbows and spine. Finger contractures have also been described (Pollitt et al., 2001).

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Table 6.1

Clinical features suggestive of limb-girdle muscular dystrophy type 2A

- Age at onset is between 8 and 16 years
- Absence of vertical pattern of inheritance
- Simultaneous involvement of both girdles (difference in onset <2 years)
- No facial, oculomotor, cardiac, or predominantly distal involvement
- Presence of winging scapulae
- Contractures in calf muscles or extended to the spine
- Preferential involvement of posterior compartment of the thighs
- Involvement of posterior superficial calves group visualized by computed tomography or magnetic resonance imaging
- 5–20-fold increase in creatine kinase level
- Peripheral eosinophilia could be present in the presymptomatic phase
- Dystrophic pattern in muscle biopsy (if biopsy is performed at start or in asymptomatic state an inflammatory pattern composed mainly of an eosinophilic infiltrate)
- Wheelchair-bound before 35 years of age or after no more than 25 years of progression

Based on Beckmann and Bushby (1996) and other published studies (Fardeau et al., 1996b; Urtasun et al., 1998; Mercuri et al., 2005; Krahn et al., 2006a).

In a series of patients from the Réunion islands, electrocardiography (ECG) and echocardiography findings were normal, except in one patient who presented with a slight diminution of left ventricle contractility (Fardeau et al., 1996b). In the later stages of the disease, cardiac abnormalities were considered to correlate with the respiratory insufficiency. A deficiency in diaphragmatic function was found in the majority of the patients, resulting in a reduction of forced vital capacity (FVC) to 30–50% at stages V–VII. One patient displayed a severe respiratory insufficiency that led to death at age 19 years.

In another study, a reduction in FVC below 80% of the predicted value was found in 12 of 31 patients (Urtasun et al., 1998). However, all the patients with normal lung vital capacity showed a reduction in maximal expiratory pressure, suggesting weakness in thoracic and abdominal muscles. The ECG was normal, although nonspecific conduction abnormalities were found in five patients and repolarization abnormalities in another two. Bidimensional echocardiographic examination performed in 29 patients was also normal.

In a series from the UK, all patients showed normal heart function on ECG and echocardiography, except two patients who showed atrial fibrillation and mildly impaired left ventricular function (Groen et al., 2007). Although respiratory function declined over time, it was still well preserved with values around 80% of predicted FVC at later stages and no indication of diaphragmatic involvement. In only two patients were values significantly lower than 80%.

Affected individuals usually become symptomatic between 8 and 15 years of age, with a range of about 2–50 years. The disease is slowly progressive, although the rate of progression varies among patients, with evident interfamilial variability. Loss of independent ambulation usually occurs between 15 and 25 years after the onset of the symptoms, but may occur earlier in patients with infantile onset of the clinical manifestations. However, age of onset is not an exact predictor of progression or use of a wheelchair.

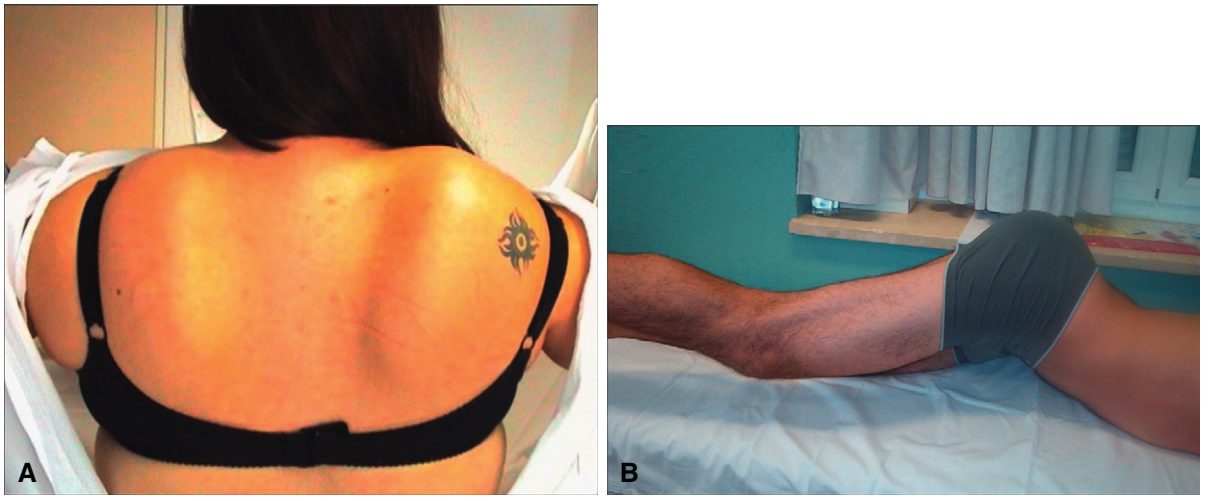

Figure 6.1. Clinical phenotype. (**A**) Scapular winging in a 17-year-old patient. (**B**) Weakness and atrophy predominantly on the glutei and posterior thigh muscles.

Atypical clinical presentations

Occasionally patients present in early childhood with severe weakness resembling Duchenne muscular dystrophy. Others present with a pseudometabolic picture with complaints of myalgia, muscle stiffness, or exercise intolerance (Penisson-Besnier et al., 1998; Pollitt et al., 2001; Fanin et al., 2004; Hermanova et al., 2006). Recently, a group of young patients (3–11 years old) with hyperCKemia, either isolated or in association with mild weakness of the lower limbs and an eosinophilic infiltrate in the muscle biopsy that led to a diagnosis of eosinophilic myositis, were in fact found to have *CANP3* mutations (Krahn et al., 2006b).

The number of patients with calpainopathy is likely underestimated as clinicians do not often think of investigating for LGMD2A in such patients. Moreover, western blot may be more likely to be normal in these more benign LGMD2A cases.

EPIDEMIOLOGY

Fardeau et al. (1996a), in a small community from the Réunion Island with a high degree of inbreeding, found an estimated prevalence of 48 per 10^6 population. Urtasun et al. (1998) reported the highest prevalence rate of LGMD to date (69 per 10^6) in a province of the Spanish Basque Country. This prevalence rate, as that reported within the Amish population in Indiana (13000 per 10^6), may have been overestimated owing to the size of the communities studied and as an artifact due to inbreeding present in ethnic or religious-based communities. In the Réunion Island, as well as in the Basque Country, LGMD2A is the most prevalent and almost the only type of LGMD. In the Amish population, LGMD2A and LGMD2E appear to be the most frequent subtypes.

In other regions with high prevalence of LGMD, as in North Africa, the presence of the calpainopathies is lower, owing to a higher presence of mutations in the genes encoding for proteins of the sarcoglycan complex (Bönnemann et al., 1998; Dincer et al., 2000). In the UK, LGMD2I has been reported to be one of the most frequent entities after the 2A type (Poppe et al., 2003).

In open populations with low rates of genetic inbreeding, few comprehensive epidemiological studies taking into account the genetic basis have been conducted in the recent years (Table 6.2). The studies performed so far are not comparable and are not real epidemiological studies. Given that the estimations of prevalence are limited, in order to establish the specific weight of the calpainopathies in different populations various approaches have been performed. According to studies carried out on familial cases in different countries, calpain-deficient families were estimated to represent 20–50% (see Table 6.2). Additional molecular studies in patients with LGMD2A have been undertaken in other countries in Europe, such as Russia (Pogoda et al., 2000), Croatia (Canki-Klain et al., 2004; Milic and Canki-Klain, 2005), Czech Republic (Chrobakova et al., 2004; Hermanova et al., 2006), Germany (Hanisch et al., 2007), and Denmark (Duno et al., 2008).

LABORATORY FEATURES

Muscle enzymes

In a recent series of patients from the UK, the range of creatine kinase (CK) levels found in patients with two mutations was 193–13000 IU/l, with one mutation 399–11000 IU/l, and with no mutations 298–38620 IU/l (not controlled for disease duration) (Groen et al., 2007). Serum CK concentration is markedly raised in the first stages of the disease and gradually decreases as the

Table 6.2

Relative proportion of calpainopathies in limb-girdle muscular dystrophy (LGMD)

Reference	Country	Calpain-deficient/LGMD families	Proportion
Richard et al. (1997)	Different origins: France, Israel, Italy, Turkey, Lebanon, Switzerland, USA	9/23	39%
Zatz et al. (2003)	Brazil	38/120	32%
Chae et al. (2001)	Japan	21/80	26%
Meznaric-Petrusa et al. (2002)	Slovenia	9/22 (cases)	41%
Piluso et al. (2005)	Italy	93/265 (cases)	35%
Balci et al. (2006)	Turkey	21/93	23%
Moore et al. (2006)	USA	17/83 (cases)	20%
Todorova et al. (2007)	Bulgaria	20/48	42%

muscular atrophy and weakness progresses and the muscles become more and more atrophic (Urtasun et al., 1998). CK levels are not as high as in other common muscular dystrophies such as Duchenne muscular dystrophy, dysferlinopathies, or LGMD2I.

Muscle imaging

Computed tomography (CT) of the thighs usually shows marked atrophy of the hamstrings and hip adductors, and moderate atrophy in quadriceps with sparing of the sartorius (Fardeau et al., 1996b; Urtasun et al., 1998). A study using muscle MRI in seven patients with LGMD2A and early contractures showed involvement of the posterior thigh muscles (Mercuri et al., 2005). With progression of the disease, other thigh muscles were also affected, depending on clinical severity. The adductors and semimembranosus muscles were also involved in young patients with minimal functional motor impairment. However, in patients with restricted ambulation a diffuse involvement of the posterolateral muscles of the thigh and of the vastus intermedius was found, with relative sparing of the vastus lateralis, sartorius, and gracilis. Finally, MRI of the legs showed involvement of the soleus muscle and the medial head of the gastrocnemius, with relative sparing of the lateral head.

When these findings were compared with those in patients with LGMD2A without contractures, similar MRI patterns were found (Figure 6.2). However, the pattern of involvement on MRI was different from that seen in other muscular dystrophies with contractures, such as Emery–Dreifuss muscular dystrophy (EDMD) or Bethlem myopathy. Although the differential involvement of the medial and lateral head of the gastrocnemius observed in the calf muscles in patients with LGMD2A was similar to that reported in patients with the dominant form of EDMD (Mercuri et al., 2002), the pattern of muscle involvement at thigh level was different. Although patients with LGMD2A had a more striking involvement of the adductor muscles and relative sparing of the vastus lateralis, the vasti muscles were selectively affected in the dominant form of EDMD. In addition, patients with the X-linked form of EDMD, in contrast to patients with LGMD2A, show a more prominent involvement of the soleus and relative sparing of the

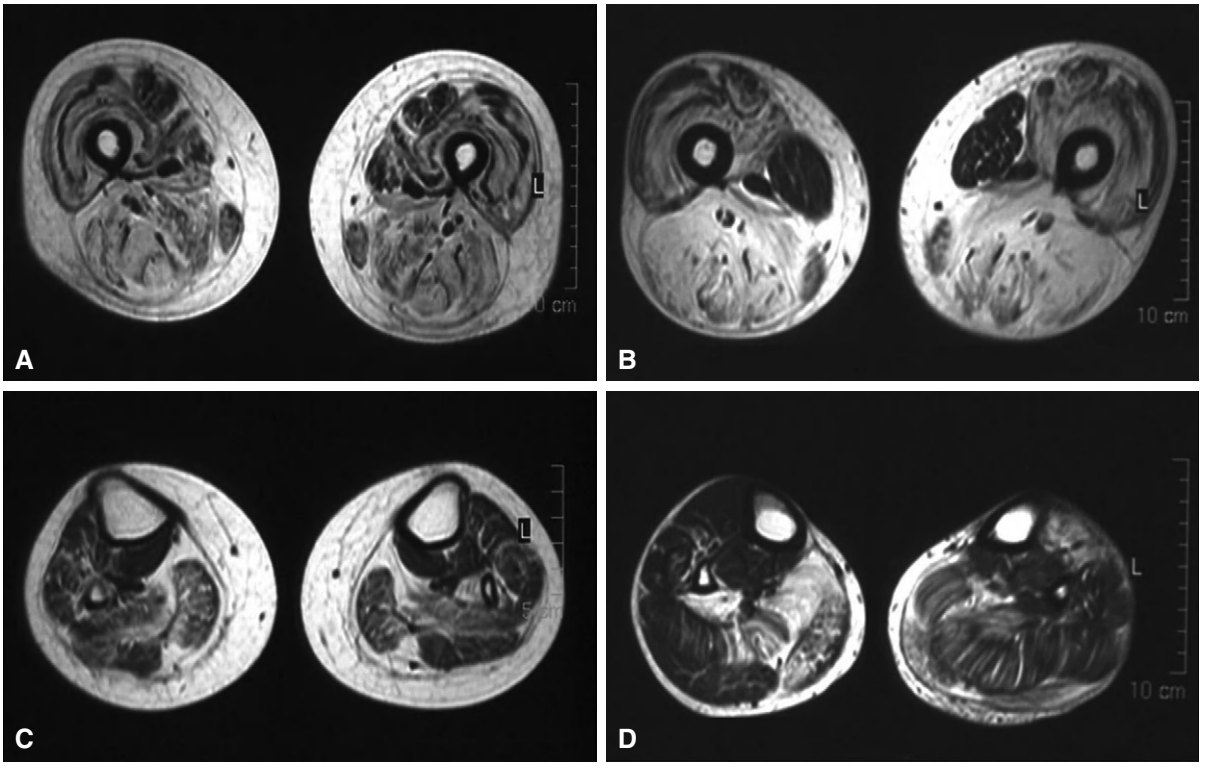

Figure 6.2. Transverse T_1-weighted images through thigh and leg muscles in two different patients. Note the predominant involvement of the posterior group muscles and of the vastus intermedius and medialis in (**A**) and the diffuse involvement of all thigh muscles with relative sparing of the sartorius (**B**). Note the differential involvement between medial and lateral head of the gastrocnemius and the involvement of the soleus (**C**) and the asymmetrical involvement of soleus, gastrocnemius medial and lateral, and tibialis anterior (**D**).

gastrocnemii muscles. Finally, patients with Bethlem myopathy show a typical pattern of muscle involvement with concentric atrophy of rectus and vastus lateralis. Mercuri et al. (2005) also suggest that these specific MRI changes support the mutational screening of the *CAPN3* gene, even in patients with normal expression of calpain-3 in the western blot.

In another study in which the authors compared the muscle MRI pattern of LGMD2I with that of other muscular dystrophies including LGMD2A, marked signal changes were found in the adductor, posterior thigh, and posterior calf muscles in both muscular dystrophies (Fischer et al., 2005). However, in LGMD2A there was selective involvement of the medial gastrocnemius and soleus muscle in the lower legs which was not seen in LGMD2I.

Neurophysiology

Electromyography (EMG) demonstrates increased insertional activity and spontaneous activity in the form of fibrillation potentials and positive sharp waves. In end-stage muscle that is replaced by adipose and connective tissue, insertional activity may be diminished. Motor unit action potentials are small in amplitude, short in duration, polyphasic, and recruit early.

Muscle biopsy

Histochemistry

The muscle biopsy of patients with LGMD2A shows variability of fiber size and interstitial fibrosis, an increased number of internal nuclei, and the presence of some necrotic and regenerating fibers (Figure 6.3A). ATPase staining shows a normal distribution of type I and type II fibers in mildly affected patients, but a conversion to type I predominance is observed in more severely affected patients. Electron microscopy does not show specific changes other than disorganization of myofibrils (Fardeau et al., 1996b).

Initially, the presence of lobulated fibers (see Figure 6.3B) was reported as typical of this dystrophy and their number seems to correlate with progression of the disease, increasing in the endstage of the condition (Guerard et al., 1985). However, the presence of lobulated fibers is also observed in other muscular dystrophies. In a retrospective clinicopathological study of 17 patients displaying a myopathy with lobulated fibers, the authors found this feature not only in LGMD2A but also in a carrier of Duchenne muscular dystrophy, α-sarcoglycanopathy, and facioscapulohumeral dystrophy, amongst others, and their presence did not correlate with the severity of the disease (Figarella-Branger et al., 2002). These fibers were also observed in one pediatric patient, indicating that they can also appear early in the pathology. Finally, in patients with repeated biopsies from different muscles, the number of lobulated fibers was higher in proximal muscles. The authors suggested that muscle sampling is very important to detect lobulated fibers (Figarella-Branger et al., 2002).

It has been reported that muscles with high numbers of lobulated fibers display a differential gene expression pattern compared with that in nonlobulated fibers in muscle biopsies from patients with LGMD2A (Keira et al., 2007). Microarray analysis of muscle biopsies demonstrated differential expression of 29 genes specifically in muscle biopsies rich in lobulated fibers compared with biopsies showing mainly necrotic and

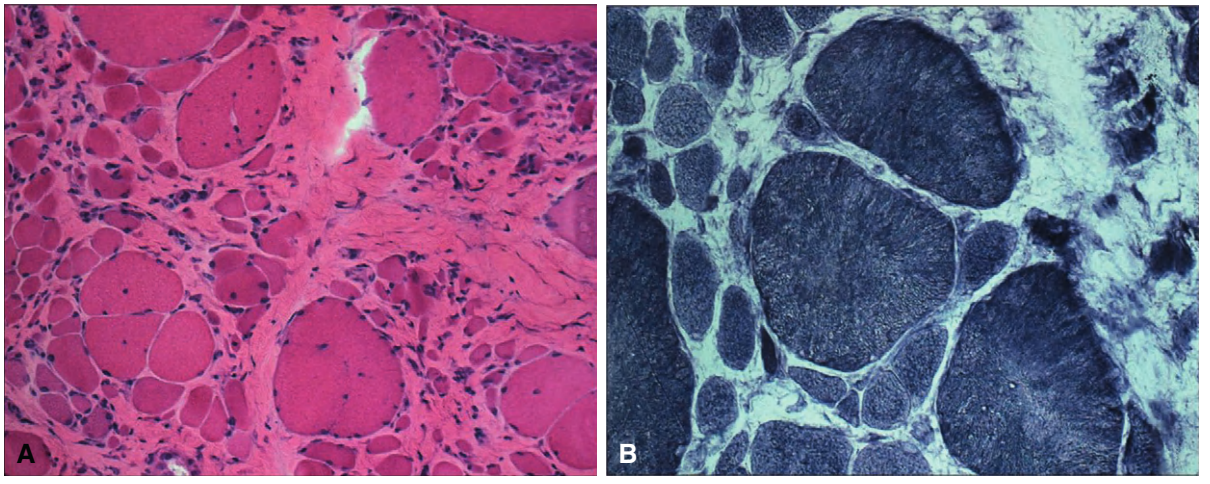

Figure 6.3. Muscle biopsy, cryostat sections. (**A**) Hematoxylin and eosin variation in muscle fiber size, increased number of internal nuclei, necrotic and regenerating fibers, and increased connective tissue. (**B**) Reduced nicotinamide–adenine dinucleotide (NADH) staining showing lobulated fibers.

regenerating fibers. Upregulation of genes associated with actin were observed in lobulated fibers, in agreement with the disorganization of myofibrils previously described in LGMD2A (Keira et al., 2007).

Additionally, some patients with eosinophilic myositis have mutations in the *CAPN3* gene (Krahn et al., 2006b). In addition to muscle biopsies demonstrating inflammatory cell infiltrates containing many eosinophils, there may also be peripheral eosinophilia. Thus, the diagnosis of a calpainopathy should be considered in any patient with eosinophilic infiltrates in the muscle biopsy (Amato, 2008).

Western blot

Immunoblot or western blot analysis of muscle biopsies using monoclonal antibodies specific for calpain-3 can be performed, although the results must be interpreted with caution (Figure 6.4). Some affected individuals with confirmed mutations in the *CAPN3* gene have normal levels of calpain-3 on western blot, as discussed below (Anderson et al., 1998). Furthermore, a secondary reduction of calpain-3 can be observed in other forms of LGMD. In a study of 16 patients with dysferlinopathy, a reduction of calpain-3 was observed in 50% (Anderson et al., 2000). However, mutations in the *DYSF* gene were available only for three patients and the mutational screening was still in progress in the other five. By contrast, in another series of six patients with dysferlinopathy, diagnosed by absence of dysferlin on immunohistochemistry and western blot, all displayed normal levels of calpain-3 (Prelle et al., 2003). In symptomatic carriers of a mutation in the dysferlin gene, normal levels of calpain-3 were observed (Illa et al., 2007). Conversely, a reduction of dysferlin expression has been observed in the muscle biopsies of patients with LGMD2A (Chrobakova et al., 2004; Hermanova et al., 2006). It has been proposed that calpain and dysferlin may interact and play a role in sarcolemma repair (Lennon et al., 2003). These studies suggest that, depending on the mutation in the *CAPN3* gene, expression of dysferlin may or may not be disrupted.

A secondary reduction of calpain-3 has also been reported in patients with tibial muscular dystrophy caused by mutations in the gene that encodes for titin (Haravuori et al., 2001; Hackman et al., 2002). In myofibrils, calpain-3 binds to titin (Sorimachi et al., 2000), and it is reasonable to hypothesize that the impairment of titin in these patients alters calpain-3 expression. Recently, it has been reported that a homozygous titin deletion in exons encoding the C-terminal M-line caused a congenital, purely recessive, titinopathy involving both cardiac and skeletal muscle (Carmignac et al., 2007). Using immunofluorescence the authors observed that titin was able to incorporate into the sarcomeres in these patients, in accordance with the normal muscle bulk and the presence of ultrastructurally normal sarcomeres. These data suggest that the last exons of titin are not important for sarcomere assembly and myogenesis in humans. However, western blot of the muscle biopsies showed absence of calpain-3, indicating disruption of the M-line protein complex and confirming the proposal that titin stabilizes calpain-3 from autolytic degradation. Although these data would explain the myopathy observed in these patients, as *CAPN3* is not expressed in heart muscle, this mechanism cannot explain the cardiomyopathy (Carmignac et al., 2007).

Fanin et al. (2003) showed in a series of patients with LGMD2A that up to 20% had normal levels of calpain-3 in the western blot. These findings have also been shown by other authors (Talim et al., 2001; Lanzillo et al., 2006). It has been proposed that certain mutations in the *CAPN3* gene suppress the normal autocatalytic function of the enzyme, either by affecting interdomain protein interaction or by lowering Ca^{2+} sensitivity (Talim et al., 2001; Fanin et al., 2003, 2007; Lanzillo et al., 2006). Furthermore, it has been reported recently that 32% of a series of patients with mutations in the *CAPN3* gene showed normal proteolytic activity, suggesting that other functions of the protein must be impaired in these patients (Milic et al., 2007). These studies suggest that western blot can be an initial screening test for LGMD2A, although normal calpain-3 levels do not rule out the diagnosis of calpainopathy and mutational analysis of the *CAPN3* gene must be performed.

The use of immunohistochemistry has been also explored for the diagnosis of LGMD2A (Charlton et al., 2009). These authors concluded that the monoclonal antibody Calp3–2C4 (recognizing exon 1) is almost as useful by immunohistochemistry and western blot. However, this last technique is necessary when CAPN3 is detected in muscle frozen sections to show secondary CAPN3 reduction and to identify LGMD2A with variable reduction of CAPN3 bands.

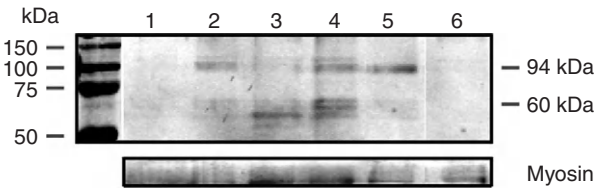

Figure 6.4. Western blot analysis using skeletal muscle extracts in five genetically confirmed patients with limb-girdle muscular dystrophy type 2A (lanes 1, 2, 3, 5, and 6) and a control (lane 4). Note the different expression pattern of calpain-3, from normal levels in lanes 2 and 5, reduction in lane 3, and absence in lanes 1 and 6.

PATHOGENESIS

Molecular genetics

LGMD2A is caused by mutations in the *calpain-3* or *CAPN3* gene located in the chromosome region 15q15.1–q21.1 (Richard et al., 1995). The cDNA is 2466 base pairs (bp) and extends through a 40-kilobase region of genomic DNA. *CAPN3* contains 24 exons of variable length, from 12 bp in exon 13 bp to 309 bp in exon 1 (Richard et al., 1995). The promoter contains consensus sequences that could be regulatory elements specific for skeletal muscle, such as E-boxes. These are binding sites for helix–loop–helix proteins found in members of the family of muscular transcription factors such as MyoD that probably regulates *CAPN3* expression (Richard et al., 1995; Sorimachi et al., 1995; Sorimachi et al., 1996).

The molecular analysis of this gene, given its size, is usually performed by denaturing high-performance liquid chromatography (DHPLC) or single-strand conformational polymorphism (SSCP) and then confirmed by sequencing. DHLPC and SSCP are less expensive than direct sequencing but the number of mutations may be underestimated (Hayashi, 1992; Krahn et al., 2006a). A correct diagnosis is also limited by the clinical variability of the LGMD2A phenotype and by the expression of calpain-3 in muscle, which can be normal in patients with LGMD2A and secondarily reduced in other forms of LGMD (Saenz et al., 2005). Thus, nearly 25% of patients with a phenotype suggestive of LGMD2A do not have mutations in the *CAPN3* gene and another genetic locus may be involved (Saenz et al., 2005); in up to 22% of patients only one mutation is identified (Richard et al., 1995; de Paula et al., 2002; Saenz et al., 2005).

To date more than 300 *CAPN3* mutations have been identified (Kramerova et al., 2007), with a distribution that varies according to specific regions or ethnic group (Leiden muscular dystrophy database, http://www.dmd.nl/capn3_home.html). For example, the frameshift mutation 550delA is considered to be the most frequent mutation in the *CAPN3* gene in Eastern Europe, including Croatia (Milic and Canki-Klain, 2005), Russia (Pogoda et al., 2000), Bulgaria (Todorova et al., 2007), and Turkey (Balci et al., 2006). This mutation is also common in Italy (Fanin et al., 2004, 2005) and has been found in a few families from France and the Netherlands, although less frequently. The 550delA mutation may have originated in the eastern Mediterranean region (Hermanova et al., 2006).

Ten of 13 mutations found in Japanese patients were not observed in other populations (Chae et al., 2001). Others specific mutations are prevalent in isolated communities, such as the Basque mutation, c.2362_2363delinsTCATCT. This mutation is also that most prevalent in Brazil as a result of a founder effect (Urtasun et al., 1998; De Paula et al., 2003).

The analysis of mutations at mRNA level is a rapid and informative method, less expensive than genomic analysis, and could corroborate the pathogenicity of synonymous changes located in coding and noncoding regions that modify pre-mRNA splicing (Chrobakova et al., 2004; Dehainault et al., 2007). It has been reported that full transcripts of *CAPN3* are most common. However, isoforms lacking exon 6 or both exons 15 and 16 are expressed at high levels in adult brain, adult smooth muscle, and embryonic lens (Herasse et al., 1999). In 2003, de Tullio et al. described the presence of calpain-3 protein in adult human tissue other than skeletal muscle. This isoform, produced by the removal of exons 6 and 15, was expressed in peripheral mononuclear blood cells (de Tullio et al., 2003). Along the same lines, molecular study of the dysferlin protein and gene using mRNA from peripheral blood monocytes has been undertaken (Ho et al., 2002; De Luna et al., 2007). Blázquez et al. (2008) examined CAPN3 expression in white blood cells in order to develop a new approach to the molecular diagnosis of LGMD2A at mRNA level in a tissue more accessible than muscle.

However, diagnosis of LGMD2A by analysis of *CAPN3* mRNA must be confirmed in DNA because the results of mRNA and DNA analysis could be discordant. When two null mutations are present, the transcripts produced by both alleles are degraded, but the polymerase chain reaction (PCR) is sensitive enough to amplify residual quantities from both of them. In contrast, in those cases with a null mutation combined with a missense or an inframe mutation, the transcript of the allele that contains the null mutation is degraded, and in the PCR the fragment that maintains the reading frame is amplified and usually seen as homozygous. In muscle, null mutations that introduce a premature translation-termination codon (PTC) in the *CAPN3* transcript are degraded by nonsense-mediated decay (NMD) (Krahn et al., 2007; Stehlikova et al., 2007).The phenomenon of NMD (Frischmeyer and Dietz, 1999; Frischmeyer et al., 2002) has been proposed in some cases of LGMD2A to explain reduced levels of CAPN3 associated with certain mutations (Krahn et al., 2007; Stehlikova et al., 2007).

Clinical–molecular correlations

It might be expected that missense mutations would lead to a less severe phenotype than frameshift mutations, although a severe phenotype associated with a missense mutation (S86F) has been reported (Richard et al., 1997). However, no clinical differences were found depending on the type of mutation present in the *calpain-3* gene (Saenz et al., 2005). Patients homozygous for null/null mutations showed a phenotype similar to that in compound heterozygotes for null/missense, and to that in patients harboring missense/missense

mutations. Nevertheless, in the latter two groups the number of patients was relatively small, and a difference in the severity of the disease depending on the type of mutation cannot be excluded definitively (Saenz et al., 2005).

In the present authors' experience, patients carrying at least one missense mutation have an older mean age of onset than patients with two null mutations. The risk of becoming wheelchair-bound at a determined age is not significantly different in patients with at least one missense mutation (null–missense and missense–missense) compared with that in individuals with two stop codon mutations. However, where the disease has been present for more than 25 years, patients with two null mutations are more likely to be reliant on a wheelchair than those with at least one missense mutation (Saenz et al., 2005).

Structure of calpains

CAPN3 was initially cloned by Sorimachi et al. (1989) and encodes calpain-3, a nonlysosomal intracellular cysteine protease. Calpain-3 belongs to a large family of proteases and is expressed specifically in skeletal muscle. The large subunit is similar in all members of the family, and is composed of four domains (Sorimachi and Suzuki, 2001) (Figure 6.5). The specific function of domain I is not known and shows no similarity with other known proteins. The N-terminal region of this domain has autolytic activity, but it is not fully understood whether this step is necessary for its activation. Domain II contains three active site residues that confer the protease activity of calpain-3. Domain III contains a calcium and phospholipid-binding C2 domain which are important to allow the structural changes necessary for calpain-3 activation. There is no consensus on how domain III contributes to Ca^{2+}-dependent calpain activation, but it appears to amplify the signal from domain IV. It would also regulate enzyme activity and regulation of activity through Ca^{2+}-mediated translocation to the membrane (Tompa et al., 2001). The C-terminal domain, or domain IV, is a calmodulin-like Ca^{2+}-binding domain. It contains five EF-hand sequences that bind the calcium ions and allows interaction with domain VI (or IV′) in the small subunit (Sorimachi et al., 1997). An EF hand is a common structure for binding Ca^{2+} in calmodulin and other Ca^{2+}-binding proteins consisting of a helix (E), a loop, and another helix (F) (Kretsinger, 1976). Domain II is necessary for the protease activity, suggesting that Ca^{2+} requirements are related to the structural organization (Suzuki and Sorimachi, 1998) (see Figure 6.4). In contrast to ubiquitous calpains, CAPN3 does not interact with a small subunit and appears to form homodiomers (Ravulapalli et al., 2005).

Calpain-3 also has three additional unique sequences that show no homology with other known sequences in other proteins, including large subunits in μ- and m-calpains. Due to these insertions, the large subunit of calpain-3 weighs 94 kDa rather than the 80 kDa observed for the ubiquitous calpain isoforms. Sequence NS (or new sequence) is rich in proline residues at the N-terminal domain of the protein, and its function is unknown (Sorimachi and Suzuki, 1992; Richard et al., 1995). Sequence IS1 (or insertion sequence) is located just before the histidine active center of p94 (Richard et al., 1995). It includes three sites that are involved in the regulation of its autocatalytic activity and this could explain why calpain-3 is unstable compared with the other calpains (Kinbara et al., 1998). Finally, sequence IS2 (or insertion sequence), which is located between domains III and IV, is rich in lysine residues near the N-terminal domain and contains a nuclear translocation signal (PVKKKKNKP). This suggest that calpain-3 may have a function in the nucleus processing transcription factors. It has been posited that calpain-3 could be involved in IκBα proteolysis. Lack of calpain-3 would increase cell levels of this protein, which normally inactivates NFκB, which in turn may predispose nuclei to apoptosis (see below) (Sorimachi et al., 1993; Baghdiguian et al., 1999). Deletion of IS2 shows normal levels of calpain-3, suggesting that this sequence is important for the autocatalysis of the protein (Sorimachi et al., 1993).

A two-step model has been proposed to explain the activation mechanism of calpain-3 based on a truncated form of CAPN3 that consists only of domains I and II. In the first step, calcium is bound, promoting a conformational change that favors the formation of the triad cysteine, histidine, and asparagine. This active site allows intramolecular cleavage at the N-terminal region of NS and IS1. In the second step, the enzyme is fully activated by the intermolecular cleavage at the C-terminal regions of NS and IS1 (Kramerova et al., 2007).

Figure 6.5. Diagram of the different domains of calpain-3 (p94). Black strips in domain IV indicate EF hands. IS, insertion sequence; NS, new sequence.

Apoptosis in calpainopathy

The role of apoptosis in muscular dystrophies is controversial. However, using TdT-mediated dUTP nick end labeling (TUNEL) a small percentage of apoptotic nuclei were found in deltoid biopsies from patients with LGMD2A but not in muscle samples from patients with

other muscular dystrophies (Baghdiguian et al., 1999). Furthermore, all TUNEL-positive nuclei also stained with an antibody directed against IκBα. In addition, whereas in control biopsies NFκB was expressed in some nuclei, in LGMD2A biopsies the staining was found in the subsarcolemmal region surrounding myonuclei. The authors proposed a model in which IκBα accumulates due to a lack of calpain-3 proteolytic activity and translocates to the nucleus. This would block the transcriptional activity of NFκB, sensitizing cells to apoptosis (Baghdiguian et al., 1999). However, the number of TUNEL-positive nuclei was too small to explain the dystrophic changes observed in late stages of the disease. In a recent paper, the same authors showed that the antiapoptotic factor cellular FLICE inhibitory protein (c-FLIP) is downregulated in the muscle biopsies of patients with LGMD2A (Benayoun et al., 2008). Although the authors observed similar findings in their mouse model (Richard et al., 2000), similar studies in a different mouse model (Kramerova et al., 2004) demonstrated that apoptotic nuclei were found mainly in or near necrotic lesions and located outside the sarcolemma, and corresponded to immune cells. The authors concluded that apoptosis in murine calpainopathy is secondary to muscle inflammation (Kramerova et al., 2004). It may be possible that the milder phenotype observed in the mouse models compared with the human phenotype accounts for the lack of apoptotic nuclei that is found late in the disease in the human pathology (Kramerova et al., 2007).

Interactions of calpain-3 with other proteins of skeletal muscle

A secondary reduction of calpain-3 occurs in dysferlinopathies, and dysferlin may be reduced in LGMD2A, suggesting an interaction between these two proteins. A model has been proposed in which calpain-3 and dysferlin, together with caveolin-3 and annexins-1 and -2, form a membrane complex important for muscle membrane repair (Lennon et al., 2003). In a mouse model of LGMD2A it was demonstrated that only some fibers lost their integrity in experiments using Evans Blue dye (Richard et al., 2000). In fact, in another study using the same mice, downhill run tests, treadmill training, and wire testing after being injected with Evans Blue showed no accumulation of dye within the muscle fibers, demonstrating the absence of membrane alteration (Fougerousse et al., 2003). The authors suggested that the action of titin as a stabilizer of calpain-3 (see above) may play an important role in the pathophysiology of LGMD2A. A signal transduced through titin could free calpain-3, which could then participate in an adaptative response of the cell, modifying its contractile properties (Sorimachi et al., 2000; Fougerousse et al., 2003).

As stated above, in myofibrils, calpain-3 binds to titin and the activity of calpain-3 is probably suppressed by this binding (Sorimachi et al., 2000). Mutations in titin are responsible for tibial muscular dystrophy (TMD) and LGMD2J, and the authors suggested that the pathophysiology of these diseases may in part be due to secondary calpain-3 deficiency (Haravuori et al., 2001; Hackman et al., 2002; Udd et al., 2005). The secondary calpain-3 defect found in TMD was also observed in the Mdm (muscular dystrophy with myositis) mouse, which bears a mutation in the N2A domain of titin and is suggested to be a mouse model for TMD (Haravuori et al., 2001; Huebsch et al., 2005). Homozygous mutation at the 2q locus may thus be capable of producing yet another LGMD (Haravuori et al., 2001). The binding of CAPN3 to titin is thought to stabilize the protease and prevent its autolysis, perhaps in a manner analogous to that of calpastatin and the ubiquitous calpains (Garvey et al., 2002).

Calpain-3 can cleave filamin C *in vitro*, suggesting that this protein may be an *in vivo* substrate for calpain-3, functioning to regulate protein–protein interactions with the sarcoglycans (Guyon et al., 2003). Thus, calpain-mediated remodeling of cytoskeletal–membrane interactions, such as those that occur during myoblast fusion and muscle repair, may involve regulation of filamin C–sarcoglycan interactions (Guyon et al., 2003). Calpain-3 may also cleave fodrin (Ono et al., 1998), talin, filamin A, vinexin, and ezrin (Taveau et al., 2003). However, these findings have not been observed *in vivo.* In one study, Cohen et al. (2006) set up a model to investigate potential calpain-3 substrates *in vivo*. Using bidimensional electrophoresis and mass spectrometry, these authors compared the proteome of transgenic mice that overexpress CAPN3 and of their nontransgenic counterparts. The proposal was that high expression of calpain-3 would increase proteolysis of the enzyme substrate *in vivo*. This would be readily detected as spots that would become faint or even disappear in the overexpressers. They found 10 protein spots differentially expressed in the transgenic mice, corresponding to myofibrillary and metabolic proteins. Using this methodology these authors were able to demonstrate that myosin light chain 1 (MLC-1) was clearly reduced in the transgenic mice, suggesting that this myofibrillar protein is a substrate for calpain-3 *in vivo* (Cohen et al., 2006).

FUTURE AND CURRENT RESEARCH

Mouse models

There are two mouse knockout models of *CAPN3* (Richard et al., 2000; Kramerova et al., 2004). The first model consisted of a deletion of exons 2 and 3 of

CAPN3 and was generated in two different genetic backgrounds (129Sv and C57BL/6). Both *CAPN3* knockout animals showed a progressive mild muscular dystrophy with central nuclei, fiber splitting, areas of necrosis/regeneration, and small infiltrates of mononuclear cells (Richard et al., 2000). The histopathological abnormalities appear earlier in the 129Sv mouse, suggesting that modifier genes would shape the phenotype. Pathological changes were always clustered in particular areas and were demonstrated by colocalization in the same fibers that take up Evans Blue dye, used to check membrane permeability, by immunodetection of IκBα, and by TUNEL, used to detect apoptotic nuclei. As observed in the muscle biopsies of patients with LGMD2A, expression of the dystrophin–sarcoglycans complex was preserved in the mice (Richard et al., 2000).

In another study employing this model, a small but significant global muscle atrophy was found (Fougerousse et al., 2003). Studies of mechanical properties revealed that slow-twitch muscles were significantly weaker. However, Evans Blue dye uptake by muscle fibers after the downhill run test and treadmill training protocol did not disclose any differences between exercised and nonexercised animals. Eccentric contraction protocols showed no differences between *CAPN3* knockout and wild-type mice (Fougerousse et al., 2003).

Muscle histology in the second *CAPN3* knockout model also demonstrated features similar to those observed in human LGMD2A (Kramerova et al., 2004). Ultrastructural analysis revealed abnormal sarcomere organization. However, in contrast to the findings observed in the previous model, apoptotic nuclei were present only in or near necrotic lesions and outside the sarcolemma, and corresponded to infiltrating immune cells. The authors inferred that the apoptosis seen in calpainopathy is secondary to muscle inflammation and not a primary feature of its pathogenesis. *In vitro*, they observed that myotubes displayed an abnormal sarcomere assembly. They also found that some pathogenic mutations impaired the interaction of calpain-3 and titin. The authors concluded that the loss of interaction between these two proteins can lead to pathogenesis in LGMD2A (Kramerova et al., 2004).

Apart from knockout mice, other mouse models have been generated in an attempt to understand further the pathophysiology of LGMD2A (Tagawa et al., 2000; Spencer and Mellgren, 2002; Cohen et al., 2006 – the model of Cohen and colleagures has been described above). Three lines of transgenic mice expressing inactive p94 (with a mutated or deleted active site) were obtained with variable mRNA expression of mutated p94. One line showed decreased grip strength. Histopathological studies of skeletal muscle revealed increased numbers of lobulated fibers, central nuclei, tubular aggregate-like structures, and fiber splitting in older mice (106 weeks) but not in 40-week-old mice. The authors suggested that accumulation of the mutated protein causes the myopathy phenotype (Tagawa et al., 2000).

In addition, an interesting model of overexpression of three different isoforms showed that increased levels of the protein are neither toxic nor impair normal skeletal muscle function (Spencer and Mellgren, 2002). However, the isoform lacking exon 6 has abnormal muscles. Surprisingly, in the third isoform lacking exon 15, only the soleus muscle was affected. The abnormal muscles do not present signs of degeneration/regeneration, serum CK levels are normal, and no evidence of membrane damage was observed. The authors suggest that this phenotype must be related to a defect in muscle maturation. Importantly, the results obtained with the truncated isoforms suggest that they may produce deleterious effects if used in gene therapy (Spencer and Mellgren, 2002).

Experimental therapeutics

Mouse models of calpainopathy have been used to test different possible therapies for the treatment of LGMD2A. In gene therapy study, use of a modified adeno-associated virus (AVV) vector bearing a promoter specific for skeletal muscle administered intra-arterially resulted in improvement not only at the histological level, with a decrease in fiber size variability, but also restoration of the force and atrophy specifically of the soleus muscle, one of the most affected muscles in this mouse model (Richard et al., 2000). Normal levels of the human calpain-3 and proteolytic enzyme activity were achieved in another study (Bartoli et al., 2006). A study of *CAPN3* knockout mice injected with AAV-mediated expression of a mutated myostatin propeptide resulted in an increase in muscle mass and absolute force in calpain-3-deficient mice (Bartoli et al., 2007).

In conclusion, AAV vectors seem to be the best approach due to their efficiency and persistency of gene transfer in all the animal models tested so far. In fact, two phase I/II clinical gene therapy trials using this kind of vector are currently being prepared at Genethon, France (Daniele et al., 2007).

Gene expression profiling in LGMD2A

In a recent study, the present authors looked at gene expression profiling in 10 muscle samples from patients with LGMD2A genetically confirmed using array technology, and compared the results with 10 normal muscle

samples. They found upregulation of genes related to extracellular matrix (different collagens), cell adhesion (fibronectin), muscle development (myosins and melusin), and signal transduction. The authors proposed that different proteins located or participating in the costameric region are implicated in processes regulated by calpain-3 during skeletal muscle development. In addition, frizzled-related protein (*FRZB*) is upregulated in LGMD2A muscle samples, suggesting that β-catenin regulation is also altered at the Wnt signaling pathway, leading to an incorrect myogenesis. Finally, the authors also found upregulation of interleukin-32 and immunoglobulin genes, perhaps explaining the eosinophilic infiltration observed in presymptomatic stages (Saenz et al., 2008).

DIAGNOSIS AND TREATMENT

Diagnosis

The diagnosis should be considered in patients with the pattern of muscle involvement on clinical examination or skeletal muscle MRI, particularly patients of eastern or southern European backgrounds or from Brazil. A muscle biopsy can be done to confirm the diagnosis of a muscular dystrophy of some type, and western blot analysis can be performed to see whether there is a deficiency of calpain-3. However, as western blot can be normal in approximately 20% of patients with LGMD2A, and calpain-3 can be secondarily deficient in other types of dystrophy, a definite diagnosis of LGMD2A requires genetic confirmation. In a recent study, Fanin et al. (2009) showed that the probability of diagnosing calpainopathy was very high in patients showing either a quantitative (80%) or a functional (88%) calpain-3 protein defect.

Treatment

There are no curative treatments for this disease, although there are possibilities for therapy in the future. Although there are no specific reports on supportive care, physical and occupational therapies are important (Eagle, 2002). Functional ability can be improved by preventing contracture development through stretching and splinting orthoses. There is no agreement on the benefit of exercise; however, in accordance with guidelines for other muscular dystrophies, gentle exercise and avoidance of prolonged immobility would be recommended. Monitoring of possible respiratory problems is important to prevent complications and to apply the ventilatory support needed for every stage of the disease (Norwood et al., 2007).

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Chapter 7

Dysferlinopathies

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INTRODUCTION

Dysferlin is a sarcolemmal protein that appears to have an important role in repairing defects in skeletal membrane by regulating vesicle fusion with the sarcolemma. It does not have any major interaction with the dystrophin–sarcoglycan complex. The clinical phenotype of the dysferlinopathies is quite variable. Affected individuals usually present with early involvement of the posterior calf muscles (Miyoshi myopathy), but can present with proximal greater than distal weakness, similar to other limb-girdle muscular dystrophies (LGMD2B), and less commonly with anterior tibial weakness. Furthermore, patients may have any combination of proximal, anterior tibial, or posterior calf weakness (Illarioshkin et al., 1996; Mahjneh et al., 1996; Illa et al., 2001; Mahjneh et al., 2001; Suzuki et al., 2004). Occasional patients manifest with an axial myopathy with rigid spine syndrome or hyperkyphosis resembling bent spine syndrome. The dysferlinopathies typically present in the late teens or early twenties, although onset as late as the seventies has been reported (Klinge et al., 2008).

In a study of 407 muscle biopsies from patients with unclassified myopathies (nondystrophinopathy and nonsarcoglycanopathy), 6.5% had abnormal dysferlin by western blot and immunostaining (Fanin et al., 2001). Dysferlinopathy accounted for 1% of patients with an unknown LGMD and 60% of patients with a distal myopathy. Clinically, 80% of the patients with dysferlinopathy presented with distal weakness, 8% had a LGMD phenotype, and 6% had asymptomatic hyperCKemia. A more recent study of 40 patients from 35 families in France revealed the following clinical phenotypes: Miyoshi myopathy in 25%, LGMD in 25%, proximodistal in 35%, pseudometabolic (distal leg swelling with myalgia) in 10%, and asymptomatic hyperCKemia in 5% (Nguyen et al., 2007). Although typically inherited in an autosomal recessive fashion, a recent report noted that carriers may rarely become symptomatic (Illa et al., 2007).

CLINICAL FEATURES

Miyoshi myopathy

This myopathy was initially reported in Japan (Miyoshi et al., 1986). Patients with Miyoshi myopathy generally present in their teens or twenties with atrophy and weakness of the posterior calf muscles. An early sign is the inability of affected individuals to stand on their tiptoes. This is in contrast to many other forms of LGMD that more typically have calf muscle hypertrophy or pseudohypertrophy. Occasionally, however, at the onset patients may have enlarged, painful calf muscles that subsequently rapidly atrophy (Diers et al., 2007; Nguyen et al., 2007). Not uncommonly, involvement of the calf muscles is asymmetrical.

Over time, the weakness progresses to involve the posterior compartment of the thighs (e.g., the hamstrings), and later the hip girdle. The extensor muscles of the forearms may also become weak and atrophy, but the brachioradialis and hand intrinsics are typically spared. The rate of progression can be quite variable with some individuals progressing rapidly over a few years to being nonambulatory, whereas others have weakness and atrophy restricted to the posterior calf muscles for prolonged period of time. One-third of patients are in a wheelchair within 10 years of onset (Linssen et al., 1997). Katz and colleagues (2003)

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described patients with a Miyoshi phenotype that did not have a primary dysferlinopathy. These patients differed from those with dysferlinopathy by their later age of onset (usually over the age of 30 years) and only slightly to moderately raised serum creatine kinase (CK) levels.

Anterior tibial myopathy

Illa and colleagues reported a family that presented with a severe distal weakness, predominately affecting the anterior tibial muscles, with only mild proximal leg weakness in their twenties (Illa et al., 2001; Vilchez et al., 2005). Over a period of 5–8 years, the patients with anterior tibial myopathy due to dysferlinopathy developed weakness in wrist and finger flexors as well as in the biceps brachii. They progressed such that they lost the ability to ambulate between the ages of 30 and 50 years. A similar clinical phenotype with anterior tibial greater than gastrocnemius weakness was reported previously in small group of Russian patients with dysferlinopathy (Ilarioshkin et al., 1996). Furthermore, a "scapuloperoneal myopathy" manifested by early steppage gait has been reported in Japanese patients (Nakagawa et al., 2001).

This distal pattern of weakness resembles Charcot–Marie–Tooth disease, except that the bulk of extensor digitorum brevis muscle in the feet and sensation is spared. This phenotype also mimics the Laing-type distal myopathy associated with mutations in the gene encoding for myosin heavy chain 1. However, Laing distal myopathy is inherited in an autosomal dominant fashion, is associated with normal or only slightly raised CK levels, and has hyaline bodies formed by large deposits of myosin heavy chain 1 on biopsy. Early involvement of the anterior tibial muscles is also typical of the Nonaka form of distal myopathy (also known as autosomal recessive hereditary inclusion body myopathy), but dysferlinopathies are distinguished by the markedly increased serum CK levels and lack of rimmed vacuoles on muscle biopsy in anterior tibial myopathy due to dysferlinopathy. Other forms of distal myopathy (Udd type, and Markesbury–Griggs) are associated with early anterior tibial weakness but usually present later in life in an autosomal dominant fashion, and biopsies reveal rimmed vacuoles or myofibrillar abnormalities not seen in dysferlinopathies.

Limb-girdle muscular dystrophy type 2B

As with other limb-girdle dystrophies, patients with LGMD have early and prominent involvement of the proximal muscles of the legs worse than the arms. Although from a symptomatic standpoint the proximal muscles are more affected, it is not uncommon to find atrophy and weakness of the posterior calf or anterior muscles. Some groups have termed this pattern as limb-girdle distal, proximodistal, or scapuloperoneal myopathy (Ilarioshkin et al., 1996; Nakagawa et al., 2001; Nguyen et al., 2007). Onset is usually between the second and fourth decade of life.

Axial myopathy

Occasional patients manifest with symptoms and signs referable to the paraspinal muscles. A recent report described a 52-year-old woman with 5 years of low back pain, mild hyperlordosis, and thoracic kyphoscoliosis who had extensive fatty degeneration of the erector spinae muscles on computed tomography (CT) (Seror et al., 2008). CT also demonstrated involvement of the posterior thigh and calf muscles. Another case of marked fatty replacement of the paraspinal muscles was reported in a 50-year-old man who presented with rigid spine syndrome (Nagashima et al., 2004). The patient also showed CT involvement of shoulder and pelvic girdle, hamstrings, and calf muscles. CT may reveal low density in the paraspinal muscles at an early age, even in patients with a Miyoshi myopathy clinical phenotype. Anecdotally, we have assessed for dysferlin mutations in several patients with neck extensor myopathy and camptocormia, but have found none with a dysferlinopathy.

LABORATORY FEATURES

Muscle enzymes

Serum CK levels are usually markedly raised (usually 35–200 times normal).

Muscle imaging

CT and magnetic resonance imaging (MRI) demonstrate areas of decreased attenuation in the gastrocnemius muscles and to a lesser extent the adductor magnus, hamstrings, and gluteus minimus muscles in Miyoshi myopathy (Linssen et al., 1997; Illa et al., 2001). In patients with anterior tibial myopathy, an early predilection for fatty replacement in the anterior tibial muscles is appreciated (Illa et al., 2001). As noted above, CT also may reveal low density in the paraspinal muscles in patients with axial myopathy (Nagashima et al., 2004; Seror et al., 2008), but also in more typical Miyoshi myopathy. Skeletal muscle MRI is more sensitive than CT; MRI abnormalities are evident prior to symptomatic involvement (Brummer et al., 2005; Okahashi et al., 2008). In Miyoshi myopathy, initial MRI scans demonstrate signal abnormalities characteristic of myoedema in posterior compartment muscles of the distal lower extremities legs followed by fatty degeneration. Further MRI changes indicate the clinical

progression of the myopathy (Brummer et al., 2005; Okahashi et al., 2008).

Skeletal muscle MRI findings in patients with the LGMD2B phenotype are more variable (Fischer et al., 2005). Mild to severe diffuse hyperintense signal changes are seen in the gluteus maximus and medius muscles in the pelvic girdle (Figure 7.1A). Moderate to severe hyperintense signal changes are evident in both the anterior and posterior thighs (Figure 7.1B). In the distal legs, scans typically show severe involvement of the gastrocnemius and soleus muscle, as seen in Miyoshi myopathy. Moderate to severe hyperintense signal abnormalities in the anterior lower leg compartment are appreciated, particularly in subjects with the anterior tibial phenotype. A recent study demonstrated that MRI findings in the thighs and distal legs in patients with LGMD2B presentation and those with Miyoshi phenotype are actually quite similar, suggesting that we avoid splitting these disorders and just use the term dysferlinopathy (Paradas et al., 2010).

Neurophysiology

Electromyography demonstrates fibrillation potentials and positive sharp waves in affected muscle groups. Over time, as muscle is replaced by adipose and connective tissue, the insertional activity may become decreased. Early recruitment of small-amplitude, short-duration, motor unit action potentials are observed in weak muscles. Motor and sensory nerve conduction studies should be normal.

Muscle histopathology

Histochemistry

Muscle biopsies demonstrate variability in fiber size, increased endomysial connective tissue, and scattered necrotic and regenerating fibers, as in other muscular dystrophies (Figure 7.2). Immunostaining reveals normal dystrophin, sarcoglycan, α-dystroglycan, and merosin. Utilizing dysferlin autoantibodies, absent or diminished

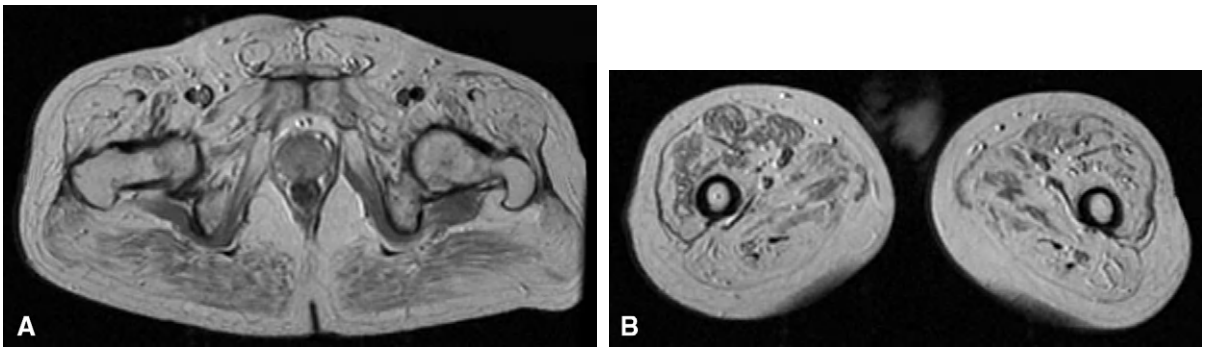

Figure 7.1. Skeletal muscle magnetic resonance imaging (MRI). (**A**) Skeletal muscle MRI scan (T_1-weighted/proton density) through the pelvis demonstrates relative preservation of the gluteus muscles at this level. (**B**) Scan through the thigh reveals severe fatty infiltration of all the muscles in the thigh, worse in the posterior compartment.

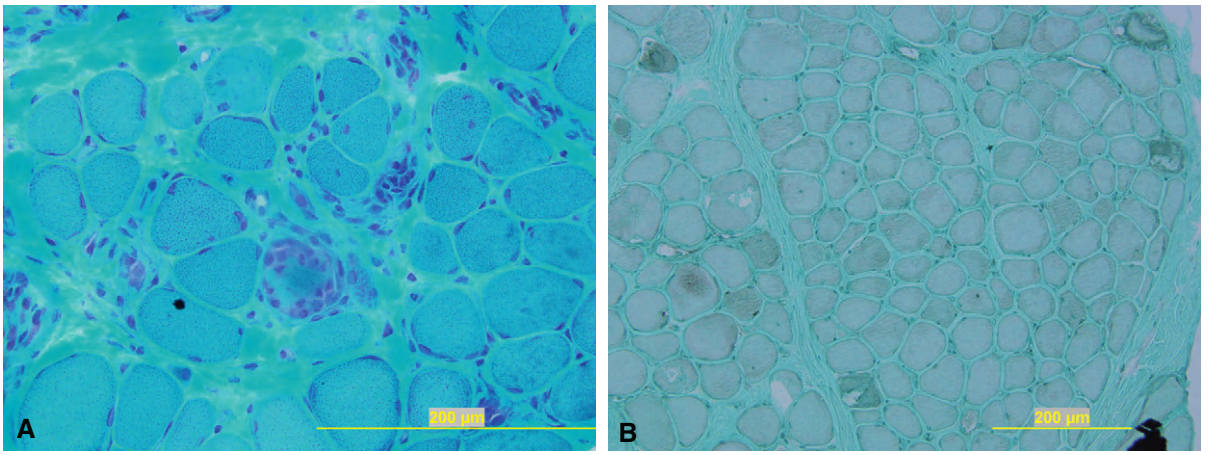

Figure 7.2. Histopathology. Muscle biopsy demonstrates variability in fiber size, increased endomysial connective tissue, and an inflammatory cell infiltrate surrounding and appearing to invade a non-necrotic muscle fiber (**A**, modified Gomori trichrome stain). Immunohistochemistry studies demonstrate diminished sarcolemmal staining with antidysferlin antibodies; however, there may be increased cytoplasmic staining (**B**).

sarcolemmal staining is evident; however, there may be increased cytoplasmic staining. Reduced sarcolemmal dysferlin immunostaining is not specific for a primary dysferlinopathy as this may occasionally be observed in other types of dystrophy (e.g., calpainopathies, LGMD2A) (Piccolo et al., 2000). Thus, confirmatory tests need to be performed to insure a primary deficiency.

Notably, the muscle biopsy may demonstrate marked mononuclear inflammatory cell infiltrate in the endomysium and perivascularly, such that patients are not uncommonly misdiagnosed as having polymyositis (Rowin et al., 1999; McNally et al., 2000; Gallardo et al., 2001). A helpful feature is that the inflammatory cells usually do not appear to invade non-necrotic fibers, unlike the observation in polymyositis. Another helpful feature on immunohistochemistry is the demonstration of membrane attack complex (MAC) on the sarcolemma of non-necrotic muscle fibers in dysferlinopathies and other dystrophies with inflammation (Selcen et al., 2001). Such deposits of MAC are not appreciated in biopsies of patients with any of the primary inflammatory myopathies (e.g., polymyositis, dermatomyositis, and inclusion body myositis). Interestingly, a recent report also demonstrated that some patients with dysferlinopathy have sarcolemmal and interstitial amyloid deposits (Spuler et al., 2008). On electron microscopy, the basement membrane is reduplicated, the sarcolemma invaginations are disrupted, papillary exophytic defects of the muscle membrane are apparent, and subsarcolemma vesicles accumulate (Selcen et al., 2001).

Western blot

Immunoblot or western blot analysis of muscle biopsies demonstrates a marked reduction or absence of dysferlin. In addition, a secondary reduction of calpain-3 can be seen (Anderson et al., 2000). However, other small series of patients with primary dysferlinopathy have noted normal calpain-3 content (Prelle et al., 2003). In addition, a reduction of dysferlin expression has been observed in the muscle biopsies of patients with LGMD2A (Chrobakova et al., 2004; Hermanová et al., 2006).

PATHOGENESIS

Molecular genetics

Mutations within the dysferlin gene, *DYSF*, are the cause of Miyoshi myopathy, LGMD2B, distal myopathies with anterior tibial weakness, and rare axial myopathies (Bashir et al., 1998; Liu et al., 1998; Illarioshkin et al., 2000; Illa et al., 2001; Weiler et al., 1999). There does not appear to be any correlation in regard to the type and location of mutation and the clinical phenotype. *DYSF* contains 55 exons of variable length that are transcribed into an approximately 8.5-kb mRNA molecule. This mRNA encodes the 2080-amino-acid dysferlin protein.

Dysferlin is a 239-kDa protein that shares amino-acid sequence homology with *Caenorhabditis elegans* spermatogenesis factor, FER-1 – hence the origin of its name. Dysferlin is widely expressed in various tissues but is particularly abundant in skeletal and cardiac muscle. Dysferlin is also present within circulating monocytes; western blot of these cells isolated from blood samples of affected individuals can confirm the deficiency (Ho et al., 2002). In muscle, dysferlin is located predominantly on the subsarcolemmal surface on the muscle membrane, but it has a small transmembrane spanning tail. It is also found in cytoplasmic vesicles (Glover and Brown, 2007).

The intracellular portion of the dysferlin protein appears to have seven C2 domains and nested repeat sequences termed DysfN and DysfC, of unknown function (Figure 7.3) (Glover and Brown, 2007). These C2 domains are named for "conserved 2" sequences that contain approximately 130 amino acids which bind anionic phospholipids in a calcium-dependent manner (Nalefski and Falke, 1996). It is believed that these sequences are important for fusion of vesicles and the muscle membrane during repair, as discussed in the next section.

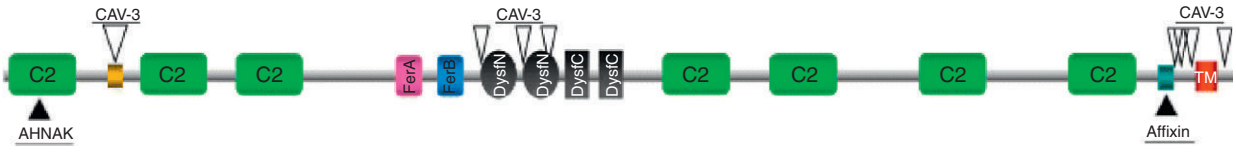

Figure 7.3. Annotation of dysferlin protein structure. Seven putative dysferlin C2 domains are represented, along with ferlin protein central domains (designated FerA and FerB) as well as four Dysf domains (two each of DysfN and DysfC). Predicted caveolin-3 (CAV-3)-binding sites at an N-terminal WW domain-binding motif and at central and C-terminal scaffolding domain-binding motifs are depicted, and experimentally determined binding sites to affixin and AHNAK are shown (arrowheads). (Reproduced with permission from Glover and Brown, 2007.)

Interactions with other proteins and membrane repair in dysferlinopathy

Dysferlin does not appear to have a significant interaction with the dystrophin–glycoprotein complex and thus immunostaining for dystrophin, dystroglycans, merosin, and the sarcoglycans is normal. Recent studies suggest that dysferlin plays an important role in patching defects in skeletal membrane by regulating vesicle fusion with the sarcolemma (Bansal et al., 2003; Cenacchi et al., 2005). Normally, increased dysferlin expression is present at sites of membrane injury. It is hypothesized that dysferlin-harboring cytoplasmic vesicles aggregate and form a "patch" by fusing with the injured membrane (McNeil et al., 2001; Glover and Brown, 2007). Elegant studies involving microinjury to isolated myofibers from normal and dysferlin-null mice have demonstrated that the time course and extent of membrane repair are significantly impaired in dysferlin-deficient muscle tissue (Bansal et al., 2003; Lennon et al., 2003). A model has been proposed in which calpain-3 and dysferlin, together with caveolin-3, annexin A1 and A2, affixins, and AHNAK, form a membrane complex important for muscle membrane repair (Figure 7.4) (Lennon et al., 2003; Glover and Brown, 2007; Huang et al., 2007).

Increased expression of annexin A1 and A2 protein are evident in muscle biopsies from patients with dysferlinopathy (LGMD2B and Miyoshi myopathy), caveolinopathy (LGMD type lC), and dystrophinopathy (Cagliani et al., 2005). Of note, these annexins are

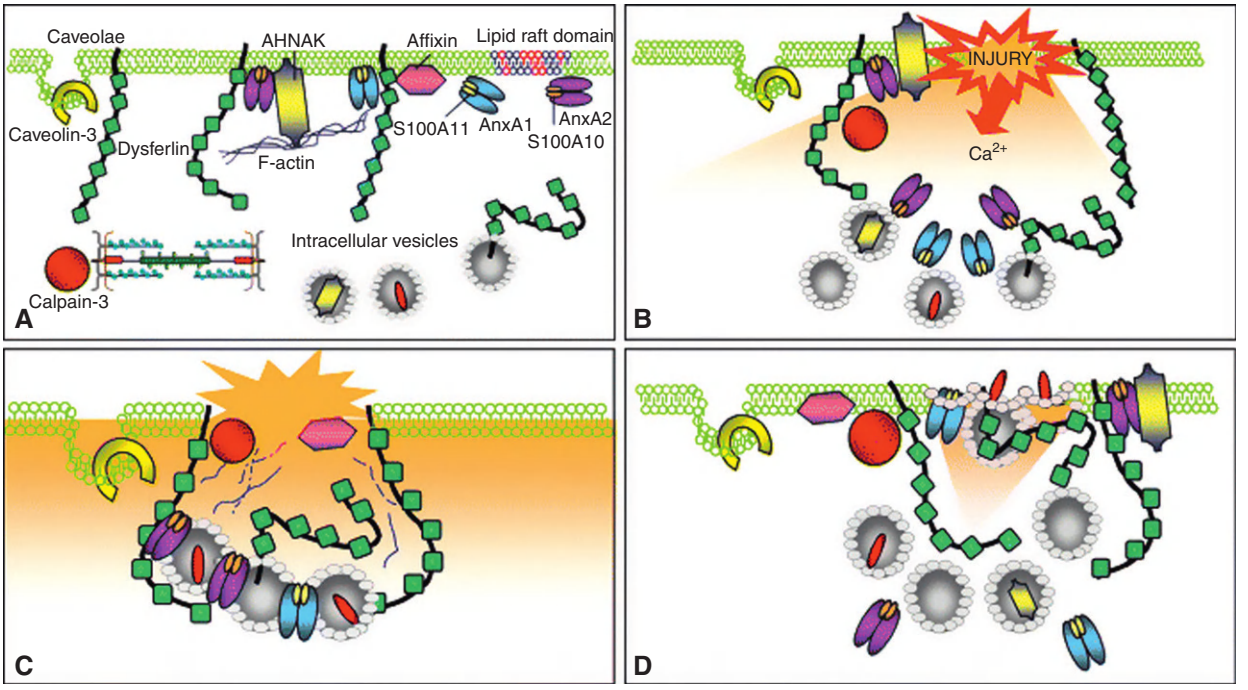

Figure 7.4. Dysferlin and the membrane repair complex in skeletal muscle. (**A**) In normal myofibers, dysferlin is localized by its C-terminal transmembrane domain to cytoplasmic vesicles and the sarcolemma, where it is associated with AnxA1 and AnxA2, AHNAK, affixin, and caveolin-3. Calpain-3 is rendered inactive through interaction with titin. Annexins A2 and A1 are associated with lipid raft and nonraft membrane domains, respectively, in heterotetrameric complexes with their respective S100-binding proteins. Annexin A2/S100A10 also forms a Ca^{2+}-regulated multimeric complex with AHNAK and actin at the plasma membrane. Affixin is found in both the cytoplasm and the sarcolemma, where it binds to dysferlin. Caveolin-3 is a principal component of caveolar membranes in skeletal muscle and may play a role in post-Golgi dysferlin trafficking. (**B**) On injury of the membrane, Ca^{2+} influx raises the intracellular Ca^{2+} concentration locally, triggering the patch repair response. Ca^{2+} influx is also the primary activation signal for calpain-3. Calpain may contribute to the patch repair response by cleaving annexin proteins at their N-termini or by cleaving cytoskeletal proteins such as talin and vimentin to mediate disassembly of the damaged actin cytoskeleton. (**C**) Intracellular vesicles are aggregated by AnxA1 and AnxA2 to form an endomembrane patch that is trafficked to the site of disruption. Vesicle trafficking is facilitated by affixin, through cytoskeleton reorganization in the site surrounding the injury. (**D**) Dysferlin molecules present on repair vesicles and the plasma membrane mediate docking and fusion of the patch, sealing the membrane breach, and preventing further influx of Ca^{2+} ions. After repair, some antigens previously exposed only to the interior of cytoplasmic vesicles are now exposed to the extracellular face of the muscle cell. AnxA1, annexin A1; AnxA2, annexin A2. (Reproduced with permission from Glover and Brown, 2007.)

widely expressed proteins that appear to be involved in vesicle trafficking and fusion (Raynal and Pollard, 1994). The annexins normally colocalize with dysferlin at the sarcolemma. However, in dysferlinopathies, annexin A1 does not seem to associate with dysferlin, although annexin A2 still retains this interaction. Notably, resealing of damaged membranes in cultured cells is impaired by blocking annexin A1 (McNeil et al., 2006). Annexins A1 and A2 are thought to be possible targets for calpain cleavage, thus possibly tying in calpain-3 with muscle membrane repair. As mentioned above, a secondary reduction in calpain-3 concentration occurs in some patients with dysferlinopathy (Anderson et al 2000), whereas reduced dysferlin levels can also be observed in patients with calpainopathy (LGMD2A) (Chrobokova et al., 2004).

Affixin (β-parvin), an adhesion molecule, also colocalizes with dysferlin as well as with α-actinin (Matsuda et al., 2005). It is possible that this dysferlin–affixin–α-actinin interaction may be important in the cytoskeletal reorganization necessary for vesicle patch trafficking and exocytotic membrane sealing (Yamaji et al., 2004).

Caveolin-3 is a major protein found in caveolae, which are 50–100-nm invaginations of the plasma membrane (Parton, 1996; Parton et al., 1997). Mutations in the gene that encodes for caveolin-3 are responsible for LGMD1C. Of note, caveolin-3 colocalizes with dysferlin on the sarcolemma as well as on developing T-tubules, particularly in regenerating fibers (Parton et al., 1997; Shaul and Anderson, 1998; Hernandez-Deviez et al., 2006). Likewise, AHNAK, a protein considered to be important in cell membrane differentiation, repair, and signal transduction, also colocalizes with dysferlin on the sarcolemmal membrane and on T-tubules (Huang et al., 2007; Klinge et al., 2007). Dysferlin also coprecipitates by immunoprecipitation with the dihydropyridine receptor, a protein complex localized in T-tubules. In primary caveolinopathies, sarcolemmal expression of both dysferlin and AHNAK are secondarily reduced. Dysferlin, at least, appears to be retained in the Golgi complex (Hernandez-Deviez et al., 2006). Thus, impaired membrane repair may be a pathogenic mechanism in LGMD1C as well.

FUTURE AND CURRENT RESEARCH

Mouse models

Mouse models for dysferlinopathy exist, including two that arose spontaneously. In the SJL mouse, a deletion within the mouse dysferlin gene deletes sequences that code for 57 amino acids (Bittner et al., 1999). This leads to a subtle reduction in the size of the protein and a substantial (85%) reduction in levels, as assessed on western immunoblot. It is therefore of interest that this mouse has a slowly progressive myopathy and is reported to develop myositis spontaneously (Bernard and Carnegie, 1975; Rosenberg et al., 1987). In the A/J mouse, a unique ETn retrotransposon insertion within intron 4 of the mouse dysferlin gene sufficiently disrupts the gene that expression of dysferlin is essentially eliminated. These mice develop a progressive myopathy (Ho et al., 2004). At least two groups have developed mice with targeted dysferlin gene inactivation (Bansal et al., 2003; Ho et al., 2004).

Experimental therapeutics

Although no specific therapies exist for dysferlinopathies, these disorders entail multiple pathways to muscle cell death, each of which is potentially a target for intervention. As in other loss-of-function disorders, there are potential approaches to correcting dysferlin deficiency using gene therapy; at least one group has approached this using adeno-associated virus strain AAV5. Another approach is to correct stop codon mutations. As in Duchenne muscular dystrophy (DMD) and cystic fibrosis, this strategy may be achievable in dysferlin deficiency using PTC124, a 1,2,4-oxadiazole compound that permits read-through of nonsense stop translation signals to produce full-length proteins. PTC124 is being investigated in phase II clinical trials of DMD and LGMDs associated with stop codon mutations, and is under consideration for trial in dysferlinopathies. As in other muscular dystrophies, in dysferlinopathy there is hope that gene replacement mediated by stem cell therapy may also be therapeutic.

DIAGNOSIS AND TREATMENT

Diagnosis

A primary dysferlinopathy should be considered in patients with onset of weakness in their late teens or early twenties, early atrophy and weakness of the posterior calf muscles, and markedly raised serum CK levels (e.g., Miyoshi myopathy). In addition, patients with anterior tibial weakness or limb-girdle weakness with superimposed gastrocnemius or anterior tibial involvement should also be evaluated for a dysferlinopathy. Because dysferlin is present on white blood cells, western blot analysis of these cells for dysferlin represents a noninvasive method of reaching the diagnosis (Ho et al., 2002). If the patient has already had a muscle biopsy, the tissue can be immunostained to see whether dysferlin is present on the sarcolemma, and a western immunoblot can be done to assess the size and amount of dysferlin present. The diagnosis can be confirmed by sequencing the *DYSF* gene for mutations using a commercially available kit.

Treatment

Unfortunately, there are no medical therapies known to alter the course of any of the dysferlinopathies at the present time. However, physical and occupational therapy can be of benefit, as in other forms of dystrophy.

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Chapter 8

Other limb-girdle muscular dystrophies

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INTRODUCTION

This chapter discusses remaining limb-girdle muscular dystrophies (LGMDs) not covered in other chapters (Table 8.1). Although mentioned in Chapter 4 on congenital muscular dystrophies, mutations causing secondary glycosylation defects in α-dystroglycan can also present in later childhood and adult life (LGMD2I, LGMD2K, LGMD2M, LGMD2N, and LGMD2O). LGMD2G is a rare dystrophy caused by mutations in the *TCAP* gene, which encodes for telethonin. A newly discovered dystrophy that can present as a limb-girdle syndrome (LGMD2L) or with a Miyoshi myopathy phenotype is believed to be caused by mutations in *ANO5*, which encodes for anoctamin 5.

LIMB-GIRDLE MUSCULAR DYSTROPHIES ASSOCIATED WITH IMPAIRED GLYCOSYLATION OF α-DYSTROGLYCAN

As discussed in detail in Chapter 4, mutations in various genes leading to secondary glycosylation defects in α-dystroglycan can cause muscular dystrophy. The spectrum can range from congenital onset of severe muscle weakness associated with central nervous system (CNS) abnormalities to adult onset of mild weakness with normal intelligence, isolated cardiomyopathy, to asymptomatic hyperCKemia.

Clinical features

LGMD2I

LGMD2I was initially described in Tunisia (Driss et al., 2000), but occurs worldwide and is the most common form of LGMD in England and northern Europe. The myopathy is caused by mutations in the gene that encodes for fukutin-related protein (*FRKP*) (Brockington et al., 2001a, b). Weakness associated with mutations in the *FKRP* gene can present in infancy (congenital muscular dystrophy type 1C or MDC1A) to the fourth decade of life or later (LGMD2I) (Brockington et al., 2001a, b; Mercuri et al., 2003; Poppe et al., 2003; Boito et al., 2005; Sveen et al., 2006; Wahbi et al., 2008; Bourteel et al., 2009). The pattern of weakness and course are variable. The proximal legs are the most severely affected muscle groups, although some patients have more weakness in the proximal arms. Hypertrophic calves similar to those in Becker muscular dystrophy are often apparent. Some patients just have asymptomatic hyperCKemia.

Approximately one-half of patients develop a dilated cardiomyopathy or ventilatory muscle weakness (Mercuri et al., 2003; Poppe et al., 2003; Boito et al., 2005; Wahbi et al., 2008; Bourteel et al., 2009). In a study of 23 patients with LGMD2I, echocardiography revealed reduced left ventricular ejection fraction in 60%, including 8% with a severe reduced left ventricular ejection fraction of less than 30% (Wahbi et al., 2008). The site of the specific gene mutation and the severity of the muscle disease are not predictive of cardiac involvement. Cardiac magnetic resonance imaging (MRI) revealed myocardial functional abnormalities, fatty replacement, and fibrosis in affected patients. Occasional patients manifest primarily with a cardiomyopathy with minimal skeletal muscle involvement. Thus, all patients with LGMD2I should undergo a cardiac evaluation (e.g., electrocardiography and echocardiography) and pulmonary function tests.

Unlike MDC1C, there are many fewer CNS abnormalities appreciated in LGMD2I. However, some patients have cognitive deficiency, and subclinical abnormalities may be demonstrated with neuropsychological testing

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Table 8.1

Other limb-girdle dystrophies

Dystrophy	Chromosome	Gene	Protein
LGMD2G			
LGMD2K*	9q34.1	*POMT1*	Protein *O*-mannosyltransferase 1
LGMD2I*	19q13	*FKRP*	Fukutin-related protein
LGMD2L	11p14.3	*ANO5*	Anoctamin 5
LGMD2M*	9q31-33	*FKTN*	*Fukutin*
LGMD2N*	14q24.3	*POMT2*	Protein *O*-mannosyltransferase 2
LGMD2O*	14q24.3	*POMGnT1*	*O*-linked mannose-β1,2-*N*-acetylglucosaminyltransferase

*Secondary α-dystroglycanopathies that are also associated with forms of congenital muscular dystrophy. LGMD, limb-girdle muscular dystrophy.

(Bourteel et al., 2009). Further, MRI of the brain may reveal cerebral abnormalities.

A 826C>A (L276I) mutation in the *FKRP* gene is the most common mutation seen in patients from North America, Europe, Canada, and Brazil (Mercuri et al., 2003; Poppe et al., 2003; Boito et al., 2005; Frosk et al., 2005; Sveen et al., 2006; Kang et al., 2007). In one study, patients homozygous for this mutation had later onset, milder clinical progression, and less muscle weakness compared with compound heterozygotes, who were all wheelchair-bound by their mid twenties (Sveen et al., 2006). However, there is no correlation with presence or absence of cardiac or ventilatory weakness.

LGMD2K

LGMD2K is caused by mutations involving protein *O*-mannosyltransferase 1 (*POMT1*). Mutations in this gene usually are associated with Walker–Warburg syndrome (WWS). The mutations have been identified genes that encode for POMT2, *fukutin*, and *FKRP* in patients with WWS, but they account for only a minority of cases (Cormand et al., 2001; Beltran-Valero de Bernabe et al., 2002; Diesen et al., 2004; Muntoni & Voit, 2004; van Reeuwijk et al., 2005). Mutations in *POMT1* account for the 20% of WWS. This is the most severe α-dystroglycanopathy and is usually associated with a life expectancy of less than 3 years. WWS presents as severe generalized weakness hypotonia in infancy (Cormand et al., 2001; Beltran-Valero de Bernabe et al., 2002; Diesen et al., 2004). In addition, the infants are usually born blind secondary to ocular malformations which include fixed pupils, hypoplasia of the optic nerves, micro-ophthalmia, corneal opacities, cataracts, shallow anterior chambers, ciliary body abnormalities, iridolental synechiae, and retinal dysplasia and detachment. WWS is associated with lissencephaly, polymicrogyria, hydrocephalus, hypomyelination of the subcortical white matter, and hypoplasia of the brainstem and vermis. However, rare cases with *POMT1* mutations are associated with a much milder clinical phenotype, LGMD2K, in which affected patients usually present later in childhood syndrome with limb-girdle weakness and only mild intellectual impairment (Balci et al., 2005; D'Amico et al., 2006; Godfrey et al., 2007).

LGMD2M

LGMD2M is caused by mutations in the gene that encodes for *fukutin*. Mutations in this gene usually causes Fukuyama congenital muscular dystrophy (FCMD) (Toda et al., 2000; Muntoni & Voit, 2004, 2005). In addition to the myopathy, FCMD is associated with severe structural abnormalities of the brain, including microcephaly, cortical dysplasia, lissencephaly, pachygyria, polymicrogyria, and hydrocephalus (Toda et al., 1995, 2000). However, mutations in the *fukutin* gene have also been associated with a milder myopathy (LGMD2L), particularly outside Japan (Murakami et al., 2006; Godfrey et al., 2007; Puckett et al., 2009; Vuillaumier-Barrot et al., 2009). Affected individuals may have normal intelligence and brain structure, have a mild limb-girdle weakness, present with only a cardiomyopathy, or have asymptomatic hyperCKemia. Some patients reportedly have remarkable steroid responsiveness (Godfrey et al., 2006).

LGMD2N

LGMD2N is caused by mutations in *POMGnT1,* which encodes protein *O*-mannose-β-1,2-*N*-acetylglucosaminyltransferase that is also responsible for causing muscle–eye–brain disease (MEB) (Haltia et al., 1997; Santavuori et al., 1989, 1998; Cormand et al., 2001; Diesen et al.,

2004; Vervoort et al., 2004). As in WWS, brain and eye abnormalities accompany the muscle weakness, although less severely compared with MEB. Although there is motor delay, most affected children eventually can sit and stand and some ambulate. Many children have cognitive impairments associated with structural abnormalities in the brain (e.g., pachygyria, polymicrogyria, abnormal midline structures, and hypoplasia of the vermis and pons). MEB is also associated with progressive myopia, glaucoma, and late cataracts. Mutations in *POMGnT1* can be associated with milder allelic variants of muscular dystrophy and normal intelligence (LGMD2N) (Godfrey et al., 2007; Clement et al., 2008).

LGMD2O

LGMD2O is caused by mutations in the gene encoding protein *O*-mannosyltransferase-2 (*POMT2*). Mutations in this gene can be a cause of WWS as noted above, but rarely can cause a milder limb-girdle syndrome with absence of brain involvement (Biancheri et al., 2007; Godfrey et al., 2007).

Laboratory features

Serum creatine kinase (CK) levels are markedly raised, particularly in younger patients, but may be normal in older individuals. Brain MRI may be normal or reveal abnormalities in any of the secondary α-dystroglycanopathies, although usually not as severely as seen with mutations resulting in congenital muscular dystrophy (Bourteel et al., 2009). Electromyography demonstrates short-duration, low-amplitude, polyphasic motor unit action potentials.

Muscle histopathology

Nonspecific dystrophic features are evident on muscle biopsy. Of note, immunohistochemistry demonstrates normal dystrophin and sarcoglycan staining. However, α-dystroglycan and occasionally merosin are reduced or absent on immunostaining (Figure 8.1) (Wewer et al., 1995; Cormand et al., 2001; Beltran-Valero de Bernabe et al., 2002; Diesen et al., 2004). Inflammatory cell infiltration may be seen and can lead to the erroneous diagnosis of polymyositis in any of these disorders (Biancheri et al., 2007; Darin et al., 2007). Western blot demonstrates reduced size of α-dystroglycan owing to the decreased glycosylation of the protein.

Pathogenesis

LGMD2I, LGMD2K, LGMD2M, LGMD2N, and LGMD2O are caused by mutations in the genes that encode for FRKP, POMT1, *fukutin*, *POMGnT1*, and POMT2, respectively. These enzymes are important in glycosylation of α-dystroglycan and are necessary for its normal binding to merosin (Kim et al., 2004; Muntoni and Voit, 2004, 2005). Normal glycosylation of α-dystroglycan is important not only for proper muscle function but also for neuronal migration in the CNS. There appears to be a correlation between a reduction in α-dystroglycan, the mutation, and the

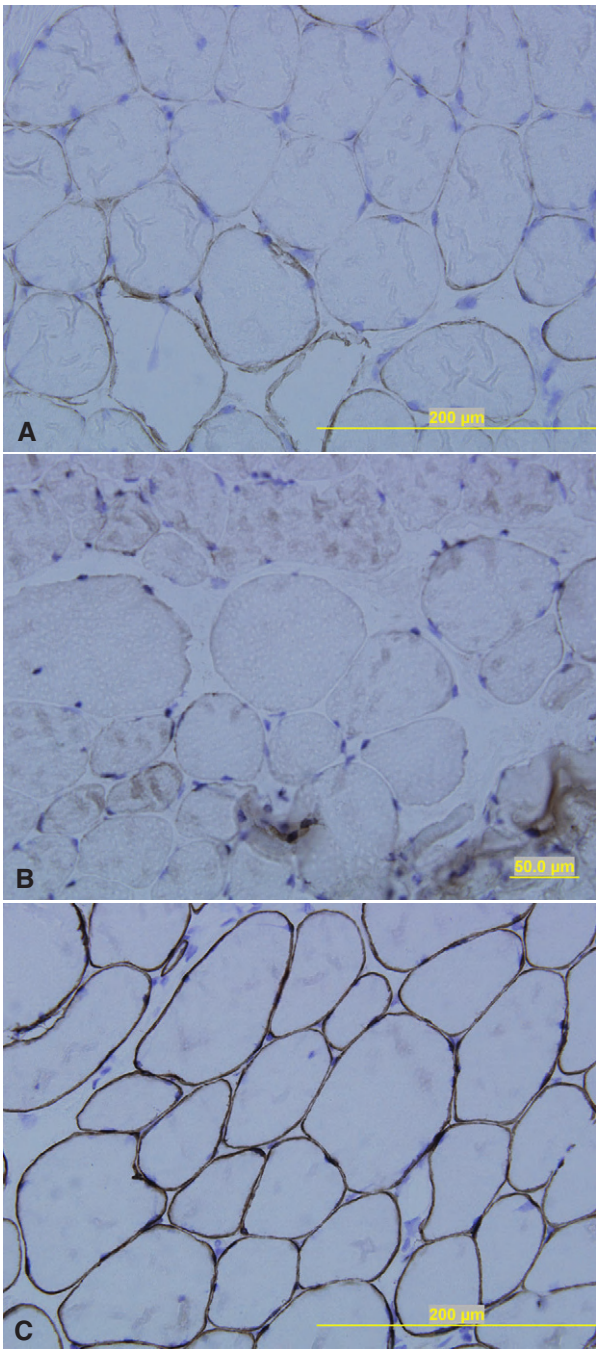

Figure 8.1. LGMD2I. Muscle biopsies demonstrate reduced or patchy merosin staining (**A**), absent α-dystroglycan staining (**B**), but normal dystrophin staining (**C**) around the sarcolemma. Immunoperoxidase stain.

severity of the clinical phenotype in these disorders (Brown et al., 2004). See Chapter 4 on congenital muscular dystrophies for greater detail.

Treatment

There is no proven medical therapy for these disorders. There are reports that some patients with prominent inflammation benefit from corticosteroids (Darin et al., 2007). Whether or not corticosteroids are associated with slowing down of the course, as in Duchenne muscular dystrophy, is unclear. Because some patients manifest with cardiomyopathy or ventilatory muscle weakness that may be out of proportion to the muscle weakness, it is important to assess patients periodically with electrocardiography, echocardiography, and pulmonary function tests, and to treat as necessary. Patients also benefit from physical and occupational therapies. like other patients with dystrophy.

LGMD2G (TELETHONINOPATHY)

Clinical features

This myopathy usually presents in the second decade of life with weakness and atrophy of the quadriceps and anterior tibial muscle groups (Moreira et al., 2000). Mild weakness of the shoulder girdle with scapular winging and calf hypertrophy has been appreciated (Olivé et al., 2008). Further, some affected individuals have early calf atrophy and their condition resembles Miyoshi myopathy (LGMD2B) (Zatz et al., 2003), whereas others have calf hypertrophy (Olivé et al., 2008). Additionally, some patients with a telethoninopathy manifest only with a cardiomyopathy (Knoll et al., 2002).

Laboratory features

Serum CK levels can be slightly to markedly raised.

Histopathology

In addition to typical dystrophic features, rimmed vacuoles are often evident in muscle fibers. As such, the biopsy could also be interpreted as a type of inclusion body myopathy or myofibrillar myopathy. Immunohistochemistry and western blot analysis demonstrate a deficiency of telethonin (Moreira et al., 2000).

Pathogenesis

LGMD2G is caused by mutations in the *TCAP* gene located on chromosome 17q11–12 (Moreira et al., 2000). The name, *TCAP*, refers to the fact that the encoded protein, telethonin, caps titin and binds it to the Z-disk. Telethonin is a 19-kDa sarcomeric protein that is expressed in skeletal and cardiac muscles, where it localizes to the central parts of the Z-disk (Mues et al., 1998). It is a ligand for the giant sarcomeric protein titin, which itself phosphorylates the C-terminal domain of telethonin in early differentiating myocytes. Telethonin may also to overlap with myosin. It is amongst the most abundant proteins in muscle. X-ray crystallography studies reveal that titin is assembled into an antiparallel (2 : 1) sandwich complex by the Z-disk ligand telethonin (Zou et al., 2006). The pseudosymmetrical structure of telethonin mediates a unique palindromic arrangement of two titin filaments (Zou et al., 2006).

The interaction of telethonin with titin is considered to be important in the normal assembly of the sarcomere, sarcomere–membrane interaction, and stretch sensing (Mayans et al., 1998; Mues et al., 1998). Much of the structural integrity of the Z-disk hinges upon this titin–telethonin bond, which may function to resist ultrahigh forces as they are applied in the direction along which it is loaded under physiological conditions (Bertz et al., 2009). Zebra fish with mutant *TCAP* have a muscular dystrophy-like phenotype, including deformed muscle structure and impaired swimming ability (Zhang et al., 2009). Interestingly, telethonin appears to integrate into the sarcomere at a stage after the Z-disk becomes periodic, and the sarcomere remains intact in *TCAP* morphants, suggesting that defective sarcomere assembly does not contribute to the pathogenesis (Zhang et al., 2009). Instead, a defective interaction between the sarcomere and plasma membrane has been detected, which may indicate impaired ability to withstand physiological forces applied to the sarcomere and muscle membrane during contractions. Given the interaction with titin and other Z-disk proteins, it is not surprising that, as in titinopathy and myofibrillar myopathy (see Chapters 11 and 16), there is early tibialis anterior weakness and rimmed vacuoles are evident on muscle biopsy.

LGMD2L (ANOCTAMINOPATHY)

Clinical features

LGMD2L was initially described in 14 French-Canadian patients from eight different families (Jarry et al., 2007). Affected individuals had onset ranging from 11 to 50 years of limb-girdle weakness associated with quadriceps atrophy and myalgia. Weakness of the quadriceps was often asymmetrical. Four patients were wheelchair-bound after an average disease duration of 12 years. Less common findings included facial weakness in two patients and calf hypertrophy in four. Recently, two patients from a Finnish family presented with early involvement of the calves in the second decade of life such that the disease clinically resembled Miyoshi myopathy (I. Mahjneh, personal communication).

Laboratory features

Serum CK levels can be normal or markedly increased. Electromyography studies showed myopathic changes. Skeletal muscle MRI studies of four patients showed atrophy of the biceps brachii and quadriceps femoris muscles with fatty infiltration (Jarry et al., 2007).

Histopathology

Muscle biopsies showed nonspecific dystrophic features with increased endomysial connective tissue associated with basal lamina duplication and collagen disorganization infiltration (Jarry et al., 2007).

Pathogenesis

The disorder is caused by mutations in the maps to 11p13–p12 and the candidate gene is *ANO5*, which encodes for anoctamin 5. This is a member of the anoctamin family of transmembrane proteins and may be a calcium-activated chloride channel. Mutations in this gene have been associated with gnathodiaphyseal dysplasia. How mutations in this gene lead to muscular dystrophy is not clear.

CONCLUSION

The clinical spectrum of these dystrophies is diverse and diagnosis challenging. No doubt many new genes responsible for various dystrophies will be discovered in the future.

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Chapter 9

Limb-girdle muscular dystrophy 2H and the role of TRIM32

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INTRODUCTION

The prevalence of limb-girdle muscular dystrophy (LGMD) worldwide has been estimated to be about one in 15 000. In the genetically isolated Hutterite population in Manitoba, Canada, however, LGMD was estimated to have a prevalence greater than 1 in 400. The first identification of LGMD in the Hutterites occurred in 1976 when Shokeir and Kobrinsky reported 11 patients with a limb-girdle syndrome in the Schmiedeleut subdivision of the Hutterite population (Shokeis MH, Kobrinsky NL 1976). This group was found to have slowly progressive proximal weakness and wasting with mildly raised serum creatine kinase (CK) values, ranging from 57 to 562 units/l. Facial muscles were also affected. A follow-up study by Weiler et al. (1997) reported an age of onset that varied from age 8 to 27 years. Common signs and symptoms in these individuals included fatigue, falling, waddling gait, and difficulty rising from a squatting position. Serum CK values also varied greatly, ranging from about 250 to 3000 units/l and muscle biopsies were observed to be dystrophic. The high prevalence of LGMD among Hutterites motivated investigators to study this population more closely. Eventually, Weiler and co-workers reported that the causative gene for LGMD in Hutterites was mapped to chromosomal region 9q31–q33 (Weiler et al., 1998) and subsequently identified a 1459 G>A (D487N) mutation in the tripartite motif-containing protein gene 32 (*TRIM32*) (aka *HT2A*) as responsible for this condition we now refer to as LGMD type 2H (Frosk et al., 2002).

Patients with LGMD2H that are homozygous for the D487N mutation in *TRIM32* demonstrate significant phenotypic variability. The age of onset, in particular, is widely variable, ranging from the first to the fourth decade. Symptoms at onset include exercise-induced myalgias, fatigue, and very mild proximal weakness. Serum CK levels are slightly raised. Neck flexor weakness, facial weakness, scapular winging, hypertrophied calves, and Achilles tendon contractures have also been commonly observed in these patients. Presymptomatic patients have been found to have normal serum CK levels, suggesting that the plasma membrane is not compromised as an early feature of the disease. A muscle biopsy performed at younger ages (before patients are symptomatic) may show characteristic dystrophic changes that are observed in symptomatic patients with LGMD2H.

After the mutation in *TRIM32* was identified in the Hutterites, this isolated population was genetically stratified further with the finding that many Hutterites with a limb-girdle syndrome did not map to the *TRIM32* locus (Frosk et al., 2005b). These patients were subsequently found to have a 825C>A (L276I) mutation in *FKRP* (fukutin-related protein), the most common mutation responsible for LGMD type 2I. Frosk et al. (2005a) also reported on two Hutterite brothers both with homozygous mutations at two LGMD loci, LGMD2H and LGMD2I, which did not result in a more severe phenotype at young age.

Recently, Saccone et al. (2008) studied *TRIM32* in 310 patients with LGMD with no mutations identified from other known LGMD loci. They identified four patients with mutations in *TRIM32*. As with the Hutterite patients with LGMD2H, these novel mutations all occurred in the carboxy-terminal, NHL domains of TRIM32. These patients were characterized as follows. Two of the patients (proband #2 and proband #3) were found to carry a mutation for 1180G>A (R394H). Proband #2 was heterozygous for this mutation. He was 73 years old with asymptomatic, elevated serum

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CK levels (2.5 times normal). Proband #3 was homozygous for this mutation. Disease onset was in the third decade of life with symptoms consisting of progressive difficulty rising from the floor, climbing stairs, and walking. His examination demonstrated proximal weakness with scapular winging. Electrocardiography revealed a right bundle branch block. A muscle biopsy demonstrated dystrophic changes with normal dystrophin staining. Proband #1 was a 44-year-old woman who was homozygous for 1559delC (T520TfsX13) with slowly progressive proximal weakness, muscle wasting, respiratory weakness, and chronic keratitis. Serum CK levels were normal. Facial muscles also appeared to be involved. Proband #4 was a 15-year-old boy who was heterozygous with a 1761–1763delGAT (D588 del). His only symptom was that of muscle cramps with exercise. His examination showed scapular winging and he was found to have increased CK levels, 4–5 times normal.

Another *TRIM32* homozygous frameshift mutation 1753–1766dup14 (I590LfsX38) was identified using genome-wide homozygosity mapping (Cossée et al., 2009). The patient of Turkish origin was born from consanguineous parents. Disease onset was at age 25 years with symptoms of proximal upper limb myalgia, which deteriorated progressively. At age 42 she manifested walking difficulties and proximal weakness in all four limbs. Serum CK levels were 4 times normal.

In 2009 a Swedish family consisting of two parents that were carriers and three affected offspring was described in which heterozygous or compound heterozygous family members were identified (Borg et al., 2009). The mother (patient II.2) carried a 1560delC (C521VfsX13) mutation. A similar 1559delC (T520TfsX13) mutation had been identified previously in Saccone et al. (2008). The father (patient II.1) carried a novel 30-kb deletion plus a 2-bp insertion at the *TRIM32* locus, abolishing all TRIM32 expression as the deletion encompasses the entire open reading frame. The offspring of these parents were symptomatic either at infancy (seen in a daughter, patient III.2) and at age 22 years (seen in the second daughter, identified as patient III.1). Patient III.2 also had an affected daughter who was a floppy infant with delayed motor milestones, and biopsy at age 2 years showed a vacuolar myopathy with variation in fiber size, atrophic type I fibers, and fiber type grouping. Both patients III.1 and III.2 had electromyography (EMG) abnormalities consisting of both myopathic and neurogenic changes. In addition, muscle biopsies from these individuals showed muscle fiber size variation with some scattered atrophic fibers and vacuolar changes. Interestingly, in both generations I and III, there were signs of a vacuolar myopathy and other indications of muscle weakness in family members that carried only one mutant allele. Although LGMD2H has been considered largely an autosomal recessive myopathy, these studies suggest that it may also be classified as a mild autosomal dominant disorder, much like mutations in the gene *Titin* can cause a mild autosomal dominant distal myopathy (Udd distal myopathy) but, when homozygous, can result in LGMD 2J.

Thus far, all mutations identified to result in LGMD2H disrupt the conserved NHL domains at the C-terminal end of TRIM32 (Table 9.1), which is thought to be important for proper dimerization of the protein. Therefore, stability of the C-terminal region of TRIM32 is essential for muscle function. Future studies are necessary to determine whether these mutations disrupt stability, substrate recognition, or another unknown function of the protein.

TRIM32 PROTEIN

The *TRIM32* gene is composed of two exons, with the entire open reading frame contained in the second exon, which codes for a protein of 653 amino acids with predicted molecular weight of 72 kDa. TRIM32 is a member of the tripartite motif (TRIM) family of proteins (Reymond et al., 2001). The protein sequence contains several conserved motifs including a RING finger domain, a B-box, a coiled-coil region, and six NHL repeats (Figure 9.1). The RING finger was shown to be responsible for the enzymatic activity of the protein (Kudryashova et al., 2005), and the NHL domains are believed to be protein–protein interaction motifs, possibly responsible for homodimerization (Saccone et al., 2008). The coiled-coil domain has also been shown to be responsible for homodimerization and heterodimerization in the TRIM proteins (Reymond et al., 2001). Interestingly, the D487N mutation and all newly identified *TRIM32* mutations causing LGMD2H occur in the NHL repeats (see Figure 9.1). Modeling of the mutations suggests that the β-propeller structure is disrupted by all mutations studied (Saccone et al., 2008). Thus, future studies are necessary to identify the effect of the mutations in the NHL domain and how they impact dimerization and/or protein–protein interaction.

TRIM32 protein is expressed widely in numerous tissues (Frosk et al., 2002; Horn et al., 2004), but is particularly well represented in the brain (Frosk et al., 2002; Horn et al., 2004; Kudryashova et al., 2005), where it was shown to be expressed at 100-fold the level in muscle (Kudryashova et al., 2009). TRIM32 protein possesses E3 ubiquitin ligase activity, demonstrated by *in vitro* ubiquitination assays (Kudryashova et al., 2005), consistent with the presence of the RING finger domain (Meroni and Diez-Roux, 2005). Ubiquitination is a post-translational modification of proteins that covalently links an 8.5-kDa ubiquitin moiety to a

Table 9.1

***TRIM32* mutations and the associated phenotypes**

TRIM32 Mutations		Clinical Phenotype	Onset of weakness	CK level	References
1459 G>A (p.D487N)	homozygous (41 patients)	LGMD2H	variable age (first to forth decade)	variable	Frosk et al., 2002
	homozygous (4 patients)	STM	variable age (congenital-third decade)	normal to 20 ×	Schoser et al., 2005
1180G>A (p.R394H)	homozygous (1 patient)	LGMD2H	third decade	not reported	Saccone et al., 2008
	heterozygous (1 patient)	Asymptomatic hyper-CKemia	N/A	2.5× normal	
1761–1763delGAT (p.D588del)	heterozygous (1 patient)	LGMD2H	age 8	4–5× normal	Saccone et al. 2008
1559delC (p.T520TfsX13)	homozygous (1 patient)	LGMD2H	not reported	normal	Saccone et al. 2008
c1753–1766dup (p.I590LfsX38)	homozygous (1 patient)	LGMD2H	age 25	4× normal	Cossée et al. 2009
1560delC (p.C521VfsX13)	heterozygous (1patient)	LGMD2H	age 51 (only one allele)	normal	Borg et al., 2009
del30,586 bp + insert2bp (30kb deletion + 2 bp insertion) (removes entire *TRIM32* ORF)	heterozygous (4 patients)	LGMD2H	age 47 (only one allele with 30 kb deletion)	normal	Borg et al., 2009
			age 22 (het for 30 kb deletion and 1560delC)	6× normal	
			infancy (het for 30 kb deletion and 1560delC)	3× normal	
			infancy (only one allele with 30 kb deletion)	2× normal	
388C>T (p.P130S)	homozygous	BBS (type 11)	N/A	N/A	Chiang et al., 2006

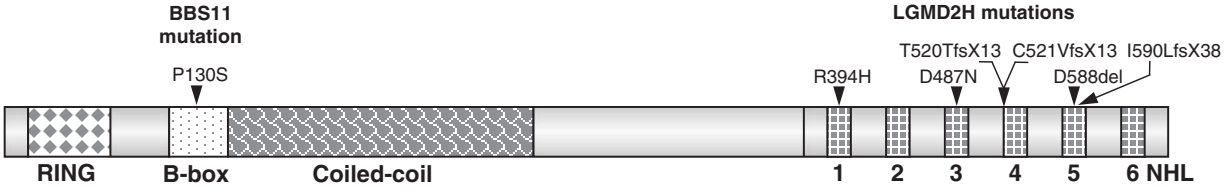

Figure 9.1. TRIM32 domain structure and localization of the human pathogenic mutations. BBS, Bardet–Biedl syndrome; LGMD, limb-girdle muscular dystrophy.

target protein. Ubiquitination serves numerous functions in the cell, including cell cycle regulation, chromatin remodeling, protein trafficking, and overall protein degradation. The process of conjugating a ubiquitin moiety to a target involves three different enzymes, E1, E2, and E3. Both the conjugation of the ubiquitin to its substrate and the degradation of the substrate in the 26S proteasome are ATP dependent. The function of the E1 enzyme is to activate the ubiquitin and transfer it to an E2-conjugating enzyme. E3 enzymes are bridging factors that bring the E2–ubiquitin complex and substrate together, resulting in the transfer of the activated ubiquitin to a lysine residue of the substrate (Pickart, 2004). Either monoubiquitination or polyubiquitination can occur on a given protein. The specificity in this system comes from the E3 ligases, which must bind to proteins that are targeted for ubiquitination through specific protein–protein interaction motifs. Hundreds of E3 ubiquitin ligases have been identified and it is known that at least three of these E3 ligases are muscle specific (MuRF1, Atrogin-1, and OzzE3). This list is likely to grow because each E3 ligase recognizes a distinct and specific set of substrates (Bodine et al., 2001; Gomes et al., 2001; Bromann et al., 2004; Nastasi et al., 2004).

SARCOTUBULAR MYOPATHY

Sarcotubular myopathy (STM) has been demonstrated to result from the same D487N mutation in TRIM32 possessed by patients with LGMD2H (see Table 9.1). It was first reported in 1973 by Jerusalem et al., who described two South Dakota Hutterite brothers, aged 11 and 15 years, with congenital nonprogressive weakness (Jerusalem F et al., 1973). Both had mild-to-moderate symmetrical, proximal weakness and atrophy, and both had difficulty with strenuous activity. Their muscle biopsies demonstrated sarcotubular changes; this condition thus became known as "sarcotubular myopathy." Subsequently, Müller-Felber et al. (1999) described two brothers from a small village in southern Germany with similar pathological changes. At the time of their report, the brothers were 33 and 35 years of age. The older brother had begun experiencing symptoms at about age 6 years, with weakness and pain on exertion. These symptoms progressed slowly. At age 11 years, he was not able to rise from the floor unassisted. Although he was still ambulatory at age 31 years, he could not walk more than 20 meters and experienced severe exercise-induced myalgias. His examination demonstrated profound proximal weakness (1/5 on the Medical Research Council (MRC) scale) with relative sparing of distal muscles (4/5 MRC). Reflexes were absent and serum CK levels were mildly raised. The younger brother did not begin to experience symptoms until age 32 years, when he started to complain of aching in the legs after strenuous exercise. He also began to experience mild weakness after jogging for more than 30 minutes. His examination at that time demonstrated mild proximal weakness (4/5 MRC) but he was able to rise from a seated position, from the floor, or from a prone position without assistance. Serum CK levels were moderately increased (995 and 1546 units/l in the two brothers). EMG showed no spontaneous activity. The muscle biopsy from both of these brothers demonstrated rounded muscle fibers with increased fiber size variation and internalized nuclei. Small vacuoles without glycogen or fat were seen. Electron microscopy showed numerous vacuoles and degenerative changes in the myofibrils with Z-disk streaming.

The finding of two pairs of affected brothers with unaffected parents led investigators to postulate that the condition was an autosomal recessive condition. Because of the high prevalence of mutations in *TRIM32* and *FKRP* in the Hutterite population, Schoser et al. (2005) hypothesized that sarcotubular myopathy may be a result of mutations in one of these two genes. Their study demonstrated that both the South Dakota Hutterite brothers and the southern German brothers carried the D487N mutation in *TRIM32* (Schoser et al., 2005). None of the brothers carried the L276I mutation in *FKRP*. The common mutation found in LGMD2H and STM suggested that these two conditions represented different forms of the same disease and that a possible common founder mutation was responsible for both the German STM and the Hutterite LGMD2H/STM cases.

Examination of the origin and migration patterns of Hutterites may provide insight into the theory of a common founder. Hutterites are an isolated communal branch of Anabaptists who trace their roots to the Austrian province of Tyrol during the Radical Reformation of the 16th century. As Hutterites are pacifists, their migration through the centuries has been driven largely by the local laws requiring military service. Over the years, their migration followed a path through Moravia, Transylvania, and then the Ukraine. Their migration from Russia to North America occurred between 1874 and 1879, after Russia installed a compulsory military service law. The three groups, Schmiedeleut, Dariusleut, and Lehrerleut, settled initially in the Dakota Territory. Dariusleut colonies were later also established in central Montana. During World War I, Hutterites who were drafted by the military refused to comply and were imprisoned and mistreated, resulting in the deaths of two Hutterite men while in US military custody. This resulted in the migration of most of the American colonies to the Canadian provinces of Alberta, Manitoba, and Saskatchewan. The Schmiedeleut group ultimately returned to the Dakotas in the 1930s after laws protecting conscientious objectors were passed. Today, most of the estimated 50 000 Hutterites live in Alberta, Manitoba, Saskatchewan, South Dakota, and Montana. LGMD2H has been reported in both the Schmiedeleut and Lehrerleut groups.

TRIM32 AND BARDET–BIEDL SYNDROME

In 2006, a third disease was identified to be associated with *TRIM32* mutations. Chiang et al. (2006) mapped the disease locus in a family with Bardet–Biedl syndrome (BBS) to the *TRIM32* gene. The homozygous 388C>T (P130S) mutation occurred in the B-box domain of the protein (see Figure 9.1). Based on this genetic mapping, the investigators designated this form of BBS as type 11. BBS is a pleiotropic disorder characterized by obesity, pigmentary retinopathy, diabetes, polydactyly, renal abnormalities, learning disabilities, and hypogenitalism. Although no muscular dystrophy has been reported in these patients, it is estimated that approximately 50% have cardiomyopathy. BBS displays significant genetic heterogeneity, with the syndrome being associated with mutations in at least 10 genes that have been mapped and identified to date. Of interest is the observation that many of the genes responsible for BBS are linked to cellular roles in ciliary or microtubular function (Beales, 2005). This observation coincides with a separate demonstration that MuRF3, another TRIM family member, has been shown to play a role in microtubule dynamics (Spencer et al., 2000). Consistent with these findings, studies by Short and colleagues revealed a novel motif necessary for microtubule binding in several TRIM members, including MuRF1 (TRIM63), MuRF2 (TRIM55), and MuRF3 (TRIM54) (Short and Cox, 2006). Thus, a theme of TRIM proteins as microtubule regulators is becoming apparent in many TRIM family members.

MOUSE MODEL OF LGMD2H

A knockout mouse has recently been created, in which the expression of *TRIM32* is ablated (Kudryashova et al., 2009). This mouse completely lacks TRIM32 protein in all tissues including muscle and brain. *TRIM32* knockout mice have reduced muscle strength and mild, dystrophic muscle changes, which include variation in fiber diameter, internalized nuclei and, central pallor on reduced nicotinamide–adenine dinucleotide (NADH) and succinate dehydrogenase (SDH) staining. On electron microscopy, a dilated sarcotubular system and vacuoles were observed. Thus, the phenotype of the *TRIM32* knockout mouse resembled features of both LGMD2H and STM. The brains of these animals showed greatly reduced neurofilament proteins, leading the authors to investigate closely the axonal diameter of motor neurons. These studies revealed a reduction in axon diameter and a subsequent muscle fiber type conversion from fast to slow (Kudryashova et al., 2009). They revealed a neurogenic aspect of LGMD2H that was later shown to be evident in the patients (Borg et al., 2009).

BIOLOGICAL ROLE OF TRIM32

Initial studies of TRIM32 showed that it bound to the human immunodeficiency virus (HIV) Tat protein by yeast two-hybrid analysis (Fridell et al., 1995), and in a separate study that TRIM32 was upregulated in skin cancer cells with antiapoptotic properties (Horn et al., 2004). Interestingly, several TRIM family members (e.g., TRIM1, 5a, 19, 22, and 45) have been implicated in retroviral restriction and antiviral defense (Nisole et al., 2005). For example, expression of MuRF1/TRIM63, another TRIM family member, is increased in the heart of transgenic rats overexpressing several HIV-1-related proteins (Tat, gp120, Nef) (Otis et al., 2008). It is not clear how these findings relate to the role of TRIM32 in muscle. Future studies are necessary to determine whether TRIM32 is a cell survival factor in muscle and its role in acquired immune deficiency syndrome myopathy.

Several different biological functions have been attributed to TRIM32, but it is not clear how disruption of these functions by pathogenic mutations results in

the observed human condition. Many studies have implicated TRIM32 as a survival factor and oncogene. For example, in keratinocytes (Albor et al., 2006) and HEp2 cells (Kano et al., 2008), it has been shown to promote cell viability. It was demonstrated that TRIM32 regulates NFκB (nuclear factor κ-light-chain-enhancer of activated B cells) activity through ubiquitination (and degradation) of protein inhibitor of activated STAT Y (PIASy), a member of the PIAS (protein inhibitors of activated STATs (signal transducers and activators of transcription proteins)) family (Albor et al., 2006). PIASy is a SUMO ligase, one of only six known proteins that serve this function in the cell. Sumoylation is a recently discovered post-translational modification that results in the placement of a small ubiquitin-like modifier (SUMO) moiety on a target protein (Wang and Dasso, 2009). Similar to the ubiquitination process, sumoylation involves a set of E1, E2, and E3 enzymes. However, sumoylation results in the addition of a single SUMO molecule to the substrate and it is readily reversible. It serves in the functional regulation of proteins involved in transcription, signal transduction, DNA repair, control of cell cycle, and autophagy. Many transcriptional regulators are modified by sumoylation, including MEF2A, an important myogenic protein that is sumoylated by PIAS1 (Riquelme et al., 2006). In addition, TRIM32/PIASy regulation of the psoriatic chemokine CCL20 through NFκB implicates TRIM32 in the pathogenesis of psoriasis (Liu et al., 2010). Another SUMO ligase, PIAS3, has been shown to be a ubiquitination target for TRIM32 (Qu et al., 2007). Nitric oxide promoted TRIM32-mediated PIAS3 ubiquitination and enhanced the stimulatory effect of PIAS3 on TRIM32 autoubiquitination. Thus, this study revealed crosstalk between *S*-nitrosation, sumoylation, and ubiquitination. Nevertheless, the relationship between TRIM32 and its effects on PIAS3, PIASy, and NFκB in muscle and muscular dystrophy is still not clear.

TRIM32 was also suggested as a novel oncogene that promotes tumor growth, metastasis, and resistance to anticancer drugs by mediating ubiquitination and degradation of Abl-interactor protein 2 (Abi2), which is known as a tumor suppressor and a cell migration inhibitor (Kano et al., 2008).

Recently, dysbindin was identified as substrate for TRIM32 E3 ubiquitin ligase activity (Locke et al., 2009). Dysbindin is a protein expressed by neurons and by muscle cells, both tissues affected in LGMD2H. The D487N mutation (found to cause LGMD2H and STM) disrupts the ability of TRIM32 to degrade both dysbindin (Locke et al., 2009) and PIASy (Albor et al., 2006). Much work is needed to create a deeper understanding of how TRIM32 regulates these substrates and why they are necessary for proper muscle integrity and function.

Given the high levels of expression in brain, TRIM32 is expected to play a role in nervous tissue. Indeed, levels of TRIM32 are increased in occipital lobes of patients with Alzheimer's disease, whereas suppression of TRIM32 by small interference RNA duplexes results in reduced neuronal survival (Yokota et al., 2006). Furthermore, TRIM32 is required and sufficient for suppressing self-renewal and inducing neuronal differentiation (Schwamborn et al., 2009). It ubiquitinates and degrades the transcription factor c-Myc, resulting in cell cycle exit and induction of neuronal differentiation. In addition, TRIM32 has been suggested to be a positive regulator of microRNA (miRNA) function in brain. It interacts with Argonaute-1, an endonuclease participating in RNA interference gene silencing, and enhances the activity of the miRNA let-7a, a known stem cell regulator (Schwamborn et al., 2009). However, the effects of TRIM32 on skeletal muscle-specific miRNAs are obscure.

POTENTIAL ROLES OF TRIM32 IN NORMAL AND DISEASED MUSCLE

The identification of TRIM32 as an E3 ubiquitin ligase suggested that it may function like other E3 ligases in the regulation of muscle protein turnover, for the following reasons. It is known that the majority of muscle protein turnover during muscle atrophy occurs at a site in the cell called the proteasome (Solomon et al., 1998; Lecker et al., 2004). The proteasome is a cellular structure composed of numerous proteins with proteolytic enzyme activity, making it the final degradative site of many cellular proteins. These proteins are targeted to the proteasome through the post-translational attachment of a ubiquitin chain. Two E3 ubiquitin ligases have emerged as important regulators of muscle protein turnover through this mechanism. These two E3 ligases, MuRF1 (TRIM63) and Atrogin-1 (MaFbox), are highly upregulated during many different forms of muscle atrophy that occur following denervation or hindlimb unloading. MuRF1 has been shown to target numerous myofibrillar proteins for ubiquitination, such as myosin heavy chain (Clarke et al., 2007) and cardiac troponin I (Kedar et al., 2004). Additionally, several other myofibrillar proteins are ubiquitinated by both MuRF1 and a related protein, MuRF2 (TRIM55) (Witt et al., 2005). Furthermore, like MuRF1, the E3 ligase Atrogin-1 was shown to target muscle proteins such as the myogenic regulator MyoD (Tintignac et al., 2005) and the translation initiation factor eIF3-f (Lagirand-Cantaloube et al., 2008). Studies of these E3 ligases may provide

clues to the biological function of TRIM32. For example, it was recently demonstrated that, similar to MuRF1 and Atrogin-1, TRIM32 is upregulated during muscle unloading (Kudryashova et al., 2005), suggesting that TRIM32 may share a common biological function with MuRF1 and Atrogin-1 in the regulation of myofibrillar protein turnover. Strong support for a role for TRIM32 in the regulation of myofibrillar protein turnover was demonstrated in studies identifying the myosin heavy chain as a binding partner and actin as a substrate of ubiquitination (Kudryashova et al., 2005). Surprisingly, the D489N mutation (engineered into the mouse TRIM32 protein, corresponding to human LGMD2H mutation) does not disrupt the ability of TRIM32 to ubiquitinate actin; therefore, the mutation disrupts another function of TRIM32 that does not depend on enzymatic activity (Kudryashova et al., 2005).

An alternative, or possibly additional, mechanism by which mutations in enzymes involved in protein turnover might be disease-causing is if these proteins have specific roles in protein regulation. This type of regulation can be found in one E3 ligase called OzzE3, which specifically controls β-catenin levels in muscle cells. Disruption of this function was shown to lead to abnormal sarcomere formation *in vitro* (Nastasi et al., 2004). Furthermore, null mutation of UBR1, another E3 ubiquitin ligase, shows a phenotype of slow growth and smaller muscles (Kwon et al., 2001). MuRF1 and MuRF2 have also been shown to play a role in titin kinase-based signaling in response to mechanical loading (Centner et al., 2001; Lange et al., 2005) and in maintenance of M-line integrity (Gregorio et al., 2005), whereas MuRF3 was shown to play a role in microtubule dynamics (Spencer et al., 2000). It is possible that these regulatory roles for E3 ligases have a relationship with muscle protein turnover, but this has not yet been established.

HYPOTHETICAL MECHANISM BY WHICH IMPAIRED PROTEIN TURNOVER MIGHT LEAD TO CELL DYSFUNCTION

Muscle is a highly metabolically active tissue. As such, any defect affecting cellular physiology and metabolism may potentially have a selective effect on muscles. Selective involvement of skeletal muscle tissue has been observed in other metabolic conditions, most notably mitochondrial cytopathies. Thus, mild defects in a ubiquitously expressed protein involved in protein degradation may potentially result in selective involvement of muscle, for similar reasons. The intracellular milieu provides conditions under which cellular proteins are continually damaged and then degraded by the proteasome. Processes such as oxidation can damage proteins, which can then become denatured, leading to protein aggregation and cellular toxicity. Numerous examples exist in which loss of proteolytic capability via the ubiquitin/proteasome pathway leads to disease. Alzheimer's disease, juvenile recessive Parkinson's disease, Angelman syndrome, and several forms of cancer can result from mutations in proteins associated with the ubiquitin–proteasome pathway (Jiang and Beaudet, 2004). Excessive accumulation of damaged or misfolded proteins can lead to a cell stress response in which induction of chaperone proteins, reductions in protein synthesis, and increased proteolysis can occur. Increases in protein degradation and decreases in protein translation could lead to muscle atrophy, a characteristic observed in numerous muscular dystrophies. For example, previous studies have shown that a null mutation in calpain-3, an intracellular, cytosolic, calcium-dependent protease, results in evidence of aggregate formation and muscle atrophy (Kramerova et al., 2005). Thus, in light of these previous findings, one could speculate that *TRIM32* mutations may in some manner (e.g., through loss of substrate recognition) lead to protein accumulation and a cellular stress response.

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Chapter 10

Caveolinopathies: translational implications of caveolin-3 in skeletal and cardiac muscle disorders

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INTRODUCTION

Caveolae (literally "little caves"), first described in 1953 by George Palade from electron micrographs of endothelial cells, are flask-shaped vesicular invaginations of the plasma membrane of 50–100 nm in size (Parton, 1996; Williams and Lisanti, 2004). Caveolae are constituted mainly by small integral membrane proteins (18–24-kDa) encoded by the caveolin gene family (*CAV*), which includes caveolin-1 (Cav-1), caveolin-2 (Cav-2), and caveolin-3 (Cav-3) (Galbiati et al., 2001b; Mercier et al., 2009). Cav-1 and Cav-3 are highly homologous and share a signature structural motif (FEDVIAEP) that is conserved across species (Hnasko and Lisanti, 2003).

The caveolin genes are expressed in distinct cell types in skeletal, cardiac, and smooth muscle tissue. Cav-1 and Cav-2 have been identified in satellite cells/myogenic precursors and in cultured skeletal myoblasts during the proliferative phase of their life cycle, whereas they become nearly undetectable in terminally differentiated myocytes (Volonte et al., 2005; Schubert et al., 2007). In contrast, the muscular isoform Cav-3 is upregulated as the cells approach the differentiation process and becomes highly expressed in fully differentiated muscle cells (Parton, 1996; Serra and Scotlandi, 2009). In mature skeletal and cardiac myocytes, Cav-3 is involved in the structural maintenance of sarcolemma integrity; it modulates the trafficking and protein–protein interactions of muscle cell membrane proteins; it regulates the activity of distinct signaling molecules and is implicated in the maintenance of muscle glucose homeostasis (Table 10.1).

Preservation of physiological Cav-3 levels is essential for normal muscle development and for postnatal skeletal muscle function. Cav-3 activities in muscle tissue differentiation were clarified in zebrafish, a readily accessible model for muscle disease. Embryos injected with Cav-3 morpholino antisense oligonucleotides display decreased and disorganized myoblast fusion. In myotube precursors, the contractile apparatus is characterized by chaotic filament bundles with interspersed many mitochondria and a poorly developed T-tubule network. This phenotype results in embryos with slowed or completely uncoordinated movements (Nixon et al., 2005). On the other hand, adult Cav-3 knockout mice, which lack muscle cell caveolae, display defects of the T-tubule system (Galbiati et al., 2001a). Accordingly, transgenic mice overexpressing the Pro104Leu mutant Cav-3 (Cav-3 P104L) show severe skeletal muscle atrophy associated with a significant increase in neuronal nitric oxide synthase and with an overactivation of the myostatin signaling pathway (Sunada et al., 2001).

Until recently, the research on caveolae biology has focused almost exclusively on caveolins and their translational implications. However, recent studies have highlighted the existence of a new additional step of regulation. Indeed, these data have unveiled the role of a family of proteins, the cavins, that seem to play a critical role in caveolar formation and function. In the cytosol, cavins form a complex, that is recruited to membrane caveolae with which cavins establish mutually stabilizing interactions. Polymerase I and transcript release factor (PTRF)/cavin-1 is one of the members of this family, and will be described in this review because it is involved in a form of muscular dystrophy (Hill et al., 2008). On the other hand, muscle-restricted coiled-coil protein (MURC)/cavin-4 is expressed in skeletal and cardiac muscle where it

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Table 10.1

Mechanisms of action of caveolin-3 in muscle cells

Biogenesis of T-tubule system
Interaction with dystrophin–glycoprotein complex through β-dystroglycan
Regulation of cellular trafficking and membrane recruitment of the membrane protein dysferlin
Platform for distinct signaling pathways and membrane receptors (Gi2α, Gβγ, c-Src, and Src-like kinases, β_2-adrenoreceptors, and myostatin type I receptors)
Regulation of insulin receptor expression on muscle membrane and of translocation upon insulin induction of the glucose transporter (GLUT) 4.
Guidance of cell membrane targeting of phosphofructokinase (PFK).

regulates extracellular signal-regulated kinase (ERK) and Rho-ROCK signaling pathways, thus modulating skeletal muscle differentiation and cardiac function. Interestingly, caveolar disruption induces the release of the cavin complex and cavin-4 is decreased in patients with Cav-3 deficiency (Bastiani et al., 2009).

CAV-3 DEFICIENCY AND SKELETAL MUSCLE DISEASES

Mutations in the *CAV3* gene are associated with four different but often overlapping neuromuscular diseases: limb-girdle muscular dystrophy (LGMD) 1C, rippling muscle disease (RMD), asymptomatic hyperCKemia (H-CK), and distal myopathy (Woodman et al., 2004).

In a screening of 663 patients affected by primary myopathies of unknown etiology, *CAV3* mutations represented the 1% of unclassified LGMD and other phenotypes, including isolated H-CK, RMD, and proximal and distal myopathy (Fulizio et al., 2005).

Many patients show such an overlap of the four skeletal muscular diseases described above that some authors have suggested that caveolinopathies should be considered as a "clinical continuum" (Kubisch et al., 2003). In this group of diseases, moreover, genotype does not predict phenotype: studies have shown that the same mutation, even in the same family, can lead to heterogeneous phenotypes and muscle histopathological changes.

In spite of this clinical variability, the immunohistochemistry (IHC) and immunoblot analysis of the muscle biopsies from caveolinopathic patients invariably show a reduction of Cav-3 expression in the muscle fibers (Figure 10.1).

The clinical findings that may suggest the diagnosis of a caveolinopathy are:

- Onset usually in the first two decades
- Progressive, proximal, symmetrical muscle weakness
- Positive Gowers' sign
- Calf hypertrophy (Figure 10.2)
- Myalgias, cramps, and/or stiffness after exercise
- Muscle hyperirritability manifest as percussion-induced rapid contraction (PIRC), in which tapping the muscle belly results in rapid contraction of the muscle, and percussion-induced muscle mounding (PIMM), in which a visible localized swelling of the muscle is caused by contraction at the point of contact.

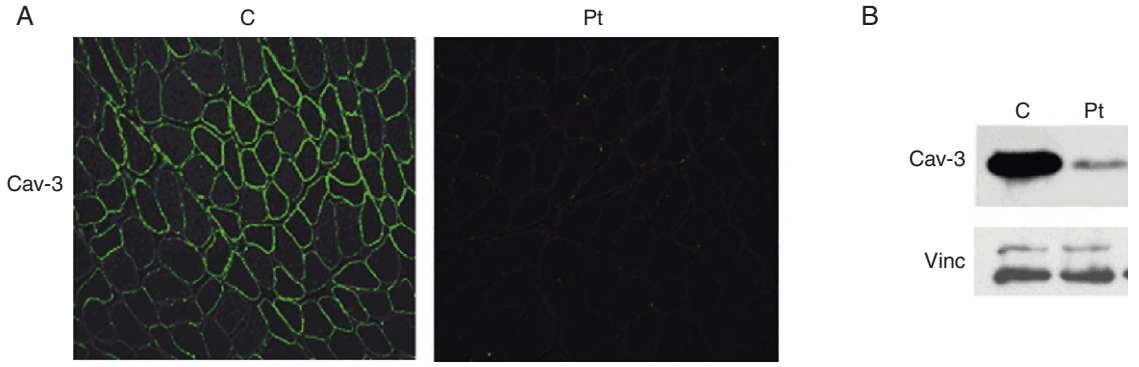

Figure 10.1. *CAV3* mutations cause a severe decrease in caveolin-3 (Cav-3) expression and membrane localization. (**A**) Frozen sections of the skeletal muscle biopsy from a representative patient (Pt) and an age-matched control (C) were prepared and immunostained with specific antibodies against Cav-3. In control muscle, Cav-3 shows a uniform pattern at the sarcolemma. In the patient, the amount of Cav-3 is markedly reduced (original magnification ×40). (**B**) Protein lysates prepared from the skeletal muscle biopsy from a patient and an age-matched control were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and subjected to western immunoblot analysis using antibodies against Cav-3. In the patient, Cav-3 protein levels are reduced by approximately 80%. Vinculin (Vinc) was utilized as a protein loading control. (Modified from Traverso et al., 2008.)

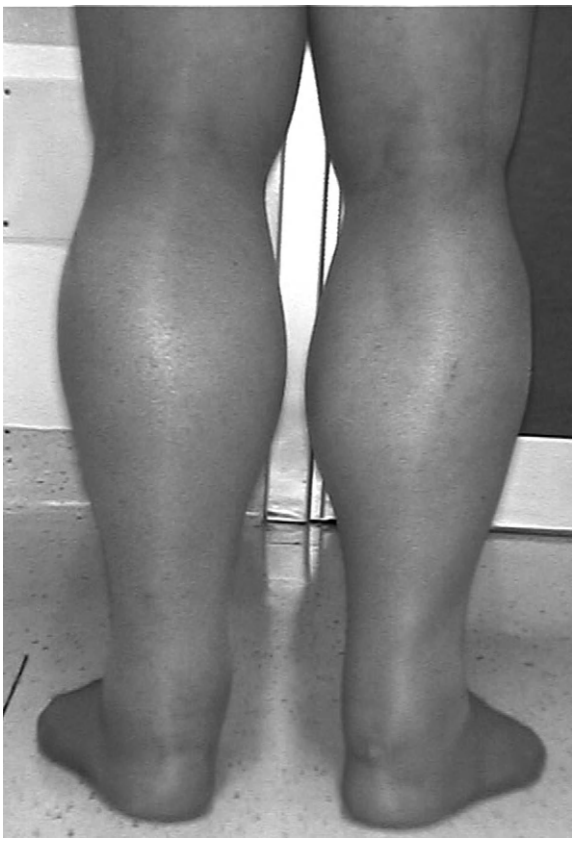

Figure 10.2. Representative picture of the calf pseudohypertrophy observed in patients affected by caveolin-3 deficiency.

- Muscle rippling – a silent (absence of action potentials) wave of muscle contractions that occurs on mechanical stretching of the muscle
- Increased serum creatine kinase (CK) levels (5–25-fold higher than the normal range).

The diagnosis is completed by:

- Evidence of Cav-3 deficiency at IHC of the muscle biopsy.
- Genetic analysis by direct sequencing – at present, 24 different missense mutations, 1 base-pair insertion, 3 distinct base-pair deletions of few nucleotides, 1 genomic macrodeletion, and 1 splice-site substitution have been reported.
- Related genetic counseling – it is appropriate to offer clarification of carrier status and genetic counseling (including discussion of potential risks to offspring and reproductive options) to young adults who are affected or at risk. Most caveolinopathies are transmitted with an autosomal dominant inheritance, and only six autosomal recessive *CAV3* mutations have been described (Gazzerro et al., 2010).
- Cardiac examination – 24-hour electrocardiography and echocardiography.

In the absence of complications, the patients are re-examined for these parameters once a year.

Limb-girdle muscular dystrophy type 1C

The term LGMD describes childhood- or adult-onset muscular dystrophies characterized by weakness and wasting restricted to the limb musculature, proximal greater than distal. In a recent analysis conducted on a large sample of genetically diagnosed Italian patients with LGMD, *CAV3* mutations were found in 5% of the probands (Guglieri et al., 2008).

The clinical features of LGMD1C include mild-to-moderate proximal muscle weakness, a positive Gowers' sign, calf pseudohypertrophy, myalgias, and serum CK concentrations approximately 4–25-fold higher than normal. In LGMD1C, electromyographic studies range from a normal to a myopathic pattern. Muscle biopsy analysis may show an increase in the size variability index of the myofibers, degenerating/regenerating muscle cells, an increased number of central nuclei, and a mild substitution of connective tissue.

This disorder is characterized by a benign clinical course. There is no evidence of respiratory impairment and life expectancy is not reduced (Minetti et al., 1998; Kunkel, 1999; Matsuda et al., 2001; Figarella-Branger et al., 2003; Sugie et al., 2004).

HyperCKemia

Isolated H-CK indicates an increased serum concentration of CK in the absence of any clinical findings of muscular disease. Serum CK levels are approximately 4–10 times higher than normal. The disease can occur in sporadic (a single occurrence in a family) or familial forms. Histological muscle analysis may reveal mild fiber size variability, and few centralized nuclei. No other myopathic features are usually evident (Carbone et al., 2000; Merlini et al., 2002; Fischer et al., 2003; Alias et al., 2004; Fee et al., 2004; Reijneveld et al., 2006).

Rippling muscle disease

RMD is characterized by signs of increased muscle irritability, such as PIRC, PIMM, and/or electrically silent muscle contractions (rippling muscle). The clinical descriptions of the patients indicate that the age of onset of disease is variable (3–36 years), whereas the presenting symptoms and signs are, usually fatigue, tiptoe walking, myalgias, upper limb muscle hypertrophy, and muscle stiffness. Calf pseudohypertrophy may be detected.

Muscle biopsy shows increased fiber size variability, an increase in central nuclei, and mild type I fiber predominance (Betz et al., 2001; Vorgerd et al., 2001; Schara et al., 2002; Kubisch et al., 2003; Van den Bergh

et al., 2004; Madrid et al., 2005; Dotti et al., 2006; Bae et al., 2007; Lorenzoni et al., 2007).

Distal myopathy

There are only two reports of distal myopathy due to *CAV3* mutations. Interestingly, both cases are associated with the same Cav-3 amino acid change, p.R26Q.

One 25-year-old Japanese woman was found with muscle atrophy of her hands and feet and decreased distal muscle function, with normal proximal muscle strength. Her serum CK level was increased 25-fold. Histological analysis of her biceps brachii showed mild variation in fiber size, an increased number of centralized nuclei, and a predominance of type I fibers (Tateyama et al., 2002; González-Pérez et al., 2009).

The second description was a Spanish family whose members developed an autosomal dominant hand-involved distal myopathy. Interestingly, the youngest members displayed signs of muscle hyperexcitability and H-CK before the distal myopathy became apparent clinically. Muscle biopsies revealed no dystrophic changes, but slight variation in fiber size and an increased number of central nuclei (González-Pérez et al., 2009).

Differential diagnosis

The differential diagnosis of caveolinopathies includes the following.

Dystrophinopathies

Mildly affected patients display calf pseudohypertrophy, H-CK, and myalgia. Although the morphological analysis of the muscle biopsy can show similar histopathological dystrophic features, IHC testing for dystrophin and caveolin-3 and the resulting genetic analysis will differentiate the diagnosis.

Limb-girdle muscular dystrophies

Biochemical testing (protein testing by immunostaining) performed on the muscle biopsy will establish the diagnosis of the LGMD subtypes (sarcoglycanopathy, calpainopathy, and dysferlinopathy). In some cases, demonstration of complete or partial deficiencies for any particular protein can then be followed by mutation studies of the corresponding gene.

Secondary hyperCKemia

Secondary hyperCKemia may occur as a result of other diseases or conditions, such as metabolic and inflammatory disorders, hypothyroidism, malignant hyperthermia, alcoholism, drug use, as well as following intramuscular injections.

Myotonic disorders

Muscle stiffness and clinical signs of increased muscle irritability may be present in myotonic disorders such as myotonic dystrophy type 1 and type 2, myotonia congenita, and hyperkalemic periodic paralysis type 1. However, the percussion or rapid pressing of selective muscles (biceps, forearm extensor and flexor, anterior tibial) inducing rapid contraction can be suggestive of RMD. In addition, electromyography in persons with RMD does not show the typical myotonic runs of myotonia.

Treatment

No specific treatment is currently available for caveolinopathies. Aggressive supportive care is essential to preserve muscle function, to maximize functional ability, and to treat complications, especially in patients with features of LGMD. Management should include the following:

- Weight control to avoid obesity
- Physical therapy to promote mobility and prevent contractures
- Social and emotional support and stimulation to maximize a sense of social involvement and productivity and to reduce the sense of social isolation common in these disorders
- Only rarely is the use of mechanical aids such as canes, walkers and orthotics required to help ambulation and mobility.

In individuals with isolated H-CK, special precautions during surgical procedures and anesthesia should be considered, despite the fact that malignant hyperthermia has not been reported in association with *CAV3* mutations.

CAV-3 DEFICIENCY AND HEART DISORDERS

Hypertrophic cardiomyopathy

Although both Cav-3 knockout and transgenic mouse models show reproducible signs of heart involvement with hypertrophic cardiomyopathy (HCM) progressing to diastolic dysfunction, the vast majority of *CAV3* mutations reported do not seem to cause cardiac phenotypes. Indeed, Cav-3 is expressed in cardiomyocytes where it colocalizes with molecules that are involved in the development of myocardium hypertrophy, such as heterotrimeric G-proteins, protein kinase C, Ras, ERK kinase, and constitutive nitric oxide synthase (cNOS). As Cav-3 inhibits the activation of these signaling pathways, it could be hypothesized that caveolae might serve as regulators of cardiomyocyte hypertrophy (Kikuchi et al., 2005).

Consistently, rats locally overexpressing Cav-3 show inhibition of agonist-induced cardiomyocyte hypertrophy, whereas the dominant-negative Cav-3 enhances this process (Koga et al., 2003). Transgenic mice overexpressing both the Cav-3 wild-type or Cav-3 P104L mutant display features of HCM such as increased thickness of the interventricular septum and left ventricular posterior wall, hypercontractility, and diastolic dysfunction (Aravamudan et al., 2003; Ohsawa et al., 2004). Accordingly, 4-month-old Cav-3 knockout mice start presenting myocardium hypertrophy, dilatation, and reduced fractional shortening (Hnasko and Lisanti, 2003). In spite of these reproducible experimental observations, the association between myopathy and cardiac involvement in caveolinopathies has been reported only rarely and the descriptions in literature are controversial.

Hayashi et al. (2004) described two siblings affected by HCM associated with a *CAV3* c.T63S substitution. Neither had any skeletal muscle manifestations and serum CK concentrations were normal. In contrast, Cav-3 expression and caveolar structures were normal in the cardiac muscle tissue of a patient affected by a *CAV3* c.F96 deletion, leading in his family to LGMD1C, H-CK, and RMD, and to a severe Cav-3 deficit in skeletal muscle (Cagliani et al., 2003).

More recently, Traverso et al. (2008) reported a 58-year-old patient affected by LGMD1C and dilated cardiomyopathy (p.T78M mutation). Similarly, Catteruccia et al. (2009) described a family affected by RMD in which the proband displayed dilated cardiomyopathy with atrioventricular conduction defects and his son presented an initial dilatation of the left ventricle (mutation p.A46T).

It is possible that compensatory mechanisms to Cav-3 deficiency are different in cardiac and skeletal muscle tissues, but the molecular basis for this difference is not known. However, these reports emphasize the need for extensive clinical examination of cardiac function of Cav-3 in these patients. Moreover, mutational screening of large populations will be necessary to assess the role of *CAV3* mutations in heart disease.

Caveolin-3 and heart hereditary arrhythmogenic disorders

Over the past few years, the interest in Cav-3 regulation of the intracellular trafficking and post-translational modifications of different cardiac ion channels and β_2-adrenoreceptors has been growing continually. In cardiomyocytes, the ion channels HCN4, Cav1.2, Kv1.5, kir6.2/Sur2a, Nav1.5, and NCX are targeted to caveolae, and Cav-3 directly interacts with β_2-adrenoreceptors with which it regulates the sarcolemmal targeting of the cyclic adenosine monophosphate (cAMP) signal (Martens et al., 2001; Yarbrough et al., 2002; Barbuti et al., 2007).

Accordingly, four *CAV3* mutations were identified in 905 patients referred for long QT syndrome (p.T78M, p.A85T, p.F97C, p.S141R). Electrophysiological studies revealed that at least two of these Cav-3 mutants altered the function of the ion channel Nav1.5 (Vatta et al., 2006). These patients did not exhibit signs of skeletal muscle involvement or primary cardiomyopathy, thus indicating a possible role for Cav-3 and caveolae in cardiac excitability. It is important to underline that when homozygous the *CAV3* T78M was associated with dilated cardiomyopathy, thus enhancing the idea that Cav-3 dysfunction could be involved in the occurrence of a heart phenotype.

These results were further developed by Cronck et al. (2007), who identified three *CAV3* mutations (p.V14L, p.T78M, p.L79R) in a population of 133 infants who died from sudden infant death syndrome. Voltage clamp analysis revealed that all three Cav-3 mutants resulted in a five-fold increase in late sodium current (Cronk et al., 2007).

PTRF MUTATIONS AND SECONDARY CAV-3 DEFECTS

Hayashi et al. (2009) recently identified two different frameshift mutations of the *PTRF* gene in five patients affected by Cav-3 deficiency but without *CAV3* mutations. The patients presented with mild muscle weakness, but hypertrophy of muscles, and PIMMS. Generalized loss of subcutaneous adipose tissue/lipodystrophy was noticed in infancy or early childhood. Serum CK levels were moderately raised in all patients and none of the patients displayed a marked increase in fasting glucose levels. Muscle biopsies showed all the key histological features of muscular dystrophy, and PTRF and Cav-3 immunoreactivity were greatly reduced in the sarcolemma. Interestingly, when transfected in a heterologous system, the PTRF mutants lost the ability to associate with Cav-3 and they induced the disruption of caveolar structures, then activating downstream effectors such as the myostatin signaling pathway. This effect is similar to that observed in muscle cells of patients with primary Cav-3 deficiency.

PATHOGENETIC MECHANISMS OF MUSCLE DEGENERATION IN CAV-3 DEFICIENCY

The secondary mechanisms that, consequent to the loss of caveolae, may lead to the degenerative aspects observed in the skeletal muscles of patients affected by Cav-3 deficiency are multiple and still only partially clear. They can be summarized briefly.

Caveolae are essential in the development and maintenance of skeletal muscle membrane architecture. Loss of caveolae at the sarcolemma is associated with the formation of large subsarcolemmal membranous vacuoles and "honeycomb" membranous structures seen during abnormal proliferation of the T-tubule system, as observed in LGMD1C, distal myopathy, or RMD (Minetti et al., 2002).

A few *CAV3* mutations (c.P104L, c.ΔTFT63–65, c.R26Q, and c.T77K) form unstable high-molecular-mass aggregates that are retained in the Golgi complex and are not correctly targeted to the plasma membrane. Consistent with their autosomal dominant inheritance, these mutations can cause retention of wild-type Cav-3 in the Golgi compartment, thereby inducing the proteolysis of wild-type Cav-3 by ubiquitination and proteasomal degradation (Galbiati et al., 1999). Accordingly, stable overexpression of Cav-3 P104L in C2C12 cells induces a significant upregulation of the ubiquitin ligase atrogin-1 (Fanzani et al., 2007).

A few Cav-3 mutants (p.ΔTFT63–65) reduce the membrane targeting of the molecule Src, and cause abnormal perinuclear accumulation of Src, thus increasing the incidence of apoptosis (Smythe et al., 2003; Smythe and Rando, 2006).

In vivo overexpression of the Cav-3 P104L mutant leads to an overactivation of myostatin signaling pathway, as evidenced by the increased phosphorylation of the myostatin downstream effectors Smad2/3 (Sunada et al., 2001).

Patients with LGMD1C and experimental models of c.P104L and p.ΔTFT63–65 *CAV3* mutations also manifest mislocalization of dysferlin, a muscle membrane protein that is decreased in Miyoshi myopathy and LGMD2B (Matsuda et al., 2001). In physiological conditions, dysferlin interacts with Cav-3 on the muscle sarcolemma, whereas in Cav-3 deficiency it accumulates in the cytoplasm or displays an irregular "patchy" distribution on the membrane. Indeed, Cav-3 modulates sarcolemmal levels of dysferlin by inhibiting its endocytosis via a clathrin-independent pathway (Hernández-Deviez et al., 2006).

The exact molecular cascade of these events has not been clarified yet and it is possible that they engage in feedback with one another, thereby amplifying their adverse effect on muscle cells.

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Chapter 11

Myofibrillar myopathies

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DEFINITION AND BASIC PRINCIPLES

The term myofibrillar myopathies (MFMs) was proposed in 1996 as a noncommittal designation for a group of chronic neuromuscular diseases associated with common morphological features (De Bleecker et al., 1996; Nakano et al., 1996). These consist of a distinct pathological pattern of myofibrillar disorganization that begins at the Z-disk, followed by accumulation of myofibrillar degradation products and ectopic expression of multiple proteins. The diagnosis of MFM is established by muscle biopsy. In trichrome-stained sections of diseased muscle, the abnormal fibers harbor an admixture of amorphous, granular, or hyaline deposits that vary in shape and size, and are dark blue or blue–red in color (Figure 11.1A & D). Many abnormal fiber regions, and especially the hyaline structures, are devoid of, or have diminished, oxidative enzyme activity (Figure 11.1B). Some hyaline structures are intensely congophilic (Figure 11.1C). Some muscle fibers harbor small vacuoles containing membranous material. Electron microscopy shows that disintegration of the myofibrils begins at the Z-disk. This is followed by accumulation of degraded filamentous material in various patterns, aggregation of membranous organelles and glycogen in spaces vacated by myofibrils, and degradation of dislocated membranous organelles in autophagic vacuoles. The clinical features of MFMs are somewhat more variable. Distal muscles are often involved, and cardiomyopathy and peripheral neuropathy are frequent associated features. Electomyography (EMG) studies of affected muscles reveal mostly myopathic motor unit potentials and abnormal electrical irritability, often with myotonic discharges. Rarely, neurogenic motor unit potentials and small motor unit potentials, or slowing of nerve conduction velocities, are seen.

The disease is transmitted by autosomal dominant inheritance, except in one patient with desminopathy with homozygous mutation in whom hemizygosity could not excluded (Munoz-Marmol et al., 1998). Although some cases are sporadic, late-onset, slow disease progression or early parental death may prevent recognition of affected parents. Some sporadic cases may also arise from *de novo* germline mutation.

MORPHOLOGY

The muscle fibers vary abnormally in size. Some abnormal fibers contain large vesicular nuclei. Groups of small fibers, with three or more small fibers per group, are a common finding. In most cases, however, the atrophic fibers account for only a small proportion of the total. Some atrophic fibers arise by fiber splitting. Sparse perivascular or endomysial mononuclear inflammatory cells were present in about 10% of the biopsy specimens in the Mayo Clinic cohort.

In trichrome-stained sections, the abnormal fibers harbor an admixture of amorphous, granular, or hyaline deposits that are dark blue or blue–red in color (Figure 11.1A & D). In a given fiber, these areas are single or multiple, vary in shape and size, are superficial or deep in position, and encompass from a small fraction to nearly the entire extent of the cross-sectioned fiber. Typical cytoplasmic bodies are conspicuous in less than 10% of the specimens. Occasional abnormal fibers also contain nemaline rods. Some abnormal fibers harbor small vacuoles rimmed or filled by membranous material. Infrequently, the vacuolar change is prominent. Pathological alterations occur in both atrophic and nonatrophic fibers. Oxidative enzyme activity is sharply reduced in fiber regions occupied by the hyaline structures and in many other abnormal fiber regions

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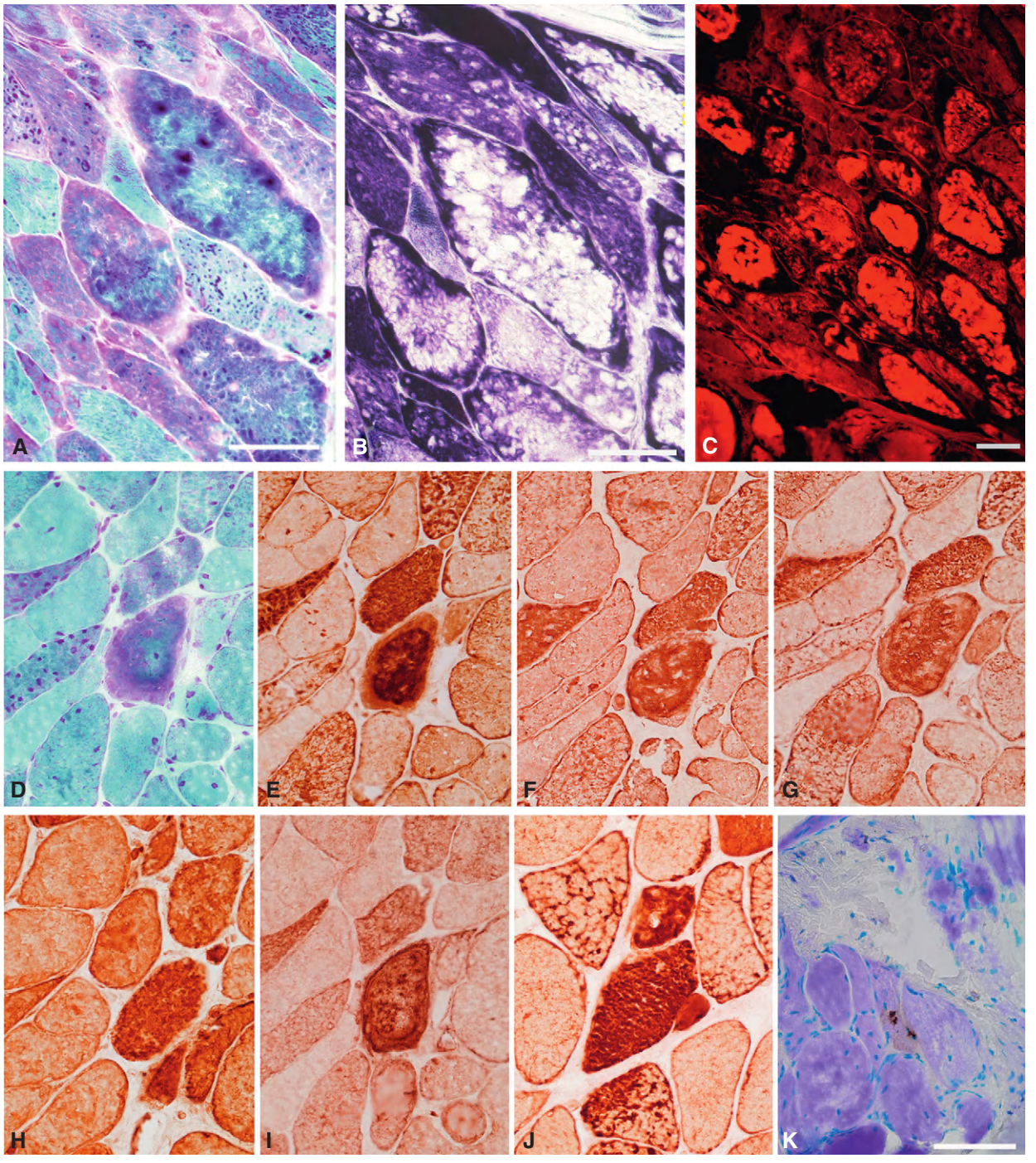

Figure 11.1. Characteristic histological findings for a patient with Bag3opathy in sections stained with trichrome (**A**), reduced nicotinamide–adenine dinucleotide (NADH) dehydrogenase (**B**), and Congo red (**C**). Nonconsecutive sections in the same series stained trichromatically (**D**) and immunoreacted for Bag3 (**E**), αB-crystallin (**F**), desmin (**G**), gelsolin (**H**), neural cell adhesion molecule (**I**), and heat-shock protein 27 (**J**). Note abnormal accumulation of each protein in the structurally abnormal fibers. (**K**) Modified TUNEL stain reveals apoptotic nuclei in two adjacent fibers. For panels (**A–C**), bar = 100 μm; for panels (**D–K**), bar in **K** = 50 μm. (Reproduced with permission from Selcen et al., 2009.)

(Figure 11.1B), but may be accentuated around the larger inclusions. Some hyaline structures are intensely congophilic (Figure 11.1C), and congophilia is best observed in Congo red-stained sections viewed under rhodamine optics. The intensity of the fluorescent signal varies from mild to very intense in different specimens. The congophilic inclusions are not metachromatic with crystal violet stain (V. Askanas, personal communication and confirmed by the present authors) and do not display apple-green birefringence in polarized light. Therefore, they are unlike classical amyloid. Nevertheless, the large congophilic deposits are an important diagnostic feature of MFM biopsies. Increased levels of acid phosphatase appear in some vacuoles and in small foci of many abnormal fibers. Preapoptotic (Selcen and Engel, 2003) and apoptotic nuclei (Figure 11.1K) (Selcen et al., 2009) are present in some cases of MFM.

Signs of denervation, consisting of groups of atrophic fibers composed of fibers of either histochemical type, and increased reactivity of atrophic fibers for nonspecific esterase are observed in some patients. Fiber-type grouping is present in few patients. Many abnormal fibers contain small lakes of periodic acid–Schiff-positive material. The muscle fiber lipid content is normal.

Immunocytochemical features

The affected fibers display abnormal ectopic accumulation of multiple proteins, including myotilin, αB-crystallin (Figure 11.1F), desmin (Figure 11.1G), dystrophin, sarcoglycans, caveolin, neural cell adhesion molecule (Figure 11.1I), plectin, gelsolin (Figure 11.1H), ubiquitin, filamin C, Xin, Bag3 (Figure 11.1E), and heat-shock protein (Hsp) 27 (Figure 11.1J). In addition, abnormal accumulation of phospho-tau, actin, amyloid-βA4, clusterin (Olivé et al., 2005), a mutant nonfunctional ubiquitin, p62, and a multimeric signal protein (Olivé et al., 2008), as well as glycoxidation and lipoxidation markers and neuronal, inducible, and endothelial nitric oxide synthases, superoxide dismutase, neuron-related proteins such as ubiquitin carboxy-terminal hydrolase L1, synaptosome-associated protein 25, synaptophysin, and α-internexin, were observed in myotilinopathy and/or desminopathy muscle specimens (Barrachina et al., 2007; Janue et al., 2007). The increase in the neuron-related proteins was attributed to decreased activity of the neuron-restrictive silencer factor (NRSF)/RE1 silencing transcription factor (REST) (Barrachina et al., 2007).

Ultrastructural features

Electron microscopy reveals that the earliest pathological alterations in MFM characteristically occur at the Z-disk (Figures 11.2 & 11.3). These changes consist of

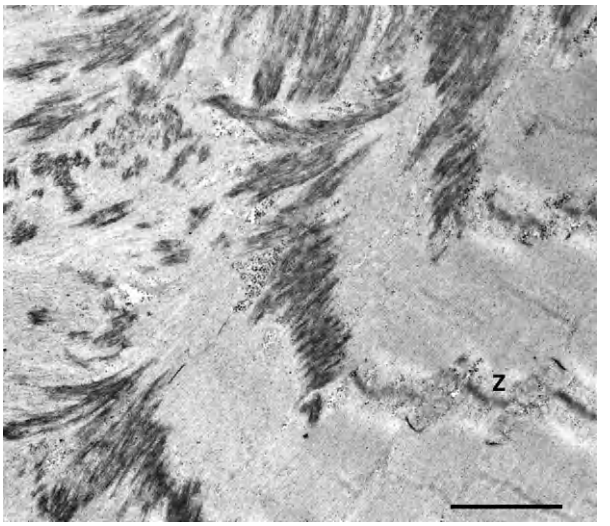

Figure 11.2. Electron micrograph from a patient with zaspopathy. Normal Z-disks are replaced by stripes of dense material. The myofibrils are out of register. Z, Z-disk. Bar = 1 μm. (Reproduced with permission from Selcen et al., 2004.)

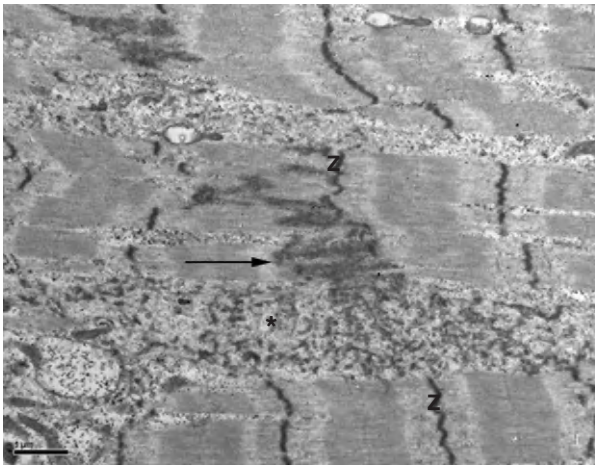

Figure 11.3. Electron micrograph from a patient with Bag3opathy. Z-disk (Z) streaming (arrow) and accumulation of small pleomorphic dense structures between the myofibrils (asterisk). (Reproduced with permission from Selcen et al., 2009.)

streaks of dense material (arrows in Figure 11.4B) or of less dense material dappled with spots of denser material (asterisks in Figures 11.3, 11.4A & B) at or around the Z-disks. In fiber regions in which the Z-disks have disintegrated, the sarcomeres fall apart and myofibrils are no longer recognizable (Figure 11.5A). Dislocated membranous organelles and glycogen accumulate in spaces vacated by disintegrating myofibrils (Figure 11.5B). At a still more advanced stage, large fiber regions harbor fragmented filaments and Z-disk remnants which aggregate into pleomorphic inclusions (Figures 11.5A & 11.6). In other fiber regions, the

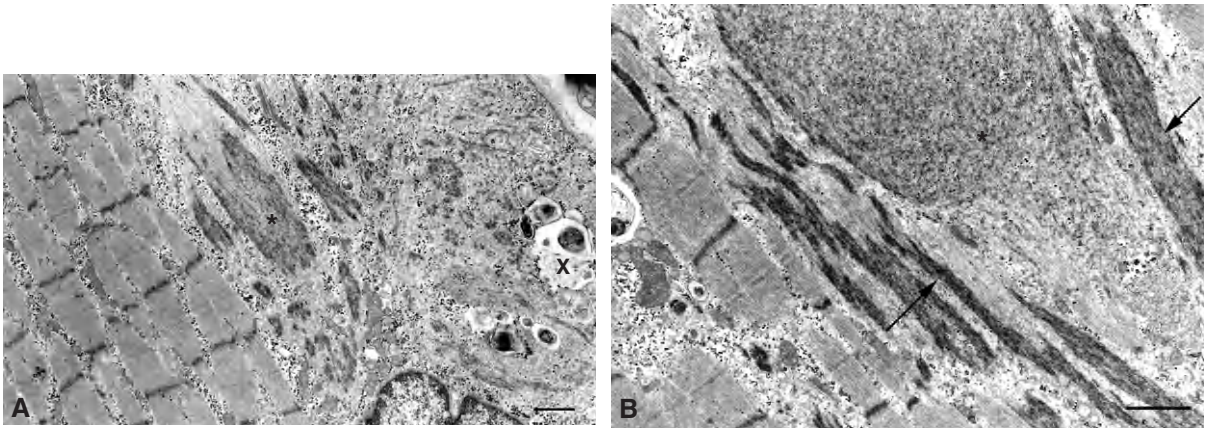

Figure 11.4. Electron micrograph from a patient with myotilinopathy. Streaks of dense material (arrows in **B**) and less dense material are interspersed with dappled small spots of dense material (asterisks in **A** and **B**) of Z-disk origin. Degraded material is accumulating in small autophagic vacuoles (X in **A**). Bars = 1 μm. (Reproduced with permission from Selcen and Engel, 2004.)

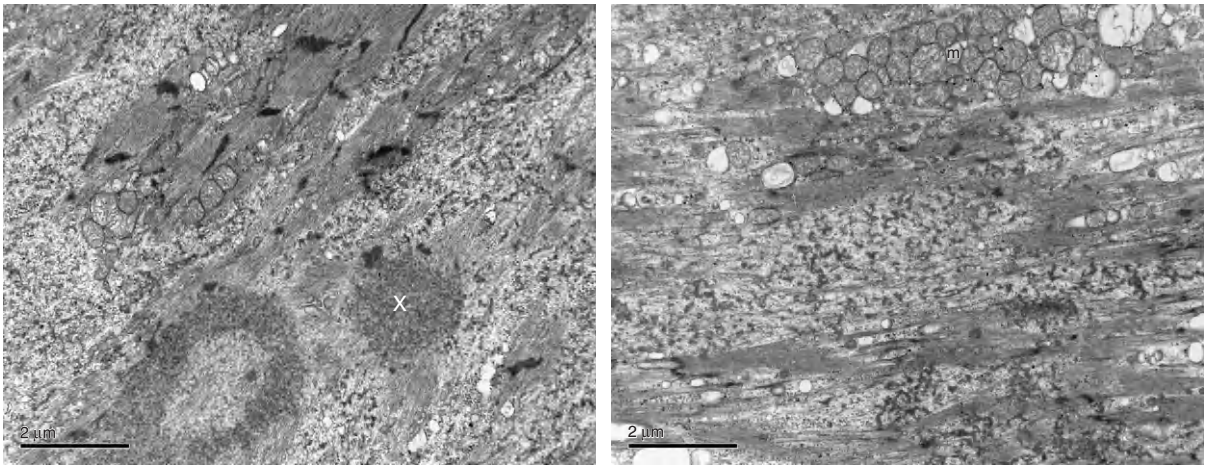

Figure 11.5. Electron micrograph from a patient with Bag3opathy. (**A**) Advanced alterations include disintegration and disarray of the myofibrils, aggregation of fragmented and degraded filaments into dense inclusions (X), and accumulation of granular debris. (**B**) Further destructive changes result in loss of myofibrillar integrity, appearance of dilated vesicles, and aggregation of mitochondria into clusters (m). (Reproduced with permission from Selcen et al., 2009.)

degraded and fragmented filaments accumulate in hyaline structures of variable electron density that entrain glycogen granules (Figure 11.6A & B). The dislocated membranous organelles are trapped and degraded in autophagic vacuoles (Figure 11.7), and some of these undergo exocytosis.

DESMINOPATHY

Desmin is encoded by a single gene, *DES*, located at 2q35. In skeletal muscle, desmin is detected at the periphery of Z-disks, under the sarcolemma, and at the myotendinous and neuromuscular junctions. In cardiac muscle, it is abundant at intercalated disks and Purkinje fibers. Desmin is a type III intermediate filament (IF) protein expressed primarily in skeletal, cardiac, and smooth muscle cells. IFs are 10 nm in diameter, intermediate in size between thick (15 nm) and thin (5–6 nm) filaments. They serve to maintain structural integrity and resist externally applied mechanical stress (Fuchs and Weber, 1994). Desmin and other associated IFs form a heteropolymeric lattice that organizes the myofibrils and links them to nuclei, mitochondria, and the sarcolemma (Fuchs and Weber, 1994; Herrmann and Aebi, 2000; Schroder et al., 2000).

Since the first description of mutations in desmin by Goldfarb and coworkers (1998) and Munoz-Marmol and colleagues (1998), more than 40 mutations have been reported (Figure 11.8). The scapuloperoneal syndrome

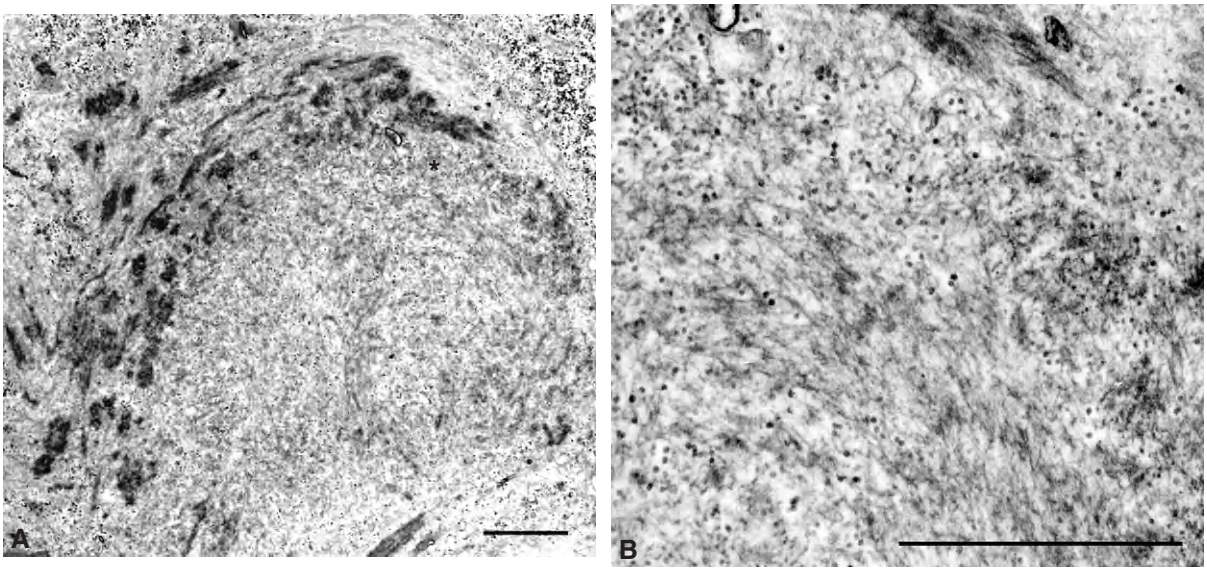

Figure 11.6. Electron micrograph from a patient with myotilinopathy. Large hyaline structures observed by light microscopy consist of compacted and fragmented filaments of variable electron density (**A**). (**B**) A higher magnification of region marked by an asterisk in (**A**) resolves filamentous profiles. Bar = 1 μm. (Reproduced with permission from Selcen and Engel, 2004.)

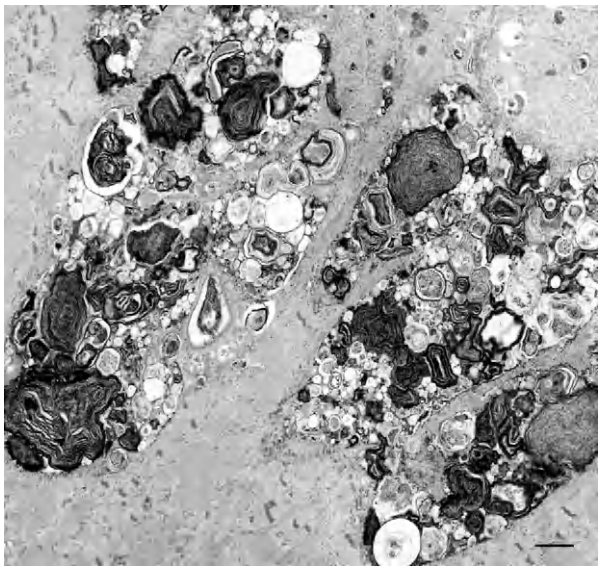

Figure 11.7. Large autophagic vacuoles harbor myeloid structures and debris. Bar = 1 μm. (Reproduced with permission from Selcen et al., 2004.)

of Kaeser has now been traced to an autosomal dominant mutation in desmin in a large kinship (Walter et al., 2007).

The findings in patients with desminopathy are similar to those of other patients with MFM in terms of the distribution of weakness, serum creatine kinase (CK) level, and EMG findings. The age of onset is between 10 and 61 years. The distribution of weakness is distal or both proximal and distal. Muscle atrophy, mild facial weakness, dysphagia, dysarthria, and respiratory insufficiency can occur. The serum CK level is normal or mildly raised. EMG studies usually show myopathic motor unit potentials and abnormal electrical irritability, including myotonic discharges. Cardiomyopathy, especially the arrhythmogenic type, is a common manifestation. With one possible exception (Munoz-Marmol et al., 1998), the family history is consistent with dominant inheritance. Some cases are sporadic (Goldfarb et al., 2004; Selcen et al., 2004).

Some desmin mutants fail to assemble into filaments *in vitro*, but other mutants are able to do so (Goldfarb et al., 1998; Bar et al., 2005, 2006), and in some cases combined expression of mutant and wild-type filaments has produced normal-appearing filaments. The hybrid filaments, however, likely have reduced ability to tolerate mechanical stress (Bar et al., 2007). Recently, abnormal cytoplasmic reactivity for caveolin-3 and aggregates of vesicular and tubular structures were shown in a patient with desminopathy (Shinde et al., 2008). It was hypothesized that the accumulation of multiple proteins could be partially due to inhibited cellular trafficking, as the caveolae are transported normally from the Golgi complex to the surface membrane. However, the increased expression of caveolin could be yet another nonspecifically expressed protein, like dystrophin.

αB-CRYSTALLINOPATHY

The α-crystallins are small heat-shock proteins that associate into 15–20-nm high-molecular-weight soluble aggregates to become functional. At a resolution of 3.6 nm, the aggregates appear as asymmetric globules

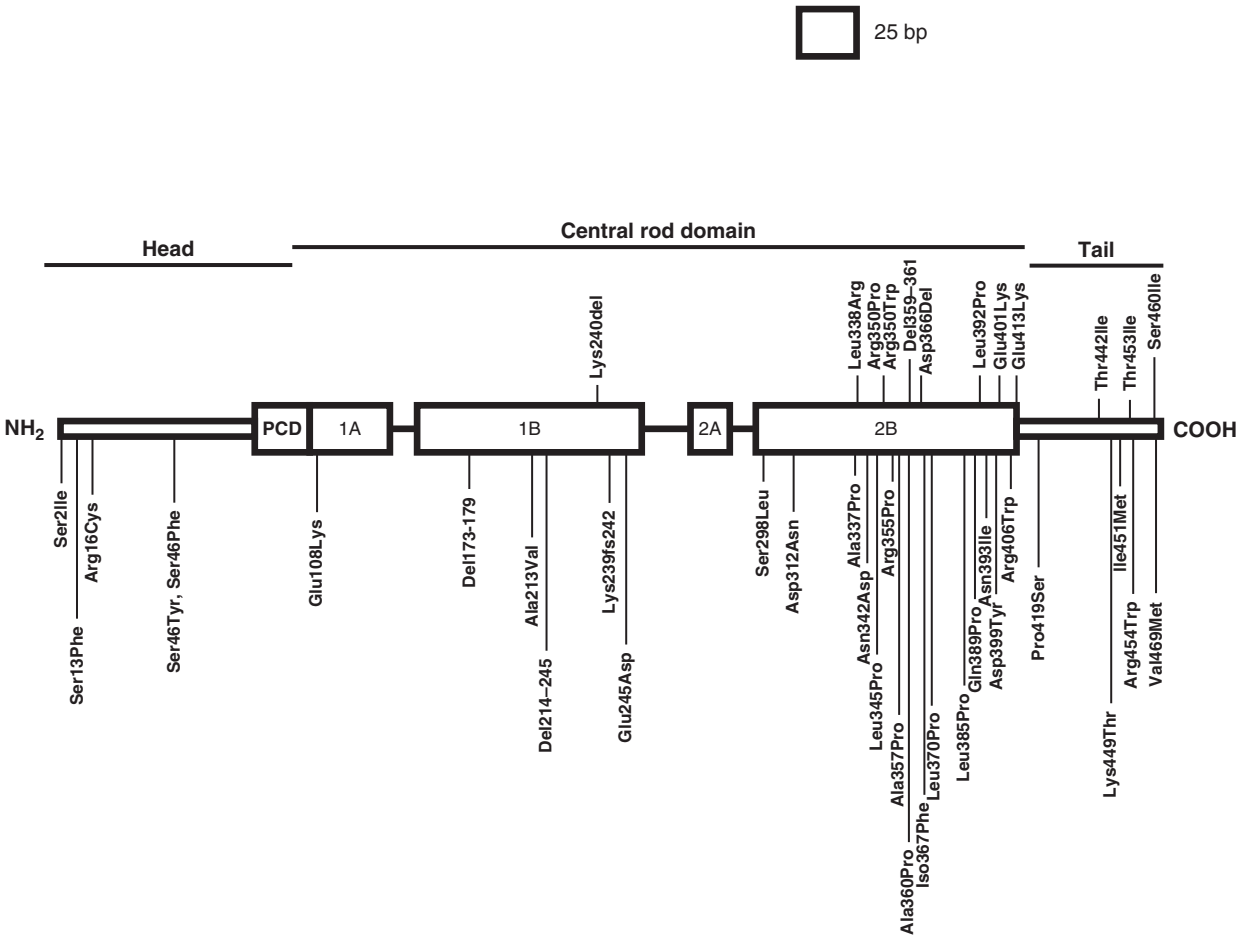

Figure 11.8. Schematic diagram of desmin and the mutations identified to date. PCD, precoiled-coil domain.

with a central cavity (Haley et al., 1998; Derham and Harding, 1999). The aggregates represent homo-oligomers, or even hetero-oligomeric complexes between αB-crystallin and another small heat-shock protein (Narberhaus, 2002). The primary role of α-crystallins is to bind to unfolded and denatured proteins to suppress their nonspecific aggregation. Like other members of small heat-shock protein family, αB-crystallin monomers contain an N-terminal domain (residues 1–63), an α-crystallin domain (residues 64–105), and a C-terminal extension (residues 106–175) (Derham and Harding, 1999).

The A and B forms of α-crystallin are encoded by different genes but have highly homologous amino acid sequences (Derham and Harding, 1999). Both forms are abundant in the lens, where they prevent cataract formation. αB-crystallin is also found in nonlenticular tissues, with highest levels in cardiac and skeletal muscle. In these tissues, αB-crystallin is immunolocalized to the Z-disk and its expression is enhanced after stress (Djabali et al., 1997) and exercise (Neufer et al., 1998). αB-crystallin chaperones actin and desmin filaments (Bennardini et al., 1992), tubulin subunits of microtubules (Arai and Atomi, 1997), and a variety of soluble enzymes (Muchowski and Clark, 1998; Bova et al., 1999), protecting them from stress-induced damage.

In 1998, a heterozygous missense mutation of Arg120Gly in αB-crystallin was identified by Vicart and coworkers (1998) in a previously reported French kinship. Subsequently, two heterozygous truncating mutations were observed in two patients in the Mayo MFM cohort (Figure 11.9) (Selcen and Engel, 2003). Affected patients present in adult life, have symmetrical

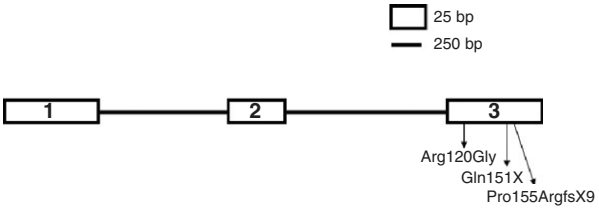

Figure 11.9. Schematic diagram of αB-crystallin and the mutations identified that cause myofibrillar myopathy.

proximal and distal muscle weakness and atrophy, and respiratory involvement. Some patients also have hypertrophic cardiomyopathy, palatopharyngeal weakness, and cataracts.

Transgenic mice with cardiac-specific expression of Arg120Gly αB-crystallin develop cardiomyopathy within 3 months and die from heart failure at 6–7 months (Wang et al., 2001). Interestingly, exercising these mice delays the appearance of heart failure and prolongs survival. A possible explanation is that exercise decreases the accumulation of the preamyloid oligomers, which are the potent mediators of cytotoxicity in protein-misfolding neurodegenerative diseases (Maloyan et al., 2007). Recently, Simon et al. (2007) showed that when mutant αB-crystallin is transfected into COS cells it produces cytoplasmic aggregates, and the expressed mutant protein is more highly phosphorylated than the wild-type protein. Moreover, the mutant proteins interact abnormally with both wild-type and mutant αB-crystallins and other small heat-shock proteins.

MYOTILINOPATHY

Myotilin is a 57-kDa Z-disk-associated protein expressed strongly in skeletal and weakly in cardiac muscle (Salmikangas et al., 1999). It contains a serine-rich amino-terminal region (residues 28–124) that also comprises a hydrophobic stretch (residues 57–79), two immunoglobulin (Ig)-like domains (residues 252–341 and 351–441) and a carboxy-terminal tail. The Ig-like repeats are required for the formation of antiparallel myotilin dimmers (Salmikangas et al., 1999, 2003). Myotilin binds to α-actinin, the main component of the Z-filaments that crosslink actin filaments at the Z-disk, and to filamin C, a peripheral Z-disk protein. The α-actinin binding site resides between myotilin residues 79 and 150 (Hauser et al., 2000, 2002), and the filamin C binding site is located in the second Ig-like domain (van der Ven et al., 2000). In addition, myotilin crosslinks actin filaments and plays a role in the alignment of myofibrils during the later stages of myofibrillogenesis (Salmikangas et al., 2003).

In 2000, a Thr57Ile mutation in myotilin was detected in a large kinship that had previously been linked to the myotilin locus at 5q31 (Hauser et al., 2000). The disease was identified as limb-girdle muscular dystrophy type 1A (LGMD1A). In this kinship, proximal leg and arm muscle weakness appeared in the third decade of life; distal muscle weakness became apparent later in the course of the disease. Tight heel cords and a nasal dysarthric speech were frequently observed. The serum CK level was normal to 15-fold increased. Some patients had hypoactive tendon reflexes. Two years later, a second kinship with a similar phenotype was found to have a Ser55Phe mutation in myotilin (Hauser et al., 2002). One of the reports (Hauser et al., 2002), however, did not assess the pathology of the disease, and the other report (Hauser et al., 2000) showed Z-disk alteration only in some muscle fibers.

Subsequently, six myotilin mutations were identified in eight unrelated patients in the Mayo MFM cohort (Selcen and Engel, 2004), indicating that the morphological substrate of LGMD1A is MFM pathology. In the identified patients, the mean age of onset was 60 years. In three patients the weakness was more prominent distally than proximally. Cardiac involvement without signs of coronary artery disease was evident in three patients. Peripheral neuropathy, reflected by clinical, EMG, and histological criteria, or by a combination of these, was apparent in all patients. Subsequent studies by other investigators identified further patients with mutations in myotilin, including members of a kinship originally described under the rubric of "spheroid body myopathy." These patients also had progressive weakness of proximal and/or distal limb muscles, dysarthric, nasal speech, hypoactive tendon reflexes, and respiratory failure. Dominant inheritance, cardiomyopathy, and intrafamily phenotypic variability were present in some kinships (Foroud et al., 2005; Olivé et al., 2005; Penisson-Besnier et al., 2006; Berciano et al., 2008).

All myotilin mutations reported to date are heterozygous, missense, amino acid changes, and all but one mutation fall in *MYOT* exon 2, a sequence of unknown structure and function (Figure 11.10) (Hauser et al., 2000, 2002; Selcen and Engel, 2004; Foroud et al., 2005; Olivé et al., 2005; Penisson-Besnier et al., 2006; Shalaby et al., 2007).

Targeted deletion of myotilin in mice does not lead to obvious abnormalities, and morphology and muscle strength are normal (Moza et al., 2007). On the other hand, transgenic mice expressing a disease-causing

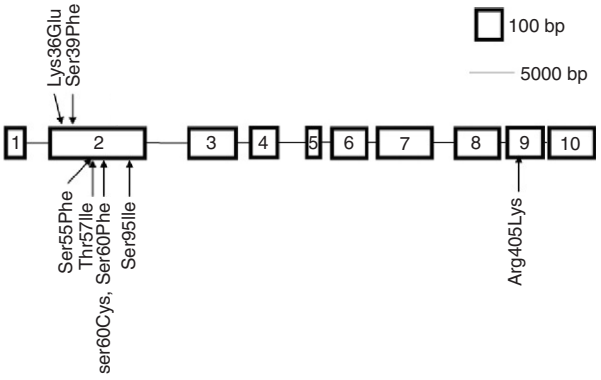

Figure 11.10. Schematic diagram of myotilin and the mutations identified to date.

mutant myotilin, Thr57Ile, reproduce the morphological and functional features of human myotilinopathy, and the morphological changes become progressively larger and more numerous with age (Garvey et al., 2006). Interestingly, double-transgenic mice expressing both wild-type and Thr57Ile myotilin exhibit significantly more severe muscle degeneration with an earlier onset of muscle pathology compared with single Thr57Ile transgenic mice (Garvey et al., 2008). This observation suggests that the presence of wild-type myotilin protein enhances the disease process.

ZASPOPATHY

ZASP (Z-band alternatively spliced PDZ motif-containing protein) is expressed predominantly in cardiac and skeletal muscle (Faulkner et al., 1999). It binds to α-actinin (Zhou et al., 1999), the structural component of the Z-filaments that crosslink thin filaments of adjacent sarcomeres. cDNAs encoding mouse and human *ZASP* were obtained in 1999 (Faulkner et al., 1999). Sixteen *ZASP*-associated exons have been detected in genomic DNA, and splice variants of these exist in cardiac and skeletal muscle. Skeletal muscle harbors three isoforms (Figure 11.11). The longest isoform lacks exons 4 and 9; another long isoform lacks exons 4, 9, and 10; and a short isoform lacks exon 4 and carries a stop codon in exon 9. In the mouse and human, three cardiac isoforms resemble those in skeletal muscle but contain exon 4 instead of exon 6 (Huang et al., 2003). Recently, however, exon 6 was also detected in the human heart. Mutations in exon 6 cause cardiomyopathy in humans, but the composition of the cardiac transcripts harboring exon 6 has not been determined (Vatta et al., 2003). All ZASP isoforms have an N-terminal PDZ domain important for protein–protein interactions (Harris and Lim, 2001) and a 26-residue ZM motif in exons 4 and 6 needed specifically for interaction with α-actinin (Klaavuniemi et al., 2004). The long isoforms have three C-terminal LIM domains that interact with protein kinase C subtypes (Arimura et al., 2004). Targeted deletion of *ZASP* in the mouse – referred to as Cypher (Zhou et al., 1999) or Oracle (Passier et al., 2000) in the mouse – causes skeletal and cardiac myopathy with fragmented Z-disks (Zhou et al., 2001).

Zaspopathy causing MFM was first described in 2005 by Selcen and Engel in 11 patients with MFM who carried heterozygous missense mutations in *ZASP*. The mean age of onset was in the sixth decade. Most patients presented with muscle weakness but one patient, whose father had muscle weakness, presented with palpitations and mild hyperCKemia. Seven of the 11 patients had a family history consistent with autosomal dominant inheritance. In five patients the weakness

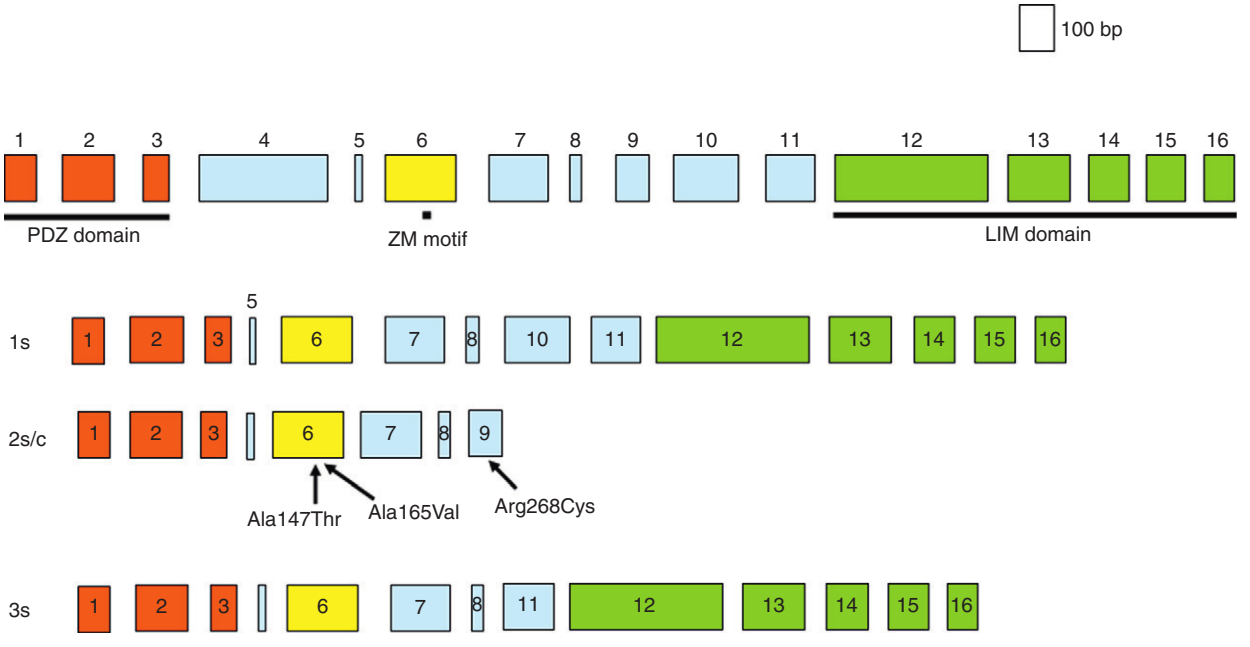

Figure 11.11. Diagram of the genomic structure of *ZASP* and the mutations identified to date. Top row shows the 16 *ZASP* exons. Not all exons are transcribed in cardiac and skeletal muscle. 1s, 2s, and 3s are expressed in skeletal muscle. The PDZ domain and the ZM motif appear in all three transcripts; the three LIM domains are present only in the two long transcripts. The Ala147Thr and Ala165Val mutations are predicted to appear in all three ZASP isoforms, whereas the Arg268Cys mutation is predicted to appear only in the short isoform.

was more prominent distally than proximally, and it was distal in only one patient. Two patients had only proximal muscle weakness. The three remaining patients had both proximal and distal muscle weakness. Three patients had cardiac involvement without signs of coronary artery disease, and in one of these the cardiac symptoms antedated the muscle weakness by 10 years. Peripheral nerve involvement by clinical, EMG, or histological criteria was detected in five patients.

The mutations detected by Selcen and Engel (2005) were Ala147Thr and Ala165Val in exon 6, and Arg268Cys in exon 9 (see Figure 11.11). The Ala165Val mutation is within, and the Ala147Thr mutation is immediately before, the ZM motif needed for interaction with α-actinin (Klaavuniemi et al., 2004).

Subsequently, a large kinship, originally described by Markesbery et al. in 1974, as well as five other kinships with distal myopathy and MFM pathology were shown to carry the same Ala165Val mutation. These six kinships and three of the zaspopathy kinships observed at the Mayo Clinic may have a common founder (Griggs et al., 2007).

FILAMINOPATHY

In 2005, Vorgerd and coworkers detected a dominant Trp2710X mutation in the last exon of filamin C in 17 affected individuals of a large German kinship. Eight examined patients presented between 37 and 57 years of age with slowly progressive distal leg weakness. The serum CK level was increased up to 8-fold. Four patients had signs of respiratory insufficiency, one had an incomplete right bundle branch block, and three also had signs of a peripheral neuropathy. Recently, two additional German families with the same nonsense mutation in filamin C were reported. All three families may have a common founder (Kley et al., 2007). Subsequently, three MFM kinships carrying the same mutation were identified in the Mayo Clinic MFM cohort. The age of onset and the clinical presentation were similar to those reported by Vorgerd and colleagues (2005).

In experimental studies, the dimerization domain of the mutant filamin C is less stable and more susceptible to proteolysis than that of the wild-type protein. As a consequence, the mutant protein does not dimerize properly and forms aggregates *in vitro* and in cultured cells (Vorgerd et al., 2005; Lowe et al., 2007). Mice carrying a deletion of the last eight filamin C exons have a severely reduced birthweight, a reduced number of muscle fibers and primary myotubes, and die shortly after birth from respiratory failure (Dalkilic et al., 2006). These findings emphasize the important role of filamin C in muscle development.

BAG3OPATHY

Bag3, also referred to as CAI stressed-1 (CAIR-1) or Bis, is a multidomain co-chaperone protein interacting with many other polypeptides. Like other members of the Bag family, it harbors a C-terminal BAG domain (residues 418–498) that mediates interaction with Hsp70 and the antiapoptotic protein Bcl-2, and a proline-rich region (residues 302–417) that interacts with WW domain proteins implicated in signal transduction and with Src-3 homology (SH3) domain proteins such as phospholipase Cγ1, which also participates in antiapoptotic pathways (Figure 11.12). Bag3 also has a unique N-terminal WW domain (residues 21–55) that binds proline-rich sequences. Bag3 forms a stable complex with the small protein Hsp8 and thereby participates in the degradation of misfolded or aggregated proteins (Takayama and Reed, 2001; Doong et al., 2002; Carra et al., 2008). Bag3 is expressed strongly in skeletal and cardiac muscle, and at a lower level in other tissues. Its targeted deletion in mice results in a fulminant myopathy with early lethality (Homma et al., 2006).

Bag3opathy causing MFM was described by Selcen and coworkers (2009) in three patients with MFM who were heterozygous for Pro209Leu in exon 3 (see Figure 11.12). All three presented in childhood with severe progressive muscle weakness. All had cardiomyopathy and developed respiratory insufficiency with diaphragm paralysis. Two also had a rigid spine. The muscle weakness was proximal in only one patient, both proximal and distal in the second patient, and distal more than proximal in the third. The serum CK level ranged from 3 to 15 times above the upper limit of normal. One patient studied by EMG had features of both axonal and demyelinating polyneuropathy.

Nuclear apoptosis had been reported to be absent in patients with MFM (Amato et al., 1999), but in αB-crystallinopathy 8% of the nuclei have preapoptotic features (Selcen and Engel, 2003) and in Bag3opathy 8% of the nuclei are frankly apoptotic (Selcen et al., 2009) (see Figures 11.1K & 11.13). The enhanced nuclear apoptosis in Bag3opathy is consistent with the known antiapoptotic effect of Bag3 (Liao et al., 2001; Doong

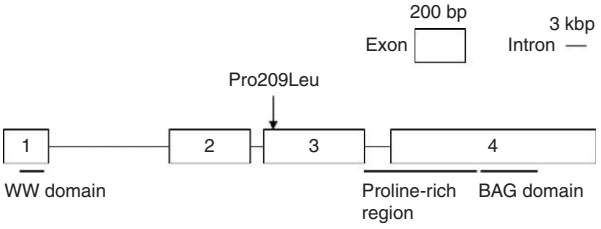

Figure 11.12. Diagram of the genomic structure of *BAG3* and the identified mutation.

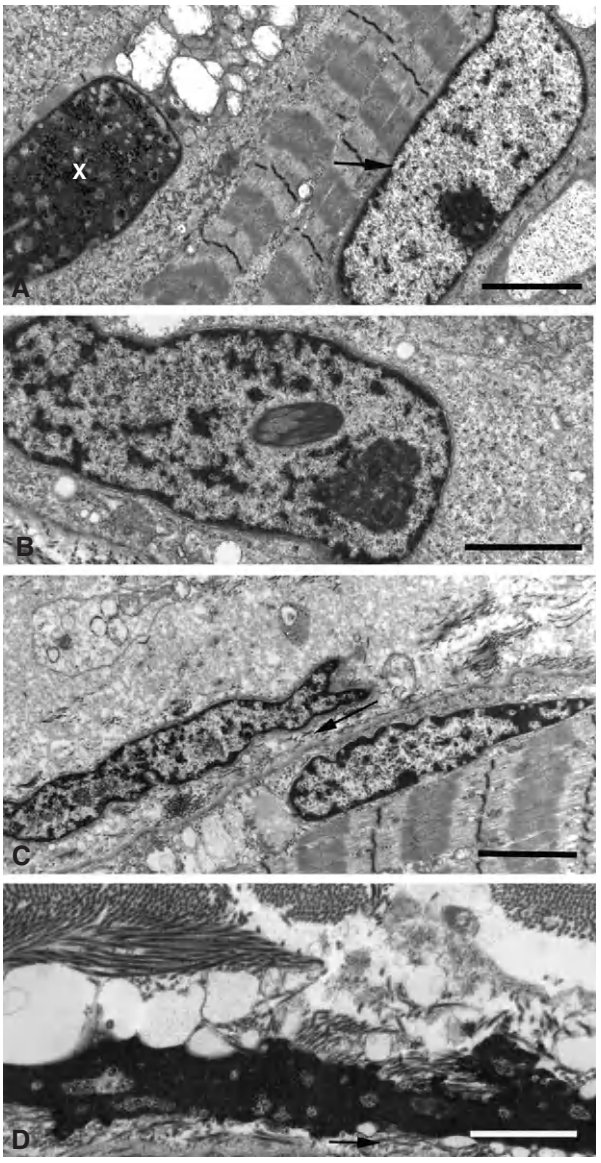

Figure 11.13. Nuclear alterations in a patient with Bag3opathy. (**A**) Ovoid nucleus with prominent nucleolus suggesting increased transcription (arrow) and apoptotic nucleus (X). (**B**) Large nucleus harboring clumps of heterochromatin. (**C**) A superficially positioned, shrunken (pyknotic) nucleus in a muscle fiber is positioned under an exocytosed pyknotic nucleus. Arrow points to collagen fibrils in the extracellular space. (**D**) An exocytosed apoptotic nucleus. Arrow points to collagen in the extracellular space. Bar = 2 μm in (**A**) and (**C**) and 1 μm in (**B**) and (**D**). (Reproduced with permission from Selcen et al., 2009.)

et al., 2002; Bonelli et al., 2004) and indicates that Pro209 contributes to this effect.

On nondenaturing electrophoresis of patient and control muscle extracts, the Bag3 complex from patients migrates faster than that from controls. The faster migration of the mutant complex could be due to a change in the size or charge. Because a proline to leucine mutation results in no change in charge, the faster mobility of the mutant complex is likely due to its smaller size. A plausible reason for this is failure of some of the binding partners of Bag3 proteins to associate with the complex. Transfection of COS-7 with FLAG-labeled mutant and wild-type Bag-3 revealed a marked tendency of the mutant protein to aggregate into small granules. A likely reason for this is altered folding, and predictably altered function, of the mutant protein (Selcen et al., 2009).

THERAPEUTIC APPROACHES

No known measures mitigate the slow but relentless progression of MFM. Physical therapy, consisting of passive exercises, orthoses, and other supporting devices, is helpful in the more advanced cases. Respiratory support consisting of continuous (CPAP) or bilevel (BIPAP) positive airway pressure ventilation, initially at night and later in the daytime, are indicated in patients with respiratory failure and signs of hypercapnea.

Periodic monitoring of patients for the appearance of cardiomyopathy should be done in all patients, and a pacemaker or implantable cardioverter defibrillator (ICD) should be considered in individuals with arrhythmia and/or cardiac conduction defects. Patients with progressive or life-threatening cardiomyopathy are candidates for cardiac transplantation.

CONCLUDING COMMENT

That the initial pathological change in MFMs is centered on the Z-disk accounts for the nearly stereotypical pathology and implicates Z-disk-related proteins as culprits. Thus, a careful observation of the morphological features of MFM has enabled the candidate gene approach. It can be predicted confidently that further studies will uncover additional MFM disease genes. A major unsolved issue is deciphering the signaling mechanism between the Z-disk and the nucleus that results in abnormal transcription, translation, or both, of multiple genes.

ACKNOWLEDGMENTS

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Chapter 12

Emery–Dreifuss muscular dystrophy

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INTRODUCTION

Emery–Dreifuss muscular dystrophy was originally described as an X-linked recessive (XLR) form of muscle disease uniquely associated with early contractures that affect the Achilles tendon, spine, and elbows, and with abnormalities of the cardiac conduction system. Like the cardiac conduction system disease, muscle weakness in EDMD is also progressive; however, the specific cardiac involvement in EDMD is associated with a high frequency of sudden cardiac death. EDMD is less frequent than Duchenne or Becker muscular dystrophy (DMD or BMD) but is the third most common form of XLR muscular dystrophy.

In this chapter, we review the clinical findings in EDMD with emphasis on the cardiac findings. In addition, we cover autosomal dominantly (AD) inherited EDMD as well as the phenotypic spectrum that is now associated with mutations in the genes associated with EDMD. Although many of these disorders do not fulfill the clinical features ascribed to EDMD, these disorders inform the clinician caring for patients with EDMD and extend the biological underpinnings of these disorders now referred to as "nuclear envelopathies."

HISTORICAL REVIEW

A series of European Neuromuscular Centre workshops on EDMD have charted the genetic and clinical progress of the field. The first of these, in 1991, defined EDMD as follows:

1. Progressive myopathy with early contractures;
2. Slowly progressive muscle weakness targeting the humeral and peroneal distributions;
3. Atrioventricular cardiac conduction system disease and possible cardiomyopathy;
4. Myopathic features, and possibly dystrophic features, on muscle biopsy.

In 1991, it was noted that this diagnosis required XLR inheritance (Yates, 1991). However, in 1998 a conference was held in which both sporadic and autosomally inherited forms of disease resembling EDMD were clearly described (Wehnert and Muntoni, 1999). In 2000 and 2002, workshops highlighted the genetic progress in understanding the molecular overlap between XLR and AD-inherited EDMD (Ellis, 2001; Bonne et al., 2002). To date, mutations in two genes are known to cause EDMD. X-linked EDMD is caused by mutations in the emerin gene. AD and autosomal recessive (AR) EDMD are caused by mutations in the *LMNA* gene. These genes code for proteins that are found in the nuclear membrane. Recently, mutations in the nuclear membrane-associated proteins, the nesprins, were also described with EDMD-like phenotypes, potentially extending the spectrum of nuclear envelope disease (Q. Zhang et al., 2007; Puckelwartz et al., 2010).

In 1966, Emery and Dreifuss studied a family in the USA with affected males in three generations. This disorder was considered to be benign compared with DMD and BMD. Elbow and spine contractures were noted along with proximal upper extremity and distal lower extremity weakness. Atrial flutter with atrioventricular block was described in one patient. In 1975, two families were described with 37 affected males with distal lower extremity and proximal upper extremity weakness (Waters et al., 1975). Bradycardia and syncope occurred in 15 individuals before the age of 50 years. A range of cardiac conduction system disease was noted, ranging from first-degree atrioventricular (AV) block to atrial standstill. Notably, cardiac failure was not commonly seen. Initially, there was some confusion whether this disorder represented an allelic variant of BMD. Ultimately, through clinical and genetic studies, EDMD was mapped to Xq28, distinct from the dystrophin locus.

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Thus, EDMD is genetically distinct from BMD and distinctive because of its frequent association with sudden cardiac death.

With the appreciation of both XLR and AD forms of EDMD, it is now clear that the AD form of EDMD is more common than XLR EDMD. Over 300 different mutations in the *LMNA* gene have been associated with dilated cardiomyopathy, often accompanied by conduction system disease and varying degrees of muscle weakness. *LMNA* encodes lamins A and C; the 3′ end of the *LMNA* gene is variably spliced, producing either lamin A or lamin C. Clinical genetic testing is now performed routinely for *LMNA* and emerin gene mutations, and should be considered for individuals with cardiomyopathy and conduction system disease, particularly when a family history of similar findings is present. As outlined below, the first presentation of EDMD may be sudden death. Family screening to identify those at risk for cardiac arrhythmias may be lifesaving.

CLINICAL FEATURES

Muscle disease and clinical testing

Most patients with EDMD, both XLR and AD, are normal at birth and in their first few years of life. Contractures often develop in the second decade of life, affecting the elbows or the ankles. Contractures can also affect the posterior cervical muscles, limiting neck flexion. Eventually, forward flexion of the spine may become limited. The muscle weakness that accompanies EDMD is slowly progressive and typically targets the peroneal and humeral distribution. Muscle wasting is common and may affect the biceps and triceps muscles concomitant with contractures that limit the ability to reach above the head. The deltoid muscle may be preserved initially. Scapular winging may be present. Early findings in the lower extremities can include toe-walking, slow running, and loss of balance or falling. Achilles tendon contractures are often present. With time, a limb-girdle distribution of muscle weakness may develop and lead to difficulty climbing stairs or rising from a chair. Unlike other muscular dystrophies, early elbow contractures greatly limit the ability of the patient to utilize arm strength. Early hyporeflexia or areflexia may help distinguish EDMD from BMD.

EDMD is variable in both age of onset and disease progression. In severe forms of EDMD, patients may lose ambulation in the late second to third decade. With these severe forms, there may be more widespread contractures affecting the wrists and spine, leading to a rigid spine. The cardiac features may be the earliest presentation of EDMD and may limit the skeletal findings, particularly if early sudden death occurs.

The genetics of EDMD has expanded the clinical phenotype by documenting the range of findings with mutations in emerin or the gene encoding lamin A and C, *LMNA*. Limb-girdle muscular dystrophy, or progressive muscle weakness in the absence of contractures, may arise from *LMNA* gene mutations (Figure 12.1) (Muchir et al., 2000). Quadriceps myopathy associated with dilated cardiomyopathy has been associated with a *LMNA* gene mutation (Charniot et al., 2006). AR-EDMD has been reported in one patient with a severe myopathy but no cardiac involvement (Raffaele Di Barletta et al., 2000). Overall, the phenotype including the degree of skeletal muscle involvement and degree of cardiomyopathy is highly variable (Mercuri et al., 2005). This variability relates to individual mutations, or alleles, but also can be associated with the identical allele, suggesting that other factors contribute to this clinical range. Conditions such as nonsense-mediated decay of the mutant allele may partially explain variable expressivity (Geiger et al., 2008).

The muscle biopsy in EDMD is similarly variable, typically showing myopathic changes of centrally placed nuclei and variable fiber size. Hypertrophic fibers may be present and fiber splitting is often evident. Fiber atrophy, increased fibrosis, and scattered necrosis may occur. The large focal regions of damage that typify DMD do not occur in EDMD. Fiber typing may be skewed but either type I or type II fibers can predominate. In some cases, dystrophic changes including fibrofatty infiltration may be present. The serum creatine kinase (CK) level is mildly raised or normal. The CK value distinguishes EDMD from DMD/BMD, because in DMD/BMD serum CK levels are usually increased by at least 10-fold. In later-stage DMD or BMD, the decline in muscle mass

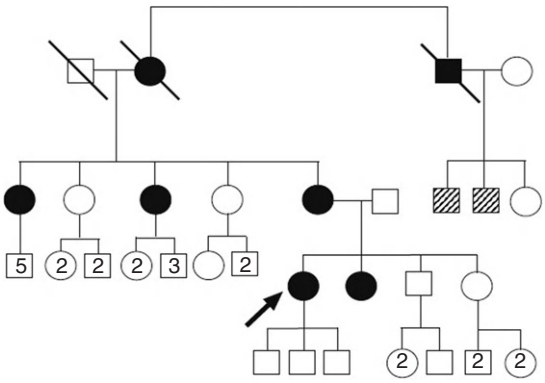

Figure 12.1. Mutations in *LMNA* are inherited in an autosomal dominant manner. Typical pedigree associated with *LMNA* gene mutations showing a family that carries a 2-bp deletion that led to a truncation at amino acid 302. The Δ302 mutation is inherited in an autosomal dominant manner and causes both cardiac and skeletal muscle disease. The proband has atrioventricular block and is indicated by an arrow.

may be associated with a fall in CK concentration. If the CK level is increased, the MB fraction may be raised; this may reflect skeletal muscle turnover and is not a sign of cardiac muscle degeneration.

Needle electromyography usually shows myopathic changes, including features consistent with reinnervation. Irregular motor unit potentials have been noted, and, in other studies, these findings have been thought to reflect neurogenic abnormalities. However, irregular motor unit potentials may reflect fiber splitting and hypertrophy, and variable fiber size (Rowinska-Marcinska et al., 2005). Nerve conduction studies are usually normal.

Cardiac findings and clinical testing

The cardiac conduction system is the earliest target of disease and may be evident long before muscle weakness develops. The risk of sudden death is present before the onset of muscle weakness; thus, upon ascertainment of an affected family member, the genetics must be determined and family members must be screened to limit cardiac sudden death (Fatkin et al., 1999). Surface electrocardiography findings include first-degree AV block, broad flat P waves, absent P waves, right bundle branch block, atrial fibrillation or atrial flutter (Figure 12.2). When atrial fibrillation or atrial flutter is present, a rapid ventricular response is often not present. This lack of rapid ventricular response reflects the underlying pathology of the AV node and caution should be used with drugs that further block the AV node. Holter monitoring or event monitoring can be used to detect paroxysmal atrial fibrillation or flutter.

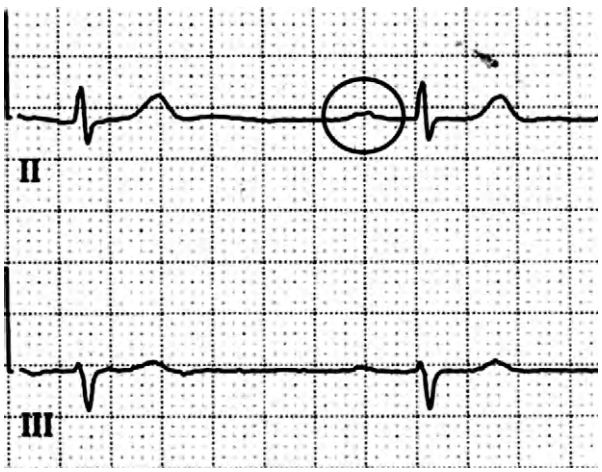

Figure 12.2. Mutations in *LMNA* can cause cardiac conduction system disease. Twelve-lead electroencephalograph of proband with the 302Δ mutation showing sinus node dysfunction leading to bradycardia and atrioventricular node dysfunction manifesting as first-degree heart block.

The major risk with EDMD is that of sudden death (van Berlo et al., 2005). It has been recognized in EDMD that bradyarrhythmias may contribute to this risk of sudden death but that pacemaker implantation is insufficient to protect fully against this risk (Sanna et al., 2003). Many of the studies on *LMNA* mutations include patients with little to no skeletal muscle involvement, but these studies are illustrative of what may occur in EDMD (Taylor et al., 2003). Pacemaker implantation does not always prevent sudden death. In one large family, 38 affected family members died suddenly. Of these, 10 had pacemakers but still had sudden death (Nelson et al., 1998). More recently, Meune et al. (2006) studied patients with *LMNA* mutations; of 19 patients with *LMNA* mutations, eight (42%) received appropriate defibrillation from an internal device over a 3-year follow-up period. Of note, the average left ventricular ejection fraction was 58% in this group, indicating that life-threatening tachyarrhythmias may occur with normal heart function. Of this group, over half had accompanying muscle disease. The conclusion from this study is that primary prophylactic placement of internal cardioverter defibrillators (ICDs) may be warranted for patients with *LMNA* gene mutations associated with EDMD. ICDs, if placed, should also include pacing function because of the high likelihood of high-grade AV node block. The age at which ICD implantation should be considered has not been determined. Sudden death with *LMNA* gene mutations can occur in the second decade, but the average age of in the study of Meune et al. (2006) was 42 years. In the work of van Berlo et al. (2005), describing 299 subjects with a range of *LMNA* mutations, 92% had some form of irregular heart rhythm by the age of 30 years.

The echocardiogram is frequently normal early in EDMD, but progressive left and right ventricular dilatation may occur along with reduced systolic function, manifested as reduced shortening fraction or ejection fraction. More sensitive imaging modalities including tissue Doppler or cardiac magnetic resonance imaging can be used to detect preclinical disease in EDMD (Smith et al., 2006). Clinical correlates of heart failure may develop, including progressive dyspnea on exertion, paroxysmal nocturnal dyspnea, and peripheral edema. These findings may be accentuated by concomitant hypoventilation from pulmonary musculature weakness, so pulmonary function testing should be conducted routinely, and hypoventilation and hypoxia should be treated.

Cardiac pathology, in EDMD, includes marked fibrosis (van Tintelen et al., 2007) or left ventricular noncompaction (Hermida-Prieto et al., 2004). In left ventricular noncompaction the developmental pattern of left ventricular trabeculation remains prominent in the mature

heart instead of undergoing the normal remodeling and compaction that occurs during development. Left ventricular noncompaction is frequently associated with cardiomyopathy and reduced cardiac function. Cardiac aneurysm formation has also been described due to *LMNA* gene variants (Forissier et al., 2003).

GENETICS AND MOLECULAR PATHOLOGY

The inner and outer nuclear membranes comprise the nuclear envelope. The nuclear envelope serves to separate DNA replication, mRNA transcription and processing, and ribosome assembly from cytoplasmic functions. The outer and inner nuclear membranes are linked at the edge of each nuclear pore complex, resulting in one contiguous membrane (Figure 12.3). The outer nuclear membrane is also contiguous with the endoplasmic reticulum so that the perinuclear space between the outer and inner membranes is an extension of the endoplasmic reticulum. The inner nuclear membrane faces the nucleoplasm and interacts with the lamina. The lamina is a fibrous network composed of intermediate filament proteins, lamins A, B, and C. The lamina associates with the inner nuclear membrane through integral membrane proteins, including emerin and nesprin-1 and -2. The lamina is responsible for supporting the nuclear envelope and maintaining nuclear shape. The lamina also serves as a scaffold for chromatin through direct and indirect interactions, and may play a role in the regulation of gene transcription. One hypothesis to explain the etiology of EDMD is that a complex exists between the nucleus and the cytoplasm that is perturbed when the genes causing EDMD are mutated.

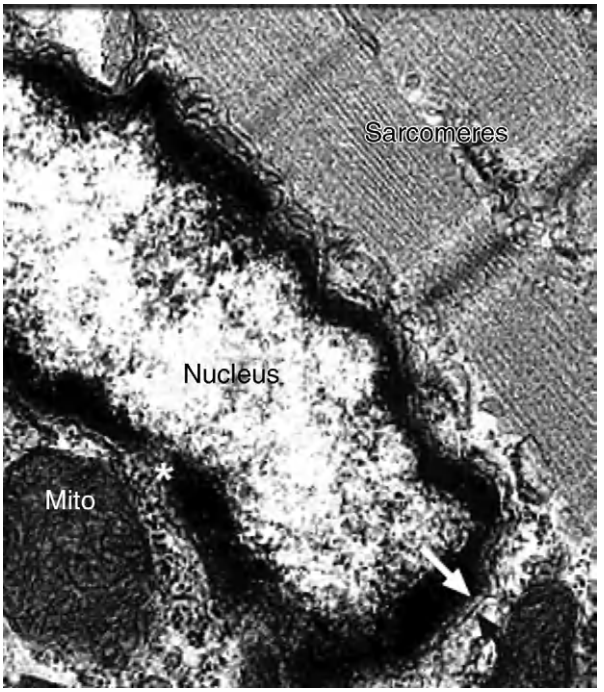

Figure 12.3. Electron micrograph of the nuclear envelope in skeletal muscle. An electron micrograph of quadriceps muscle shows a nucleus surrounded by sarcomeres. The electron-dense region at the periphery contains condensed heterochromatin and the nuclear lamina. Bordering the electron-dense material is the double-bilayer structure of the nuclear envelope, where the outer lipid bilayer is the outer nuclear membrane (black arrow) and the inner nuclear membrane (white arrow). A nuclear pore traversing the inner and outer nuclear membranes is indicated by a white asterisk. A mitochondrion is indicated (Mito).

Emerin

EMD, the gene encoding emerin, was first linked to EDMD in 1994 (Bione et al., 1994). Over 100 mutations have been identified throughout the emerin gene, with no indication of mutational "hotspots" (Figure 12.4) (see The Universal Mutation Database (UMD)/*EMD* mutations database at www.umd.be/EMD/) (Beroud et al., 2000). The majority of the mutations are nonsense, missense, frameshift, or putative splice-site mutations (Yates et al., 1999). Most of these mutations produce premature stop codons in the open reading frame and result in the absence of emerin protein. Approximately 15% of known mutations result in expression of modified forms of emerin (Manilal et al., 1996; Nagano et al., 1996; Yates et al., 1999). Female carriers of *EMD* mutations may have conduction abnormalities without muscle

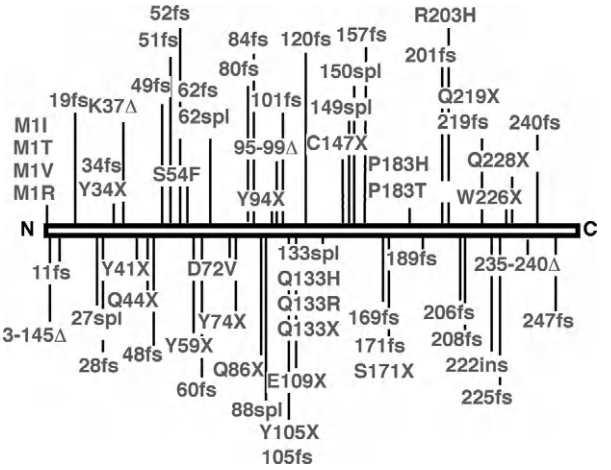

Figure 12.4. Mutations in the nuclear envelope protein emerin cause EDMD. Shown is a schematic of the 254 amino acid protein, emerin. Protein variants responsible for X-linked EDMD are labeled. Mutations were compiled using The Universal Mutation Database at www.umd.be.

weakness due to skewed X-inactivation, and therefore should be assessed for cardiac defects (Manilal et al., 1998).

The emerin gene is located on chromosome Xq28 and encodes a 29-kDa serine-rich transmembrane protein that is localized to the nuclear rim in all tissues examined, including skeletal and cardiac muscle. Emerin is a type II integral membrane protein anchored to the inner nuclear membrane near its carboxy-terminus, with its amino-terminus projecting into the nucleus (Manilal et al., 1996). *L*amin-associated protein (LAP), *E*merin and *M*AN1 are nuclear envelope proteins that share a common protein domain, the LEM domain. LEM domains bind to BAF (barrier to autointegration factor), a small protein that can aggregate and bind directly to DNA. BAF is hypothesized to link the inner nuclear membrane to the lamins and to chromatin (Lee et al., 2001). Emerin has a number of binding partners including the transcriptional repressors GCL (germ cell less) and Btf (death promoting transcriptional repressor), YT521-B, an RNA-associated splicing factor, inner nuclear membrane isoforms of nesprin-1 and -2, MAN1, nuclear actin, nuclear myosin I, nuclear αII spectrin, and lamins A, B, and C (Bengtsson and Wilson, 2004). Emerin interacts directly with lamin A and nesprin-1α, a splice form of nesprin-1, at the inner nuclear membrane, and this interaction is required for localization of emerin to the nuclear membrane (Sullivan et al., 1999; Clements et al., 2000; Vaughan et al., 2001; Mislow et al., 2002a; Muchir et al., 2003).

Lamin A/C

In 1999, Bonne et al. identified mutations in the *LMNA* gene in families with autosomal dominant Emery–Dreifuss muscular dystrophy (AD-EDMD). Mutations in *LMNA* cause a wide variety of diseases called the laminopathies. The laminopathies include skeletal and cardiac muscle disease (AD-EDMD, AR-EDMD, dilated cardiomyopathy with conduction system defects, limb-girdle muscular dystrophy 1B), a peripheral neuropathy (Charcot–Marie–Tooth syndrome type 2b), lipodystrophies (Dunnigan-type familial partial lipodystrophy, mandibuloacral dysplasia), and premature aging disorders (Hutchinson–Gilford progeria, atypical Werner syndrome, and restrictive dermopathy). Approximately 15% of all *LMNA* mutations causing striated muscle disease are nonsense and may lead to haploinsufficiency of lamin A/C due to degradation of the amino-terminus (see UMD/*LMNA* mutations database at www.umd.be/LMNA/) (Beroud et al., 2000). For *LMNA* mutations that affect heart and skeletal muscle (Figure 12.5), there is marked interfamilial and intrafamilial variability in clinical expression, indicating that genetic modifiers may be involved in disease progression (Bonne et al., 2000). Additionally, there is no clear genotype–phenotype correlation for *LMNA* mutations and striated muscle dysfunction. Unlike emerin, mutations in the lamin A/C gene are inherited predominantly in an AD manner. A haploinsufficient mechanism has been suggested for a subset of *LMNA* mutations whereby a half-dose of lamin A/C may lead to disease

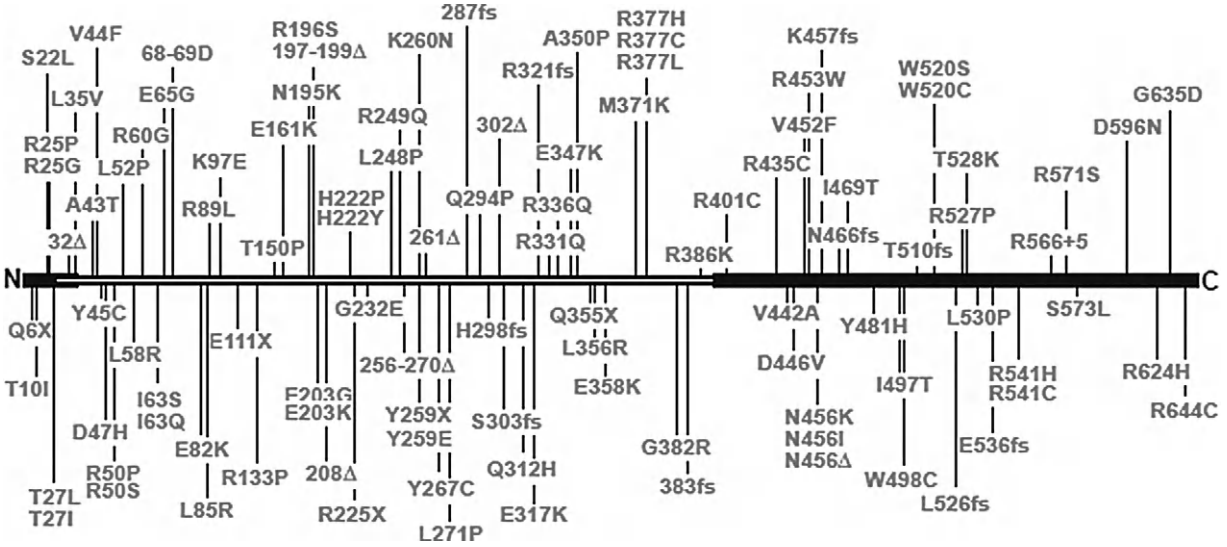

Figure 12.5. Mutations in lamin A/C cause cardiac and skeletal muscle disease. Shown is a schematic of the lamin A/C protein. The amino-terminal rod domain is shown in white, the carboxy-terminal globular domain is shown in black. Protein variants that cause cardiac and skeletal disease are labeled. Mutations were compiled using the Leiden Muscular Dystrophy Pages at www.dmd.nl.

(Bonne et al., 2000). However, most mutations appear to act by a gain or change of function evidenced by proper localization of lamin A/C protein at the nuclear rim in affected tissues (Fatkin et al., 1999; Bonne et al., 2000). Lamin A/C mutations may be clinically more severe than XLR *EMD* mutations, with more severe heart involvement and earlier age of onset. The heart phenotype often requires implantation of an ICD, rather than a pacemaker as recommended for X-linked EDMD (Vytopil et al., 2003; Meune et al., 2006).

The *LMNA* gene is located on chromosome 1q21 and encodes the A-type lamins, lamin A and C, and two minor isoforms, C2 and AΔ10, which arise through alternative splicing. Lamins A and C are identical for the first 566 amino acids. At the carboxy-terminus, prelamin A, a precursor of lamin A, contains 98 unique amino acids. The last 18 amino acids, which contain a farnesyl group, are cleaved. Lamin C has an additional six amino acids after divergence (Figure 12.6) (Fischer et al., 1986). Lamin B is the dominant lamin of mitotically active, dividing cells, where lamins A and C are generally expressed only during or after differentiation. The level of expression of lamin A and C increases with terminal differentiation and growth arrest. In addition, during the mitotic disassembly of the nuclear membrane, lamin A/C solubilize and are dispersed throughout the cell (Moir et al., 2000).

During interphase, the lamins provide structural support to and determine the shape of the nucleus. It is also believed that lamins play a role in DNA replication and transcription through stabilization of heterochromatin. Favreau and colleagues (2003) have shown that the carboxy-terminal ends of lamin A/C associate with chromatin. In addition, lamin A/C null mice have regions of localized heterochromatin detachment from the nuclear lamina (Sullivan et al., 1999). Other studies have shown that lamin A/C colocalizes with RNA splicing factors within the nucleus. It has been proposed that these lamin A/C speckles in the nucleus are part of a dynamic structure that can act, through specific signaling events, to organize spatially mRNA splicing and polymerase II transcription (Muralikrishna et al., 2001; Kumaran et al., 2002). Lamin A/C has also been shown to interact with transcription factors and signaling molecules (Kapiloff et al., 1999; Dreuillet et al., 2002).

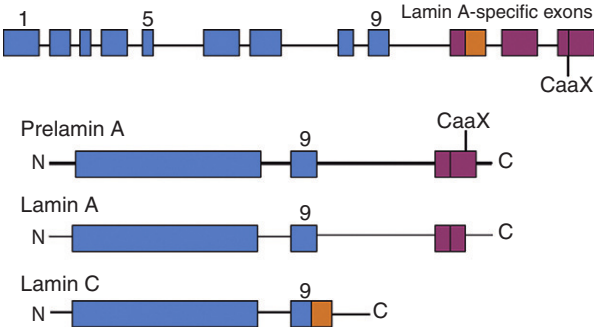

Figure 12.6. The *LMNA* gene is alternatively spliced. Shown is a schematic of the 12 *LMNA* exons that are alternatively spliced at the 3′ end to create prelamin A and lamin C. Lamin A and C are identical for the first 566 amino acids. Prelamin A has 98 unique amino acids at the carboxy-terminus, 18 of these are cleaved to produce mature lamin A. Lamin C has six alternate amino acids at the carboxy-terminus.

Nesprin-1 and -2

Approximately 60% of patients with EDMD do not have mutations in *EMD* or *LMNA* (Bonne et al., 2003). Mutations are likely to be found in other genes encoding proteins that interact with lamin A/C and emerin at the nuclear membrane, especially those expressed in muscle tissue. Recently, mutations were identified in two such genes, *SYNE1* and *SYNE2*, encoding nesprin-1 and -2 in subjects with EDMD and EDMD-like phenotypes (Figure 12.7) (Q. Zhang et al., 2007; Puckelwartz et al., 2010). In total, five amino acid changes have been described, four in nesprin-1α and one in nesprin-2β, that were not identified in controls. The missense mutations were in evolutionarily well-conserved regions that are in the lamin A/C- and emerin-binding domains of nesprin-1 and -2 (Q. Zhang et al., 2007; Puckelwartz et al., 2010). The patients used in these studies have a wide range of phenotypes, from raised CK levels to muscular dystrophy with heart block requiring a heart transplant in the third decade of life. Like mutations in *LMNA*, mutations in nesprin-1 and -2 appear to be influenced by genetic modifiers, as is evidenced by the phenotypic variability of these mutations. Further screening of patients with EDMD is necessary to determine the degree to which nesprin-1 and -2 are responsible for EDMD and EDMD-like phenotypes. Mutations were also identified in the large isoform

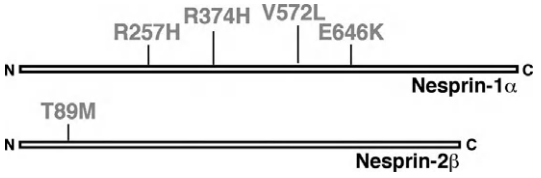

Figure 12.7. Mutations in the *nesprin-1* and *-2* genes cause EDMD-like phenotypes. The nesprins are large proteins of the nuclear membrane that, through alternate transcriptional initiation and splicing, yield a variety of amino-terminal truncated isoforms. Mutations that cause both cardiac and skeletal muscle phenotypes resembling EDMD have been identified in smaller isoforms of both nesprin-1 and -2.

of nesprin-1 that did not cause a muscle phenotype, but resulted in an autosomal recessive cerebellar ataxia (ARCA1) (Gros-Louis et al., 2007). Mutations causing EDMD have not been found in the largest isoforms and this may indicate that they play a different role in the cell than their smaller counterparts.

Nesprin-1 and -2 (*n*uclear *e*nvelope *sp*ectrin *r*epeat proteins) belong to a recently identified family of spectrin-repeat containing proteins (Apel et al., 2000; Zhang et al., 2001; Mislow et al., 2002b; Zhen et al., 2002). Nesprin-1 and -2 are transcribed from two genes, *SYNE1* on chromosome 6q24 and *SYNE2* on chromosome 14q23. Alternate initiation and splicing of the *SYNE* genes results in a wide variety of nesprin isoforms, the smaller of which contain variable numbers of spectrin repeats and are truncated at the amino-terminus (Zhang et al., 2001; Warren et al., 2005). Nesprin-1α and -β were first identified in the mouse as nuclear envelope proteins highly expressed in skeletal, cardiac, and smooth muscle cells (Apel et al., 2000). Nesprin-1 and -2 are orthologs of *Caenorhabditis elegans* ANC-1 and *Drosophila* MSP-300; these proteins contain more than 6000 amino acids and amino-terminal calponin homology domains that bind actin, a large central spectrin repeat rod domain, and a 60-amino-acid carboxy-terminal KASH (Klarsicht-ANC-Syne homology) domain, which has been shown to be important in anchoring nesprins in the nuclear membrane (Zhang et al., 2001, 2002). Nesprins are ubiquitously expressed, with the largest isoforms anchored in the outer nuclear membrane and the smallest isoforms anchored in the inner nuclear membrane. Specific inner nuclear membrane short isoforms are highly expressed in cardiac and skeletal muscle (Apel et al., 2000; Zhang et al., 2001). These shorter isoforms form dimers and bind lamin A/C and emerin *in vitro* (Mislow et al., 2002a).

MECHANISMS OF DISEASE

Many muscular dystrophies are caused by mutations in plasma membrane or extracellular matrix-associated proteins that are important for the structural integrity of the cell. Two hypotheses exist to explain how ubiquitously expressed proteins of the inner nuclear membrane specifically affect skeletal and cardiac muscle: the nuclear fragility hypothesis and the gene expression hypothesis. *In vitro* studies and animal models of EDMD suggest that these two hypotheses are not mutually exclusive and may together explain the pathology of EDMD (Wilson, 2000). The nuclear fragility model postulates that, when disrupted due to mutations in nuclear membrane proteins, the lamina can no longer act to protect the cell from mechanically induced stress. This fragility is most damaging in cells under high mechanical load, such as skeletal and cardiac muscle. The gene expression model proposes that, because the lamina plays a role in chromatin scaffolding, mutations in the nuclear membrane proteins will disrupt the chromatin and lead to altered gene expression. It is hypothesized that the skeletal and cardiac muscle is affected when tissue-specific lamina interactions are disrupted because of mutations in nuclear membrane proteins.

MODELS OF EDMD

Mice have been engineered with a targeted disruption of exons 8 through 11 of the *LMNA* gene (Sullivan et al., 1999). *LMNA* null mice are indistinguishable from their wild-type littermates at birth, but develop muscular dystrophy and dilated cardiomyopathy at approximately 3–4 weeks and die by 8 weeks (Sullivan et al., 1999; Nikolova et al., 2004). *LMNA* null fibroblasts have altered nuclear envelopes with detached chromatin and mislocalized emerin, indicating that loss of lamin A/C alters the structure of the inner nuclear membrane (Sullivan et al., 1999). Only one individual, a fetus that died late in gestation, has been described as completely lacking lamin A/C (Muchir et al., 2003). This fetus was produced from two individuals carrying a *LMNA* truncation, $LMNA^{259X}$. Fibroblasts derived from the $LMNA^{259X/259X}$ fetus had abnormally shaped nuclei with no lamin A or C and mislocalized emerin and nesprin-1 (Muchir et al., 2003). However, in patients with heterozygous lamin A/C mutations, emerin and nesprin-1 localize normally to the nuclear membrane. To determine whether the nuclear abnormalities are caused by a weakened nucleus, Lammerding and colleagues mechanically strained mouse embryonic fibroblasts from $LMNA^{-/-}$ and wild-type mice (Lammerding et al., 2004; Lammerding and Lee, 2005). These nuclear strain experiments showed that external strain on a cell results in increased nuclear strain, indicating that the extracellular matrix is coupled to the nucleus (Lammerding et al., 2004). Embryonic fibroblasts from $LMNA^{-/-}$ mice are more fragile in response to mechanical stress than wild-type fibroblasts. Upon stretching, $LMNA^{-/-}$ fibroblast nuclei and cytoplasm are less rigid and more deformable than wild-type fibroblasts. These data indicate that loss of lamins A and C weakens the nucleus and, in turn, weakens the entire cell, indicating that a complex exists linking the cytoplasm and nucleus. These experiments also showed that transcriptional activation in response to mechanical stimulation is attenuated in $LMNA^{-/-}$ cells, supporting the hypothesis that gene expression is altered in lamin A/C cells (Lammerding et al., 2004).

Due to the scarcity of patient tissue available for study, cell culture and mouse models expressing *LMNA* mutations have been generated to determine the effect

of the various missense mutations commonly found in AD-EDMD. Expression of missense *LMNA* mutations in mouse and human cell lines disrupts the structure of the nuclear envelope (Ostlund et al., 2001; Raharjo et al., 2001). Fibroblasts from patients with EDMD revealed similar nuclear envelope defects (Muchir et al., 2004). Muscle biopsies from patients with *LMNA* mutations reveal nuclear alterations in a small percentage of muscle fibers, including peripheral heterochromatin loss (Sabatelli et al., 2001). Mouse models have been generated to determine the effect of expressing a *LMNA* missense mutation in place of wild-type lamin A/C. It is important to note that these mouse models have a phenotype in the homozygous state, whereas human patients are generally heterozygous. One such model expresses the H222P mutation in place of wild-type lamin A/C. These mice are normal at birth, but homozygous mutant male mice display an abnormal walking posture, chamber dilatation, and conduction defects in the heart, and die by 9 months of age. Female mice have a similar phenotype with a later onset (Arimura et al., 2005). Gene expression analysis of homozygous mutant H222P hearts revealed activation of the mitogen-activated protein kinase (MAPK) cascade and downstream targets that are hypothesized to be important in the development of cardiomyopathy (Muchir et al., 2007). This model supports the hypothesis that changes in gene expression, especially muscle-specific gene expression, can result from mutations in lamin A/C. A transgenic mouse was generated that overexpressed the AD-EDMD causing mutation, M371K, in the heart. These mice are born at a lower than expected frequency and die between 2 and 7 weeks of age (Wang et al., 2006). *LMNA*-M371K transgenic mice also have alterations in nuclear morphology, including convoluted nuclear envelopes, intranuclear inclusions, and chromatin clumps in heart cells (Wang et al., 2006). This model demonstrates that overexpression of mutant lamin A/C can cause changes in nuclear morphology that result in tissue and organ damage. Lastly, a lamin C-only mouse was generated that expressed lamin C but no lamin A (Fong et al., 2006). Although $LMNA^{-/-}$ mice have muscular dystrophy and cardiomyopathy, the lamin C-only mice appear normal throughout life (Fong et al., 2006). In addition, the lamin C-only mice have a normal distribution of emerin, suggesting that lamin C is sufficient for a functional nuclear membrane.

Mice lacking emerin have no overt muscle pathology, but have delayed muscle regeneration (Melcon et al., 2006). Microarray analysis of regenerating muscle in hemizygous emerin null mice revealed a misregulation of molecular pathways upstream of retinoblastoma (Rb)/MyoD relative to wild-type controls (Melcon et al., 2006). Rb is also destabilized in $LMNA^{-/-}$ fibroblasts (Johnson et al., 2004). Muscle biopsies from patients with either X-linked EDMD or AD-EDMD also revealed a disruption in the Rb/MyoD cascade (Bakay et al., 2006). Rb is responsible for suppression of proliferative transcription programs and exit of myoblasts from the cell cycle (Puri et al., 2001). Cultured primary myoblasts from hemizygous emerin null mice were delayed in fusing to form myotubes, suggesting that the gene expression data are representative of pathologies in the muscle (Melcon et al., 2006). Cell strain experiments were conducted on emerin null fibroblasts and the amount of nuclear deformation was not different from that in wild-type controls (Lammerding et al., 2005). In common with human patients with EDMD, fibroblasts from emerin null mice have irregularly-shaped nuclei with blebbing of the nuclear membranes (Lammerding et al., 2005). These experiments also revealed that, like $LMNA^{-/-}$ cells, emerin null fibroblasts exhibit altered expression of mechanosensitive genes (Lammerding et al., 2005). Gene expression studies in hearts of emerin null mice revealed an activation of the MAPK pathway as well as activation of downstream target genes involved in the pathogenesis of cardiomyopathy (Muchir et al., 2007). A second emerin null mouse was generated and displayed a different phenotype. This null mouse had minimal motor and cardiac deficits with structural fragility of myonuclei (Ozawa et al., 2006). Together, these models support both the nuclear fragility and the gene expression models and suggest that loss of emerin in the mouse is not as devastating as loss of lamin A/C.

Multiple mouse models have been generated to study nesprin-1 and -2. The first model is a transgenic overexpressing the carboxy-terminal domain of nesprin-1 in muscle. The transgene acted in a dominant negative fashion, partially displacing endogenous nesprin-1 from the nuclear envelope (Grady et al., 2005). The mice had normal localization of the inner nuclear membrane proteins lamin A/C, emerin, and SUN2, and ultrastructural analysis revealed that the nuclear envelopes were grossly normal in these animals (Grady et al., 2005). Adult myofibers contain hundreds of nuclei that are evenly spaced, except under the neuromuscular junction (NMJ) where three to eight functionally specialized nuclei cluster to aid in the development and maintenance of NMJs. These mice were found to have a reduced number of nuclei under the NMJs (Grady et al., 2005). They do not have an overt neuromuscular phenotype, indicating that the reduction in nuclei under the NMJ does not alter muscle function. However, this transgene may have limited expression levels and the dominant negative effect was not sufficient to eliminate endogenous nesprin-1 from the nuclear envelope. Therefore, this animal may not be the ideal model for overexpression of nesprin-1. Nesprin-1 null and nesprin-2 null (also called *SYNE1*

and *SYNE2* null) models were generated that targeted the carboxyl-terminal KASH domain of each gene (X. Zhang et al., 2007). These mice lacked nuclei under the NMJs in muscle and had disorganized myonuclei in muscle fibers. *SYNE1*$^{-/-}$ mice also had aberrant innervation sites and elongated motor nerve branches. A greater proportion of muscle cells in *SYNE1*$^{-/-}$ animals exhibit centralized nuclei than in wild-type controls (X. Zhang et al., 2007). It is unclear whether these centralized nuclei represent regenerating muscle fibers or mislocalized nuclei. No phenotype was ascribed for the *SYNE2*$^{-/-}$ null animal. To determine whether *SYNE-1* and *-2* have redundant function, a mouse lacking both *SYNE1* and *SYNE2* was evaluated. These mice die shortly after birth from respiratory failure (X. Zhang et al., 2007). These models illustrate the importance of nesprins in properly anchoring myonuclei, and that these myonuclei play a role in proper motor innervation and respiration. Although these models aid in the understanding of the molecular function of nesprin-1 and -2, they do not shed light on the role nesprins may play in EDMD.

Other nesprin-1 mutant animal models with overt muscle phenotypes have been generated. The first of these, termed Δ/ΔKASH, replaces the carboxy-terminal KASH domain of nesprin-1 with an unrelated stretch of amino acids (Puckelwartz et al., 2009). The Δ/ΔKASH mice have 50% perinatal lethality, with surviving mice exhibiting cardiac conduction defects and progressive muscle wasting similar to phenotypes seen with EDMD (Puckelwartz et al., 2009, 2010). Components of the nuclear membrane localize normally in the Δ/ΔKASH mice; however, they fail to assemble functionally. It is hypothesized that this misassembly results in loss of communication between the largest isoforms of nesprin in the cytoplasm and smaller isoforms of nesprin and lamin A/C in the nucleus, ultimately affecting gene expression (Puckelwartz et al., 2009). Another nesprin-1 model deletes all isoforms containing the carboxy-terminal spectrin repeat region (Zhang et al., 2010). These mice, referred to as Nesprin-1$^{-/-}$, also exhibit perinatal lethality, but do not have cardiac defects and only mild muscle defects, illustrated by reduced exercise tolerance (Zhang et al., 2010). Both the Δ/ΔKASH and Nesprin-1$^{-/-}$ models also have defective anchoring and mislocalization of the myonuclei, similar to the *SYNE1*$^{-/-}$ mice (X. Zhang et al., 2007; Puckelwartz et al., 2009; Zhang et al., 2010). Each of these models may express different isoforms of nesprin, resulting in various perturbations of the components of the nuclear envelope. The phenotypes of these models may reflect the importance of the different splice forms in nesprin function. Together these models illustrate the complexity of nesprins and their role in muscle disease.

CONCLUSIONS

Data from patient samples and mouse models suggest that EDMD is caused by a combination of nuclear fragility leading to signaling defects in response to mechanical stress and altered gene expression caused by a disorganized lamina. Recently, the LINC complex has been described for its role *LI*nking the *N*ucleus to the *C*ytoskeleton and may play an important role in signaling and structural integrity. The LINC complex consists of large isoforms of nesprin-1 and -2 facing the cytoplasm. The nesprins are tethered to the outer nuclear membrane by interaction with SUN proteins. SUN proteins span the inner nuclear membrane with a nucleoplasmic amino-terminus and a carboxy-terminus located in the perinuclear space located between the outer and inner nuclear membranes (Crisp et al., 2006). SUN proteins interact with smaller inner nuclear membrane-bound isoforms of nesprin-1 that, in turn, interact with lamin A/C in the nucleus (Figure 12.8). Given that the largest isoforms of nesprin-1 and -2 bind actin, the LINC complex provides a direct molecular connection between the actin cytoskeleton and the nuclear lamina (Crisp et al., 2006). Previous studies

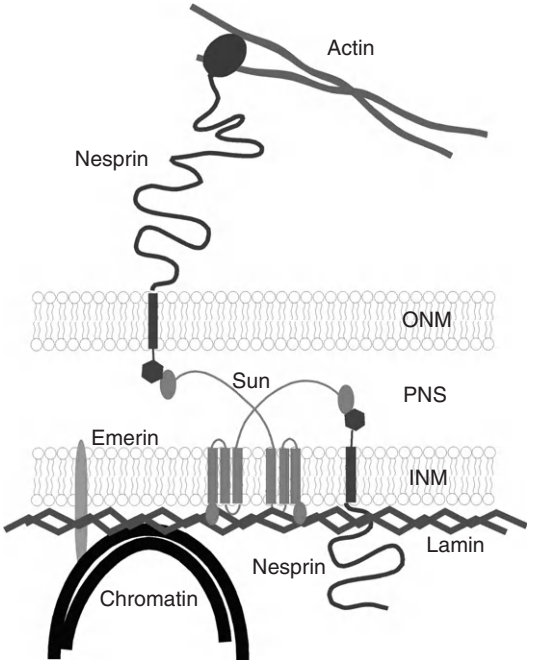

Figure 12.8. The LINC complex consists of actin-bound nesprin isoforms in the cytoplasm, tethered to the outer nuclear membrane (ONM) by their transmembrane KASH domain. The KASH domain interacts with the SUN proteins in the perinuclear space (PNS). The SUNs bind to lamin/chromatin complexes in the nucleus. It has been hypothesized that the LINC complex forms a bridge between the nucleus and cytoplasm and may be important for communication between the two cell compartments.

have shown that *LMNA*$^{-/-}$ fibroblasts have weakened cytoplasm and, like emerin null fibroblasts, misregulated signaling pathways (Lammerding et al., 2004). Based on data presented here, it can be hypothesized that the LINC complex serves to communicate stress to the nucleus to affect gene regulation and that this "mechanosensor" complex would be important in tissues under high stress loads such skeletal and cardiac muscle. Therefore, disruption of the LINC complex, through mutations in any of its components, may lead to chromatin reorganization, changes in gene expression, and nuclear disorganization leading to signaling pathway defects, and may explain the etiology of EDMD (Tzur et al., 2006). More than half of patients with EDMD do not have *LMNA* or emerin mutations, and therefore components of the LINC complex are excellent candidates for contributing genetically to the EDMD phenotype.

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Chapter 13

Facioscapulohumeral dystrophy and scapuloperoneal syndromes

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INTRODUCTION

Facioscapulohumeral dystrophy (FSHD) is the third most common muscular dystrophy. It is named for its characteristic involvement of the muscles of the face and upper arm. Although long recognized, it is only in relatively recent times that the genetic basis has been identified. Like many conditions, this has proved more complex than anticipated, and the molecular pathogenesis remains unclear. Nevertheless, the possibility of molecular genetic diagnosis has allowed a wider understanding of the diversity of clinical presentation, and has clinical implications for patients. A number of therapeutic clinical trials have been performed, but without significant benefit.

EPIDEMIOLOGY

FSHD is inherited as an autosomal dominant condition, with 95% clinical penetrance by the age of 20 years. Around 30% of patients have new mutations, with no clinical abnormality in their parents. The disease is found worldwide with an estimated incidence of 1 in 20 000 in the European population. The prevalence is around 4.4 per 100 000 (Padberg et al., 1995b). Total lifespan is not significantly affected (Lunt and Harper, 1991; Padberg, 2004).

PATHOGENESIS AND GENETICS

In the first description of FSHD by Landouzy and Dejerine in 1885, nine affected individuals were identified in four generations of one kindred. A number of smaller families were identified, consistent with autosomal dominant inheritance. A large family from Utah, USA, originating from England in 1775, included 1249 individuals, 58 with features of FSHD, and a typical mendelian dominant pattern of inheritance, complete penetrance, but variable expression (Tyler and Stephens, 1950).

Molecular genetic studies have identified that the vast majority of patients have an abnormality on chromosome 4q, although the precise molecular genetic pathogenesis remains uncertain (van der Maarel et al., 2007). Family studies have demonstrated linkage to chromosome 4q35, in the telomeric region (Wijmenga et al., 1990). Subsequent studies have shown that patients with FSHD have a reduction in the number of repeats of a 3.3-kilobase (kb) tandem repeat *Kpn*I fragment termed D4Z4 (Wijmenga et al., 1992; van Deutekom et al., 1993). These are recognized by the reduction in size of an *Eco*RI fragment on genomic DNA when hybridized with probe p13E-11 (Figure 13.1). In general terms, normal individuals have between 12 and more than 100 copies of this repeat (>48-kb *Eco*RI fragment) and patients with FSHD have only 1–10 copies (10–41-kb *Eco*RI fragment). This can be used as a diagnostic DNA test by performing enzymatic digestion of genomic DNA, electrophoresis, visualization with probe p13E-11, and measurement of size (Wijmenga et al., 1992; Orrell et al., 1999). Complete absence of D4Z4 repeats is not associated with FSHD, and monosomy 4q does not cause FSHD, suggesting a critical role for D4Z4 (Tupler et al., 1996).

Further studies have demonstrated a number of potential complications in the molecular genetic diagnosis. Some of these relate to the technique used; pulsed-field gel electrophoresis is preferred because of the large size of these regions. In addition, an almost identical repeat is found in the subtelomere of

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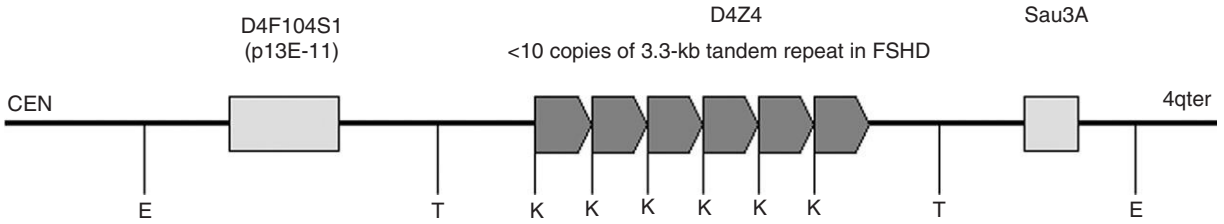

Figure 13.1. Restriction map of the facioscapulohumeral dystrophy (FSHD) locus at chromosome 4q35, including restriction sites used in molecular diagnostics (see text). The *Eco*RI fragment is detected by probe p13E-11, and predominantly comprises an array of 3.3-kb tandem repeats (D4Z4). There are usually 12–100 copies of D4Z4 in normal individuals, and fewer than 10 in patients with FSHD. E, *Eco*RI restriction site; K, *Kpn*I restriction site; T, *Tru9*1 restriction site; CEN, centromeric; 4qter, telomeric.

chromosome 4q (Bakker et al., 1995; Deidda et al., 1995). Contractions of the repeats on chromosome 10q do not cause FSHD (Lemmers et al., 2001; Zhang et al., 2001).

In addition, there may be exchange of repeat units between chromosome 4q and 10q. This requires more detailed laboratory analysis, and explains some of the initially confusing results. Approximately 10% of the population have exchange of repeats between the chromosomes, but it is only the changes on 4q that cause disease. Contractions of 4q-derived repeats on 10q appear to not cause disease (van Deutekom et al., 1996a; Lemmers et al., 1998; van Overveld et al., 2000).

Further, a biallelic variation has been identified in the 4q telomere (van Geel et al., 2002) (Figure 13.2). Normal individuals have an equal distribution of the two variants – 4qA and 4qB – but FSHD alleles are usually 4qA (Lemmers et al., 2002, 2004). Further haplotyping of the subtelomeric domain of chromosome 4q has shown nine distinct haplotypes, two of which are not associated with FSHD. Three of the haplotypes are 4qA, but one is not associated with FSHD (Lemmers et al., 2007).

In a small number of patients with FSHD the deletion extends proximally to include the probe region p13E-11, and the shortened allele may not be identified using standard methods (Lemmers et al., 1998, 2003). It remains uncertain how many individuals, if any, with a FSHD phenotype are not linked to 4q35, but the number appears to be very low.

Despite identification of the genetic locus, and increasing understanding of the molecular genetic changes, the specific genes affected and pathogenesis remain uncertain. A potential double-homeobox gene 4 (*DUX4*) is present within the D4Z4 region (see Figure 13.2), but expression or function of this gene is unproven at present

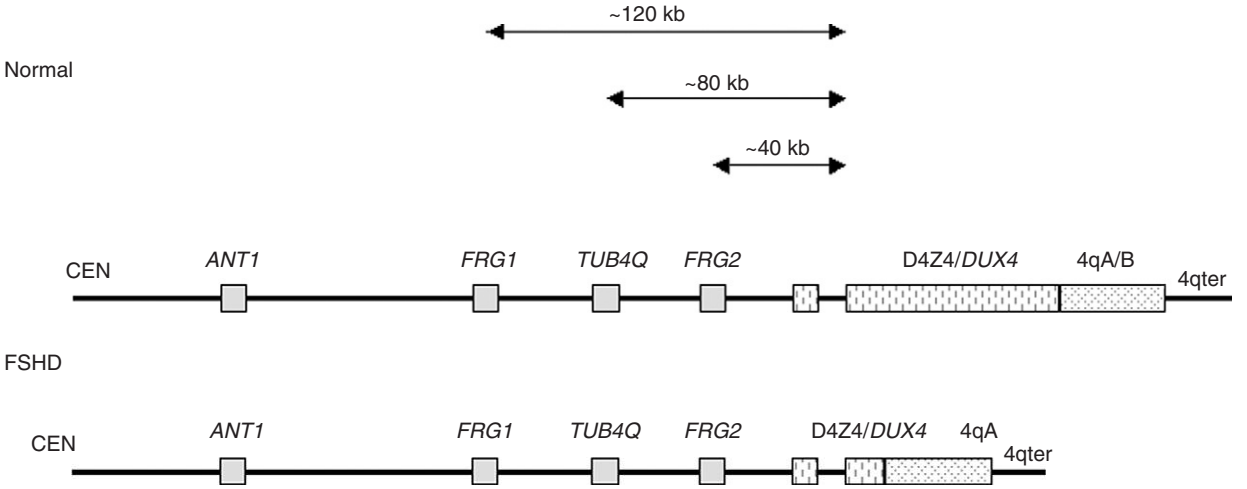

Figure 13.2. Representation of the facioscapulohumeral dystrophy (FSHD) locus on chromosome 4 (not to scale). CEN, centromeric; 4qter, telomeric. There are usually 12–100 copies of D4Z4 (containing the *DUX4* gene) in normal individuals, and fewer than 10 in patients with FSHD (see text). FSHD is usually associated with the 4qA telomeric variant. Proximal genes to the D4Z4 repeats, which may be affected by the reduced number of repeats, include *FRG2* (FSHD region gene 2), *TUB4Q* (β-tubulin, a pseudogene), *FRG1*, and *ANT1* (adenine nucleotide translocator 1) – distance of approximately 3 Mb.

(Lyle et al 1995; Gabriels et al., 1999). When comparing genome-wide gene expression data from muscle biopsies of patients with FSHD to those of 11 other neuromuscular disorders, PITX1 (paired-like homeodomain transcription factor 1) was specifically upregulated in patients with FSHD. DUX4 was upregulated in patient myoblasts, at both mRNA and protein level (Dixit et al., 2007). Another putative double-homeobox gene (*DUX4c*), of uncertain function, is present in an inverted and truncated copy of D4Z4, around 40 kb proximal to D4Z4, and is absent on chromsome 10 (Coppee et al., 2004). A recent study demonstrated conservation of the *DUX4* open reading frame for more than 100 million years, with a suggestion of selection for maintenance of a protein-coding function. There was also evolutionary conservation of the tandem-array organization and high repeat copy number (Clapp et al., 2007). A number of new sense and antisense RNA transcripts, novel mRNAs, and micro/small interfering RNA-sized fragments generated from D4Z4 units have been identified. In particular, these relate to the C-terminal polypeptide of DUX4, which inhibits myogenesis between MyoD transcription and activation of MyoD target genes (Snider et al., 2009).

In the absence of a clearly affected gene within the affected chromosomal region, closely related genes were sought, with the possibility of altered gene regulation related to the chromatin structure, a phenomenon called position–effect variegation. At low copy number, D4Z4 has been demonstrated to act as an insulator, protecting transgenes from position effect, dependent on CTCF (CCCTC-binding factor) and A-type lamins. It also interferes with enhancer–promoter communication. These effects are lost at higher copy number (Ottaviani et al., 2009). In FSHD muscle, three genes – *FRG1*, *FRG2* (FSHD region genes 1 and 2) and *ANT1* (adenine nucleotide translocase type 1, also known as SLC25A4) – were noted to be transcriptionally upregulated, with the gene closest to D4Z4 being most significantly upregulated, and patients with smaller repeat numbers showing greater upregulation. A DNA-binding repressor complex of high mobility group 2B (HMG2B), Yin Yang-1 (YY1), and nucleolin was proposed to be involved, supported by upregulation of FRG2 in cell cultures lacking the components of this repressor complex (Gabellini et al., 2002). Unfortunately this has not been supported by further studies (Jiang et al., 2003; Winokur et al., 2003). DNA methylation has been studied. Normal alleles show D4Z4 methylation levels of approximately 50%, with a significant reduction in disease-causing alleles. There is a suggestion that hypomethylation of D4Z4 is necessary but not sufficient to cause FSHD (van Overveld et al., 2003, 2005). In 15 FSHD families without a D4Z4 contraction, but at least one 4qA161 haplotype (FSHD2), D4Z4-restricted hypomethylation was observed on chromosomes 4q and 10q, suggesting a common mechanism for developing FSHD with or without a D4Z4 contraction (de Greef et al., 2009).

The *FRG1* gene is 120 kb from the D4Z4 repeat. *FRG1* encodes a nuclear protein, which appears to be spliceosomal, involving pre-mRNA splicing (van Deutekom et al., 1996b; Grewal et al., 1998; van Koningsbruggen et al., 2004). Studies of FRG1 expression in human FSHD muscle have shown variable results, including no change, upregulation, and downregulation. A comprehensive expression analysis of FSHD candidate genes, at mRNA and protein level, in primary myoblasts, myotubes, and quadriceps muscle, did not identify any consistent change compared with normal and disease controls, other than a selective increase in *FRG2* mRNA expression in FSHD myotubes (Klooster et al., 2009). Transgenic mice were generated selectively overexpressing in skeletal muscle the 4q35 genes *FRG1*, *FRG2*, or *ANT1* (Gabellini et al., 2006). The *FRG1* transgenic mice developed a muscular dystrophy, but the *FRG2* and *ANT1* transgenic mice were normal. The degree of dystrophy on histology, and kyphosis, correlated with the extent of FRG1 expression. The *FRG1* mice were said to show a similar differential involvement of muscle groups to that of patients with FSHD. Most muscular dystrophies involve sarcolemmal proteins, and animal models of these diseases show alteration of sarcolemmal integrity. As might be expected for FSHD, no alteration of sarcolemmal integrity was found in the *FRG1* mice. The characteristic loss of contractile strength of muscle fibers and preferential loss of fast fibers, occurred independent of alterations of the plasma membrane (D'Antona et al., 2007). Alternative splicing of two pre-mRNAs – TNNT3 (fast skeletal muscle troponin T, which regulates muscle contractility) and MTMR1 (myotubularin-related protein 1, which can regulate muscle atrophy) in the muscle of *FRG1* mice and FSHD was demonstrated. The proposal that FSHD is caused by FRG1 overexpression is supported by the observation that some patients with FSHD have deletions that include *FRG2* and a portion of D4Z4 (Lemmers et al., 2003), but individuals with deletions of D4Z4, *FRG2*, and *FRG1* appear not to show features of FSHD (Tupler et al., 1996). The full significance of *FRG1* in the pathogenesis of FSHD remains uncertain at present.

An alternative hypothesis relates to the nuclear organization of chromosomes and the possible commonality with the nuclear envelope dystrophies. 4qter is preferentially located in the outer nuclear rim, with sequences proximal to D4Z4 being necessary and sufficient for perinuclear localization (Masny et al., 2004; Tam et al., 2004). 10qter is not similarly localized.

CLINICAL FEATURES

The classical presentation is subclinical, in that individuals may have features of muscle weakness without realizing they have the condition for many years, or for life. Individuals may be identified at an early stage when more severely affected family members are found to be affected. Weakness of eye closure, and mouth closure, may not be noticed by the patient. Typically the orbicularis oculi, zygomatic, and orbicularis oris muscles are affected in the face, and the masseter, temporalis, extraocular, and pharyngeal muscles are spared. The muscle weakness may be asymmetrical. The patient may be unable to whistle, or drink through a straw.

Weakness of the scapular fixation muscles is a typical early feature (Figure 13.3). This involves latissimus dorsi, lower portion of trapezius, rhomboids, and serratus anterior muscles. The scapula may ride upward and forward at rest. Scapular winging is apparent on forward movement of the arms. Deltoid is characteristically spared in the early stages, and relatively spared in comparison to other shoulder muscles as the condition progresses. The difficulty with arm elevation and shoulder weakness is usually the problem for which patients seek attention, sometimes being referred to orthopedic surgeons or other specialties, when the primary muscle weakness is not recognized. Biceps and triceps weakness and wasting develop next (Figure 13.4). Pelvic muscles are also affected. Tibialis anterior may be affected around the same time as the shoulder girdle, with difficulty walking on the heels, or foot drop. Other muscle groups may also be affected (Figure 13.5).

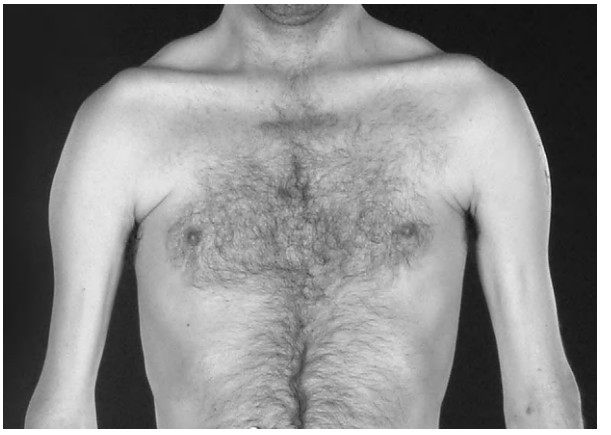

Figure 13.4. Wasting of pectoral, shoulder girdle, and humeral muscles in a patient with facioscapulohumeral dystrophy.

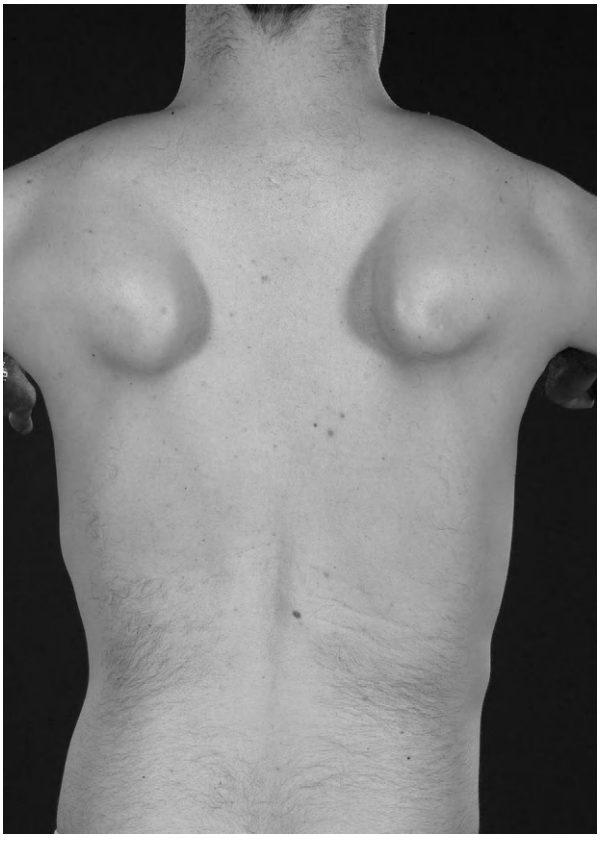

Figure 13.3. Winging of the scapula in a patient with facioscapulohumeral dystrophy.

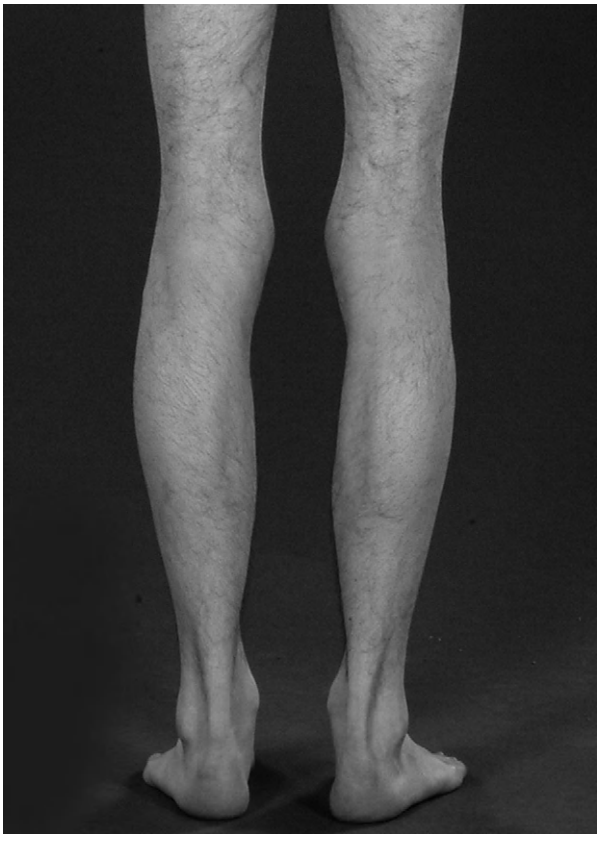

Figure 13.5. Wasting of the leg muscles in a patient with facioscapulohumeral dystrophy.

Beevor's sign is often present: an upward deflection of the umbilicus on flexion of the neck, reflecting selective lower abdominal muscle weakness. In a study of patients with neuromuscular diseases, 19 of 20 patients with FSHD had a positive Beevor's sign, compared with 2 of 28 with other muscle diseases, and 0 of 20 in a neurological control group (Shahrizaila and Wills, 2005). In another study, 27 of 30 patients with FSHD had a positive Beevor's sign, and 0 of 40 controls (Awerbuch et al., 1990). Although the muscle weakness is generally slowly progressive, there may be a perception of a stepwise progression. Around half of patients have symptoms limited to the shoulders and arms, with no difficulty walking. Some patients are severely affected, but most patients remain independent of a wheelchair, and lifespan is not significantly reduced.

Diagnostic criteria have been proposed but these predate and are now largely overridden by molecular diagnostics (Padberg et al., 1997). The key proposed features are given in Tables 13.1 and 13.2. Not all patients with the 4q35-reduced fragment size fulfill these criteria. For example, six patients were described with foot extensor, thigh, or calf muscle weakness. None had apparent facial weakness, only one complained of shoulder weakness, and none had a positive family history. On "expert" physical examination, however, typical facial expression, abnormal shoulders, or scapular winging were identified (van der Kooi et al., 2000).

Other symptoms include auditory, cardiac, retinal, and epilepsy problems.

Table 13.1

Four diagnostic clinical criteria for facioscapulohumeral dystrophy (Padberg et al., 1997)

1	Onset of the disease in facial or shoulder girdle muscles; sparing of the extraocular, pharyngeal, and lingual muscles, and the myocardium
2	Facial weakness in more than 50% of affected family members
3	Autosomal dominant inheritance in familial cases
4	Evidence of myopathic disease on electromyography (EMG) and muscle biopsy in at least one affected member without biopsy features specific to alternative diagnoses

Table 13.2

Diagnostic criteria for facioscapulohumeral dystrophy

Inclusion	Weakness of face or scapular stabilizers (in familial cases, facial weakness is present in >90% of affected individuals)
	Scapular stabilizer weakness greater than hip-girdle weakness (applicable in mild to moderate cases)
	Autosomal dominant inheritance in familial cases
Exclusion	Extraocular or pharyngeal muscle weakness
	Prominent and diffuse elbow contractures
	Cardiomyopathy
	Distal symmetrical sensory loss
	Dermatomyositic rash or signs of an alternative diagnosis
	Electromyographic evidence of myotonia or neurogenic potentials
Supportive features	Asymmetry of muscle weakness
	Descending sequence of involvement
	Early, often partial, abdominal muscle weakness (positive Beevor's sign)
	Sparing of deltoid muscles
	Typical shoulder profile: straight clavicles, forward sloping of shoulders
	Relative sparing of neck flexors
	Selective weakness of wrist extensors in distal upper extremities
	Sparing of calf muscles
	High-frequency hearing loss
	Retinal vasculopathy

Source: Facioscapulohumeral Consortium of the International Conference on the Cause and Treatment of Facioscapulohumeral Dystrophy, Boston, 1997.

Hearing

A multicenter study of 73 patients with FSHD identified 49 patients with no other risk factors for deafness. Clinical assessment, including pure-tone audiometry, did not identify any higher prevalence of hearing loss in FSHD than in the normal population (Trevisan et al., 2008). Earlier reports had suggested an increased incidence of high-frequency hearing loss in FSHD (Brouwer et al., 1991; Padberg et al., 1995a), especially in more severe early-onset forms (Hobson-Webb and Caress, 2006). Rogers et al. (2002) also found normal audiometry in 21 patients with FSHD.

Cardiac

Cardiac arrhythmias have been found to be increased in patients with FSHD in some studies (Stevenson et al., 1990; Laforet et al., 1998; Trevisan et al., 2006), but not in others (De Visser et al., 1992). Echocardiography is generally normal, and the cardiac alterations do not appear related to the degree of skeletal muscle involvement or 4q35 fragment size (Trevisan et al., 2006). In this Italian study, supraventricular tachycardia was the main abnormality, present in 3.6% patients compared with 0.22% of the normal population. In another Italian study, a range of subclinical electrocardiographic and echocardiographic abnormalities were identified in patients with FSHD (Galetta et al., 2005).

Retinal

Coats' disease is a congenital idiopathic retinal telangiectasia that can progress to retinal exudation and detachment (Shields et al., 2001). Similar findings may be found on ocular examination of patients with FSHD, but rarely progress to advanced Coats' disease (Fitzsimons et al., 1987). There is some question as to whether Coats' disease is the correct term for use in FSHD, as classically this is unilateral, whereas in FSHD the findings are bilateral; consequently, the term retinal telangiectasia may be preferred. In a study of 75 patients with FSHD, 64 studied with fluoroscein angiography, 75% demonstrated peripheral retinal telangiectasia. Some 1% had related visual acuity loss (Fitzsimons et al., 1987). In clinical practice this is not usually a clinically important issue in adult patients. Extreme examples include a reported 2-year-old girl with sudden onset of glaucoma, with findings consistent with Coats' disease. The findings were bilateral, the right eye was enucleated, and the left eye was treated with laser photocoagulation and cryotherapy of telangiectasia. A diagnosis of FSHD was made at age 5 years (Shields et al., 2007). Two Norwegian children with FSHD presented with Coats' disease, deafness, intellectual impairment, and possible epilepsy, with enucleation of an eye in one child and cryotherapy in the other eye. Their father was a somatic mosaic, with an *Eco*RI fragment size of 12 kb (Bindoff et al., 2006). In support of a link between the muscular dystrophy and retinal vasculopathy in FSHD, 32% of genes identified as being differentially expressed in FSHD muscle were involved in vascular smooth muscle or endothelial cells (Osborne et al., 2007).

Epilepsy

Intellectual impairment and epilepsy may be a feature of more severe forms of FSHD, especially with small fragment size (usually less than 11 kb) (Matsuzaka et al., 1986; Funakoshi et al., 1998; Miura et al., 1998; Saito et al., 2007). The presence of intellectual impairment and epilepsy may interfere with the diagnosis of FSHD, and with assessment of other complications such as auditory impairment.

INVESTIGATION

The clinical presentation should lead to suspicion of FSHD, and molecular genetic analysis is the preferred diagnostic investigation. The creatine kinase level is often, but not always, raised. Lactic dehydrogenase, aldolase, serum glutamic oxaloacetic transaminase and serum glutamic pyruvic transaminase levels are less commonly elevated (Munsat et al., 1973). Electromyography (EMG) typically shows short-duration low-amplitude polyphasic potentials, with increased interference pattern. As with all EMG analyses, the interpretation is observer dependent, and more typical neurogenic features may sometimes be reported. The differential diagnosis (Table 13.3) is discussed below, in the section on scapuloperoneal syndromes.

Table 13.3

Differential diagnosis of facioscapulohumeral dystrophy (Padberg, 2004)

Facioscapulohumeral muscular dystrophy
Scapuloperoneal muscular dystrophy
Limb-girdle muscular dystrophy
Proximal myotonic myopathy
Facioscapulohumeral spinal muscular atrophy
Scapuloperoneal spinal muscular atrophy
Scapuloperoneal syndromes with cardiomyopathy
Davidenkow syndrome
Polymyositis
Inclusion body myositis
Acid maltase deficiency
Mitochondrial myopathy
Congenital myopathy

With molecular genetic analysis, muscle biopsy is not usually required as a primary diagnostic. Muscle biopsy may still be performed in difficult cases, or where the diagnosis of FSHD has not been suspected. There remains substantial information on muscle histology from before the molecular diagnostic era. There is also the research indication for muscle biopsy. As with many muscular dystrophies, there may be replacement of muscle with adipose and connective tissue, impeding the assessment of muscle histology. Typically, features of a myopathy are found. Inflammatory infiltrates are well described in FSHD, and may be found in other muscular dystrophies. The significance of these is uncertain. At present there is no specific immunocytochemical staining for a proteinopathy.

PROGNOSIS

The natural history of FSHD was studied by the FSH-DY Group (1997). As expected, there was a slow but detectable decline in muscle strength and function over the 3-year study period. This did not appear to be associated with age, gender, age at onset, or duration of disease. The clinical progression of disease in individual patients and muscle groups may, however, appear stepwise, with periods of stability followed by more rapid progression. The muscle involvement in FSHD is usually asymmetrical, with suggestions this may relate to handedness and use of the dominant arm (Brouwer et al., 1992). This was not supported in subsequent studies, where the right–left asymmetry was not related to handedness, and more likely was secondary to intrinsic disease processes (Tawil et al., 1994). Prognosis is variable, being more severe if early onset. Around 20% of patients eventually become wheelchair dependent. Women tend to be less severely affected than men (Zatz et al., 1998). There has been a suggestion of clinical anticipation, with increasing severity with successive generations, but this is not commonly observed in practice (Tawil et al., 1996). There is a relationship, although not absolute and reliable, between the extent of D4Z4 repeat contraction, and the age at onset and severity of disease (Lunt et al., 1995; Tawil et al., 1996).

MANAGEMENT

Prednisone

Prednisone has been demonstrated to improve strength in patients with Duchenne dystrophy. A pilot, open-label trial of prednisone in eight patients with FSHD, 1.5 mg/kg daily for 12 weeks, demonstrated no significant change in strength or muscle mass. The study was not designed to detect any effect on slowing or arrest of disease progression (Tawil et al., 1997).

Albuterol

Albuterol is a β_2-adrenergic agonist. Animal and human studies have suggested an anabolic effect of albuterol on muscles. An initial open-label trial in 15 patients was performed (Kissel et al., 1998), followed by a randomized, double-blind, placebo-controlled trial in 90 patients with FSHD (Kissel et al., 2001). There were three groups: placebo, 8 mg and 16 mg sustained-release albuterol given twice daily for 1 year. There was no improvement in the primary outcome measure – global strength measured by maximum voluntary isometric contraction (MVIC) testing. There was, however, an increase in muscle mass, assessed by dual-energy X-ray absorptiometry, and grip strength.

In another study, 65 patients with FSHD were randomized to strength training of elbow flexors and ankle dorsiflexors or to no training. A randomized, double-blind, placebo-controlled design was used. After 26 weeks, sustained-release albuterol, 8 mg twice daily, was added (Van der Kooi et al., 2004). Both the training and albuterol were concluded to be well tolerated. Training of elbow flexors did not result in a significant effect on MVIC, but dynamic strength improved. Elbow flexor MVIC strength increased significantly in albuterol-treated patients. Ankle dorsiflexor strength decreased in all groups. Eleven of 12 untrained muscles showed a positive effect on MVIC when treated with albuterol. Muscle volume decreased in the placebo-treated group and increased in albuterol-treated patients. The conclusion was that strength training and albuterol appear safe in FSHD, but with limited positive effect on muscle strength and volume. The duration of the study was short, and the effects of long-term intervention uncertain, with no indication for routine treatment with these methods at present.

The effects of albuterol on pain and fatigue were studied further (Van der Kooi et al., 2007). Some 80% of patients reported chronic persistent or periodic, multifocal pain; 34% of patients were severely fatigued. Strength training and albuterol did not have a significant effect on these outcomes.

Diltiazem

An open-label pilot trial of diltiazem was conducted in 20 patients with FSHD. Diltiazem is a calcium ion influx inhibitor. The trial was initiated on the basis of anecdotal reports and patient testimonials suggesting benefit in FSHD. Diltiazem was given for 24 weeks. No significant improvement was seen in muscle strength, function, or mass (Elsheikh et al., 2007).

MYO-029

MYO-029 is a recombinant human neutralizing antibody to myostatin, an endogenous inhibitor of muscle growth. Absence of myostatin leads to muscle growth two to three times greater than normal. Loss of myostatin protein due to a splice-site mutation has been identified in a child with muscle hypertrophy (Schuelke et al., 2004). A double-blind, placebo-controlled, multinational, randomized trial evaluated MYO-029 in patients with FSHD and other dystrophies (Wagner et al., 2008). MYO-029 was administered intravenously every 2 weeks for 6 months. Only 42 patients with FSHD were studied, and the design was not powered to detect arrest of disease progression. MYO-029 was considered to be safe, although there was hypersensitivity at the higher dose.

Aerobic training

Eight patients with FSHD were trained for 12 weeks on a cycle ergometer. Maximal oxygen uptake (V_{O_2}max) was increased in both patients with FSHD and normal individuals by up to 16%, most probably reflecting an improvement in cardiovascular fitness. There was a positive effect on self-reported strength, endurance, and level of activity. Although a short study, regular aerobic exercise to maintain cardiovascular fitness was recommended (Olsen et al., 2005).

Scapular fixation

Scapular winging is a frequent and characteristic finding in FSHD. During elevation of the arm, the scapulothoracic muscles are required to stabilize the scapula to the rib muscles. The first 90° of shoulder abduction occurs largely at the glenohumeral joint, but stabilization of the scapula on the chest is important for the remaining 90° of shoulder abduction. With increasing weakness, patients become unable to hold heavy objects or perform overhead activities, such as combing hair or shaving. A number of interventions are possible. A Cochrane Systematic Review found no randomized trials of scapular fixation in muscular dystrophy, but acknowledged the difficulty in performing these (Orrell et al., 2010). An orthotic device may be used to press the scapula to the rib cage, but is rarely used as it is awkward and has poor efficacy (Barnett et al., 1995). Surgical options include a scapulothoracic fusion or arthrodesis (scapulodesis), and scapulothoracic fixation without arthrodesis (scapulopexy). A study of the outcome of 26 fixations of the scapula by scapuloplexy (using metal wires to four ribs), in 13 patients with FSHD, concluded that there was a low rate of complications, the procedure was safe and effective, and resulted in clinical and functional improvement (Giannini et al., 2007). Another study of scapulothoracic arthrodesis (although the procedure appears similar to the previous with wire fixation to the ribs), in nine shoulders of six patients with FSHD, concluded that this was a successful treatment, improving appearance, function, and tolerance to exercise. The patients were selected to have nearly normal function of shoulder abduction, but showed shoulder instability and scapular winging due to weakness in the trapezius, latissimus dorsi, serratus anterior, and pectoralis major muscles. The average preoperative active flexion was 71°, improving to 109° with surgery (Rhee and Ha, 2006). The procedure may lead to loss of internal rotation posteriorly and cross-body adduction, and the range of motion achieved after the procedure may deteriorate with time.

Ventilatory support

Although ventilatory insufficiency is a common complication of several neuromuscular disorders, it is a relatively unusual feature in FSHD. In a Dutch population, 10 patients were identified on nocturnal ventilatory support at home, representing around 1% of the FSHD population. The risk profile included severely affected, wheelchair-bound patients with moderate to severe scoliosis and lumbar hyperlordosis, and pectus excavatum. All patients reported an improved quality of life on ventilatory support.

Respiratory support is not usually required in FSHD. A patient with an obstructive respiratory disorder during sleep, who also had FSHD, was treated with BiPAP (bilevel positive airway pressure) ventilation. There were problems with mouth leaks due to facial weakness, and the weakness of orbicularis oculi muscles caused additional conjunctival irritation due to the air leak. A home-made elastic bandage to cover the mouth made the BiPAP tolerable (Della Marca et al., 2009).

Dysphagia

FSHD is not usually associated with neuromuscular dysphagia. A number of abnormalities in the swallowing process were observed in a study of 20 patients with FSHD, although it was not clear that these related to the dystrophic process (Stubgen, 2008). None of the patients complained spontaneously of swallowing problems, but on direct questioning there was some suggestion in 40%. In a study of 151 Japanese patients with FSHD, 4.6% had an atrophic tongue associated with early onset of disease, and two of these had difficulty swallowing (Yamanaka et al., 2001). Dysphagia has been reported to occur in advanced FSHD, with mild involvement of the jaw and lingual muscles,

but no involvement of the pharyngeal or laryngeal muscles. The symptoms are seldom life-threatening (Wohlgemuth et al., 2006). This may cause some confusion, as dysphagia is often considered an exclusion criterion for FSHD (Padberg et al., 1997).

Pain

In a community survey of 127 patients with FSHD, 82% reported pain, 23% with severe pain, of average pain duration of 13 years. The most frequent sites of pain were lower back (74%) and legs (72%). A wide range of pain treatments were used, the most effective being ibuprofen, aspirin, opioids, massage, chiropractic manipulation, nerve blocks, heat, and marijuana (Jensen et al., 2008).

SCAPULOPERONEAL SYNDROME

The clinical presentation of FSHD is often typical, and is confirmed by molecular genetic analysis. There remain patients who may present with a similar pattern of weakness, especially involving scapular muscles, who may present some diagnostic confusion. There is another group of patients, who may not have typical features of FSHD, but present with a scapuloperoneal syndrome. FSHD may also present as a facial-sparing scapular myopathy (Jardine et al., 1994; Felice et al., 2000). In one study of 14 unrelated patients, the FSHD 4q35 deletion was found in 10 (71%) (Felice et al., 2000). Scapular winging may occur in conditions including scapuloperoneal dystrophies, Emery–Dreifuss muscular dystrophy, congenital myopathies, myotonic dystrophy, and acid maltase deficiency (Felice et al., 2000). These include child- and adult-onset conditions; with autosomal dominant, recessive, X-linked inheritance, or sporadic; primary neuropathic or myopathic.

A search of Online Mendelian Inheritance in Man (OMIM) for scapuloperoneal yields 17 conditions, excluding FSHD (Table 13.4). Many of these are very rare, and have distinguishing features. Scapuloperoneal syndrome was first described by Jules Broussard in 1886 as "*une forme hereditaire d'atrophie musculaire progressive*," beginning in the lower legs and affecting the shoulder region earlier and more severely than the distal arm (OMIM). Although exclusion of the FSHD locus is not excluded for all the described cases, some have other genetic abnormalities defined, and in some absence of linkage to the 4q35 locus has been defined (Tawil et al., 1995).

Davidenkow (1939) described patients with a scapulohumeral distribution of weakness or muscle atrophy,

Table 13.4

Scapuloperoneal syndromes (with sample references)

OMIM reference	Name	Gene locus	Reference
#181430	Scapuloperoneal myopathy, MYH7 related; SPMM	14q12	Pegoraro et al. (2007)
#181400	Scapuloperoneal syndrome, neurogenic, Kaeser type	2q35	Walter et al. (2007)
%181405	Amyotrophy, neurogenic scapuloperoneal, New England type (scapuloperoneal spinal muscular atrophy, SPSMA)	12q24.1–q24.31	Isozumi et al. (1996)
#300695	Scapuloperoneal myopathy, X-linked dominant	Xq27.2	Quinzii et al. (2008)
#310300	Emery–Dreifuss muscular dystrophy, X-linked, EDMD	Xq28	Bione et al. (1995)
271220	Spinal muscular atrophy, scapuloperoneal		Feigenbaum and Munsat (1970), Emery (1971)
#608358	Myopathy, myosin storage	14q12	Tajsharghi et al. (2003)
*300163	Four-and-a-half LIM domains 1: FHL1	Xq27.2	Quinzii et al. (2008)
%255160	Myopathy, hyaline body, autosomal recessive	3p22.2–p21.32	Onengut et al. (2004)
+ 160760	Myosin, heavy chain 7, cardiac muscle, beta: MYH7	14q12	Pegoraro et al. (2007)
*125660	Desmin myopathy; DES	2q35	Walter et al. (2007)

Continued

Table 13.4

Continued

OMIM reference	Name	Gene locus	Reference
#611067	Spinal muscular atrophy, distal, autosomal recessive, 4: DSMA4	1q36	Maystadt et al. (2007)
#181350	Emery–Dreifuss muscular dystrophy, autosomal dominant (scapuloilioperoneal atrophy with cardiopathy); EDMD2	1q21.2	Jennekens et al. (1975)
#300257	Danon disease (vacuolar cardiomyopathy and myopathy, X-linked)	Xq24	Bergia et al. (1986)
%606071	Hereditary motor and sensory neuropathy, type IIC	12q23–q24	McEntagart et al. (2005)
#167320	Inclusion body myopathy with early-onset Paget disease and frontotemporal dementia; IBMPFD	9q13–p12	Waggoner et al. (2002)
%182970	Spinal muscular atrophy, facioscapulohumeral type		Fenichel et al. (1967), Furukawa and Toyokura (1976), Kazakov et al. (1977)

together with sensory involvement, a neurogenic (amyotrophic) scapuloperoneal syndrome, with similarity to Charcot–Marie–Tooth disease, which has subsequently been termed Davidenkow syndrome (Schwartz and Swash, 1975). A number of similar presentations have been described, but pathologically this may be a heterogeneous condition. Davidenkow's original descriptions included patients with neurogenic and myogenic scapuloperoneal weakness, and although the descriptions were further refined (Kazakov, 2003) the use of the term "Davidenkow syndrome" seems to confuse rather than clarify the understanding of scapuloperoneal syndromes in the era of molecular genetic diagnosis. For example, a family with Davidenow syndrome has been described as having the deletion of chromosome 17p11.2, commonly found in hereditary neuropathy with liability to pressure palsies (HNPP), allelic to *CMT1A* (Verma, 2005).

CONCLUSION

FSHD is a common muscular dystrophy, with an often distinctive clinical phenotype. The reduction in D4Z4 repeats was first reported in 1992, but the molecular pathology remains difficult to define. Current evidence points to a common mechanism for FSHD1 and FSHD2, in that FSHD1 is contraction-dependent on D4Z4, and FSHD2 is contraction-independent of D4Z4 repeats. The D4Z4 hypomethylation needs to occur on the background of a specific 4qA16a haplotype (de Greef et al., 2009). Further clarification of the molecular mechanisms should lead to a better understanding of the pathogenesis and to the development of therapies, which remain lacking. The distinctive differential muscle involvement in FSHD and the scapuloperoneal syndrome remains an enigmatic challenge.

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Chapter 14

Oculopharyngeal muscular dystrophy

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DEFINITION

Oculopharyngeal muscular dystrophy (OPMD) is a late-onset muscle disease associated with progressive ptosis of the eyelids, dysphagia, and unique tubulofilamentous intranuclear inclusions (INIs) in skeletal muscle (Brais, 2003; Abu-Baker and Rouleau, 2007). OPMD is usually transmitted as an autosomal dominant trait (Online Mendelian Inheritance in Man (OMIM) #164300). A rarer allelic autosomal recessive form has also been observed (OMIM #257950) (Brais et al., 1998b; Hebbar et al., 2007; Semmler et al., 2007). Both forms are caused by short $(GCN)_{11–17}$ expansions in the polyadenylate-binding protein nuclear 1 gene (*PABPN1*, previously abbreviated as *PABP2* – polyadenylate-binding protein 2), localized on chromosome 14q11.2 (Brais et al., 1998b). The mutations cause the lengthening of an N-terminal polyalanine domain. OPMD was first clearly described by Taylor in 1915 in four members of a French-Canadian family. Taylor's clinical report was largely overlooked until Victor, Hayes, and Adams' classical description was published in 1962 (Victor et al., 1962).

CLINICAL FEATURES

OPMD usually manifests itself in the fifth or sixth decade by eyelid ptosis and dysphagia. Later, all extraocular and other voluntary muscles may become affected. In advanced stages of the disease, the eyelids become very thin and transparent. The forehead is permanently wrinkled, the eyebrows are raised, and the supraorbital ridges appear prominent (Figure 14.1). Initially, OPMD is often restricted to the levator palpebrae and pharyngeal muscles. As the disease progresses, there may be impairment of extraocular movements, occasionally associated with diplopia; nevertheless, complete external ophthalmoplegia is infrequent. Involvement of the orbicularis oculi may also occur. The intrinsic eye muscles are always spared and retinal function is not affected. The dysphagia is noticed first for solid foods and progresses insidiously. The dysphagia late in the disease often leads to malnutrition and may cause fatal aspiration pneumonia. Other symptoms and signs are associated with myopathic involvement of other muscles, particularly of the limb girdles, which can start as early as the early fifties in the legs.

When the following three criteria are met, this establishes that a patient is affected by OPMD on clinical grounds (Brais et al., 1995):

1. a positive family history with involvement of two or more generations
2. the presence of ptosis (defined as either vertical separation of at least one palpebral fissure that measures less than 8 mm at rest) or previous corrective surgery for ptosis
3. the presence of dysphagia, defined as swallowing time greater than 7 seconds when drinking 80 ml of ice-cold water.

The decade-specific penetrances of these criteria for carriers of the most common dominant $(GCN)_{13}$ mutation are: 1% (<40 years), 6% (40–49 years), 31% (50–59 years), 63% (60–69 years), 99% (>69 years) (Brais et al., 1997). The age of onset of autosomal dominant OPMD is variable and often difficult to pinpoint. A study of 72 French-Canadian symptomatic carriers of a $(GCN)_{13}$ mutation established a mean

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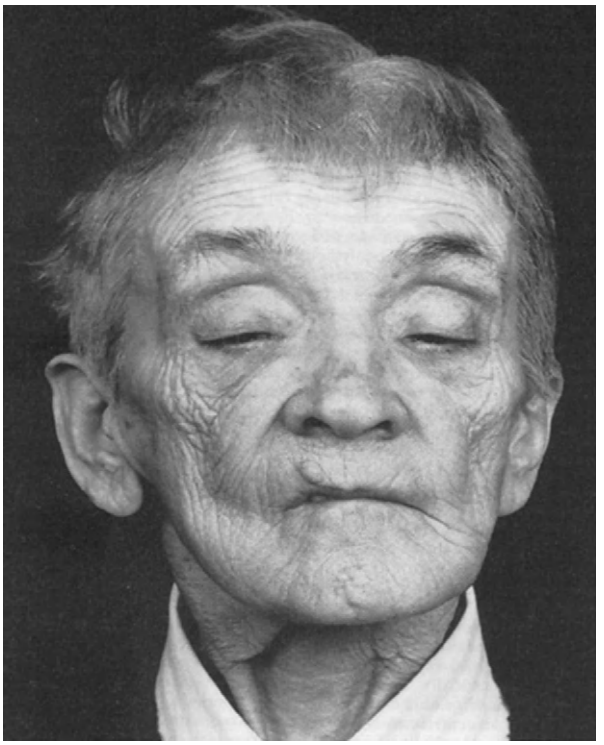

Figure 14.1. Elderly woman previously operated on for eyelid ptosis. The forehead wrinkling is typical of oculopharyngeal muscular dystrophy; its absence suggests the diagnosis of oculopharyngodistal muscular dystrophy. The cachectic state and atrophied neck muscle are typical of advanced cases. (Photograph courtesy of J.-P. Bouchard.)

age of onset for ptosis of 48.1 (range 26–65) years and for dysphagia of 50.7 (40–63) years (Bouchard et al., 1997). Other signs observed as the disease progresses are: proximal upper extremity weakness (38%), facial muscle weakness (43%), limitation of upper gaze (61%), dysphonia (67%), proximal lower extremity weakness (71%), and tongue atrophy and weakness (82%) (Bouchard et al., 1997). The relative percentage of patients with the different associated findings varies between cohorts. (Ruegg et al., 2005).

The disease has a slowly progressive course. Life expectancy is not shortened (Becher et al., 2001). Until recently, death occurred at an advanced age as a result of starvation or aspiration pneumonia. With progress in the treatment of pharyngeal dysfunction and better nutrition, the quality of life is much improved.

LABORATORY FINDINGS

Electromyographic studies of facial, limb, and pharyngeal muscles generally show a myopathic pattern (Bouchard et al., 1997). A mixed myopathic and neuropathic pattern in limb muscles was reported in some patients, but may be related to age (Boukriche et al., 2002). Sensory and motor conduction velocities are either normal or, less frequently, slightly reduced (Boukriche et al., 2002). Serum creatine kinase (CK) and aldolase levels are generally within normal limits, although the CK concentration may be increased 2–5-fold, mostly in individuals who have more limb-girdle involvement or who are taking statins for dyslipidemia (Bouchard et al., 1989). Manometric and cine-radiological studies of pharyngeal and esophageal motility show weak, prolonged, and repetitive pharyngeal contractions, but contraction of the upper esophageal sphincter is normal; however, sphincter relaxation, which depends on pharyngeal pressure, is late and incomplete (Bender, 1976; Duranceau et al., 1980). These findings explain the mechanism of the dysphagia. Computed tomography studies have disclosed low density in limb muscles, mainly in the semimembranosus, semitendinosus, and biceps femoris muscles, and fatty infiltration of the tongue (Medici et al., 1989; Chang et al., 1993; Bilgen et al., 2001).

HISTOPATHOLOGY

The changes seen in the muscle fibers of extraocular and other voluntary muscles vary according to the stage of the disease and the muscle biopsied. Probably all skeletal muscles are affected, but extraocular, lingual, pharyngeal, and diaphragmatic muscles are selectively more severely involved at autopsy (Rebeiz et al., 1969; Schmitt and Krause, 1981; Little and Perl, 1982). Classic histological methods show changes that are common to many muscular dystrophies. These include loss of muscle fibers, abnormal variation in fiber size, increase in the number of nuclei, internal nuclei, and increased interstitial fibrous and fatty connective tissues. Fibers undergoing necrosis and phagocytosis are rare. Inflammatory changes are usually not present. Histochemical studies reveal small angulated fibers that often react strongly for oxidative enzymes (more frequently type 1 than type 2) and rimmed vacuoles. Although the small angulated fibers may suggest an underlying denervation process, their occurrence may be due mostly to the advanced age of patients. The vacuoles consist of irregularly round or polygonal clear spaces lined by a ring of material that is basophilic with the hematoxylin and eosin stain and stains red with Gomori's trichrome stain (Dubowitz and Brooke, 1973; Tomé et al., 1997). Rimmed vacuoles are observed in several other disorders, and are therefore not considered specific to OPMD (Lotz et al., 1989). The rimmed vacuoles are autophagic in nature and have been reported to have acid phosphatase activity.

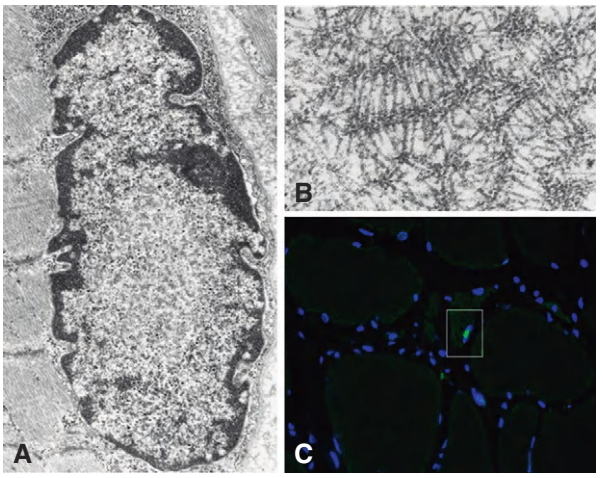

Figure 14.2. (**A**) Large collections of filaments appear as clear zones surrounded by chromatin. (**B**) The most significant ultrastructural change is the presence of intranuclear tubular filaments with 8.5-nm outer and 3-nm inner diameters. The filaments are unbranched, often course rectilinearly, and frequently form tangles or palisades. (**C**) The oculopharyngeal muscular dystrophy (OPMD) inclusions contain polyadenylate-binding protein nuclear 1 (PABPN1), as shown by immunofluorescent microscopy of PABPN1 in an OPMD nucleus of a homozygotic patient (green).

The most significant ultrastructural change is the presence of intranuclear tubular filaments with 8.5-nm outer and 3-nm inner diameters (Tomé and Fardeau, 1980). The filaments are unbranched, often course rectilinearly, sometimes striated with 7–7.5-nm periodicity, and up to 0.25 µm in length (Figure 14.2A,B). They are disposed in various directions and frequently form tangles or palisades. Large collections of filaments appear as clear zones surrounded by chromatin in the affected nuclei. These nuclei can often be identified in semi-thin epoxy sections by phase-contrast microscopy, where they appear as clear zones. Studies of serial semi-thin sections suggest that in some specimens the filamentous inclusions occur in all muscle fiber nuclei (Tomé et al., 1997). The highest percentage of muscle nuclei containing INIs were observed in seven patients who were homozygous for $(GCN)_{13}$ *PABPN1* mutations (Blumen et al., 1999). In these more severe OPMD cases, 9.4% of nuclei contained INIs, compared with 4.9% in heterozygotes for the same mutation. The inclusions were found only in muscle fibers and not in the nuclei of any other cells (including satellite cells) in muscle. Rarely, inclusion body myositis (IBM)-type tubular filaments of 16–18 nm in external diameter have also been found in OPMD muscle (Serratrice and Pellissier, 1987; Coquet et al., 1990).

Since the discovery that *PABPN1* is the gene mutated in OPMD, much work has centered on the identification of other molecules present in the INIs (Brais et al., 1998b). PABPN1 was shown to be an integral part of the muscle OPMD inclusions (Figure 14.2C) (Becher et al., 2000; Calado et al., 2000b; Uyama et al., 2000). The INIs also contain components of the ubiquitin–proteasome pathway such as ubiquitin, proteasome subunits, heat-shock protein (Hsp) 40 and Hsp70 (Bao et al., 2002; Abu-Baker et al., 2003), poly(T) RNA(Calado et al., 2000b), transcription factors such as SNW1 (SKIP) important in myogenesis (Kim et al., 2001), and other mRNA-binding proteins such as CUGP1, SFRS3, and FKP1A (Corbeil-Girard et al., 2005).

MOLECULAR GENETICS

Autosomal dominant and recessive forms of OPMD have been described (OMIM #164300 and #257950, respectively) and were found to be allelic. Although large clusters of patients with OPMD have been identified in different populations, there is clearly a great diversity of mutations, suggesting a large number of independent original mutational events. The largest cluster of OPMD was found in Québec, Canada, by André Barbeau (Bouchard et al., 1997). Other large clusters have been observed in Bukharan Jews (Blumen et al., 1997), Uruguayans (Medici et al., 1997), and Spanish Americans living in New Mexico (Becher et al., 2001) and California (Grewal et al., 1999). Patients with OPMD have now been reported in more than 35 countries (Brais, 2003). The condition is estimated to have a prevalence of 1 in 1000 in the French-Canadian population, and 1 in 600 in Bukharan Jews living in Israel (Brais et al., 1995; Blumen et al., 1997).

The OPMD locus was first mapped to chromosome 14q11.2–q13 by linkage analysis on large French-Canadian families (Brais et al., 1995). A positional cloning strategy led to the identification of short $(GCN)_{12–17}$ expansions of the *PABPN1* gene (previously abbreviated as *PABP2* – polyadenylate-binding protein 2) in all dominant OPMD cases (Brais et al., 1998b). Initially described as a $(GCG)_n$/polyalanine disease, it was later found often to be caused by cryptic GCN/alanine insertion (Brais et al., 1998b; Nakamoto et al., 2002). The number of extra codons coding for alanines varies from one in the case of the recessive mutation to between two and seven for the dominant mutations (Figure 14.3). The most common sizes of mutation worldwide are the $(GCN)_{13}$ and $(GCN)_{14}$ (Figure 14.3). Dominant and recessive OPMD are caused by mitotically and meiotically stable, short triplet repeat expansions of a $(GCN)_{10}$, and more rarely point mutations, both leading to the lengthening of an uninterrupted polyalanine domain at the N-terminus of the protein (Brais et al., 1998b; Nakamoto et al., 2002; Robinson et al., 2006). Two mutational mechanisms have been

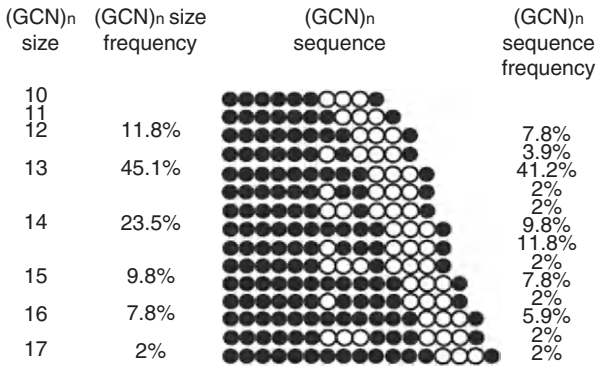

Figure 14.3. Frequency of the different oculopharyngeal muscular dystrophy *PABPN1* mutations based on a study of more than 150 unrelated families from more than 25 ethnic backgrounds and including only one French-Canadian family. The filled circles correspond to GCG/alanine and the open circles to GCA/alanine codons.

suggested: unequal crossover or slippage during recombination (Brais et al., 1998b; Nakamoto et al., 2002). Although unequal crossing over is the favored mechanisms at this time, there is still no experimental evidence to support either mechanism (Nakamoto et al., 2002). The great diversity of mutation in European populations clearly suggests that many distinct mutations have arisen independently. Two recent case studies suggest that neo mutations are still happening, and may explain some patients with no family history (Gurtler et al., 2006; Tremolizzo et al., 2007).

Gene dosage has a clear influence on the age of onset and severity of the OPMD phenotype (Brais et al., 1998a, b). The most severe OPMD phenotype is reported for individuals homozygous for two dominant OPMD mutations (Blumen et al., 1996, 1999; Brais et al., 1998b). The study of four French-Canadian and three Bukharan Jewish OPMD homozygotes documented that on average the onset was 18 years earlier than in $(GCN)_{13}$ heterozygotes (Blumen et al., 1999). Severity of the dominant OPMD phenotype is also variable (Bouchard et al., 1997). Severe cases have an earlier onset, before the age of 45 years, of ptosis and dysphagia, and an incapacitating proximal leg weakness that starts before age 60 years. In a large French-Canadian cohort of $(GCN)_{13}$ mutation carriers, 5–10% of patients have a severe phenotype (Brais et al., 1998a). Approximately 20% of these severe cases are compound heterozygotes for the dominant mutation and a $(GCN)_{11}$ recessive mutation/polymorphism on their other copy of the *PABPN1* gene (Brais et al., 1998b). This polymorphism has a 1–2% prevalence in North America, Europe, and Japan. Patients with a more severe compound heterozygote OPMD phenotype have also been observed in Italy and the UK (Mirabella et al., 2000; Hill et al., 2001). The cause of the increased severity in the other 80% is not known. Strikingly, severe cases cluster in families, suggesting that other genetic factors are modulators of severity (Brais et al., 1998a). Together the more severe phenotypes observed in homozygotes and compound heterozygotes for a dominant and a recessive mutation suggest a clear gene dosage effect (Brais et al., 1998b; Blumen et al., 1999). This being said, the recessive OPMD clinical phenotype has been found to be either milder or more severe than the dominant one (Brais et al., 1998b; Hebbar et al., 2007; Semmler et al., 2007). To date, no study has demonstrated statistically that the size of the (GCN) expansion correlates with severity, although there may be a trend towards increased severity with lengthening of the mutation that cannot reach significance because of the important variability observed between carriers of the same mutation, the limited accuracy of variables such as age of onset in these patients, the small number of different sizes of mutation, and the only limited variation in the number of extra codons.

To date, (GCN)/polyalanine expansions have been found in nine disease genes: *PABPN1* in OPMD (Brais et al., 1998b), *HOXD13* in synpolydactyly (Muragaki et al., 1996), *RUNX2* in cleidocranial dysplasia (Mundlos et al., 1997), *ZIC2* in holoprosencephaly type 5 (Brown et al., 1998), *HOXA13* in hand–foot genital syndrome (Goodman et al., 2000), *FOXL2* in blepharophimosis, ptosis, and epicanthus inversus syndrome (Crisponi et al., 2001), *PHOX2B* in congenital central hypoventilation syndrome (Amiel et al., 2003; Sasaki et al., 2003), *ARX* in infantile spasm X-linked syndrome (Bienvenu et al., 2002), and *SOX3* in mental retardation with growth hormone deficiency syndrome (Laumonnier et al., 2002). These diseases are mostly congenital and involve mutations in transcription factors (Amiel et al., 2004; Brown and Brown, 2004; Albrecht and Mundlos, 2005). OPMD stands as an exception in polyalanine diseases, being a late-onset condition that is not caused by a mutation in a transcription factor.

PATHOGENESIS

The function of PABPN1 as a ubiquitous polyadenylation factor essential for the extension of the poly(A) tails of all eukaryotic mRNA has been studied extensively (Kuhn and Wahle, 2004). The protein shuttles between the nucleus and the cytoplasm (Calado and Carmo-Fonseca, 2000; Calado et al., 2000a; Bear et al., 2003). PABPN1 is a multidomain protein that includes: a polyalanine tract (amino acids (aa) 2–11); a coil-coiled domain important for the stimulation of the poly(A) polymerase (aa 119–147) (Kerwitz et al., 2003; Verheesen

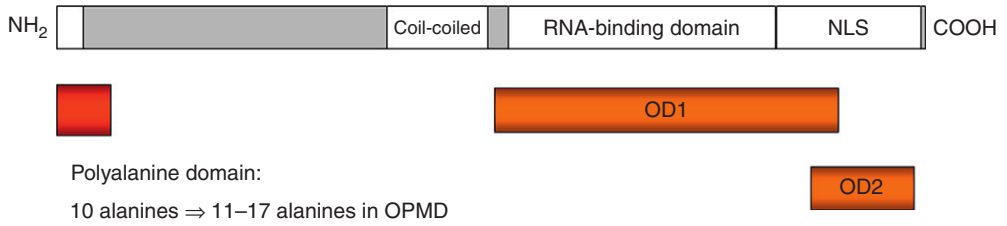

Figure 14.4. *PABPN1* has the following domains: a polyalanine tract (amino acids (aa) 2–11); a coil-coiled domain important for the stimulation of the poly(A) polymerase (aa 119–147); a ribonucleoprotein-motif RNA-binding domain essential for self-interaction (aa 161–257); a nuclear localization signal (NLS; aa 289–306); and two oligomerization domains (OD1 and OD2; aa 155–294 and 264–306) that overlap the RNA-binding domain and the NLS. OPMD, oculopharyngeal muscular dystrophy.

et al., 2006); a ribonucleoprotein-motif RNA-binding domain essential for self-interaction (aa 161–257) (Kuhn et al., 2003); a nuclear localization signal (NLS; aa 289–306) (Calado and Carmo-Fonseca, 2000; Abu-Baker et al., 2005); and two oligomerization regions (aa 155–294 and 264–306) that overlap the RNA-binding domain and the NLS (Fan et al., 2001) (Figure 14.4). To date, the role of the polyalanine tract and its expansion on PABPN1 structure and function is still unknown.

During the past 10 years our understanding of the molecular pathogenesis of OPMD has progressed, but no definitive mechanisms have yet been established. Various nuclear inclusion-dependent and -independent mechanisms have been proposed. Although most hypotheses suggest that the expansion of the polyalanine stretch leads to a gain of function of the protein, there is evidence that the intranuclear inclusions may not be responsible for the disease, and may even be protective (Messaed et al., 2007). PABPN1 is currently subdivided into three domains: the N-terminal domain containing the polyalanine stretch, the central RNA recognition motif (RRM or RNA-binding domain) that overlaps with the first oligomerization domain, and the C-terminal domain that includes a second oligomerization domain and the NLS. The precise impact of the expansion of the N-terminal polyalanine tract of PABPN1 on its structure and function has been explored only recently by structural biologists. The aggregative biophysical property of polyalanine stretches has been known for years (Brais et al., 1998b). In a series of elegant experiments published by the German group led by Elisabeth Schwarz, the increased aggregative tendency and resistance to solvent of the expanded N-terminal portion of PABPN1 has been well documented at the kinetic and structural levels (Lodderstedt et al., 2007, 2008; Rohrberg et al., 2008; Sackewitz et al., 2008a, b). The group showed that expansion from 10 to 17 alanines of the N-terminal domain of PABPN1 appears to decrease fibrillar formation, providing structural evidence that the mutated form may in fact have a greater negative impact on physiological interaction between PABPN1 molecules with itself and other partners (Sackewitz et al., 2008b). Interestingly, in their study of the influence of various substances on their fibrillar assay, they showed that doxycycline and trehalose, two molecules previously shown to diminish mutated PABPN1 toxicity in a mice transgenic model (Davies et al., 2005; Davies et al., 2006b), cause an increase in fibril formation (Lodderstedt et al., 2008). This provides indirect evidence that increased aggregation may in fact be protective, or alternatively, that these substances, by ensuring a better conformation of the soluble mutated form, may slow the disease process. These studies have clearly shown that the lengthening of the polyalanine domain does indeed have an impact on its conformation in a way that will influence the structure of soluble and fibrillar PABPN1. Other structural work led to the identification of the crystal structure of the RRM that, when mutated, clearly influences aggregation formation and toxicity (Messaed et al., 2007; Ge et al., 2008). Overexpression of PABPN1 readily produces in cellular, mice, fly, and nematode models the formation of INIs associated with cell death (Kim et al., 2001; Ravikumar et al., 2002; Abu-Baker et al., 2003; Bao et al., 2004; Hino et al., 2004; Corbeil-Girard et al., 2005; Davies et al., 2005; Chartier et al., 2006; Catoire et al., 2008). The PABPN1-containing INIs are usually filamentous and share features of OPMD muscle INIs, although they are less well structured. In different cellular and animal models of OPMD, investigators have shown that some molecules reduced cellular toxicity. In cellular models it was shown that inducing heat-shock protein expression using zinc sulfate, 8-hydroxyquinoline, ibuprofen, and indometacin (Wang et al., 2005), or exposing cells to anti-PABPN1 antibodies that interfere with oligomerization (Verheesen et al., 2006), could prevent cell death. In a mouse transgenic model of OPMD, investigators have reduced inclusion formation and cell death with agents that interfere with protein aggregation such a Congo red, doxycycline (Davies et al., 2005), and trehalose (Davies et al., 2006b). Together, these results suggest that the

progression of the toxicity may be slowed by interfering with PABPN1 aggregation.

Despite the strong evidence that the expanded polyalanine domain influenced PABPN1 aggregation and toxicity, there is mounting evidence suggesting that the larger size aggregates may not correspond to structures that play a key role in the underlining pathology, and that they may even be protective. The uncovering of dynamic aggregates in normal physiological circumstances, such as oxytocin-producing neurons in rats, first raised the possibility that such aggregation may not be pathological *per se* (Berciano et al., 2004; Villagra et al., 2008). Furthermore, different groups have reported that overexpressing PABPN1 with a normal 10-alanine-size domain, or even without an alanine domain, can lead to PABPN1 nuclear or perinuclear aggregates (Corbeil-Girard et al., 2005; Davies et al., 2008; Klein et al., 2008). In a series of experiments where different domains of PABPN1 were modified to decrease its aggregation propensity, cellular toxicity appeared to be greatest in cells where aggregates were not formed and PABPN1 stayed in a soluble form (Messaed et al., 2007). In this study, in fact, cells with aggregates that were able to divide fared better. This finding may correspond to the observation in an OPMD fly model where the expression of a PABPN1 without a polyalanine domain led also to a muscle phenotype without clear aggregation (Chartier et al., 2006). In the more extreme overexpression of PABPN1 with very large expansion, the toxicity was greatest with the largest constructs in which aggregates were not formed (Klein et al., 2008). These apparently discordant observations may be reconcilable if the soluble mutated PABPN1 is the true culprit, and the aggregates that arise through overexpression are visible bystanders of a molecular toxicity that is caused by the soluble PABPN1. Furthermore, cells able to produce the inclusion may in fact be protected against the toxicity (Messaed et al., 2007). This hypothesis may explain in part why wild-type PABPN1 is protective in a mouse transgenic model by having an antiapoptotic effect associated with an increased aggregation (Davies et al., 2008).

DIAGNOSIS

The previously mentioned strict clinical diagnostic criteria for dominant OPMD have been shown to have 100% specificity: (1) a positive family history of OPMD; (2) at least one palpebral fissure at rest smaller than 8 mm (or previous blepharoplasty); and (3) a swallowing time greater than 7 seconds when asked to drink 80 ml of ice-cold water (Brais et al., 1995). Although dominant OPMD based on these criteria is fully penetrant past the age 70 years, one-third of patients in their sixties will not meet these strict clinical diagnostic criteria. The clinical diagnosis of recessive OPMD is more problematic (Brais et al., 1998b; Hebbar et al., 2007; Semmler et al., 2007). Late-onset ptosis accompanied by dysphagia without a clear family history should raise this diagnostic possibility.

Until the identification of the OPMD *PABPN1* mutations, definitive diagnosis relied on the electron microscopy observation of OPMD INIs (Tomé and Fardeau, 1994). This approach has now been replaced by genetic testing (Brais et al., 1998b). The molecular diagnosis of dominant and recessive OPMD is straightforward. A single polymerase chain reaction (PCR) is required to establish the carrier status of an individual (Brais et al., 1998b). The region of the gene that is mutated is amplified by PCR, and the size of the DNA products are subsequently established to determine the number of (GCN) insertions. The fragment can also be sequenced to uncover the exact (GCN) sequence of the mutation and whether a point mutation leading to lengthening of the alanine stretch is present without causing an expansion of the region by converting the 12th triplet to an alanine instead of glycine codon (Robinson et al., 2006). The test has a sensitivity and specificity greater than 99%, and is available through many diagnostic laboratories worldwide. The major indications for DNA testing of a symptomatic individual are: (1) confirmation of the diagnosis in a family never tested; (2) that the clinical picture presents a diagnostic dilemma; (3) evaluation of the size of the mutation as a possible indicator of severity; (4) exclusion of a compound heterozygote status in a patient with a severe earlier-onset form of the disease; and (5) that the patient may suffer from recessive OPMD.

GENETIC COUNSELING

OPMD is inherited in both an autosomal dominant and an autosomal recessive manner. For autosomal dominant OPMD, it is expected that one of the biological parents will have had OPMD, provided they lived long enough. If a parent of a patient had OPMD, the risk to each child of having inherited an OPMD mutation is 50%. Every child of an individual heterozygous for a $(GCN)_{12-17}$ mutation has a 50% chance of inheriting the disease-causing mutation. Only a few cases of individuals with autosomal recessive OPMD homozygous for the $(GCN)_{11}$ allele have been reported (Brais et al., 1998b; Hebbar et al., 2007; Semmler et al., 2007). The offspring of an individual with autosomal recessive OPMD are obligate heterozygotes (carriers) for a mutant allele causing OPMD. The risk of their child

being affected is less than 1%, considering the 2% carrier rate for the $(GCN)_{11}$ recessive allele documented in many different populations (Brais et al., 1998b). Much care should be taken before requesting the predictive testing of an asymptomatic individual, because no preventive treatment is yet available. Presymptomatic testing should be performed in a context where genetic counseling and psychological support can be offered. Considering the late onset and relatively mild physical limitations caused by this disease, to our knowledge prenatal testing for OPMD has never been requested.

DIFFERENTIAL DIAGNOSIS

A clinical diagnosis of OPMD can readily be made based on the three previously described diagnostic criteria. The diagnosis is more difficult when the family history is uninformative. Ptosis of the eyelids and impaired ocular movements occasionally occur in advanced muscular dystrophies (e.g., myotonic dystrophy) and in some congenital myopathies (e.g., centronuclear myopathy), but the ocular palsies are never the predominant finding in these diseases. There are several different genetic causes of familial ptosis, blepharophimosis, and extraocular muscle fibrosis. Most of these entities have earlier onset and are not associated with dysphagia. Genetic testing will readily exclude the OPMD diagnosis in these cases, as in the frequent cases of late-onset isolated familial ptosis without dysphagia.

Dysphagia may be observed in other muscle disorders, such as myotonic dystrophy and polymyositis, but these diseases can easily be distinguished from OPMD by their clinical and pathological features. For example, ptosis is never observed in polymyositis. Dysphagia can also occur and is sometimes the presenting symptom in IBM (Wintzen et al., 1988; Danon and Friedman, 1989; Lotz et al., 1989; Verma et al., 1991; Litchy and Engel, 1992). Limb muscle weakness is usually prominent and there are no reports of ocular palsies in IBM. OPMD and familial recessive IBM mutation screening can distinguish the two conditions (Brais et al., 1998b; Eisenberg et al., 2001). The pathological differences between OPMD and IBM filaments can also help in distinguishing the two conditions.

Ocular myasthenia gravis is clearly the most important disease to exclude in completing the workup of patients with ptosis. In most myasthenic patients, the fluctuating diplopia, easy fatigability, and response to anticholinesterase drugs establish the diagnosis. The presence of circulating acetylcholine receptor antibodies can further support the diagnosis. However, in some myasthenic patients the ocular symptoms do not fluctuate, the limb muscles may atrophy, the response to anticholinesterase drugs is poor, and the antibody test is negative. In these patients, the diagnosis requires the demonstration of a neuromuscular transmission defect by electromyography, a detailed morphological study of the neuromuscular junction, and OPMD mutation screening in some cases.

Late-onset Kearns–Sayre syndrome (KSS) should also be entertained as a diagnosis. KSS occurring in late adult life may be difficult to distinguish from OPMD. Severe dysphagia and a dominant inheritance may exist in mitochondrial myopathies, and the visceral, cardiac, or retinal changes are frequently absent in late-onset KSS. Histochemical and ultrastructural studies of muscle can help to differentiate between the two diseases. A significant number of cytochrome *c* oxidase-negative fibers, biochemical defects in the respiratory chain, and mitochondrial DNA deletions will confirm the diagnosis of KSS. It should be stressed that some mitochondrial changes do occur in a few cases of OPMD, and also in aged patients with other muscle diseases. In the pediatric age group, ptosis and dysphagia may be caused by mitochondrial neurogastrointestinal encephalopathy syndrome (MNGIE; OMIM #603041). This condition has sometimes been referred to as pediatric OPMD, but no $(GCN)_n$ *PABPN1* mutations have been uncovered and multiple mitochondrial deletions caused by mutation in the thymidine phosphorylase gene appear to be responsible for most cases (Nishino et al., 1999).

Distal muscle involvement is rare in typical OPMD, but is a characteristic feature of late-onset autosomal dominant oculopharyngodistal myopathy (OMIM #164310) (Satoyoshi and Kinoshita, 1977). Several familial cases of a late-onset muscular dystrophy resembling OPMD but associated with more involvement of the distal limb muscles have been described in Japan. In our experience, facial diplegia is a shared feature of many of these cases, but never a dramatic feature of OPMD (see Figure 14.1). The muscle biopsies in these patients were reported to show nonspecific dystrophic features, and no *PABPN1* mutation has ever been reported (Satoyoshi and Kinoshita, 1977; Uyama et al., 1998; Minami et al., 2001). Autosomal dominant distal myopathy with vocal cord paralysis (OMIM #606070) may also enter the differential diagnosis because it is usually associated with vocal cord and pharyngeal dysfunction, although it does not present with ptosis (Feit et al., 1998).

THERAPY

There is no medical treatment yet available for OPMD (Davies 2006a). A high-protein diet is recommended. This is particularly important as the dysphagia becomes severe and the patient shies away from sources of animal protein such as meat. Special attention should be

paid to prevent the frequent social withdrawal of patients as their dysphagia progresses. They should be told to eat before or after social gatherings if necessary. They should be reassured about the very small risk of fatal choking, as confirmed by the normal life expectancy (Becher et al., 2001). Aspiration pneumonia being a frequent cause of death, patients should be advised to consult early if they have a productive cough accompanied by fever. Exercises that maintain a good cardiovascular condition should be encouraged, but strenuous exercises should not be promoted. A large proportion of patients will use either a cane or a walker late in the course of the disease. Rarely, patients with more severe disease will require a wheelchair, mostly to cover long distances. Prevention of traumatic fractures due to falls is paramount.

The surgical treatments currently available are used to correct the eyelid ptosis and improve swallowing in moderately to severely affected individuals. Two types of operation are used to correct the ptosis, with good results overall: resection of the levator palpebral aponeurosis and frontal suspension of the eyelids (Codère, 1993; Codère et al., 2001). Resection of part of the aponeurosis is performed easily, but usually needs to be repeated every 3–8 years with increasingly unsatisfactory results due to postsurgical scarring (Rodrigue and Molgat, 1997). Frontal suspension of the eyelids consists of using a synthetic or skeletal muscle fascia thread as a sling that is inserted in the tarsal plate of the upper eyelid and attached at its ends in the frontalis muscle, which is relatively preserved in OPMD (Codère, 1993; Codère et al., 2001). The major advantage of this procedure is that it is permanent, although use of the muscle fascia may require general anesthesia. Surgery is recommended when the ptosis interferes with vision or when the patient complains of recurrent cervical pain due to constant dorsiflexion of the neck (the astronomer position). Contraindications to blepharoplasty are marked ophthalmoplegia, dry eye syndrome, or poor orbicularis function.

Surgical evaluation of symptomatic dysphagia should be prompted by severe dysphagia, marked weight loss, near-fatal choking, or recurrent pneumonia. Cricopharyngeal myotomy will alleviate symptoms in most cases. Unfortunately, dysphagia will slowly reappear over years, although operated individuals appear always to do better than individuals with the same degree of dysphagia who have not undergone surgery. Severe dysphonia and lower esophageal sphincter incompetence are contraindications to surgery (Duranceau, 1997). Repetitive dilatation of the upper esophageal sphincter with bougies and botulinum toxin injections in the cricopharyngeal muscle are still at the experimental stage (Mathieu et al., 1997).

UNSOLVED PROBLEMS

Despite the identification in 1998 of the mutations responsible for OPMD, many important questions remain unanswered. It is clear that the severity of the phenotype varies even with carriers of the same size of $(GCN)_n$ *PABPN1* mutation (Bouchard et al., 1997; Brais et al., 1998b). However, no study has demonstrated conclusively that the size of the mutation influences the severity of the phenotype, at least when assessed by the age of onset (Mirabella et al., 2000; Hill et al., 2001; Muller et al., 2001). Only carriers of the smallest $(GCN)_{12}$ mutation appear clearly to have a milder phenotype, with a later age of onset in the seventh decade, with ptosis, and only mild dysphagia (Brais et al., 1998b; Hill et al., 2001; Muller et al., 2001). Compound heterozygotes for a dominant and a recessive mutation have also been shown to have more severe phenotypes (Brais et al., 1998b; Hill et al., 2001). The sequencing of the mutation has demonstrated that OPMD mutations do not consist of pure $(GCN)_n$ repeat expansions, but of (GCN)/polyalanine insertion (Scacheri et al., 1999; Nakamoto et al., 2002); however, the mechanism responsible for the genesis of the mutations is still not fully established (Brais et al., 1998b; Nakamoto et al., 2002).

In OPMD, as in most muscular dystrophies, the greater selective involvement of certain muscles has yet to be understood. This is particularly interesting in OPMD, because the mutated gene is expressed ubiquitously in all tissues. Some have proposed that the unique embryological origin of extraocular muscles and continuous remodeling play a role in the greater involvement of these muscles (Wirtschafter et al., 2004). However, this does not fully explain the early involvement of other muscles, such as the tongue. A dosage effect for dominant mutations is clearly observed in individuals who are homozygotes for two dominant mutations and compound heterozygotes for a dominant and a recessive mutation (Brais et al., 1998b; Blumen et al., 1999). This is not as clear in patients with recessive OPMD, where more severe and less severe phenotypes have been observed (Brais et al., 1998b; Hebbar et al., 2007; Semmler et al., 2007). Lastly, despite extensive knowledge of the structure and function of PABPN1, we still do not know how expansions of its short polyalanine domain cause muscle demise. As discussed above, even the pathogenic role of the INIs still needs to be elucidated. What is clear, however, is that, because of the increasing number of diseases caused by polyalanine expansions and the pathological overlap with CAG/polyglutamine diseases, pathological insights gained by the study of OPMD could lead to a better understanding of a much larger group of developmental and degenerative diseases (Gaspar et al., 2000).

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Chapter 15

Myotonic dystrophy types 1 and 2

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INTRODUCTION

Myotonic dystrophies are autosomal dominant disorders characterized by myotonia, dystrophic muscle degeneration, lens opacities, and other variably associated multisystemic manifestations. Currently, there are two genetically distinct types of myotonic dystrophy: myotonic dystrophy type 1 (DM1, dystrophia myotonica 1, Steinert's disease; Online Mendelian Inheritance in Man (OMIM) #160900) and myotonic dystrophy type 2 (DM2, dystrophia myotonica 2; OMIM #602668) (Table 15.1). DM2 is also known as proximal myotonic myopathy (PROMM) because DM2 causes prominent proximal muscle weakness, often as a presenting symptom, as opposed to predominantly distal muscle weakness and atrophy in DM1. The mutation of DM1 is an expansion of the unstable CTG trinucleotide repeat in the 3′ untranslated region (UTR) of the dystrophia myotonica protein kinase (*DMPK*) gene on chromosome 19q13.3, whereas DM2 is caused by an expansion of an intronic CCTG tetranucleotide repeat in the zinc finger 9 (*ZNF9*) gene in 3q13.3–q24. Thus, the mutations of DM1 and DM2 are strikingly similar in that both are expansions of microsatellite repeats encoding RNA transcripts containing repeated CUG motifs, which are not translated into the respective protein products. Because of these similarities, it has been predicted that these two diseases share the pathogenic mechanism involving "*trans*-dominant toxic gain of function" by repeat-containing RNA transcripts. It should be noted that Le Ber et al. (2004) reported a family with "DM3." However, this disease was later found to be a different entity known as inclusion body myopathy with early-onset Paget's disease and frontotemporal dementia (IBMPFD), caused by mutations in the valosin-containing protein (*VCP*) gene (OMIM #167320) (Udd et al., 2006). This chapter reviews the current understanding of clinical phenotypic characteristics, genetic aspects, and pathogenic mechanisms of DM1 and DM2.

CLINICAL FEATURES

Clinical features of adult-onset myotonic dystrophy type 1

The clinical entity of DM1 was first recognized by Steinert (1909) and Batten and Gibb (1909) in 1902, when they described this disease as a entity distinct from myotonia congenita (Thomsen's disease; OMIM #160800) (Harper, 2001). Early investigators found that patients with DM1 have progressive muscle deteriorations and extramuscular disorders, such as testicular atrophy (Steinert, 1909) and cataract (Greenfield, 1911; Curschmann, 1912) as distinguishing features. Furthermore, the variable phenotypic expressivity of DM1 was described in 1918 by Fleischer, who showed that individuals with cataracts without the muscle phenotype might connect apparently separate families with DM1. This observation became the basis for the genetic concept of "anticipation," which is now known to occur in many diseases caused by repeat expansion mutations (see section on Anticipation and DM1 below; reviewed in Harper, 2001). In subsequent years, involvement of many other organ systems has been recognized, firmly establishing DM1 as a multisystemic disorder.

Skeletal muscle manifestations

The most common presenting clinical features of DM1 are muscle weakness, myotonia, and iridescent lens opacities (Ashizawa and Harper, 2006). The skeletal

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Table 15.1

Myotonic dystrophies

	Dystrophia myotonica 1	Dystrophia myotonica 2
OMIM	#160900	#602668
Alternative names	Myotonic dystrophy type 1 DM1 Myotonic dystrophy Steinert's disease Myotonia atrophica Proximal myotonic dystrophy	Myotonic dystrophy type 2 DM2 Proximal myotonic myopathy PROMM Ricker–Moxley disease
Inheritance	Autosomal dominant	Autosomal dominant
Chromosome locus	19q13.2–q13.3	3q13.3–q24
Gene	*DMPK*	*ZNF9*
Mutation	CTG expansion (3′ UTR)	CCTG expansion (intron 1)
Normal repeat size	Up to 37	Up to 27
Premutations	38–50	28–75 (?)
Protomutations	51–100	NA
Full mutations	51 to several thousand	76 to 11 000
Anticipation	Yes	Yes

OMIM, Online Mendelian Inheritance in Man; UTR, untranslated region.

muscles of patients with DM1 typically show myotonia, which accompanies weakness and wasting of muscles in distal limbs, face, and neck (sternocleidomastoid muscle). The facial features are often described as "hatchet face," showing narrowed face, weakness and atrophy of facial and temporalis muscles, bilateral blepharoptosis, and frontal balding (Figure 15.1). Bulbar muscle weakness causes oropharyngeal dysphagia, which is a common complaint and may become an aspiration risk.

Myotonia is detected clinically as a delayed relaxation of a muscle after forceful contraction of the muscle. Myotonia in DM1 is readily elicited by asking the patient to grip the examiner's fingers firmly and then to let go (grip myotonia), or by firm percussion of the thenar eminence or brachioradialis muscle (percussion myotonia); in either case, relaxation of the muscle is delayed by several seconds. Unlike exercise-induced muscle cramps, myotonia is usually not painful, although patients may complain of discomfort. Percussion of the edge of a tongue blade perpendicularly placed across the tongue may also show a persistent furrow (tongue myotonia). Myotonia is also present in many other skeletal muscles in patients with DM1 and may be detectable as a formation of a "dimple" after percussion of the muscle belly. However, myotonia may be minimal when muscle wasting is severe, and it is usually clinically undetectable in very young children. Myotonia of DM1 usually shows the "warm-up phenomenon," in which repetitive exercise of the muscle successively decreases the severity of the myotonia. Myotonia is also detected with electromyography (EMG), which shows continuous, waxing and waning, but overall gradually waning, discharges for a few to several seconds with characteristic "dive bomber" or "motorcycle revving" sound upon insertion of the electrode or subsequent mechanical or electrical muscle stimulation. DM1 skeletal muscles also show small-amplitude, short-duration motor units in addition to the electrical myotonia on EMG. Muscle histopathology is characterized by variation in fiber size with atrophy of type 1 fibers, increased internal (centrally located) nuclei, nuclear clump fibers, sarcoplasmic masses, and ring fibers, with some moth-eaten fibers, necrotic fibers, mildly increased connective tissues, and rarely fat replacement (Figure 15.2) (Vihola et al., 2003; Dubowitz and Sewry, 2006). Although these findings are seen individually in many other myopathies, collectively they may be diagnostic of myotonic dystrophy. Generally, skeletal muscle manifestations are similar between DM1 and DM2 in the sense that both cause myotonic myopathies. However, there are important clinical, electrophysiological, and histopathological differences, which can be helpful in making a correct clinical diagnosis (Table 15.2; see also section on Clinical manifestations of DM2/PROMM).

Cardiac involvement

Abnormalities in the heart (for a review see Phillips and Harper, 1997; Sovari et al., 2007) are important clinical features of DM1, especially because sudden death in adults is not uncommon and may have been

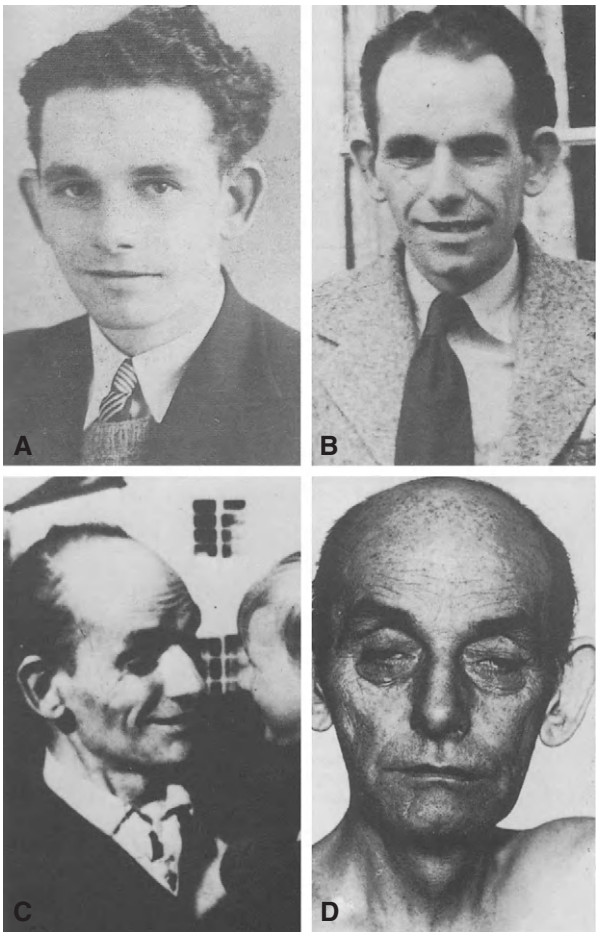

Figure 15.1. Facial features of myotonic dystrophy. Note development of myotonic dystrophy facies with age in a patient with late-onset DM1. (**A**) Aged 17 years, no facial weakness. (**B**) Aged 30 years, slight facial weakness and ptosis. (**C**) Aged 44 years, the facial features are obvious, but the patient had no symptoms. (**D**) Aged 56 years, with marked weakness and wasting of facial and sternocleidomastoid muscles, the patient was having distal weakness, myotonia, cataracts, and retinal degeneration. (From P.S. Harper, Myotonic Dystrophy, 3rd edition, WB Saunders, London, 2001, with permission.)

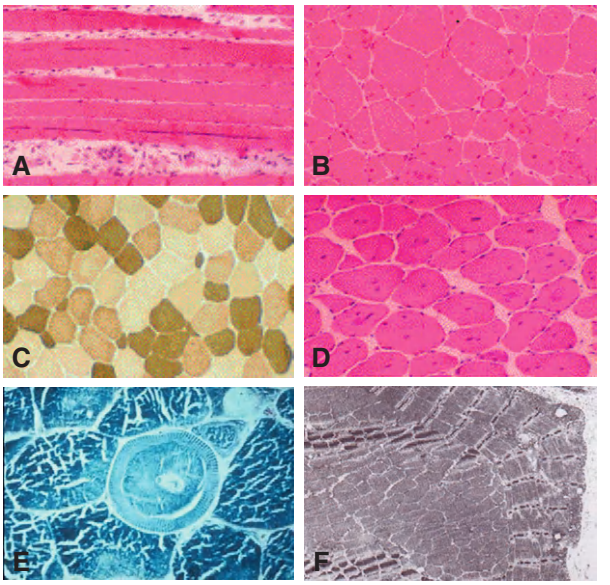

Figure 15.2. Histopathology and histochemistry of the skeletal muscle in myotonic dystrophy type 1. (**A**) Chained nuclei (hematoxylin and eosin (H&E) stain, original magnification $\times 200$). (**B**) Variation in fiber size with internal nuclei (H&E, $\times 100$). (**C**) Type 1 fiber atrophy (ATPase, pH 4.3, $\times 200$). (**D**) Internal nuclei in most fibers and sarcoplasmic masses in some fibers (H&E, $\times 200$). (**A–D** reproduced with permission from I.-N. Sunwoo (2001). Clinical application of muscle biopsy. J Korean Neurol Assoc 19: 67–87.) (**E**) Ring fiber (NADH-TR, $\times 400$) (from Pathology Department, Virginia Commonwealth University, with permission). (**F**) Electronmicrograph of a ring fiber ($\times 7400$) (from P.S. Harper, Myotonic Dystrophy, 3rd edition, WB Saunders, London, 2001, with permission).

underestimated in children (Bassez et al., 2004). Conduction defects are often found on electrocardiography (ECG) in affected adults, including those without cardiac symptoms. Invasive electrophysiological studies may be needed to detect the conduction abnormalities in the His bundle and other parts of the conduction system (Lazarus et al., 1999; Dello Russo et al., 2006) that have a predilection to degenerate. Although clinically overt congestive heart failure is a cardiac manifestation among patients with DM1 in late stages, tissue Doppler ECG studies show that up to 29% of asymptomatic patients may have left ventricular systolic dysfunction (Bhakta et al., 2004; Vinereanu et al., 2004). Various arrhythmias, notably atrial flutter/fibrillation and ventricular tachyarrhythmias, are common. These arrhythmias appear to be attributable to cardiomyopathy rather than abnormal autonomic regulation of the heart (Di Leo et al., 2004). The prevention of complete conduction block and ventricular fibrillation, which may cause sudden death, is one of the foremost important issues in the management of patients with DM1 (see section on Loss of *DMPK* function).

Although the correlation between skeletal muscle and cardiac manifestations is a controversial issue, careful examination usually shows some degree of skeletal muscle involvement, either myotonia or weakness, when cardiac muscle is significantly affected (Ashizawa and Harper, 2006). Patients with congenital or infantile-onset DM1 tend to have cardiac problems early in their lives (Forsberg et al., 1990; Morgenlander et al., 1993). However, it should be noted that young asymptomatic carriers of the *DM1* mutation may develop life-threatening cardiac events (Bassez et al., 2004). Interestingly, muscular and cardiac involvement appeared to show familial clustering in a study of

Table 15.2

Muscle involvement in myotonic dystrophy types 1 and 2 (modified from Ashizawa and Harper, 2006)

	DM1	DM2
Muscle involvement		
Facial weakness and ptosis	++	±
Jaw muscle	+ +	−
Sternomastoids	++	±
Proximal limb muscle	+	++
Distal limb muscle	++	+*
Myotonia	++	+†
Pseudohypertrophy	±	−
Muscle pain	±	++
Myotonia		
Severity	++	+
Warm-up phenomenon	++	±
Fluctuation	±	++
Myotonic discharges (EMG)	Waxing and waning	Waning
Distribution of electrical myotonia	Widespread	Proximal > distal
Characteristic features on muscle histology		
Increased central nuclei	+	+
Nuclear chains	+	±
Ring fibers	+	+
Sarcoplasmic masses	+	−
Type 1 fiber atrophy	+	−
Type 2 fiber atrophy	−	+
Rimmed vacuoles	−	+
Small angular fibers	±	+
Moth-eaten fibers	±	+

++, Prominent feature; +, may occur; ±, inconsistent or late feature; −, absent; *frequent in deep finger flexors; †electrical myotonia present in 90% of patients but clinical myotonia may be subtle.

affected sibs with DM1 (Groh et al., 2005). Cardiac manifestations of DM1 are generally more severe than those of DM2 (Table 15.3; see Section on Clinical manifestations of DM2/PROMM).

Respiratory problems

Respiratory complications in DM1 are attributable to multiple problems, including aspiration, diaphragmatic weakness, and impaired central nervous system (CNS) respiratory drive. Alveolar hypoventilation is well documented (Begin et al., 1997). Use of domiciliary assisted ventilation in patients with DM1 sustains arterial blood gas tensions (Nugent et al., 2002). Respiratory depression caused by anesthetics and sedatives is a serious perioperative complication; consequently, great care should be taken if anesthesia is required,

Table 15.3

Cardiac abnormalities in myotonic dystrophies (from Ashizawa and Harper, 2006)

Cardiac abnormality	DM1	DM2
Arrhythmias	++	+
Atrioventricular block	++	+ (11%: Day et al., 2003)
Dilated cardiomyopathy	+	± (7%: Day et al., 2003)
Sudden death	+	± (reported)
Congestive heart failure	+	− (not reported)

++, Prominent feature; +, may occur; ±, inconsistent or late feature; −, absent.

even in young and mildly affected patients (see section on anesthesiological management) (Mathieu et al., 1997).

Brain abnormalities

Abnormalities in the brain of patients with congenital DM1 have been well recognized; however, the CNS abnormalities may be found in patients of all ages with DM1. The brain phenotype may pose as a major cause of disability in DM1, especially in patients with congenital DM1 and childhood-onset DM1. In adult patients with DM1, the importance of CNS problems has been underestimated. Although major cognitive impairments are unusual in adults, frontal executive dysfunctions including apathy, reduced initiative, stubbornness, and avoidant personality trait often render these patients socioeconomically disadvantaged (Delaporte, 1998; Meola et al., 2003; Modoni et al., 2004; Gaul et al., 2006) and decrease health-related quality of life (Antonini et al., 2006). Deviant temperament and character, and impaired recognition of facial emotion, have also been noted (Winblad et al., 2005, 2006). Psychiatric disorders (axis I disorders in the Diagnostic and Statistical Manual of Mental Disorders, 4th edition) are usually not the major CNS manifestation, although depression and phobia may be encountered (Kalkman et al., 2007). Daytime hypersomnolence is frequently noted and is partly attributable to central sleep apnea, although obstructive sleep apnea may also contribute to the problem. Hypersomnolence in DM1 has been reported to accompany short sleep latency, rapid eye movements at sleep onset, and abnormalities in the hypothalamic hypocretin system (Martinez-Rodriguez et al., 2003). However, a recent study of 38 patients with DM1 showed that hypocretin levels in the cerebrospinal fluid did not differ from those of 33 control subjects, and splicing of HcrtR1 and HcrtR2 mRNA

was not altered (Ciafaloni et al., 2008). Minor degrees of atrophy with progressive focal white matter lesions have been found on imaging studies in adults (Kornblum et al., 2004). The focal areas of white matter and subregions of the corpus callosum showed abnormal signals in diffusion tensor magnetic resonance imaging (MRI) (Fukuda et al., 2005; Ota et al., 2006), and correlations between intellectual function and white matter lesions in insular and temporal lesions have been reported (Kuo et al., 2008). One study of 60 patients with DM1 from 22 families suggested familial aggregation of white matter lesions without correlation with the CTG repeat length (Di Costanzo et al., 2008). Gray matter lesions are also detected in the neocortex, independent of white matter lesions (Giorgio et al., 2006). Neurofibrillary tangles containing the tau protein, inclusion bodies, and oxidative products have been noted in different areas of the brain (Vermersch et al., 1996; Sergeant et al., 2001; Oyamada et al., 2006). Studies of evoked potentials (Arakawa et al., 2003) and central oculomotor control further suggest widespread CNS dysfunction (Shaunak et al., 1999).

Ocular abnormalities

Cataracts are the most well-recognized eye problem in DM1. Lens opacities are rarely seen in young children, but most affected adults have detectable opacities (Figure 15.3). In early stages, lens opacities have a characteristic refractile, multicolored appearance in the subcapsular region under a slit-lamp biomicroscope. As the opacities progress, they become stellate white opacities leading to mature cataracts, which are usually indistinguishable from other types of cataract. Lens opacities of this type are highly specific for DM1 and DM2. Multicolored opacities may rarely occur in individuals from the general population without the *DM1* mutation (Giordano et al., 1996); however, *DM2* mutations were not tested in these individuals. It should be noted that autosomal dominant peripheral circumferential iridescence of anterior lens capsule has recently been reported in three families (Traboulsi et al., 2005).

The retina may show macular or peripheral abnormalities, which may be detected by sensitive electroretinography even in the presence of some cataracts. Other ocular abnormalities include ptosis, weakness of orbicularis oculus muscles, and decreased ocular pressure. Saccadic eye movement abnormalities have been reported and attributed to impaired central oculomotor control (Shaunak et al., 1999). Myotonia is rarely detected in the eyelids and ocular muscles, in contrast to myotonia congenita (Versino et al., 2002). However, there may be an increased prevalence of hypermetropia, esotropia, and amblyopia (Bollinger et al., 2008). Although lens opacities of DM2 are identical to those seen in DM1, little is known about other ocular abnormalities in DM2 (Table 15.4).

Gastrointestinal abnormalities

Gastrointestinal problems may involve multiple areas of the digestive system (Table 15.5) and subjectively be the most concerning symptom to many patients with DM1 (Ronnblom et al., 1999; Harper, 2001; Harper et al., 2004). Heartburn, regurgitation, dyspepsia, abdominal pain, bloating, and changes in bowel habit are often reported. The severity and presence of gastrointestinal problems do not appear to be correlated with the degree of the skeletal muscle disease in DM1 (Bellini et al., 2006).

Dysphagia in the esophagus is attributable to the abnormal peristaltic motility of the esophageal body and reduced pressure of the upper esophageal sphincter.

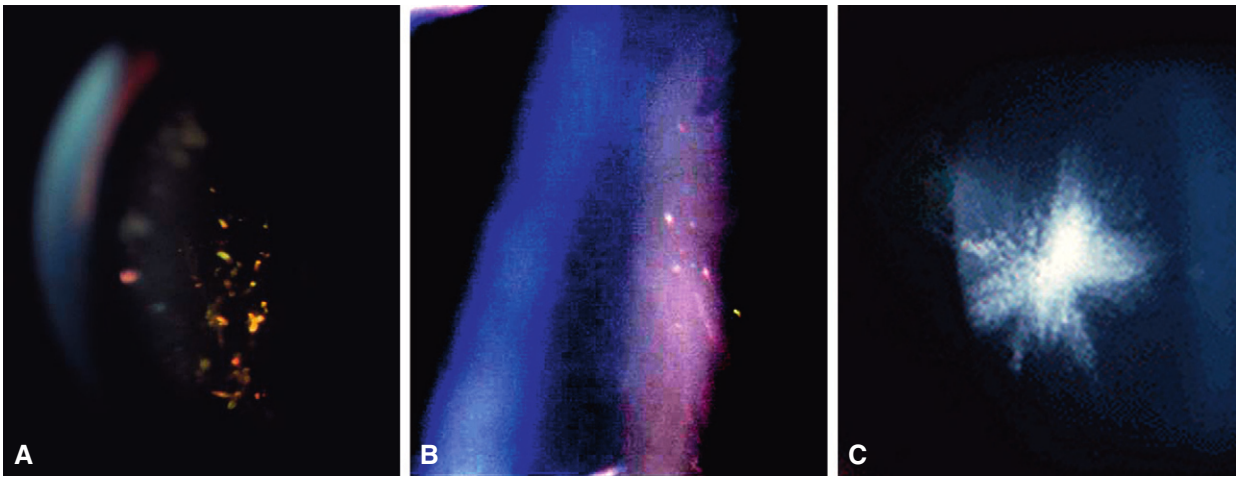

Figure 15.3. Iridescent lens opacities in myotonic dystrophy. (**A**) Multichromatic iridescent opacities (from Digital Journal of Ophthalmology, with permission). (**B**) Progression to white opacities. (**C**) Stellate cataract.

Table 15.4

Ocular abnormalities in myotonic dystrophies (from Ashizawa and Harper, 2006)

Ocular abnormality	DM1	DM2
Cataract	~85%	80–90%
Retinal degeneration	>50%	Not reported
Low intraocular pressure, enophthalmos	60–80%	Not reported
Ptosis	~50%	Absent or mild
Corneal lesions	Occasional	Not reported
Extraocular myotonia	Infrequent	Absent
Extraocular muscle weakness	Infrequent	Absent

Table 15.5

Gastrointestinal tract involvement in myotonic dystrophy type 1 (from Ashizawa and Harper, 2006)

Anatomical structure	Description
Pharynx	Delayed relaxation, retention of bolus: frequent tracheal aspiration
Esophagus	Reduced motility and pressure; dilatation: dysphagia frequent
Stomach	Dilatation, food retention (rare)
Small bowel	Usually normal
Colon	Megacolon, fecal impaction, symptoms of spastic colon; rarely volvulus
Anal sphincter	Myotonia demonstrable

None of these gastrointestinal tract involvements has been reported in patients with myotonic dystrophy type 2 except those listed in Table 15.6.

Severe peristaltic abnormalities of the esophagus may be seen in patients with relatively little weakness and could contribute to the aspiration pneumonia. In some patients, however, the impairment of gastrointestinal function is so gradual that they adapt to it with little or no awareness of any disturbance (Modolell et al., 1999). Consequently, the prevalence of dysphagia ranges from 25% to 80% in different reports, depending on whether asymptomatic dysphagia is included (Ronnblom et al., 1996; Modolell et al., 1999).

Abnormalities in the lower esophageal sphincter, which could lead to gastroesophageal reflux, are seen less consistently (Costantini et al., 1996; Modolell et al., 1999). Pharyngoesophageal myotonia or problems with postcontraction relaxation, once considered to be an important mechanism of dysphagia (Siegel et al., 1966), are no longer considered to be a main problem of the esophageal involvement (Costantini et al., 1996; Modolell et al., 1999).

Delayed gastric emptying is often recognized (Ronnblom et al., 2002). Colonic involvement is less common, although affected children may have colicky abdominal pain and some patients may develop megacolon. Intestinal pseudo-obstruction may occur in both children and adults (Brunner et al., 1992a), mimicking a surgical emergency, but usually responds to conservative treatment. However, one should watch for true surgical complications such as ileus, volvulus, and rupture of the colon in patients with megacolon.

The manifestations of impaired gastrointestinal motility have previously been attributed to smooth muscle degeneration, although histological evidence of alterations is sparse and conflicting (Costantini et al., 1996; Bellini et al., 2006). Contributions of central, autonomic, and enteric nervous system dysfunction, impaired secretion of gastrointestinal peptides, bile acid malabsorption, and bacterial contamination of the small bowel have also been hypothesized (Nowak et al., 1984; Ronnblom et al., 1998, 1999, 2001; van Engelen and Brunner, 2004; Bellini et al., 2006).

Cholelithiasis and cholestasis are common problems of adult patients with DM1, although infantile cases have been reported recently (Sumi et al., 2005). Despite the frequency of the gallbladder problems, little is known about the underlying mechanism. Delayed emptying of gallbladder due to smooth muscle dysfunction may be the major cause, and a fundamental metabolic disturbance involving cholesterol and bile acids has been postulated to contribute to the development of cholelithiasis (Harper, 2001).

Although gastrointestinal abnormalities have not been studied systematically in DM2, subjective gastrointestinal symptoms on a patient survey are comparable in patients with DM2 (Table 15.6) (Tieleman et al., 2008).

Endocrine abnormalities

Although various endocrine disturbances are known to exist in DM1 (Table 15.7; Harper, 2001), the most clinically conspicuous abnormality is testicular atrophy. Histopathologically, the testis of male patients with DM1 shows primary tubular degeneration with fibrosis and hyalinization, and hypertrophy of Leydig cells. Infertility is common in men with advanced DM1; however, even in the presence of reduced spermatogenesis, patients may be fertile. Blood levels of follicle-stimulating hormone (FSH) and, to a lesser degree, luteinizing hormone (LH), are frequently raised.

Table 15.6

Gastrointestinal symptoms in myotonic dystrophy types 1 and 2 (from Tieleman et al., 2008)

Gastrointestinal symptom	DM1	DM2
Dysphagia	62%	52%
Dysphagia for liquids	35%	38%
Dysphagia for solid food	62%	41%
Abdominal pain	45%	62%
Postprandial	17%	41%
During fasting	17%	31%
No decline after defecation	35%	41%
Epigastric pain	38%	55%
Epigastric pain in the day	35%	55%
Epigastric pain at night	24%	48%
Constipation	55%	62%

In females, the incidence of reproductive loss is high. Miscarriages occurring late in pregnancy may be attributable to problems of fetuses with congenital DM1. The early pregnancy loss is more likely to be attributable to an endocrine disturbance. Further complications at labor and delivery may be caused by both congenital DM1 of the fetus and uterine smooth muscle involvement in the mother.

Diabetes mellitus is a well-recognized problem in DM1, although clinical diabetes was seen in only 6% of patients in Harper's series (Harper, 1989). In contrast, insulin metabolism is almost universally abnormal. The main feature is hyperinsulinemia in response to a glucose load, which has been attributed to insulin resistance (Morrone et al., 1997). Excessive insulin secretion by pancreatic beta cells accounts for the hyperinsulinemia in patients with DM1. Early studies have shown decreased insulin sensitivity, suggesting abnormalities in the insulin receptor or receptor-associated processes that modulate insulin binding (Moxley et al., 1978, 1981). Whether hyperinsulinemia is a result of a negative feedback from the insulin resistance is yet to be investigated. A recent study indicated that insulin sensitivity may be preserved in DM1, and that the primary problem may reside in insulin secretory function rather than insulin resistance (Perseghin et al., 2003). Patients with DM1 also show abnormal plasma proinsulin levels in the fasting state, during clamping, and during the oral glucose tolerance test, suggesting secretory dysfunction. Furthermore, levels of leptin, tumor necrosis factor (TNF)-α, TNF receptor II, and testosterone, as well as responses of glucagon-like peptide-1 and adrenocorticotropic hormone/cortisol, are altered (Johansson et al., 2002a, b). Adrenocorticoid and mineralocorticoid metabolism is also impaired in DM1 (Johansson et al., 2000, 2001).

Table 15.7

Endocrine abnormalities in myotonic dystrophies (from Ashizawa and Harper, 2006)

Organ	DM1	DM2
Testis	Testicular atrophy (60–80% clinically); degeneration of tubular cells; hyperplasia of Leydig cells; serum testosterone slightly reduced	Testicular dysfunction (~65%) with increased serum FSH, low or low–normal testosterone levels, and oligospermia (Day et al., 2003)
Ovary	No consistent evidence of abnormality; high fetal loss	Increased pregnancy loss reported (Day et al., 1999)
Pituitary	Increased FSH levels; slightly increased LH levels; increased LHRH response Hyperresponsiveness to exogenous growth hormone* Pituitary adenoma*	None reported
Pancreas	Increased insulin response to glucose load and other stimuli Clinical diabetes*: abnormal glucose tolerance test results*	Manifest diabetes (~25%: Day et al., 2003; Udd et al., 2003) with insulin resistance
Thyroid	Inconsistent	Reported but probably coincidental in hypothyroidism
Adrenal; parathyroid	No consistent abnormality found	

FSH, follicle-stimulating hormone; LH, luteinizing hormone; LHRH, luteinizing hormone releasing hormone.
*Rare or inconsistent abnormality.

Other clinical manifestations in peripheral nerve, skin, bone, and the immune system

Several other clinical features have been noted in DM1. These patients often show loss of muscle stretch reflexes, and weakness and atrophy of the distal muscles early in the disease. However, involvement of peripheral nerves remains contentious. Neurophysiological studies show evidence for a nonprogressive axonal neuropathy in less than half of patients, whereas sural nerve biopsies have shown axonal damage and reductions in myelinated fiber density (Cros et al., 1988; Logullo et al., 1992; Mondelli et al., 1993). Interestingly, abnormal axonal excitability was observed in the median nerve after abductor pollicis brevis contraction (Krishnan and Kiernan, 2006).

Early male-pattern balding, multiple pilomatricoma of the neck and head (Chuang and Lin, 2004), and sweat gland atrophy (Ichikawa et al., 1989) are a frequent dermatological phenotype (Harper, 2001). Early male-pattern balding is mostly seen in DM1 males with muscle disease, and may also be seen in severely affected women with adult-onset DM1, although it never occurs in childhood, even with the congenital form. The mechanism of the hair loss is uncertain. Hormonal impairment could contribute to the hair loss, but male hormone levels are not raised in DM1. Interestingly, consistent abnormalities in morphology of hair, such as twisting, swelling, and high-frequency bands on the hair shaft, were reported in 25 affected patients (Amorosi et al., 1999). In the same study, an increase in glutamic acid levels and a reduction in serine content were found in all 25 patients, and an increase in cystine content in 80% of cases. It may be speculated that the hair loss could be related to myotonia of the hair follicle muscle arrector pili, as alopecia totalis is seen in Satoyoshi's disease (Satoyoshi, 1978), a neuromuscular disease with severe generalized muscle cramps.

Multiple pilomatricomas, pituitary adenomas, parathyroid adenomas, parotid adenomas, and other benign neoplasias have been associated with DM1 (Harper, 2001). Skeletal changes include cranial hyperostosis, enlargement of air sinuses, and a small pituitary fossa, which may be related to endocrine abnormalities (Harper, 2001). Temporomandibular dislocation is also common. In congenital cases, talipes and arthrogryposis are frequently found.

Hypogammaglobulinemia has been recognized since the 1950s, although the exact mechanism of reduced levels of immunoglobulin remains elusive (Harper, 2001). IgG deficiency has been attributed to increased catabolism (Wochner et al., 1966). An increased number and a greater turnover of Fc receptors have been observed in cultured monocytes and may be relevant to the abnormal immunoglobulin catabolism (Banerjee et al., 1982). Defective neutrophil and granulocyte functions have also been reported (Seay et al., 1978; Friedenberg et al., 1986; Mege et al., 1988). Although 54% and 13% of patients with DM1 failed to make antibody to tetanus toxoid and *Salmonella typhi* H antigen, respectively (Grove et al., 1973), no clinically significant immune deficiency has been reported.

Congenital and childhood-onset DM1

Congenital DM1 (CDM1)

The congenital form of DM1 (CDM1) and some cases of early childhood-onset disease appear clinically distinct from the typical "adult" form (Table 15.8). CDM1 occurs almost exclusively when the mother is the transmitting parent. Clinical features of CDM1 have been reviewed extensively by Harper (2001). Decreased fetal movements, polyhydramnios, hypoplastic diaphragma, thin ribs, hydrocephalus, talipes, and arthrogryposis are frequently detected by prenatal ultrasonography (Zaki et al., 2007). At birth, infants with CDM1 present with "floppy infant" syndrome with generalized hypotonia (Figure 15.4). Facial diplegia, jaw weakness, respiratory failure, and feeding difficulty in neonates are attributable to the hypotonia resulting from immature skeletal muscles. Many affected infants die soon after birth from respiratory problems; even now, many are not diagnosed correctly during life. The duration of assisted ventilation during the neonatal period is a significant prognostic factor for subsequent morbidity and developmental delay (Campbell et al., 2004).

If the infant survives the neonatal hypotonia, intellectual impairment becomes apparent (Modoni et al., 2004). Infants and young children with CDM1 do not show clinically evident myotonia, although EMG may

Table 15.8

Main clinical features of congenital myotonic dystrophy type 1 (from Ashizawa and Harper, 2006)

Bilateral facial weakness
Hypotonia
Delayed motor development
Mental retardation
Hydrocephalus
Neonatal respiratory distress
Feeding difficulties
Talipes and arthrogryposis
Hydramnios in later pregnancy
Reduced fetal movements

The congenital form has not been reported in DM2.

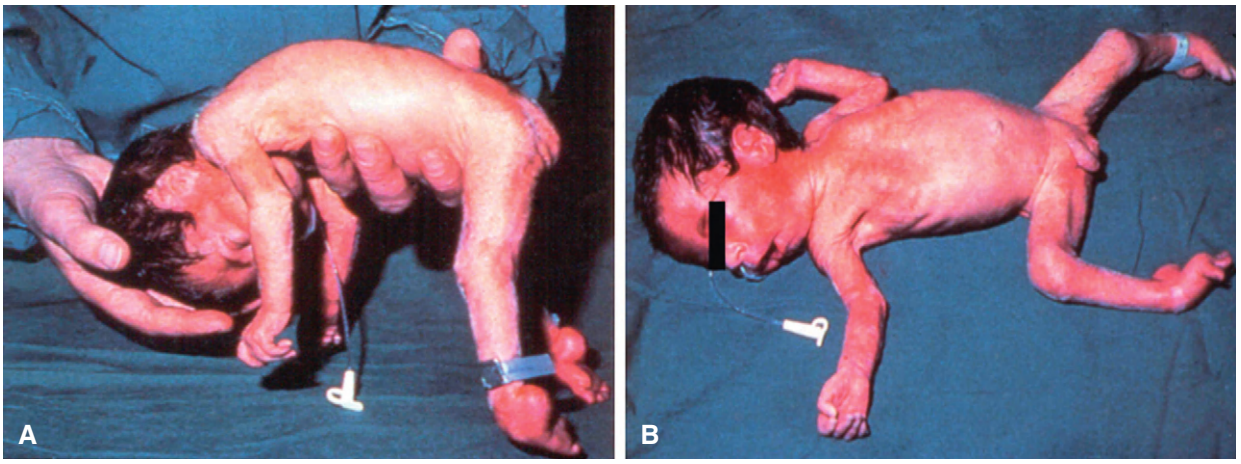

Figure 15.4. Congenital myotonic dystrophy. (**A,B**) Floppy infant syndrome with generalized hypotonia.

detect some myotonic discharges. The facial weakness and characteristic "tented mouth" should permit instant suspicion of CDM1 (Figure 15.5). These facial features become even more pronounced in later age, causing speech and eating problems, as well as difficulties in dental care (Engvall et al., 2007; Sjogreen et al., 2007). Although their developmental milestones may be delayed initially, these patients do remarkably well in the first decade of life, with rare childhood fatality and almost universal acquisition of independent gait (Harper, 1975). By the second decade of life, clinically evident myotonia and other features of adult-onset DM1 emerge. The progression of muscle weakness and atrophy may be faster in patients with CDM1. The muscle weakness and accompanying kyphoscoliosis, joint contractures, talipes, and other deformities in these children often cause ambulatory problems, which occur earlier than gait difficulties in patients with onset in adulthood.

Figure 15.5. Anticipation and congenital myotonic dystrophy. A child with CDM1 with his mother (right) and maternal grandmother (left). Note that the child shows characteristic facial weakness with a tented upper lip, whereas the mother has bilateral facial weakness with ptosis. Her temporalis muscle atrophy is hidden by her hair. The grandmother has no detectable facial features of DM1. (Reproduced with permission from P.S. Harper, Myotonic Dystrophy, 3rd edition, WB Saunders, London, 2001.)

Although the severity of intellectual impairment varies from patient to patient, it creates the greatest problem in daily living for patients with CDM1. Brain MRI typically shows ventriculomegaly and moderate-to-severe hyperintensity of the posterior white matter at all ages (Kuo et al., 2005). The long-term outlook for patients with CDM1 is poor for living an independent life, and physical deterioration and complications usually significantly shorten their lifespan. Some patients may have few neonatal symptoms but present in later childhood with variable degrees of intellectual impairment and speech problems.

Childhood-onset DM1

Patients with early childhood-onset DM1 are normal at birth but often exhibit intellectual impairment, speech problems, and other features seen in patients with CDM1. Thus, the clinical boundary between CDM1 and early-onset DM1 may not always be distinct, and childhood-onset DM1 is in many ways intermediate between the congenital and adult-onset types (De Die-Smulders, 2000; Echenne et al., 2008). Many children with early-onset DM1 have facial weakness with dysarthria, feeding difficulties, drooling, and dental care problems (Engvall et al., 2007; Sjogreen et al., 2007). The cognitive impairment in childhood DM1 may be linked to deficits in attention and executive function (Angeard et al., 2007). In this group of patients, the impact of cognitive and behavioral problems may be as prominent as those seen in patients with CDM1, most notably with attention-deficit/hyperactivity disorder and

anxiety disorder (Goossens et al., 2000), and with a high frequency of an autism spectrum disorder (Ekstrom et al., 2008). Some patients with CDM1 and childhood-onset DM1 show progressive cognitive impairment, raising the possibility of an early degenerative process (Echenne et al., 2008). Patients with an early onset also frequently complain of daytime sleepiness, and polysomnographic studies may show abnormal respiratory events and/or periodic limb movements (Quera Salva et al., 2006). Children with DM1 generally show significant muscle weakness, primarily in distal muscles; however, patients with childhood-onset DM1 were stronger than those with CDM1 (Kroksmark et al., 2005).

Clinical manifestations of DM2/PROMM

Ricker and colleagues in Germany (Ricker et al., 1995) and Thornton and coworkers in the USA (Thornton et al., 1994a) recognized a group of patients with a DM1-like autosomal dominant myotonic myopathy that does not show the CTG repeat expansion. Ricker and colleagues (1995) named this new disorder proximal myotonic myopathy (PROMM) because of conspicuous weakness and atrophy of proximal muscles. It soon became clear that the condition occurred in most Indo-European populations, although it seems to be especially prominent in Germany (Phillips et al., 1998).

A few years later a DM1-like disease with no CTG repeat expansion in Minnesotan families was designated myotonic dystrophy type 2 (DM2), and the *DM2* locus was mapped to chromosome 3q (Ranum et al., 1998). Soon afterwards, this locus was confirmed also to be the locus for PROMM in the German families (Ricker et al., 1999). These families were studied by German and American investigators, who concluded that DM2 was the same disease as PROMM. Subsequently, an expansion of a CCTG repeat in intron 1 of the *ZNF9* gene was identified as the genetic mutation in DM2/PROMM (Liquori et al., 2001). The discovery of the *DM2* mutation and the development of direct DNA testing led to the identification of additional families, including one from Japan (Saito et al., 2008).

Recently, a locus for a new autosomal dominant family with multisystemic myotonic myopathy with frontotemporal dementia was mapped to 15q21–q24 (Le Ber et al., 2004). Although this locus was proposed to be designated *DM3*, the disease mutation was later located in the valosin-containing protein (*VCP*) gene, whose mutations cause IBMPFD (OMIM #167320), and the *DM3* designation was withdrawn. Consequently, the *DM3* locus currently remains unassigned (Udd et al., 2006). There is a handful of other families with multisystemic myotonic myopathies without *DM1* or *DM2* mutations, hinting at the presence of DM3 and beyond (Udd et al., 2003).

In general, DM2/PROMM is milder and more slowly progressive than DM1, with predominant weakness and wasting in proximal muscles, together with weakness of deep finger flexor muscles (see Table 15.2). Facial and bulbar muscles are seldom involved. The presence of severe muscle and joint pain and calf muscle hypertrophy may differentiate DM2/PROMM from DM1 (George et al., 2004). However, pain of nonspecific nature is also not uncommon in patients with DM1, and low back and leg pain have also been reported in over 60% of patients with DM1 (Jensen et al., 2008). Some patients with the *DM2* mutation may present with subtle and nonspecific clinical signs, such as myalgia and fatigue. As for muscle histopathology, which shows findings similar to those seen in DM1, type 2 fiber atrophy, "denervation-like" changes, and the absence of sarcoplasmic masses are findings that distinguish DM2/PROMM from DM1 (see Table 15.2) (Udd et al., 2003; Schoser et al., 2004c). Serum creatine kinase and γ-glutamate transferase levels frequently show a mild increase (Day et al., 2003; Udd et al., 2003), and increased levels of serum creatine kinase may be the only manifestation in some patients with DM2 (Merlini et al., 2005). MRI of muscles shows abnormalities in only one-third of patients with DM2, in contrast to 100% in those with DM1, and the erector spinae and gluteus maximus muscles may be most vulnerable to degeneration in DM2, whereas the medial heads of gastrocnemius and quadriceps (sparing the rectus femoris) may be primarily involved in DM1 (Kornblum et al., 2006). Myotonia is inconspicuous or difficult to detect both clinically and electrophysiologically. Myotonia in DM2 muscles usually shows a waning pattern, in contrast to the waxing-and-waning myotonia in DM1 muscles (Logigian et al., 2007).

As in DM1, progressive frontal executive dysfunction, focal white matter changes on brain MRI, and tau pathology have been documented in DM2 (Gaul et al., 2006; Meola and Sansone, 2007; Sansone et al., 2007). However, frontal executive dysfunction and the social and cognitive disability are typically milder in patients with DM2 than in those with DM1 (Meola et al., 2003; Meola and Sansone, 2007). Brain ^{1}H magnetic resonance spectroscopic features also differ between DM1 and DM2 (Vielhaber et al., 2006). Hypersomnolence and intellectual impairment are not prominent findings in DM2 (Udd et al., 2003). In DM2, there have been limited reports of consistent manifestations of the gastrointestinal tract in the literature (see Table 15.5). Arrhythmias are frequently found in DM2, but cardiac involvement is generally

milder and atrioventricular blocks are infrequent. However, sudden cardiac deaths with abnormal cardiac histology have been reported (see Table 15.3) (Udd et al., 2003; Schoser et al., 2004b). Cataracts in DM2 are similar to those found in DM1 (see Table 15.4). In DM2, endocrine abnormalities, such as testicular dysfunction and insulin resistance, are frequently reported, but they are milder than those found in DM1 (see Table 15.7). In women, the muscle symptoms may become more noticeable during pregnancy, and the frequencies of miscarriage and preterm labor and preterm delivery are increased, although stillbirth and neonatal deaths have not been observed (Rudnik-Schoneborn et al., 2006). Deafness and hyperhidrosis may also be underreported clinical abnormalities in DM2 (Phillips et al., 1998; Day et al., 1999).

As DM1, DM2/PROMM is an autosomal dominant disorder. However, anticipation in DM2 seems less marked than in DM1, and no congenital or severe childhood cases have been found (Schneider et al., 2000; Day et al., 2003), even in rare patients with homozygous CCTG repeat expansions (Schoser et al., 2004a). Also in contrast to DM1, there are no detectable effects of the gender of the transmitting parent on the phenotype of offspring (Day et al., 1999; Udd et al., 2003).

DIAGNOSIS AND GENETIC COUNSELING OF MYOTONIC DYSTROPHY TYPES 1 AND 2

Clinical diagnosis

Making the clinical diagnosis of DM1 is not difficult when the patient displays the typical features of adult-onset disease. The hatchet face with frontal balding is often sufficient to raise a strong suspicion for the diagnosis, which can be quickly ascertained by detection of myotonia when the physician shakes hands with the patient. Subsequent confirmation of the systemic nature (mostly iridescent cataracts) of the disease establishes the clinical diagnosis.

The differential diagnosis of DM1 and DM2 includes other myotonic myopathies (Tables 15.9 and 15.10). Documentation of systemic manifestations of DM1, especially the characteristic iridescent lens opacities, is helpful in differentiating DM1 and DM2 from other myotonic myopathies. The distal muscle weakness and atrophy distinguish DM1 from most myopathies whose weakness is in proximal muscles. The "warm-up" phenomenon of myotonia in DM1 and DM2 (see section on Skeletal muscle manifestations) distinguishes myotonic dystrophy from paramyotonia of sodium channelopathies caused by *SCN4A* gene mutation. In contrast to

Table 15.9

Inherited myotonic disorders (from Ashizawa and Harper, 2006)

	Inheritance	OMIM #	Mutation/Gene
Myotonic dystrophy type 1 (DM1)	AD	160900	$(CTG)_n$ expansion in *DMPK* (chromosome 19)
Myotonic dystrophy type 2 (DM2/PROMM)	AD	602668	$(CCTG)_n$ expansion in *ZNF9* (chromosome 3)
Myotonia congenita			
Thomsen's disease	AD	160800	Muscle chloride channel (*CLCN1*) (chromosome 7)
Becker's disease	AR	255700	Muscle chloride channel (*CLCN1*) (chromosome 7)
Paramyotonia			
Paramyotonia congenita	AD	168300	Muscle sodium channel (*SCN4A*) (chromosome 17)
Hyperkalemic periodic paralysis	AD	170500	Muscle sodium channel (*SCN4A*) (chromosome 17)
Potassium-aggravated myotonia	AD	608390	Muscle sodium channel (*SCN4A*) (chromosome 17)
Chondrodystrophic myotonia (Schwartz–Jampel syndrome)	AR	255800	Perlecan (*HSPG2*) (chromosome 1)
Acid maltase deficiency	AR	232300	Acid α1,4-glucosidase (GAA) (chromosome 17)

AD, autosomal dominant; AR, autosomal recessive; OMIM, Online Mendelian Inheritance in Man; PROMM, proximal myotonic myopathy.

Table 15.10

Clinical features of myotonia congenita and myotonic dystrophies in adults (from Ashizawa and Harper, 2006)

	Myotonia congenita	Myotonic dystrophies (DM1 & DM2)
Age at onset of myotonia	Infancy or early childhood	Late childhood to adult life
Severity of myotonia	Often severe; generalized	Usually moderate or slight
Muscle weakness	Slight; nonprogressive	Very variable – may be severe; progressive
Cardiac muscle involvement	Absent	Common
Cataract	Absent	Diagnostic
Other systemic abnormalities	Absent	Widespread
Inheritance	Autosomal dominant or recessive; sporadic cases frequent	Autosomal dominant; anticipation

the "warm-up" phenomenon, paramyotonia exhibits "paradoxical" worsening of myotonia with muscle contractions – hence the name paramyotonia. However, it should be noted that the "warm-up" phenomenon is also seen in chloride (*CLCN1*) channelopathies, i.e., autosomal dominant and recessive myotonia congenita. Unlike paramyotonia, myotonia in DM1 and DM2 usually does not show worsening upon cooling of the muscle, and this provides another diagnostic clue.

The iridescent multicolored lens opacities are useful for diagnosing DM1 and DM2 because of the high specificity. Although mature opaque cataracts are nonspecific, up to 1.5% of the general population with idiopathic cataracts, including those with onset at age 40 years or above, may have small *DM1* mutations ("protomutations") (Cobo et al., 1996; Medica et al., 2007).

The differential diagnosis of CDM1 should be from other causes of infantile hypotonia, such as congenital myopathies, neonatal myasthenia gravis, spinal muscular atrophies, Pompe's disease, and various CNS disorders, including cerebral palsy. The X-linked neonatal myotubular myopathy may closely mimic CDM1, although muscle histology and molecular diagnosis provide distinctions. Young children with CDM1 or early-onset DM1 usually do not show myotonia; thus, a young child with overt myotonia is likely to have one of the nondystrophic myotonic disorders. Examining the mother for signs of DM1 is often a key to making the diagnosis of CDM1.

In DM2, differential diagnoses must be broadened because patients with DM2 often exhibit only subtle signs of myopathy or myotonia. In one series, 3% of patients with the diagnosis of fibromyalgia were found to have the *DM2* mutation (Suominen et al., 2007). Thus, the diagnosis of DM2 should be considered in patients with nonspecific neuromuscular diseases, especially when there is a family history of DM2.

EMG, nerve conduction studies, serum creatine kinase level, and muscle biopsy used to be essential for establishing the diagnosis of DM1. However, after the discovery of *DM1* and *DM2* mutations, DNA testing has become the single decisive diagnostic test. EMG for detection of electrical myotonia and myopathic units, and slit-lamp biomicroscopy for lens opacities, are still useful for determining the necessity of DNA testing in clinically equivocal cases. Other diagnostic tests may be useful for assessment of multisystemic manifestations; these include ophthalmological, neurological, cardiological, endocrinological, gastroenterological, and immunological workups as described above.

Molecular diagnosis

Molecular genetic analysis offers unsurpassed accuracy for the diagnosis of DM1. Polymerase chain reaction (PCR) and Southern blot analyses are primary methods for detecting *DM1* and *DM2* mutations, and repeat-primed PCR supplements these assays (Warner et al., 1996). In DM2, conventional PCR/Southern blot protocols detect the *DM2* mutation in only 80% of subjects with known expansions. In the remaining 20% of patients with DM2, the expanded allele may have extensive-size mosaicism. These mosaic alleles are sometimes too large to be amplified by the PCR assay and too diffuse to be detected by Southern blot analysis. Thus, performing the repeat-primed PCR-based assay is necessary to assure greater than 99% detection of expanded CCTG repeat (Day et al., 2003; Udd et al., 2003; Bonifazi et al., 2004). Detection of the *DM2* mutation by *in-situ* hybridization-based analyses has also been investigated, but the usefulness of the assay has not been documented (Sallinen et al., 2004; Bonifazi et al., 2006).

Genetic counseling

Before undertaking molecular diagnosis, it is important to clarify the purpose of the testing, which divides the molecular diagnosis in three categories: (1) predictive testing, (2) prenatal testing, and (3) diagnostic testing.

The International Myotonic Dystrophy Consortium (2000) has established guidelines for genetic testing of DM1 with these purposes. However, the guidelines should be subjected to changes as new insight is gained into the natural history of the disease and new therapeutics are developed that can alter the disease course.

Predictive DNA testing

DNA testing generally provides an accurate diagnosis, but there are some pitfalls. Full genetic counseling is particularly important in predictive (presymptomatic) testing (American College of Medical Genetics/American Society of Human Genetics, 1998; Fokstuen et al., 2001). The diagnosis must be established molecularly in one or more affected relatives of the individual who wishes to undergo predictive testing. If molecular analysis in affected family members does not show the mutation, an alternative diagnosis is likely. However, if no affected family members are available, but the clinical diagnosis in the family is secure, molecular testing of DM1 and DM2 is still highly likely to give a definitive answer. If the affected family member has an atypical or equivocal clinical diagnosis, the molecular diagnosis should be attempted first on the relative before the predictive molecular diagnosis is attempted.

In a young child at risk for DM1, clinical assessment remains the most helpful procedure; genetic analysis in such a normal child raises many issues that may best be resolved when the child reaches adulthood. There is no evidence that clinically normal mutation carriers are at any risk of surgical or other procedures. However, recognition of cardiac risks for sudden death in asymptomatic at-risk children has raised the question of whether predictive testing is justifiable before the legal adult age (Bassez et al., 2004). Unfortunately, however, the risk factors for cardiac death in asymptomatic children who carry the *DM1* mutation remain unknown. If such an at-risk child develops cardiac symptoms/signs, or syncope/near syncope, DNA testing is justifiable even in the absence of skeletal muscle or lens abnormalities.

A normal result on the molecular testing in an asymptomatic subject at risk can exclude the individual's risk of developing DM1 in the future and of transmitting the disorder (Brunner et al., 1992b). However, the uncertainty in the prognosis needs to be addressed in individuals who test positive. In DM1, statistically significant correlations have been detected between the genotype and various phenotypic variables, including age of onset. However, the genotype–phenotype correlations show relatively low correlation coefficients. There appear to be two age-dependent factors that contribute to the relatively weak correlations: one is imprecision of currently available phenotypic data, which are obtained largely retrospectively, and the other is instability of the expanded allele size in somatic tissues. The phenotypic expression of the mutation is further altered by other genetic and environmental modifiers. Thus, prediction of disease phenotype and prognosis by the expansion allele size is generally discouraged in genetic counseling. However, some degree of phenotypic predictions may be feasible with small expansion alleles. The "protomutation" expansion (55 to 100 copies) usually implies lack of significant neuromuscular disease (Gennarelli et al., 1996).

The psychosocial impact of presymptomatic DM1 testing may be minor, although life is perceived as a change for the better by noncarriers and as a change for the worse by carriers (Prevost et al., 2004). However, some subjects may go into depression and their psychological wellbeing should be monitored carefully with appropriate counseling. Social, financial, ethical, and legal ramifications of obtaining the DNA test results should also be addressed in the pretest counseling, although discrimination of patients based on genetic testing is prohibited by the Genetic Information Nondiscrimination Act. Presymptomatic testing is now feasible, but no cases have been reported in DM2.

Prenatal DNA diagnosis

Family studies have shown an increased probability for CDM1 in subsequent siblings once a severely affected child has been born (Koch et al., 1991). Thus, prenatal diagnosis by direct mutational analysis is particularly useful in DM1 families in such a situation. The size of the expanded allele should be determined by Southern blot analysis. It is generally unwise to estimate the prognosis from the size of the expansion in DM1. As repeat instability has an inherent expansion bias, it appears reasonable to assume that the fetus has an increased risk for CDM1 if the expanded allele size exceeds 1000 repeats. Unfortunately, the variable somatic instability of the repeat in different tissues during fetal development, and the imprecise correlation between the repeat size and the CDM1 phenotype, complicate the prediction of CDM1. Further data of repeat-size-based prenatal prediction of CDM1 will be useful to establish practical guidelines.

Where an affected male is the parent, the expanded repeat of the fetus may show reduction of the size into the minimal, or rarely even into the normal, range. Although the risk for delivering a CDM1 neonate is very small with paternal transmission of the expanded allele, precise prediction of the age of onset and severity is an unattainable task. These limitations should be

made clear in genetic counseling beforehand. Furthermore, it should be noted that prenatal testing should not be performed if termination of the pregnancy is not considered as an option, because the procedure to obtain fetal cells, such as amniocentesis and chorionic villus biopsy, impose risks to both the mother and the fetus. It should also be noted that if the pregnancy were allowed to continue after a prenatal diagnosis of DM1 and produced a clinically unaffected child, this would create the same concerns as predictive testing for asymptomatic minors.

In DM2, no cases of prenatal DNA diagnosis have been reported, although it is technically feasible. This is probably because DM2 is generally a milder disease than DM1, with no severe congenital or early childhood forms.

Preimplantation genetic diagnosis (PGD) is increasingly available at multiple centers for a range of genetic disorders including DM1 (Sermon et al., 2001; Kakourou et al., 2008). However, it has not been possible for DM2. Linkage marker *ApoC2* should be used in PGD with a caution that a crossover between *DMPK* and *ApoC2* could lead to a misdiagnosis (Kakourou et al., 2007).

Diagnostic DNA testing

The accuracy of direct mutational analysis makes it extremely useful in confirmation of the clinical diagnosis of DM1 or DM2, and in excluding DM1 and DM2 as a differential diagnosis in patients who have similar clinical manifestations. The presence of the expansion mutation in the diagnostic DNA testing can lead decisively to the diagnosis of DM1 or DM2, not only in the relatives of a known patient with DM1 but also in individuals with no known family history. Newborns with CDM1 present at birth as a "floppy infant" and confirm about 4% of cases of neonatal hypotonia (Laugel et al., 2008). Diagnosing CDM1 in neonates with floppy infant syndrome is often challenging in relation to other neuromuscular disorders (Weiss et al., 2007). The diagnostic DNA testing of DM1 is particularly useful for such cases.

GENETICS OF MYOTONIC DYSTROPHY TYPES 1 AND 2

Genetic aspects of DM1

Early studies established autosomal dominant inheritance for DM1 and already recognized the remarkable variability in phenotype and age at onset. Anticipation and congenital DM1 were mysteries in human genetics, and some aspects of these genetic phenomena are still elusive even after the discovery of the CTG repeat expansion. Furthermore, the genetics of DM1 is distinct in many aspects from DM2, despite both diseases being caused by repeat expansion mutations. Several excellent reviews on these issues are available (Harper and Johnson, 2001; Monckton and Ashizawa, 2004).

Identification of the *DM1* mutation

Identification of the *DM1* gene was one of the early successes of positional cloning approach, carried out over a period of almost a decade. After genetic linkage to the secretor and Lutheran blood group in 1971, and assignment to chromosome 19 in 1982, the gene locus was restricted to 19q, which underwent progressive physical mapping and cloning (reviewed in Harper and Johnson, 2001). The DNA expansion mutation was identified at the end of 1991 (Buxton et al., 1992), and rapidly confirmed (Aslanidis et al., 1992; Harley et al., 1992), soon after the discovery of expansion mutations of trinucleotide repeats in Kennedy's disease fragile X syndrome. The identity of the DNA expansion mutation was determined as the CTG repeat expansion in the 3′ UTR of the *DMPK* gene (Brook et al., 1992; Fu et al., 1992; Mahadevan et al., 1992), which is now known as the *DM1* mutation. Normal alleles contain 5–35 CTG repeats, whereas *DM1* alleles show expansions reaching several thousand CTGs.

Anticipation and DM1

Anticipation is a genetic term that denotes progressively earlier onset of the disease with an increasingly severe phenotype in successive generations. Anticipation was first recognized in 1918 by Fleischer, who found that patients with DM1 with muscle disease frequently had family members in earlier generations showing cataract as the only clinical manifestation. However, inconsistency of this phenomenon with mendelian genetics led Penrose (1948) to dispute anticipation as a result of the inherent ascertainment biases. In 1989, Howeler and colleagues published data indicating that the biases postulated by Penrose were inadequate to explain anticipation (Howeler et al., 1989). A similar anticipation, which is now known as the "Sherman paradox," in fragile X mental retardation had also been recognized (Sherman et al., 1985). Anticipation is now recognized as a hallmark of disorders caused by expansions of unstable trinucleotide repeats. However, although it has become a fashionable concept, most reports of apparent anticipation in a wide range of other disorders are likely to reflect the biases documented by Penrose many years ago, although currently unknown genetic or epigenetic explanations could emerge in some of these diseases in the future (Ashizawa and Conneally, 1999).

Genotype–phenotype correlation in DM1

Correlation between the CTG repeat expansion size and age of onset was recognized soon after identification of the *DM1* mutation (reviewed in (Harper and Johnson, 2001; Nagamitsu, 2002; Monckton and Ashizawa, 2004). The severity of the DM1 phenotype has also been shown to correlate with the repeat expansion size. The repeat expansion in peripheral blood is also correlated with each of the organ-specific phenotypes (Kinoshita et al., 1996) and more general socioeconomic status of the patient (Laberge et al., 2007). Although these correlations are, at best, modest, careful application of the correlation based on a large data set may be clinically useful (Salehi et al., 2007). The extremes of the distribution for age and severity are of particular interest. Amongst the most severe cases of CDM1, remarkably large expansions are usually detected, sometimes exceeding 2000 repeats (Geifman-Holtzman and Fay, 1998). In contrast, minimal cases of older individuals with cataract alone show consistently small expansions; repeats of less than 100 are associated only with cataracts, with no significant neuromuscular disease (Gennarelli et al., 1996; Arsenault et al., 2006). A study from Spain showed that no individual with fewer than 55 repeats had any clinical abnormality, but that there was significant meiotic instability in the range of 30–55 repeats (i.e., premutation alleles), especially when transmitted by males (Martorell et al., 2001). This is consistent with observations in families with *de novo* mutations, in which the earliest generation of family has an asymptomatic male carrying a premutation allele, which was passed on as a larger allele to affected members in the subsequent generations.

Intergenerational changes

The genetic instability of the *DM1* mutation is likely to be the major factor underlying the observed anticipation (reviewed in Harper and Johnson, 2001; Nagamitsu and Ashizawa, 2002; Monckton and Ashizawa, 2004). There is a modest inverse correlation between the repeat expansion size and age of onset, and the repeat tends to increase in successive generations. However, a small proportion of parent–child pairs show no anticipation, and in some cases later onset in the offspring. Furthermore, smaller repeats have been detected in the offspring in about 6% of parent–child pairs pooled from centers worldwide (Ashizawa et al., 1994). The intergenerational repeat contraction typically occurs with paternal transmissions, an observation that leads to discussion of the important sex-related differences in transmission of the *DM1* mutation. Unexpectedly, in a small subpopulation of these pairs in which the repeat expansion decreased in size, anticipation was still observed in terms of earlier age at onset. Although selection biases involved in the study of two-generation families may exist in these data, somatic instability of expanded repeats may contribute to such "uncoupling" between repeat size and age of onset.

Parental origin effects

DM1 behaves as a classical autosomal dominant trait in respect of equal incidence in, and transmission by, each sex. However, important influences of sex have been noted in terms of the phenotypic expression of the disorder. The most striking example is CDM1, which is seen almost exclusively in children born to an affected mother, although a handful of cases of CDM1 have been reported with paternally transmitted mutations (Zeesman et al., 2002). At the molecular level, many maternal transmissions result in large alleles exceeding 1000 CTGs in offspring, whereas paternal transmissions seldom give alleles larger than 1000 CTGs. A less conspicuous gender effect is the preponderance of males in the earliest affected ancestors, usually showing only minimal disease (Brunner et al., 1993a, b). Similarly, most normal individuals who have produced offspring with *de novo DM1* mutations in their children are males. Repeat expansions with 55–100 CTGs cause minimal disease, which often shows cataracts only, whereas premutation alleles (30–55 CTGs) do not express the disease phenotype (Martorell et al., 2001). Small expansion alleles in these ranges are unstable, with a propensity to expand into larger alleles in the disease-causing range in subsequent generations when transmitted paternally (Martorell et al., 2001). These alleles are also present in females, although they are underrepresented because the alleles are relatively stable in maternal transmissions. These data suggest that the gender-dependent instability of the expanded repeat with a bias toward further expansion can explain the gender-specific phenotypic differences (see Monckton and Ashizawa, 2004).

It is consistently observed that the degree of repeat instability and expansion in sperm is considerably greater than that found in blood (Monckton et al., 1995; Martorell et al., 2004). Studies of DNA samples from patients with DM1 using small-pool polymerase chain reaction (SP-PCR) suggested that sperm from DM1 males show high levels of repeat-length variation with bias toward further expansion. The largest length changes in these sperm samples were observed for premutation and protomutation alleles, whereas the highest frequency of contractions was found in full mutation alleles (Martorell et al., 2004). Embryos and gametes obtained during PGD and chorionic villus and cultured amniotic fluid samples for prenatal

diagnosis have shown that there were significant increases in the number of repeats in embryos from female patients with DM1 and in their immature and mature oocytes, whereas spermatozoa and embryos from male patients showed smaller increases (De Temmerman et al., 2004; Martorell et al., 2007). In oocytes, enlargement of the repeat had occurred at the germinal vesicle stage, that is, either during premeiotic proliferation of oogonia or during prophase I of meiosis I (De Temmerman et al., 2004). This is consistent with DNA repair-based expansion of the repeat in these cells that have undergone a long period of quiescence, as proposed by Pearson (2003). Interestingly, the repeat expansion size in sperm from two male patients with DM1 showed no changes in 4 years. In a study of siblings with DM1, the birth order, intergenesic interval, oldest sib's CTG repeat, parental age, and parental CTG repeat size did not significantly influence siblings' genotype or phenotype, suggesting that effects of the time- and gender-dependent expansion in germline cells may be concealed by the variability of other genetic and environmental factors (Brisson et al., 2002).

Congenital DM1

The molecular mechanism of CDM1 is not fully understood. However, a combination of diminished fertility of males with adult-onset DM1 and possible selection against sperm carrying very large expansion alleles could explain the maternal transmission. Although paternal transmission of a congenital case is extremely rare, several documented instances of paternally transmitted CDM1 suggest that it can occur with paternal transmission of the mutation if the expansion size is large enough in the offspring (Zeesman et al., 2002). CDM1 cases typically arise from mothers who either have, or will later develop, typical adult-onset DM1, and only very rarely from those with cataract alone (Koch et al., 1991). Although mothers with relatively small alleles can give birth to children with CDM (Redman et al., 1993), the size of the parental repeat is generally an important determinant in CDM1 cases, as well as that of the patient alone (reviewed by Harper, 2001).

The size of parental expansion may explain the origin; however, it remains unknown why very large repeats lead to the CDM1 phenotype, which differs completely from the phenotype of adult-onset DM1. An alteration of chromatin structure, methylation of the regional DNA (Steinbach et al., 1998), inhibition of binding of a zinc-finger protein, CTCF (CCCTC-binding factor) in this region (Filippova et al., 2001), and increased expression of the *DMPK* gene have been found in CDM1 at the molecular level and will be discussed later in this chapter. The effects of genes interacting with the *DM1* gene may also be relevant. However, an intrauterine maternal effect, proposed many years ago, is still not entirely obsolete in this respect.

Studies of the skeletal muscle and myoblasts from subjects with congenital DM1 have shown defective muscle differentiation during development (Filippova et al., 2001; Amack and Mahadevan, 2004). However, it remains unknown how the very large CTG repeat causes such muscle immaturity. It is noteworthy that overexpression of the 3′ UTR of the *DMPK* gene and of *CUGBP1* (CUG-binding protein 1, which has been shown to be increased in DM1 cells) causes defective muscle differentiation in cultured cells and mice (Amack et al., 2002; O'Cochlain et al., 2004; Storbeck et al., 2004). These data will also be discussed later in this chapter.

Homozygosity for the *DM1* gene

Patients with DM1 who are homozygous for the CTG repeat expansion could provide evidence that DM1 is absolutely a dominantly inherited disease, in which heterozygotes and homozygotes should express the same phenotype. In DM1, however, the issue is complicated by the variability of disease severity, which is dependent primarily on the repeat expansion size. Several cases of DM1 homozygotes have been reported in the literature. Seven homozygotes for the minimal expansion mutation appeared to be healthy (Martorell et al., 1996). In contrast, two sibs with 43/180 and 43/500 CTGs exhibited an adult-onset phenotype (Abbruzzese et al., 2002). Two homozygous patients, who had two fully expanded alleles (330/770 CTGs in one and 200/1200 CTGs in the other) resulting from incestuous mating, showed the congenital phenotype. Although the first patient showed dysmorphic features, these may be due to other recessive genes that were brought to homozygosity by the consanguinity. Thus, it is still not clear whether homozygotes generally differ from heterozygotes, as has been shown for Huntington's disease (Huntington's Disease Collaborative Research Group, 1993).

Reversal of the *DM1* mutation

Intergenerational reduction of expanded *DM1* repeats has been discussed earlier in this chapter. Reversal of the *DM1* mutation to the normal range has also been reported in several individuals (Brunner et al., 1993a, b; O'Hoy et al., 1993), who were detected based on discrepancy between a haplotype analysis suggesting transmission of the *DM1* gene and a normal mutational analysis result. All cases have been of paternal origin. These cases are clinically important in risk prediction for such individuals and their families in

genetic counseling. However, it is unclear whether the reverted normal repeat is more unstable than alleles in the normal population. It must be stressed that these cases differ from true nonpenetrance of the expanded allele.

Somatic instability and mosaicism

Intergenerational changes of the expanded CTG repeat size are based on analysis of the blood leukocyte allele. Thus, in addition to germline instability in the parent, somatic instability in the parent and offspring may contribute to changes in the expansion size shown with transmission. At the time of discovery of the *DM1* mutation, expanded alleles were noted to have a "smear" or diffuse band on Southern blot analysis (Buxton et al., 1992; Harley et al., 1992). The smear is a result of heterogeneity of the size of DNA restriction fragments containing the expanded repeat. Subsequent SP-PCR analyses of expanded *DM1* alleles confirmed that the repeat size is heterogeneous in soma (Monckton et al., 1995; Wong et al., 1995).

The expanded *DM1* CTG repeat is not only unstable within blood leukocytes but also between different tissues. Early autopsy studies showed such variation (Jansen et al., 1994), although analysis of muscle biopsy samples indicated that the expanded repeat was substantially larger in muscle than in blood (Ashizawa et al., 1993; Thornton et al., 1994b; Monckton and Ashizawa, 2004). Despite such variations, however, the repeat size in blood generally shows a closer correlation with severity than does that in muscle.

Another important observation is that the extent of somatic instability of expanded CTG repeats depends on age and expansion size. From analysis of multiple DNA samples obtained from the same patients over time, it became clear that expanded alleles show an age-dependent increase in the mean repeat size and repeat-size heterogeneity in the samples obtained at a later timepoint (Wong et al., 1995; Martorell et al., 1998). Consistent with this notion is that large expansions in chorionic villus prenatal samples show relatively little repeat-size heterogeneity (Myring et al., 1992). Thus, those showing most spread in expansion are generally older and severe cases. However, DNA samples from minimally affected older patients generally show little repeat-size heterogeneity, suggesting that time is not the only determinant of the repeat instability, but that the size of the expansion also plays an important role. The degree of instability generally correlates with the repeat size. SP-PCR studies have provided further insights into the process involved in the somatic instability. Expanded alleles from young patients with DM1 typically show an allele-size distribution consisting of a peak with a sharp lower boundary and a skew toward expansion. As the patient ages, the peak shifts toward right (larger repeat size) with gradual flattening of the peak. Based on this model, Morales and colleagues in Glasgow proposed a formula to calculate the size of the expanded allele that was inherited from the affected parent using the age and distribution of the expanded alleles on SP-PCR (personal communication). The predicted inherited allele size showed a substantially tighter correlation with age of onset.

Any true mosaicism would be expected to result from early embryonic rather than germline variation. Monozygotic twins give some indication of the likely extent and role of such developmental differences; affected DM1 twin pairs, including a pair with CDM1 (Garcia de Andoin et al., 2005), have generally shown close similarity of both clinical features and repeat size.

Genetics of DM2 (PROMM)

As already discussed, the clinical phenotype of DM2/PROMM closely resembles that of DM1, although patients with DM2/PROMM typically exhibit milder symptoms and signs. It should also be noted that the *DM2* mutation is similar to that of the *DM1* mutation; it is a large expansion of an unstable CCTG repeat, which contains transcribed but untranslated CUGs. Like the *DM1* CTG repeat, the *DM2* CCTG repeat shows a copy number polymorphism in the general population ranging from approximately 10 to 30 repeats. In patients, however, the CCTG repeat is larger, in the range from 75 to more than 11 000 repeats, with an average of around 5000 (Liquori et al., 2001; Day et al., 2003). DM2 also shares a major part of the pathogenic mechanism with DM1 (i.e., *trans*-dominant RNA gain-of-function; see below).

Despite these similarities in the mutation and the pathogenic mechanism, clinical genetics and molecular genetics of DM2 have shown a few important distinctions from those of DM1. First, anticipation has been noted (Schneider et al., 2000; Day et al., 2003), but is not striking. Second, a severe congenital form of DM2 has never been reported. Third, no obvious parental origin effects have been identified. Fourth, although the *DM2* CCTG repeat shows a considerable intergenerational repeat-size instability, there is a tendency for contraction rather than further expansion in parent–child pairs. Fifth, there is no correlation between the age of onset and the repeat expansion size. Lastly, somatic repeat-size mosaicism appears to be more striking than in DM1, and the expanded CCTG repeat typically undergoes rapid expansions throughout the lifetime of the individual (Liquori et al., 2001; Day

et al., 2003). Consequently, the most consistent correlation with repeat size was the age of the patient at the time of blood sampling. These high levels of instability may have interfered with attempts to demonstrate intergenerational biases, parental origin effects, and the correlation between repeat size and age of onset.

So far, most DM2 families identified are from northern Europe, primarily Germany, Poland, American families with ancestors from these regions (Day et al., 2003), or an Afghan family (Liquori et al., 2003; Schoser et al., 2004a). Thus, the ethnicity of these families may be categorized as Indo-European. One exception has been a single report of a Japanese family with the *DM2* mutation (Saito et al., 2008). The prevalence of the *DM2* mutation in Germany has been estimated to be 1 in 100 000 or greater (Udd et al., 2003), although these numbers may have been grossly underestimated due to difficulty in the diagnosis of DM2, particularly in mild cases with subtle clinical features. Multiple studies of haplotype/linkage disequilibrium analyses suggested that the *DM2* expansion originated from a single or a few founder mutations (Bachinski et al., 2003; Liquori et al., 2003; Saito et al., 2008). It has been estimated that the original *DM2* expansion mutation occurred approximately 200–540 generations ago (Bachinski et al., 2003), perhaps before the Aryan migration of Indo-Europeans (Liquori et al., 2003).

MUTATIONS OF MYOTONIC DYSTROPHIES

Mechanism of instability of the CTG repeat in *DM1*

Mouse models for CTG repeat instability

The molecular mechanism of the CTG repeat instability has been investigated by various *in vitro* and *in vivo* models. Earlier studies in bacteria and yeast introduced basic phenomena involved in mechanisms of instability, such as slippage of two strands containing the triplet repeat sequences, hairpin and other alternative DNA structures formed by the repeat, recombination, single- and double-stranded breaks, proofreading, DNA repair, orientation dependence, repeat length effects, location of the repeat, interruptions of the pure repeats, replication restart, and effects of transcription (Lahue and Slater, 2003; Cleary and Pearson, 2005; Pearson et al., 2005). Unfortunately, in *Escherichia coli*, CTG repeat instability is strongly biased toward deletion. Studies in yeast provided further insights into the mechanism, and showed that the repeat can expand in lower organisms (Wells and Ashizawa, 2006).

However, transgenic mouse models with expanded CTG repeats have proved to be particularly interesting. Among several *DM1* transgenic mouse models, mice with genomic nonexpression of about 160 CTGs (Dmt-D mice) (Monckton et al., 1997; Fortune et al., 2000), mice with a transgene from a human genomic clone containing a 45-kilobase genomic segment of the DM1 region with a 55-CTG repeat (DM55 mice) (Gourdon et al., 1997; Seznec et al., 2000), and knockin mice with the human *DMPK* exons 13–15 with 84 CTGs (van den Broek et al., 2002) have provided interesting data on somatic, germline, and intergenerational instability of expanded CTG repeats. Transgenic mice with up to 1440 CTG repeats, which are interrupted in the TCGA-$(CTG)_{20}$-TCGAG-$(CTG)_{20}$ configuration within the *DMPK* 3′ UTR, have stable repeats (Philips et al., 1998). Other transgenic mouse models of DM1 and DM2 have been generated for studying pathogenic mechanisms, but reports on the repeat instability in these mice are limited.

These transgenic mice recapitulate germline and somatic instability with a strong bias towards expansions (hence the intergenerational expansion) (Gourdon et al., 1997; Monckton et al., 1997; Fortune et al., 2000; Seznec et al., 2000; Zhang et al., 2002). DMT mice, DM55 mice, and knockin mice have shown gradual increases in the size of expanded repeats. Some descendants of DM55 mice have been shown to have the repeat size up to a few hundred CTGs as a result of these gradual modest intergenerational expansions, most of which were smaller than 10 CTG units per generation, although the DM300-328 line has shown intergenerational repeat gains of 60 CTGs (Gomes-Pereira et al., 2006). More recently, large CTG "big jumps" of several hundred repeats, which are often seen in intergenerational changes in human DM1, were recreated in the DM400 line (Gomes-Pereira et al., 2007).

Somatic and germline instability in mouse models

These transgenic animals show that the size of expanded CTG repeats variably increases in different somatic tissues with age, resembling the somatic instability found in humans (Fortune et al., 2000; Seznec et al., 2000). Sperm of Dmt-D mice showed that the CTG repeat in the transgene increases in size with age, suggesting that mutations accumulate with time in spermatogenic stem cells (Zhang et al., 2002). Examinations of germline tissues in $DM300^{+}$ mice showed that strong mosaicisms towards expansions occur in spermatogonia before meiosis with no detectable difference in mosaicism between spermatogonia and spermatozoa, suggesting that expansion does not continue after the meiotic

event (Savouret et al., 2004). Pedigree data showed a significant age-dependent bias toward repeat contraction in female transmissions and a trend towards expansion with age in male transmissions (Zhang et al., 2002).

Intergenerational changes of the repeat expansion size in these transgenic mice are also different between the sex of the transmitting parent. Pedigree analyses of the DM55 and DM300^{+} transgenic mouse lines have shown effects for the parental sex similar to those observed in human DM1 families (Seznec et al., 2000). In DM650^{+} mice, in which "big jump" expansions were observed, very large increments of CTG repeats seem to arise more frequently through male than female transmissions, with an overall frequency of 5% (Gomes-Pereira et al., 2007). Pedigree data for Dmt-D mice showed a significant age-dependent bias toward repeat contraction in female transmissions and a trend towards expansion with age in male transmissions, whereas the sex of the offspring had no detectable effect on the direction of the mutational length change (Zhang et al., 2002). Thus, effects of the sex of the transmitting parent exist in DM1 transgenic mouse models, although they differ in detailed characteristics of repeat-size instability from those seen in human DM1 families.

Mechanisms of CTG repeat instability in mouse models

Another important finding derived from these mouse models is that the repeat-length mutations of CTG repeats may occur without DNA replication. Studies in transgenic mouse models and in cell lines derived from these mice have shown that the repeat expansion rate does not correlate with the division rate (Lia et al., 1998; Fortune et al., 2000; Gomes-Pereira et al., 2001). Furthermore, the brains of adult mice increase the degree of mosaicism despite the fact that the great majority of cells in the brain are neurons and glias, which are postmitotic cells and stop dividing by 4 months of age (Fortune et al., 2000). The stable CTG repeat size in transgenic mice with interrupted expansions (Philips et al., 1998) is consistent with observations that interrupted repeats are generally more stable than pure repeats in various short tandem repeats in a variety of organisms.

Transgenic mouse models have also led to important observations that genes controlling the DNA mismatch repair (such as *Msh2*, *Msh3*, and *Msh6*) play important roles in the mutation mechanism (van den Broek et al., 2002). CTG repeats are known to be able to adopt unusual secondary structures, such as "hairpins," that differ from the usual double helical (B-DNA) structure, and these unusual structures may be targets of mismatch repair (Pearson and Sinden, 1996). Transgenic mice with expanded CTG repeats in the Msh2-deficient background by cross-breeding showed that the Msh2 deficiency shifts the instability towards contractions, both within tissues and through generations (Savouret et al., 2003). Msh3 deficiency has shown to block completely the somatic instability of expanded CTG repeats (van den Broek et al., 2002; Foiry et al., 2006). The Msh6 deficiency resulted in a significant increase in the frequency of somatic expansions, which may be attributable to competition of Msh3 and Msh6 for binding to Msh2 in functional complexes with different DNA mismatch-recognition specificity (van den Broek et al., 2002). Interestingly, pedigree analyses showed that the frequency of expansions decreased in maternal transmissions when Msh6 was absent, and levels of Msh2 and Msh3 proteins in Msh6$^{-/-}$ ovaries were found to be low, suggesting that the absence of Msh6 may have an indirect effect on Msh2 and Msh3 in the ovary (Foiry et al., 2006). Interestingly, flap endonuclease 1 (Fen1), whose orthologs play an important role in the stability of expanded CTG repeats in *in vitro* and yeast model systems (Callahan et al., 2003; Liu et al., 2004; Yang and Freudenreich, 2007), has been shown to have no major effects on stability of the *DM1* CTG repeat in the knockin mice (van den Broek et al., 2006). The process of removing oxidative damage to nucleotide bases is dependent on a single base excision-repair enzyme, 7,8-dihydro-9-oxoguanine-DNA glycosylase (OGG1), and plays a key role in the age-dependent somatic repeat expansion in CAG repeat of Huntington's disease (Kovtun et al., 2007). It is likely that this enzyme is involved in CTG repeat instability at the *DM1* locus.

van den Broek and colleagues (2007) studied their *DM1* knockin mouse model during aging after the onset of terminal differentiation, and found that somatic CTG repeat expansions occur almost uniquely in the fraction of cells with high cell nuclearity and DNA ploidy and are aggravated with aging, suggesting that postreplicative and terminal-differentiation events coupled to changes in cellular DNA content influence trinucleotide expansion. Changes in cell nuclearity and DNA ploidy abnormalities have not been reported in human *DM1* embryos or cells derived from them. Thus, the relevance of these observations to human DM1 remains to be investigated.

Human cells in culture have also provided some interesting findings. In lymphoblastoid cell lines derived from patients with DM1, the expanded CTG repeat alleles showed gradual further expansions over time. Eight of them had additional large repeat-size gains, and the cell population with this "big jump" mutation eventually replaced the progenitor allele population.

This growth advantage was attributable to the faster growth mediated by Erk1/2 activation, which was negatively regulated by p21 (WAF1). This phenomenon, which was designated "mitotic drive," is a mechanism that could contribute to the expansion bias of *DM1* CTG repeat instability *in vivo* (Khajavi et al., 2001).

Origins of the *DM1* mutation

Large normal alleles and premutations

As already discussed, clinically significant cases of DM1 could arise from an asymptomatic parent (usually the father) who has a small expansion of the CTG repeat in the premutation (33–55 CTGs) range. These individuals often provide the links between different DM1 families. Although cataracts are one of the most common early manifestations of DM1, screening of patients with young-onset cataracts in the general population for the CTG repeat expansion showed that 0.9–3.0% of these patients had the *DM1* mutation (Kidd et al., 1995; Cobo et al., 1996). These mildly affected patients could constitute the genetic reservoir for the *DM1* mutation. In a very large Dutch kindred, the increased repeat size, clinical anticipation, and eventual elimination of the gene occurred in all branches within five generations (de Die-Smulders et al., 1994). Premutation alleles identified both in distant relatives of probands and more rarely in unaffected spouses showed the repeat-size instability with a liability to expand in succeeding generations, particularly through paternal transmissions (Martorell et al., 2001). These findings suggest that premutation carriers are at high risk of having affected offspring within a limited number of generations, leading to eventual elimination of the mutation by anticipation. In other words, premutation carriers may not be able to sustain as a stable reservoir for *de novo* cases of DM1. There has been a report of a DM1 family in which the number of CTG repeats remained in the minimally expanded range through at least three, and possibly four, generations (Simmons et al., 1998). If such stable small expansion alleles were frequent, they could constitute a stable source of the *DM1* mutation; however, there have been no additional reports of stable small mutations. As cases with premutation alleles are usually ascertained through the proband with a full expansion allele, it is possible that small expansion alleles are stable in the general population but those that happened to have expanded to cause DM1 have been preferentially ascertained. Alternatively, the original mutational event of DM1 may be the expansion into the upper range of normal repeat size, instead of expansion into the lower abnormal range. Then, the prevalence of DM1 may be maintained by expansion of the high end of normally sized alleles. Studies of the distribution of normal repeat sizes at the *DM1* locus in different populations have shown considerable differences. Geographical variations in the current prevalence of DM1 have been attributed mostly to a relatively recent founder effect or to thoroughness of ascertainment. However, studies of sub-Saharan African populations showed a striking and peculiar absence of DM1 (Ashizawa and Epstein, 1991; Goldman et al., 1996). This led to the hypothesis that the original *DM1* mutation occurred after the migration of Caucasoid populations out of Africa about 90 000 years ago (Ashizawa and Epstein, 1991). Interestingly, normal alleles with high repeat numbers are relatively deficient in the sub-Saharan African populations (Tishkoff et al., 1998). Thus, it is tempting to hypothesize that normal alleles with high repeat numbers were enriched through a population bottleneck during the human migration out of Africa. Together, these data suggest that frequency and origins of the *DM1* mutation in a population might depend on the high frequency of large-size normal alleles predisposing to a high frequency of DM1.

DM1 haplotype

The *DM1* mutation is in complete linkage disequilibrium with a nearby two-allele insertion/deletion (Alu-1kb$^{+/-}$) polymorphism. Not surprisingly, the Alu-1kb^{+} allele associated with DM1 is also found in individuals with normal alleles in the upper range with 19–30 CTGs. The Alu-1kb^{+} allele is also associated exclusively with the allele with five CTGs, whereas the Alu-1kb^{-} allele was associated exclusively with 11–13 CTGs. Thus, it was proposed that the initial predisposing event(s) consisted of a jump from a $(CTG)_5$ allele to $(CTG)^{19–30}$, rather than a gradual increase through the intervening range (Imbert et al., 1993). However, a subsequent study of eight global populations showed dissociation of the Alu-1kb^{+} allele from both the $(CTG)_5$ and $(CTG)_{19–30}$ alleles, challenging this "jump" hypothesis, and raising the possibility that the occurrence of high–normal repeat numbers may antedate the origin of the insertion deletion polymorphism (Zerylnick et al., 1995). Haplotype analysis of genetic markers within and immediately flanking the *DMPK* gene showed that essentially all patients with DM1 share a single haplotype, suggesting that the DM1 mutation occurred on the background of a particular haplotype in which the $(CTG)_n$ repeat became inherently unstable and therefore predisposed to amplification (Neville et al., 1994).

A molecular genetics study (Krahe et al., 1995b) of a Nigerian DM1 family, originally reported by Dada et al. (1973), showed a CTG expansion on a haplotype

different from that of Eurasian patients with DM1. Similarly, expanded CTG repeats have been found in yet another haplotype background in some patients with DM1 in India, and this haplotype is uniquely prevalent among the Indian population (Basu et al., 2001). These cases may be exceptional and may not disprove a single origin of the mutation over most of the world; however, they provide evidence against the theory that the predominant DM1 haplotype is not an absolute prerequisite for the *DM1* mutation. Studies using genetic markers in populations with high frequency of DM1, such as those in Quebec, northern Sweden, South Africa, and Istria, using genetic markers around the *DM1* locus, have suggested founder effects: i.e., the origin of the disease is from one or very few ancestors on the same haplotype.

Meiotic drive

"Meiotic drive" is the term given to the preferential transmission at meiosis of a particular allele at a given locus. The existence of meiotic drive in DM1 remains controversial. Evidence for meiotic drive in DM1 has been put forward in two types of data. One suggests preferential transmission in normal individuals of the chromosome carrying the larger repeat size at the *DM1* locus (Carey et al., 1994; Chakraborty et al., 1996; Dean et al., 2006), and the other shows preferential transmission of the expanded allele in DM1 families (Gennarelli et al., 1994; Zatz et al., 1997; Magee and Hughes, 1998). Abnormal segregation consistent with meiotic drive has been reported in transgenic mouse lines carrying an expanded allele (Monckton et al., 1997). These studies suggested that the sex of transmitted parent was an important determinant of meiotic drive. However, reanalysis of some data and a new study using data from prenatal molecular studies, which are not subject to ascertainment bias, showed no convincing evidence for abnormal segregation (Zunz et al., 2004). Furthermore, which sex is associated with meiotic drive was inconsistent between studies.

The CCTG repeat expansion in DM2

The CCTG repeat expansion in the *ZNF9* gene responsible for DM2 is the largest known expansion mutation causing a human disease, and larger than the expansion seen in the congenital *DM1* CTG repeat (Liquori et al., 2001). The copy number of *DM2* CCTG repeats is less than 27 in normal chromosomes but up to about 11 000 in the affected allele in patients. The smallest reported CCTG repeat expansion in DM2 is an uninterrupted $(CCTG)_{75}$ allele found in a patient with mosaic expansion (Liquori et al., 2001). The CCTG repeat at the *DM2* locus is part of a complex polymorphic repeat tract in the configuration of $(TG)_n$ $(TCTG)_n$ $(CCTG)_n$ $(NCTG)_n$ $(CCTG)_n$. Reported normal alleles have one or more interruptions, whereas *DM2* alleles show an expansion of the CCTG repeat within the configuration of $(TG)_n$ $(TCTG)_n$ $(CCTG)_n$ without interruption, as long as the sequence can be determined.

The expanded *DM2* CCTG repeat shows extraordinary somatic instability with significant increases in length over time (e.g. 2000 base pairs (bp)/3 years) (Day and Ranum, 2005). As a result, the repeat expansion size correlates with the age in DM2. However, this remarkable somatic instability makes the analysis of genotype–phenotype correlations complicated. The inverse correlation between age of onset and repeat expansion size observed in many repeat expansion diseases is not found in DM2, presumably obscured by the somatic instability (Day et al., 2003). Anticipation, which has been implicated in DM2 families in multiple reports, does not coincide with intergenerational increases of the CCTG repeat expansion size; indeed, the repeat expansion size of the transmitting parent is often larger than that of the offspring in a given parent–offspring pair (Schneider et al., 2000; Day et al., 2003). Likewise, the effect of the sex of transmitting parents on the offspring's repeat size is likely to be obscured by the somatic instability. The mechanism behind this extraordinary instability of the *DM2* CCTG repeat is unknown.

As in DM1 families, haplotype analyses of DM2 families suggested that the critical initial expansions may have originated from one or few founder mutations (Liquori et al., 2001, 2003; Bachinski et al., 2003), which may have occurred approximately 200 to 540 generations ago prior to the Aryan migration of Indo-Europeans in 2000–1000 BC (Liquori et al., 2003; Schoser et al,, 2004a). What were the mutation events that pushed a normal allele into the mutational range then? Unlike DM1, in which a reservoir of premutation alleles in the population is thought to provide the source of *de novo DM1* alleles, there have been no reports of premutation alleles in DM2. It should also be pointed out that DM2 is a milder disease than DM1, and the lack of congenital form of DM2 may slow down the elimination rate of *DM2* mutant alleles from the population. Thus, one may predict a much lower mutation rate in DM2 than in DM1 that is necessary to maintain the steady prevalence in the population (Schoser and Ashizawa, 2009). Are there premutation alleles in DM2? Large normal alleles may be precursors of such premutation alleles, or they may be capable of functioning as premutation alleles. Large normal alleles in Caucasian and African-American populations have different allele size distributions: Caucasian chromosomes show a unimodal distribution

with a peak at 132 bp, whereas alleles in African-Americans show a secondary peak at 174 bp (Bachinski et al., 2009). In general, short tandem repeats become increasingly unstable as the repeat length increases. How do we explain that Caucasians, in whom DM2 is predominantly found, have shorter alleles in the upper normal range? The answer appears to be in the interrupted sequences in alleles of African-Americans. The presence of interruptions of a pure repeat tract has a known stabilizing effect in short tandem repeats. SP-PCR analysis of large normal alleles showed that long normal alleles with an uninterrupted CCTG repeat tract, which are found only among Caucasians, are more unstable than the long interrupted normal alleles found in African-Americans (Bachinski et al., 2009). Although whether these unstable alleles are truly *DM2* premutations will remain unclear until actual expansion of these alleles into the full mutational range has been documented, the data are consistent with the high prevalence of DM2 among Caucasians.

MOLECULAR PATHOGENESIS OF MYOTONIC DYSTROPHY TYPE 1

The underlying genetic mutation in DM1 is the expansion of trinucleotide repeats CTG in the 3′ UTR of the dystrophica myotonin protein kinase (*DMPK*) gene (Harper and Johnson, 2001). The mechanism by which expansion of CTG repeats within the noncoding region of *DMPK* leads to the multisystemic complex phenotypes in DM1 is not completely understood. As of today, there are several hypotheses regarding the molecular pathophysiology of DM1 that arise due to the massive expansion of CTG repeats. As the expanded CTG repeat is located within the 3′ UTR of *DMPK*, the function of the encoded protein remains unperturbed in DM1. Numerous recent studies show that the mutant *DMPK* is fully transcribed, and that transcripts produced from the mutant *DMPK* allele contain the expanded CUG repeat in the 3′ UTR. Thus, although a majority of human genetic diseases manifest as the result of altered or abnormal protein product, the DM1 pathogenic mechanism seems to be unique. Furthermore, various studies have also indicated that the massive expansion of the CTG repeat at the *DM1* locus leads to altered chromatin structure which may also affect the transcription of the neighboring genes, contributing to the disease phenotypes. From recent studies, two major models for DM1 disease mechanism have emerged: (1) a model based on chromatin condensation at the *DM1* locus leading to the loss of function of the genes in the vicinity of the CTG repeat, including *DMWD, SIX5*, and *DMPK* itself, and (2) a model based on a gain of toxic function by the expanded CUG repeat encoded in the mutant *DMPK* transcripts (Figure 15.6).

Loss of function of the genes flanking the *DM1* CTG expansion locus

Loss of DMPK function

The DMPK deficiency was proposed as the first pathogenic model soon after the identification of the CTG expansion mutation in *DM1*. Although studies have reported conflicting results of *DMPK* mRNA levels in skeletal muscle and other tissues from patients with DM1, most studies, especially those using adult DM1 tissues, showed decreased levels of *DMPK* mRNA (reviewed in Nagamitsu and Ashizawa, 2002). The decrease in the *DMPK* mRNA has been correlated with the size of CTG repeat expansion, and attributed to the retention of mutant *DMPK* transcripts in nuclear foci in DM1 cells rather than impaired transcription of the mutant *DMPK* gene (see Figure 15.6) (Krahe et al., 1995a; Davis et al., 1997; Furling et al., 2001). Furthermore, the actual *DMPK* mRNA levels were found to be at least 50% diminished in some patients with DM1 compared with normal levels, potentially by a dominant negative effect of the mutant *DMPK* mRNA on the wild-type *DMPK* mRNA (Wang et al., 1995). In any case, this loss-of-function model assumes that DMPK is tightly regulated in a rate-limiting step of the signal transduction pathways in affected tissues (Fu et al., 1993).

Evidence suggests that DMPK is one of the serine–threonine protein kinases. Although *DMPK* mRNA is strongly expressed in skeletal muscle, heart, brain, liver, and kidney (Fu et al., 1993), its exact physiological function is not fully understood. DMPK phosphorylates serine and threonine residues of a myosin-binding subunit of myosin phosphatase (MYPT-1) and inhibits myosin phosphatase activity (Muranyi et al., 2001). Thus, DMPK may be involved in regulating cell size and shape like other "myotonic dystrophy family of protein kinases" (MDFPK). Several other proteins, such as the β-subunit of the L-type calcium channel (Timchenko et al., 1995), phospholemman (Mounsey et al., 2000a), and CUG-binding protein (Roberts et al., 1997), are also phosphorylated by DMPK. The DMPK protein appears to be preferentially localized in muscle and heart (Lam et al., 2000). In skeletal muscle DMPK protein has been found in sarcoplasmic reticulum (Ueda et al., 1999). Whether DMPK is truly tightly regulated in the signal transduction pathway, however, remains unknown.

The DMPK loss-of-function hypothesis lost significant credibility in 1996 when initial observations of DMPK-deficient mice showed no robust abnormalities (Jansen et al., 1996; Reddy et al., 1996) except for mild

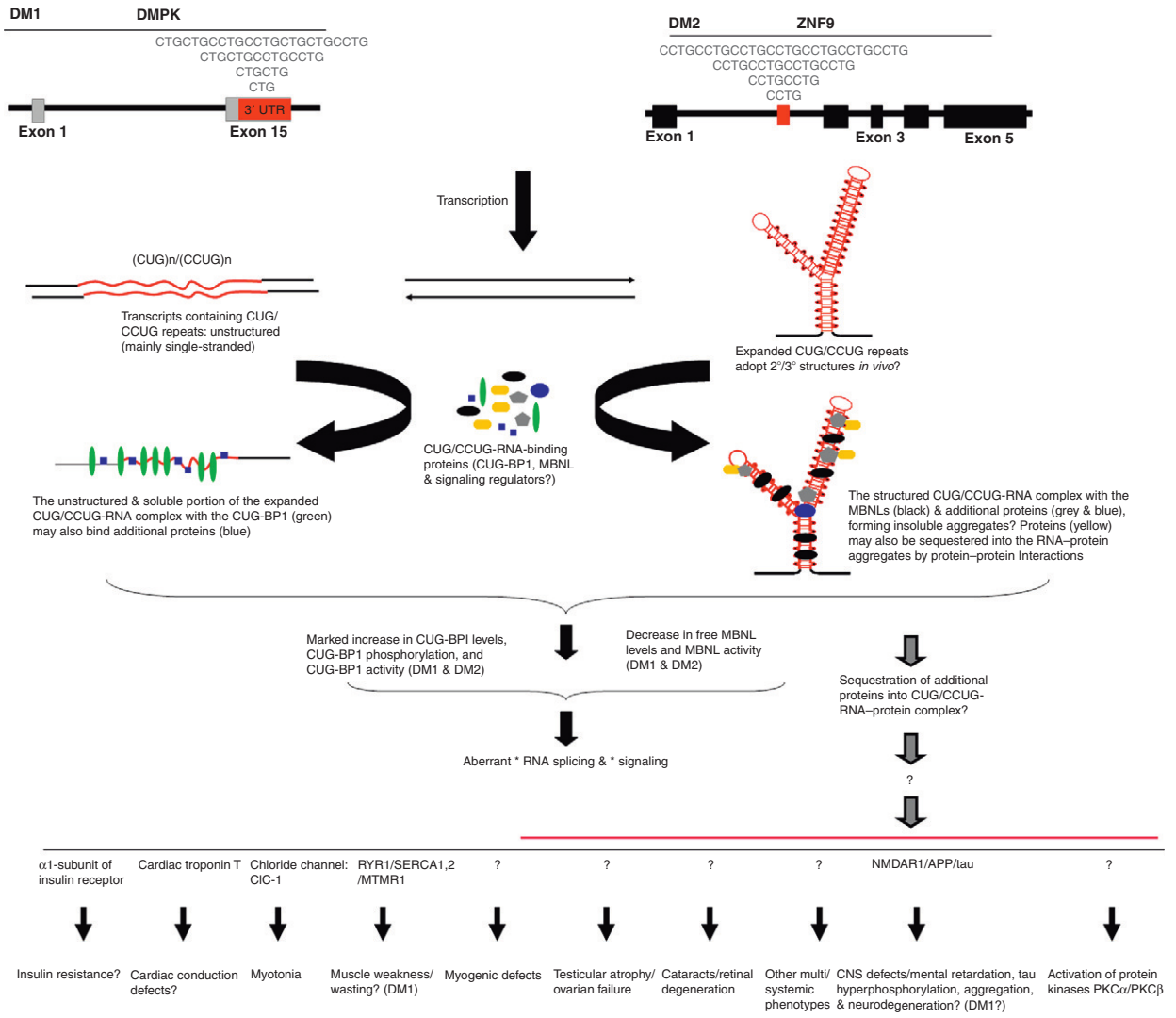

Figure 15.6. The RNA gain-of-function model for pathogenesis of myotonic dystrophy types 1 and 2. In DM1, the genetic mutation is the massive expansion of a CTG repeat in the 3′ untranslated region (UTR) of dystrophia myotonia protein kinase (*DMPK*) gene. The genetic mutation in DM2 is the expansion of a CCTG repeat in the first intron of the zinc-finger protein 9 (*ZNF9*) gene. In DM1 and DM2, the expanded CTG and CCTG repeats are transcribed and the expanded repeats complex with proteins; diminished activity of the sequestered proteins contributes to disease pathogenesis. As depicted in the figure, a portion of the expanded repeats in the transcripts may adopt secondary and tertiary structures *in vivo*, and the single-stranded as well as the structured RNA repeats may complex with a variety of proteins in the DM cell. Proteins such as CUG-RNA-binding protein (CUG-BP1) may bind specifically to the single-stranded CUG/CCUG-RNA and proteins; for example, muscle-blind like proteins (MBNLs) may bind with the mismatched hairpin structures formed by a part of the CUG/CCUG repeat RNA. Additional proteins might be sequestered into the CUG/CCUG-RNA–protein complexes by protein–protein interactions or by binding directly with the CUG/CCUG-RNA. There is a marked increase in CUG-BP1 levels and phosphorylation of CUG-BP1 and decrease of MBNL activity, probably through its sequestration in DM, resulting in aberrant splicing of a large number of transcripts in DM1. The transcripts that are aberrantly spliced in DM1 are shown in the lower panel of the figure.

muscle weakness. However, more recent studies of homozygous and heterozygous DMPK-deficient mice suggested that haploinsufficiency of *DMPK* does cause skeletal and cardiac muscle abnormalities through alterations of sodium and calcium channels. The abnormal sodium channel opening and repetitive discharges observed in the skeletal muscle membrane of these DMPK-deficient mice (Mounsey et al., 2000b) may in part be caused by silencing of muscle sodium channels (Reddy et al., 2002). Cardiac conduction block found in homozygous and heterozygous DMPK-deficient mice (Reddy et al., l996) may be attributable to abnormal sodium channel gating (Mounsey et al., 2000b). Thus, *DMPK* haploinsufficiency could account

for some electrophysiological abnormalities in DM1 skeletal and cardiac muscles.

The DMPK isoforms may add an additional twist to the haploinsufficiency theory. Alternative splicing of the *dmpk* transcript in a transgenic mouse model produced six major isoforms, with tissue-dependent expression (Groenen et al., 2000), and DMPK isoforms have cell type- and subcellular location-dependent substrate specificities, which are attributable to an alternately spliced VSGGG motif and C-terminal structures (Wansink et al., 2003). Additionally, four splicing factors have been shown to bind *DMPK* transcript at the 3′ UTR, yielding a novel mRNA isoform containing no CUG repeats, which was not retained in the nucleus of DM1 cells (Tiscornia and Mahadevan, 2000). Further studies are needed to gain insight into the functional significance of these isoforms in the *DMPK* haploinsufficiency theory.

Loss of SIX5 function

Multiple lines of evidence suggest that the expansion of CTG repeats in the 3′ UTR of *DMPK* results in altered chromatin modeling and organization in the *DM1* locus, and altered chromatin organization affects the expression of *DMPK* as well as its neighboring genes such as *SIX5* and *DMWD* in DM1. It has been predicted that the altered expression of the neighboring genes may contribute to the disease phenotypes in DM1. *SIX5*, previously known as DM-associated homeodomain protein (DMAHP) (Boucher et al., 1995), is located immediately downstream of the *DMPK* gene. *SIX5* shows homology to the *Drosophila sine oculis* locus, and is involved in muscle and gonad development (Kirby et al., 2001; Sarkar et al., 2004). The DNase I-sensitive site positioned immediately downstream of the CTG repeat was found to be eliminated when the CTG repeat was expanded, and this loss of DNAse I sensitive areas has been attributed to the formation of a condensed chromatin structure in the CTG expansion locus (Wang et al., 1994). Thus, the highly condensed chromatin structure at the *DM1* locus might impede the physical movement of RNA polymerase or diminish the accessibility and binding of transcription factors with the target DNA sequences in the CTG expansion locus. *SIX5* mRNA is found in tissues affected in DM1 such as eyes, skeletal muscle, heart, and brain (Boucher et al., 1995; Winchester et al., 1999). Subsequent studies showed that an expansion of the CTG repeat reduces expression of *SIX5*, by impeding an enhancer element for the gene (Thornton et al., 1997; Klesert et al., 2000; Frisch et al., 2001). This effect is expansion size-dependent and tissue-specific, with the greatest reductions found in muscle and liver (Thornton et al., 1997; Korade-Mirnics et al., 1999). In contrast to these results, however, other studies have shown no significant reduction in the *SIX5* levels in DM1 tissues (Hamshere et al., 1997; Eriksson et al., 1999). Two independent studies showed that *SIX5* deficiency causes cataracts in both homozygous and heterozygous knockout mice (Klesert et al., 2000, Sarkar et al., 2000). *SIX5* regulates the transcription of the Na,K-ATPase α1 subunit gene (*Atpla1*), and the increased *Atpla1* mRNA level in SIX5 deficiency may alter the regulation of the osmotic balance within the lens and contribute to the development of lens opacity (Sarkar et al., 2000). Cardiac conduction system abnormalities were also found in SIX5-deficient mice (Wakimoto et al., 2002). However, SIX5-deficient mice do not exhibit histopathological, contractile, or electrophysiological abnormalities in the skeletal muscle (Mistry et al., 2001; Personius et al., 2005). Further studies are needed to determine whether other organs show abnormalities compatible with DM1 in these knockout mice.

Loss of DMWD function

Massive expansion of the CTG repeat may also influence the expression of the immediately upstream gene *DMWD* (Alwazzan et al., 1999). The 5′ end of *DMPK* overlaps with the 3′ end of the *DMWD* gene, and the 3′ boundary of the *DMWD* has not been clearly defined. *DMWD* is highly expressed in brain and testis, which are known to be affected in DM1. The predicted protein product contains WD repeat sequences, and such motifs are found in several proteins involved in the regulation of cell division, transcription, mRNA processing, cytoskeletal assembly, vesicle fusion, and signal transduction (Jansen et al., 1995). Decreased *DMWD* mRNA levels were reported in the cytoplasm of DM1 myoblasts and adult DM1 skeletal muscles (Alwazzan et al., 1999).

Gain of function by the mutant *DMPK* mRNA and DM1 pathogenesis

DM1 is an autosomal dominant disease, and therefore, even if the expanded CTG repeats completely suppress the transcription of the *DMPK* from the mutant allele, half of the DMPK protein would still be produced from the normal allele. For most of the genes in the human genome the amount of protein produced from the normal allele would be sufficient to support normal cell and tissue functions in spite of having a defective copy of the gene. However, if the mutant *DMPK* gene produces a toxic product, this may result in cellular dysfunction regardless of the level of the DMPK protein produced from the normal allele. Therefore, a gain of function of the mutant *DMPK* allele is a viable

pathogenic model to explain the dominant inheritance pattern of DM1. As the CTG repeat is located in the 3′ UTR, the DMPK protein is unlikely to gain a toxic function as a dominant contributor of the DM1 phenotypes. However, the expanded CTG sequences is transcribed into the mutant *DMPK* mRNA as a noncoding CUG repeat, making the RNA-based gain of function a plausible model for DM1. The first observation to support such an RNA gain-of-function model was that the mutant *DMPK* mRNA accumulates in nuclear foci in DM1 cells (Krahe et al., 1995a; Taneja et al., 1995; Davis et al., 1997). This important observation led to the hypothesis that the mutant *DMPK* transcripts complex with regulatory proteins and the loss, diminished, or altered functions of these proteins could be the basis of the gain-of-function theory in DM1.

A large amount of recent evidence supports the hypothesis that the mutant *DMPK* alleles are fully transcribed and the mutant *DMPK* transcripts containing the expanded CUG sequences play a major toxic role in eliciting the complex phenotypes in DM1. Studies by Mahadevan and colleagues provided, for the first time, evidence that the expanded CUG-RNA sequences present in the mutant *DMPK* transcripts *per se* have a toxic role in myogenesis in DM1 (Amack and Mahadevan, 2001; Amack et al., 2002). C2C12 myoblasts ectopically expressing approximately 200 CUG repeats from a transgene were shown to exhibit some of the characteristic features of the molecular pathology seen in DM1, including the formation of CUG-RNA foci and inhibition of myoblast fusion and differentiation (Amack and Mahadevan, 2001; Amack et al., 2002). This study strongly supported the hypothesis that the expanded CUG-RNA sequences play a dominant role in impairing the efficacy of myogenic differentiation in DM1. The accumulation of the mutant *DMPK* transcripts as an aggregated mass in DM1 cells led to the hypothesis that the expanded CUG sequences bind with RNA-binding proteins and that diminished or altered activities of the sequestered proteins result in aberrant molecular interactions and signaling, leading to disease phenotypes in DM1.

The RNA gain-of-function hypothesis, CUG-RNA-binding protein (CUG-BP1) and DM1

Timchenko et al. (1996a) identified a novel RNA-binding protein that showed strikingly high binding affinity for CUG repeat sequences and was termed CUG-RNA-binding protein 1, or CUG-BP1. CUG-BP1, a member of the CELF family of proteins, exhibits high affinity for the 3′ UTR of the *DMPK* mRNA encoding expanded stretches of CUG sequences. The *in vitro* RNA–protein-binding studies suggested that CUG-BP1 interacts directly and complexes with the single-stranded CUG-RNA as well as with the expanded CUG sequences encoded in the mutant *DMPK* transcripts *in vitro* (Timchenko et al., 1996b). A significantly high affinity of CUG-BP1 for the expanded CUG-RNA sequences *in vitro* led to the birth of a new hypothesis, termed the RNA gain-of-function hypothesis, to describe the complex phenotypes in DM1. According to this hypothesis, a majority of the cellular CUG-BP1 exists as an RNA–protein complex in the DM1 cell, and formation of the CUG-BP1–CUG-RNA complex leads to complete or partial loss of function of CUG-BP1, and diminished or altered CUG-BP1 activity contributes toward the disease phenotypes in DM1. Although the RNA–protein-binding studies suggested the possibility that CUG-BP1 interacts directly with CUG-RNA *in vitro*, fluorescence *in situ* hybridization (FISH) studies, however, have shown that CUG-BP1 colocalizes poorly with the nuclear aggregates or foci in DM1 myoblasts or skeletal muscle (Michalowski et al., 1999; Fardaei et al., 2001). These contrasting findings raised doubts whether CUG-BP1 is indeed sequestered into the CUG-RNA nuclear foci *in vivo* and whether this sequestration leads to altered or loss of function of CUG-BP1 and DM1 phenotypes. It is noteworthy that the studies described here considered that the majority of the mutant *DMPK* transcripts form insoluble aggregates in DM1 cells and are deposited as "CUG-RNA nuclear foci," and did not take into account the possibility that CUG-BP1 might also complex with the soluble fraction of the mutant *DMPK* transcripts, which are not deposited within the nuclear CUG-RNA foci. However, further investigation is needed to confirm that the soluble fraction of the mutant *DMPK* transcript complexes with CUG-BP1 in DM1.

Importantly, contrary to the CUG-BP1 loss-of-function hypothesis, the steady-state level of CUG-BP1 was found to be significantly and consistently raised in DM1 myoblasts, skeletal muscle, and heart (Savkur et al., 2001; Timchenko et al., 2001a, b; Ho et al., 2005a, b; Orengo et al., 2008). Interestingly, CUG-BP1 levels were also found to be markedly increased in C2C12 myoblasts as well as in transgenic mice ectopically expressing expanded CUG-RNA (Timchenko, 2001a, b; Orengo et al., 2008). These data suggest that the expression of CUG-RNA is sufficient to enhance the steady-state levels of CUG-BP1 in DM1. Subsequent studies, however, have suggested the possibility that CUG-RNA is involved, directly or indirectly, in phosphorylating CUG-BP1 and that this phosphorylation might be a key molecular step in increasing the stability and steady-state levels of CUG-BP1 in DM1 striated muscles, as well as in transgenic mice overexpressing mutant *DMPK* 3′ UTR sequences.

Consistent with the CUG-RNA gain-of-function model, a large amount of experimental evidence has

supported the idea that altered/increased activity of CUG-BP1 is linked with the aberrant alternate splicing of several transcripts, such as cardiac troponin T (*cTNT*) (Philips et al., 1998), insulin receptor (*IR*) (Savkur et al., 2004), muscle chloride channel (*CLC-1*) (Mankodi et al., 2000, 2002; Charlet et al., 2002), and myotubularin-related 1 (*MTMR1*) (Buj-Bello et al., 2002) in DM1. These studies indicate that the inappropriate splicing of multiple transcripts might contribute towards the development of some of the characteristic DM1 phenotypes. Furthermore, CUG-BP1 was shown to target CCAAT/enhancer binding protein-β (C/EBPβ), which regulates the expression of many genes, and DM1 muscles show induced translation of an inhibitory isoform of C/EBPβ (Timchenko et al., 2001a). These experiments indicate that aberrant CUG-BP1 activity might be a key pathway leading to the misregulation of alternate splicing of many transcripts, and complex pathology in DM1 (Timchenko et al., 2004; Ho et al., 2005a). Further evidence to support the hypothesis that raised or altered activity of CUG-BP1 contributes towards DM1 phenotypes, at least partially, came from a recent study by Timchenko and colleagues which demonstrated that the transgenic expression of CUG-BP1 in mouse skeletal muscle results in neonatal lethality and partly DM1-like myogenic defects (Timchenko et al., 2004). Importantly, the CUG-BP1 transgenic mice display splicing abnormalities of *Clcn1*, cardiac troponin T2 (*cTnnt2*), and myotubularin-related 1 gene (*Mtmr1*), akin to the splicing defects in DM1 (Buj-Bello et al., 2002; Ho et al., 2005a). Aberrant splicing of *Mmtr1* has been demonstrated in congenital DM1 skeletal muscle, as well as myoblasts derived from patients with congenital DM1, but not in adult DM1 skeletal muscle, suggesting that the congenital muscle phenotypes in DM1 may result from the misregulation of alternate splicing mediated by CUG-BP1 (Buj-Bello et al., 2002).

Although the studies described here support the hypothesis that CUG-RNA modulates CUG-BP1 phosphorylation and probably its activation, the mechanism by which CUG-RNA triggers CUG-BP1 phosphorylation, and more specifically the extent to which this hyperphosphorylation contributes towards aberrant splicing and DM1 phenotypes, is not clearly understood and needs additional investigation. The possibility that the expanded CUG-RNA triggers hyperphosphorylation of CUG-BP1 in a mechanism that is independent of the interactions of CUG-BP1 with the CUG-RNA also cannot be ruled out from the currently available data. Importantly, more recent studies by Cooper and colleagues indicate that the transgenic expression of the mutant *DMPK* 3′ UTR results in a marked increase in CUG-BP1 and DM1-like muscle phenotypes, and is sufficient to cause an inappropriate activation of PKCα and PKCβII (Kuyumcu-Martinez et al., 2007; Orengo et al., 2008). This novel finding indicates that aberrant activities of protein kinases, triggered by the mutant *DMPK* transcripts by means of a yet unknown mechanism, cause hyperphosphorylation and stabilization of CUG-BP1, and contribute to the DM1 phenotypes. Further investigation is necessary to establish whether activated protein kinases trigger aberrant signaling in a mechanism that is independent of the activation/phosphorylation of CUG-BP1 in DM1. Collectively, the published data thus far indicate that the expanded CUG-RNA plays a dominant role in altering protein kinase as well as CUG-BP1 activities, and that aberrant activities of these key regulatory proteins have a broad pathogenic role in DM1. The mechanism by which CUG-RNA causes aberrant activation of the protein kinases leading to the hyperphosphorylation of CUG-BP1 may be critical in delineating the complex disease phenotypes in DM1, and needs further investigation. Moreover, further research is necessary to established whether interactions between CUG-RNA and CUG-BP1 are necessary and sufficient for activation of the protein kinases and CUG-BP1.

In summary, the experimental data discussed thus far suggest the possibility that the CUG-RNA molecules are directly or indirectly involved in the activation of protein kinases as well as CUG-BP1, and aberrant activation of the proteins results in inappropriate splicing of multiple transcripts, contributing to some of the phenotypes in DM1. However, the currently published data do not clearly establish whether interaction of CUG-BP1 with CUG-RNA is sufficient to activate protein kinases and CUG-BP1, and inappropriate splicing or these two distinct molecular events are mechanistically independent events mediated by CUG-RNA. It is possible that accumulation of the phosphorylated form of CUG-BP1 results in the phosphorylation of target proteins and inappropriate signaling that is sufficient to disrupt tissue homeostasis in DM1. The protein kinases that catalyze phosphorylation of CUG-BP1 might also phosphorylate additional proteins and trigger aberrant signaling in parallel. Therefore, elucidating the mechanism by which expanded CUG-RNA triggers activation of protein kinases, regulates the phosphorylation and stability of CUG-BP1, and distinctly disrupts the alternate splicing of multiple pre-mRNA transcripts will be immensely important in delineating the mechanism of abnormal signaling, splicing, and complex etiology of DM1.

Muscle-blind proteins (MBNLs) and DM1 pathogenesis

Soon after the discovery of CUG-BP1, it became increasingly evident that the expanded CUG-RNA sequences are capable of binding with more than one

protein. Consistent with this hypothesis, many studies supported the idea that sequestration of multiple proteins into the CUG-RNA–protein complex could be a major pathogenic mechanism in eliciting complex and variable phenotypes in DM1 (Bhagavati et al., 1996). It was postulated that multiple proteins are sequestered by the expanded CUG-RNA and the combinatorial loss or altered functions of the sequestered proteins might contribute towards the development of multisystemic and complex phenotypes in DM1. Further research into the possible existence of additional proteins sequestered into the CUG-RNA–protein complexes led to the discovery of a new family of MBNL proteins (Miller et al., 2000). Importantly, unlike CUG-BP1, the MBNL proteins were found to bind specifically with the mismatched hairpin structures adopted by the expanded CUG-RNA but not with single-stranded unstructured CUG-RNA *in vitro* (Michalowski et al., 1999; Miller et al., 2000; Fardaei et al., 2001). In contrast to CUG-BP1, FISH analyses on DM1 cells revealed that significant amounts of all the three isoforms of MBNL (MBNL1, MBNL2, and MBNL3) were present within the CUG-RNA nuclear foci, suggesting that the CUG-RNA molecules, at least partially, adopt hairpin structures *in vivo* and sequester MBNL proteins. It is possible that longer CUG repeats with a higher propensity to form thermodynamically stable hairpin structures bind larger amounts of the MBNL proteins, whereas the shorter CUG repeat tracts tend to remain primarily single-stranded and show a stronger affinity for CUG-BP1. It is possible that longer CUG-RNA molecules with a higher propensity to adopt higher-order nucleic acid structures bind MBNLs, whereas shorter CUG repeats, which remain predominantly in single-stranded and soluble forms, bind CUG-BP1. Given the fact that the CUG repeat lengths are mosaic in DM1 tissues, and the limitations of the current methodologies employed in characterizing the nature of the *in vivo* interactions of CUG-RNA with probable RNA-binding proteins, the possible coexistence of both CUG-BP1-CUG-RNA and MBNL-CUG-RNA complexes in DM1 cannot be ruled out. In fact, ultraviolet light-crosslinking experiments indicated that CUG-BP1 and MBNLs interact with the CUG-RNA both *in vitro* and *in vivo* (Timchenko et al., 1996a, b, 2001a, b, 2004). The important question that has emerged from these studies, and which has remained unanswered, is why MBNL proteins, but not CUG-BP1, are accumulated in the insoluble nuclear CUG foci in DM1 muscle, myoblasts, and fibroblasts (Mankodi et al., 2000; Fardaei et al., 2002; Mankodi et al., 2002). It is speculated that the unstructured and soluble form of the single-stranded CUG-RNA molecules binds preferentially with CUG-BP1; in contrast, the structured and insoluble forms of the CUG-RNA complex with MBNLs are deposited as aggregates within nuclei in DM1. Therefore, the ratio of the single-stranded soluble CUG-RNA and their insoluble aggregated form in DM1 cell may actually dictate the extent to which MBNL and CUG-BP1 are sequestered into the CUG-RNA–protein complexes and interfere with the downstream RNA processing and possibly additional signaling processes in DM1.

Various recent studies support the hypothesis that both MBNL and CUG-BP1 function antagonistically to regulate common pre-mRNA transcripts. However, the detailed mechanism by which CUG-BP1 and MBNL proteins modulate alternate splicing of transcripts, and contribute toward DM1 phenotypes, is further complicated by recent studies showing that MBNL proteins are the primary component of the nuclear foci and aberrant insulin receptor (IR) splicing in DM1 (Dansithong et al., 2005), whereas CUG-BP1 and other CELF proteins regulate the equilibrium of splice-site selection by antagonizing the EXP proteins on splicing of IR and cardiac troponin T (cTnT) (Dansithong et al., 2005; Ho et al., 2005 a, b). These studies also showed that CUG-BP1 levels are consistently and significantly raised in DM1 cells by mechanisms that are independent of MBNL1 and MBNL2 loss. Furthermore, studies by Cooper and colleagues further corroborate the hypothesis that the splicing regulations mediated by the MBNL proteins and CUG-BP1 are antagonistic, with MBNLs favoring adult isoforms and CUG-BP1 promoting the retention of the embryonic isoforms of several of the transcripts found to be misregulated in DM1 (Ho et al., 2004). It is important to note that, although MBNL proteins and CUG-BP1 play antagonistic role in terms of splicing regulation, their binding sites for target RNA and their expression are regulated independently (Dansithong et al., 2005). Consistent with these data, transgenic mice overexpressing CUG-BP1 developed signs of DM1-like muscle defects and aberrant splicing of *ClC-1* and *TNNT2*, similar to *Mbnl1* mutant mice (Timchenko et al., 2004; Ho et al., 2005a). Cell culture experiments using mini-genes further support the idea that aggregation of CUG-RNA into foci is regulated primarily by MBNL proteins, but that aberrant splicing of *TNNT2* and *IR* transcripts requires the presence of CUG-BP1-binding sites (Philips et al., 1998; Savkur et al., 2001; Ho et al., 2004). Furthermore, sequestration of MBNLs into the similar lengths of CAG-RNA foci does not correlate with the alternate splicing of transcripts known to be modulated by MBNL proteins (Ho et al., 2005b), suggesting that sequestration of MBNLs into the CUG-RNA foci is important, but may not be sufficient for diminishing the activity of MBNL proteins and inflicting aberrant

alternative splicing in DM1. This important finding also raises a fundamental question whether activity of MBNL proteins is impaired only through their sequestration into the CUG-RNA–protein complexes, or whether MBNL activity is altered, in parallel, in a mechanism that is independent of their interactions with CUG-RNA. It is also possible that a significant amount of MBNL protein complexes with the soluble fraction of the CUG-RNA and is therefore not deposited into the insoluble CUG-RNA foci or aggregates in DM1 cells. Moreover, it is not clear from the published experimental data whether a portion of MBNL proteins is also inactivated by binding with the soluble fraction of the CUG-RNA in DM1 cells; further research is needed to test this possibility.

A large number of recent studies have shown that the expanded CUG-RNA sequences form nuclear foci and recruit MBNL proteins, and that this sequestration results in aberrant splicing of at least 13 different pre-mRNA transcripts in DM1, including that of chloride channel 1 (ClC-1), cardiac troponin T (cTnT), and the α1-subunit of the insulin receptor (Philips et al., 1998; Savkur et al., 2001; Mankodi et al., 2002; Kanadia et al., 2003; Ho et al., 2004; Jiang et al., 2004). Importantly, of the 13 inappropriate splicing events observed in DM1, the irregular and aberrant splicing of only four transcripts was consistent with either loss of MBNL function and/or gain of CUG-BP1 activity. Importantly, consistent with the MBNL loss-of-function model for DM1, an *Mbnl1* mutant mouse model has been shown to develop DM1-like eye and muscle phenotypes, and aberrant alternate splicing of several transcripts (Kanadia et al., 2003). Subsequent experimental data suggest that the CUG-RNA-mediated splicing abnormalities that lead to accumulation of the embryonic form of ClC-1 cause myotonia in DM1 (Charlet et al., 2002; Mankodi et al., 2002; Kanadia et al., 2003), and, importantly, the *Mbnl1* mutant mice also showed DM1-like splicing abnormalities of ClC-1 pre-mRNA (Kanadia et al., 2003). A recent study also suggested that aberrant accumulation of the embryonic form of cTnT and of the nonmuscle-specific isoform of IR play an important role in the manifestation of cardiac phenotypes and development of insulin insensitivity, respectively, in DM1. However, the extent to which aberrant splicing of the α1-subunit of the IR contributes to the development of insulin resistance is not clear from the present data. Furthermore, recent data also suggest that insulin overshooting in the glucose tolerance test may not be due to insulin resistance in DM1, but perhaps to oversecretion of insulin. Importantly, recent studies by Thornton and colleagues further substantiate the idea that myotonia in DM1 results from CUG-RNA-mediated inappropriate alternate splicing of the muscle-specific chloride channel (ClC-1) leading to reduced conductance of chloride ions in the sarcolemma (Mankodi et al., 2002; Wheeler et al., 2007). This study showed that morpholino antisense oligonucleotides, targeting the 3′ splice site of ClC-1 exon 7a reversed the defect of ClC-1 alternate splicing in mouse models of DM1 (Wheeler et al., 2007). This important finding clearly shows that, by repressing the inclusion of this exon, the antisense oligo restores the full-length reading frame in ClC-1 mRNA, upregulates the level of ClC-1 mRNA, increases the expression of ClC-1 in the membrane, normalizes muscle ClC-1 current density and deactivation kinetics, and eliminates myotonic discharges. These critical findings further corroborate the hypothesis that myotonia in DM1 results from the CUG-RNA-mediated inappropriate splicing of ClC-1 and suggest that antisense oligo-induced exon skipping methodology might be developed into a powerful method for correcting the alternate splicing defects of ClC-1 transcripts and abnormal myotonic discharges in DM1.

Although the role of MBNL proteins in causing aberrant splicing and DM1 phenotypes is supported by the fact that *Mbnl1* mutant mice develop DM1-like myotonia and splicing anomalies of ClC-1, TNNT2, and skeletal muscle troponin T (TNNT3), akin to the aberrant splicing observed in DM1 skeletal muscle (Kanadia et al., 2003), the exact mechanism by which the expression of CUG-RNA results in diminished MBNL activity, and triggers aberrant splicing, and DM1 phenotypes needs further investigation. The studies using Mbnl1-deficient mice have shown that the loss of MBNL function is sufficient to cause aberrant splicing of ClC-1, which results in robust DM1-like myotonia. This study corroborated the hypothesis that loss of MBNL1 function is definitely an important molecular step in inflicting splicing defects of several pre-mRNA transcripts in DM1. Further analysis of DM1 patient samples, as well as *Mbnl1* mutant mice, is needed to establish the number of transcripts whose processing/splicing is affected by the diminished MBNL activity in DM1. However, another important recent study by Mahadevan and colleagues showed that expression of the 3′ UTR of the normal *DMPK* allele (containing five CUG repeats) is sufficient to recapitulate the DM1-like splicing abnormalities and cardiac conduction defects, and to enhance steady-state CUG-BP1 levels in transgenic skeletal muscle (Mahadevan et al., 2006). Importantly, although these novel transgenic mice develop splicing and cardiac conduction abnormalities akin to those in DM1, they do not show altered MBNL1 levels, detectable CUG-RNA foci, or aggregation of MBNL1 protein into discrete aggregates, which are the characteristic features of transgenic mice expressing 250 CUG

repeats (Mankodi et al., 2001, 2002). The absence of CUG-RNA foci and MBNL sequestration suggests the possibility that CUG repeats may sequester additional proteins, resulting in aberrant alternate splicing of target genes and inappropriate phosphorylation of CUG-BP1, and contributing to the development of DM1-like phenotypes. Additional research is necessary to establish the underlying molecular mechanism by which expanded CUG-RNA molecules interfere with MBNL activity, the mechanism by which expression of CUG-RNA results in enhanced protein kinase activity and hyperphosphorylation of CUG-BP1 in DM1.

Recent experimental evidence suggests that CUG-RNA-mediated aberrant splicing also contributes towards the development of CNS defects in DM1. In DM1 brains, the cortical and subcortical neurons also show the presence of a large number of the CUG-RNA nuclear foci, sequestration of MBNL proteins, and deregulated alternate splicing of the *N*-methyl-D-aspartate (NMDA) receptor, NR1 subunit, neuronal microtubule-associated protein tau, and amyloid precursor protein (APP) (Jiang et al., 2004). In DM1 brains and transgenic mice expressing the human *DMPK* with expanded CUG repeats, tau, a protein involved in many neurodegenerative diseases, shows abnormal isoforms which aggregate (Vermersch et al., 1996; Sergeant et al., 2001). These studies suggest that CUG-RNA may also play an important role in the CNS abnormalities of DM1.

Collectively, the data described thus far indicate that the gain-of-function mechanism, mediated by the expanded CUG sequences encoded in the mutant *DMPK* mRNA, is a viable model that explains both the dominant inheritance and multisystemic nature of this disease. Furthermore, a series of recent studies, described in this review, using different genetic mouse models have provided convincing evidence that CUG-RNA-mediated aberrant splicing plays a central role in the pathogenic mechanism of DM1. The transgenic mouse line expressing 250 CUG repeats (Mankodi et al., 2000, 2002) developed robust electrical myotonia and clinical and histopathological features of myopathy reminiscent of the phenotypes observed in patients with DM1. These mice showed abnormal splicing of muscle chloride channel, *Clcn1* gene, in a pattern similar to that seen in DM1 skeletal muscle (Mankodi et al., 2002). Although the DM1 mouse models described here developed myotonia, histological defects, and splicing abnormalities reminiscent of DM1, they did not develop muscle wasting and weakness, the most debilitating skeletal muscle defects developed by patients with DM1. Importantly, more recent studies by Cooper and colleagues have shown that inducible expression of the mutant *DMPK* exon 15, encoding 960 CTG repeats, is sufficient to cause severe skeletal muscle wasting reminiscent of DM1 (Orengo et al., 2008). Importantly, consistent with the previous findings, this novel DM1 mouse model also shows consistently raised CUG-BP1 levels in skeletal muscle. Understanding the unique and aberrant molecular signaling that is triggered in this inducible mouse model, sufficient to cause muscle wasting, needs further investigation. Putting these data together, the gain of toxic function by the *DMPK* mRNA through CUG-binding proteins is currently the most convincing hypothesis for the disease mechanism of DM1.

Bidirectional transcription of the CTG expansion locus and development of congenital DM1 phenotypes

The animal models that have been developed for understanding the pathophysiology of DM1 did not recapitulate the dystrophic and degenerative defects commonly observed in DM1 with large repeat expansions, particularly in patients with congenital DM1. The transgenic mice expressing 250 CUG repeats and Mbnl-deficient mice recapitulated DM1-like splicing abnormalities and several of the key DM1 phenotypes, but failed to recreate the degenerative defects associated with large expansions in congenital DM1 phenotypes (Mankodi et al., 2002; Kanadia et al., 2003). However, histological analysis of skeletal muscle from transgenic mice overexpressing CUG-BP1 indicated myogenic defects reminiscent of congenital DM1 (Timchenko et al., 2004). Furthermore, the transgenic mice overexpressing CUG-BP1 developed DM1-like splicing anomalies for Clcn1, cardiac troponin (cTnnt2), and myotubularin-related 1 gene (*Mtmr1*) (Ho et al., 2005a). The aberrant splicing of *Mtmr1* has been demonstrated in congenital DM1 skeletal muscle as well as in myoblasts derived from patients with congenital DM1, but not in adult DM1 skeletal muscle, suggesting that the congenital muscle phenotypes in DM1 may result from the misregulation of alternate splicing mediated by CUG-BP1. Importantly, various other hypotheses have been proposed recently to explain the molecular basis of more severe and complex phenotypes in DM1, more specifically in congenital DM1. Recent studies have indicated the possibility that the *DM1* loci with massive CTG repeat expansions might be transcribed in a bidirectional fashion and that bidirectional transcription contributes to the development of more severe phenotypes in DM1 (Filippova et al., 2001). The CTG repeat in DM1 has been shown to be flanked by CTCF sites that are capable of functioning as insulators of the neighboring enhancers or altered chromatin domain structure in the *DM1* locus (Filippova et al., 2001). CTCF binding was also found to be inhibited by the CpG methylation that occurs in

the CTG expansion loci in congenital DM1 but not in adult DM1 cells (Steinbach et al., 1998; Filippova et al., 2001). CTCF binding has been found to be absent in the expanded and methylated DM1 allele, and was present in the wild-type, nonmethylated allele in a congenital DM1 cell (Cho et al., 2005). Bidirectional transcription has been shown to extend across the expanded CTG repeats in the *DM1* locus, and evidence suggests that the transcribed RNA is converted into siRNA-sized fragments on both alleles. However, antisense transcription is limited by the presence of CTCF sites in the wild-type allele (Cho et al., 2005). Additional experiments are needed to establish whether the CTCF-binding and bidirectional transcription of the mutant CTG expansion loci contribute to DM1 phenotypes, specifically dystrophic and degenerative defects.

Molecular pathogenesis of DM2

Like the CTG repeat in *DMPK*, the CCTG tetranucleotide repeat in the first intron of the *ZNF9* gene (Liquori et al., 2001) is transcribed into a CCUG repeat, but not translated into the protein. The normal function of the ZNF9 protein is unknown, although it is known to be capable of binding both DNA and RNA. Mice completely deficient for *Znf9* is embryonic lethal with gross disruption of forebrain development (Chen et al., 2003). Around 40% of heterozygous Znf9-deficient mice are also born with abnormal forebrain development and craniofacial abnormalities, and die within a few hours of birth, and the remaining 60% develop normally into adulthood or with mild eye and skeletal defects. These effects are not obviously related to the classical myotonic dystrophy phenotype. No data have been reported on the transcription of the *ZNF9* gene from the mutant chromosome in patients with DM2. Therefore, whether haploinsufficiency of *ZNF9* plays any pathogenic role in DM2 remains unknown. In addition to the similarities in clinical phenotypes, significant similarities between DM1 and DM2 have also been demonstrated in the molecular mechanism of the disease at the RNA level (Tapscott, 2000; Cho and Tapscott, 2007). The MBNL proteins accumulate in the nuclear foci where RNA with CCUG repeats are deposited (Mankodi et al., 2001, 2003; Cardani et al., 2006; Lucchiari et al., 2008), and *CLC-1* and IR mis-splicing is also observed in patients with DM2 (Day and Ranum, 2005; Botta et al., 2007). These data suggest that the *trans*-dominant RNA gain of function may play a central role in DM2. However, one should be reminded that there are clinical differences between these two diseases, most notably the lack of the congenital form in DM2 (Day et al., 2003). Whether the differences are due to the lack of loss of function of DMWD/DMPK/SIX5 in DM2 or due to different gain-of-function effects by the expanded CCUG repeat and/or loss of function of ZNF9 remains to be investigated.

MANAGEMENT OF CLINICAL MANIFESTATIONS

Despite our increasing understanding of DM1 and DM2 since the discovery of the mutation in 1992 and 2001, respectively, no specific therapies to improve the course of the disorder are currently available. However, symptomatic treatments play important roles in the management of patients with DM1 and DM2. Some pharmacological, surgical, physical, and psychological therapies are available for the alleviation of many organ-specific problems. Although these treatments do not alter the disease course, they provide significant improvements in the wellbeing of patients. Prevention of cardiac death and anesthesia-related respiratory complications are a critical part of symptomatic treatments. Equally important are counseling and support for patients and their families. Multidisciplinary integrative approaches may be valuable for the management of patients with myotonic dystrophies (Gagnon et al., 2007b). Experimental therapeutics to alter the disease course may be developed in the near future based on the pathogenic mechanisms, drug screening, and empirical discovery. A recent review of the present management and future therapy describes these issues in detail (Harper et al., 2004).

Organ-specific symptomatic treatments

Management of cardiac problems

Cardiac abnormalities in DM1 and DM2 could be life-threatening when complete heart block or ventricular fibrillation occurs. Cardiological management (Table 15.11) of DM1 has been reviewed recently by Duboc and colleagues (2004) and Sovari et al. (2007). Some experts recommend that all patients should undergo ECG annually, 24-hour Holter monitoring every 2 years, and echocardiography every 5 years, as long as neither cardiac symptoms nor ECG abnormalities develop (Duboc et al., 2004). Patients with conduction defects (symptomatic or asymptomatic atrioventricular (AV) conduction abnormalities) or ventricular dysrhythmias documented by these studies should undergo electrophysiological (EP) studies (Lazarus et al., 1999). Patients with syncope or near-syncope and those in preparation for major surgery under general anesthesia should also undergo EP studies (Clarke et al., 2001). Although intra-hisian conduction may be abnormal in the absence of ECG abnormalities, the indication for EP testing in patients

Table 15.11

Evidence-based management of cardiovascular phenotype in patients with myotonic dystrophy type 1 (modified from Sovari et al., 2007)

1	Baseline ECG and follow-up ECG (every 6–12 months) for asymptomatic patients with normal ECG findings (especially the elderly)
2	Early referral to cardiologist for any conduction abnormality seen on ECG, including asymptomatic sinus bradycardia or first-degree AV block
3	Insufficient data regarding management of patients with DM1 with only first-degree AV block because of the unknown rate of progression to complete heart block. EP study or close monitoring for development of symptoms of a more advanced ECG abnormality for asymptomatic patients with these types of conduction block. At least one study supporting pacemaker placement for HV interval >70 ms
4	EP study for patients with any further ECG abnormality, including second-degree AV block, bifascicular block, LBBB, first-degree + fascicular block, frequent PVCs or junctional premature beats, and nonsustained VT
5	Pacemaker/ICD placement for patients who meet classical indications (e.g., patients with third-degree AV block or Mobitz 2)
6	An aggressive diagnostic workup for conduction abnormality/arrhythmia with Holter monitoring/SAECG/EP study for patients with symptoms suggestive of arrhythmia (e.g., syncope, palpitation, shortness of breath) and no abnormality on ECG. EP study for patients with late potentials on SAECG
7	Holter/SAECG/EP study for patients with DM1 and a family history of SCD or VT/VF
8	Uncertainty in the management of asymptomatic patients with VT induced at EP study

AV, atrioventricular; ECG, electrocardiography; EP, electrophysiological; ICD, implantable cardioverter–debrillator; LBBB, left bundle branch block; PVCs, premature ventricular contractions; SAECG, signal-averaged electrocardiography; SCD, sudden cardiac death; VF, ventricular fibrillation; VT, ventricular tachycardia.

with normal ECG findings remains controversial (Bushby et al., 2003). Mildly affected young patients could develop ventricular fibrillation, especially when they suddenly engage in vigorous physical activity (Bassez et al., 2004). New technologies such as MRI of cardiac function (Vignaux et al., 2002) and turbulence-onset analysis by 24-hour Holter recording (Casella et al., 2006) may become useful for determining the necessity for EP studies. Cardiac pacemaker should be the primary mode of treatment for serious conduction problems (Lazarus et al., 1999, 2002). Simultaneous implantation of an automatic defibrillator may be considered.

Although the incidence of ventricular tachyarrhythmias may not be sufficiently high to justify implantable defibrillator therapy routinely when permanent pacing is indicated (Bushby et al., 2003), defibrillator implantation has been proven to be life-saving in some cases (Pelargonio et al., 2002). It should be remembered that pacemakers do not treat ventricular fibrillation.

Biventricular involvement, which may be an important cause of heart failure in myotonic dystrophy, may be diagnosed early by Doppler ECG and by pulsed tissue Doppler of lateral mitral annulus and tricuspid annulus (Parisi et al., 2007). Cardiac resynchronization therapy with biventricular pacing may be useful in patients with DM1 with intraventricular conduction delay and congestive heart failure (Kilic et al., 2007). Ventricular tachycardia due to focal myocardial degeneration in the DM1 heart may respond to radiofrequency catheter ablation (Muraoka et al., 2005). Beta-blockers and amiodarone may be useful for treatment of arrhythmias, whereas procainamide, flecainide, quinidine, disopyramide, and others should be avoided because of proarrhythmogenic effects (Duboc et al., 2004).

Prevention of anesthesia-related respiratory failure and other perioperative complications

Perioperative complications in patients with DM have been well recognized in the literature (Mathieu et al., 1997; Rogers and Clyburn, 2004). The most common scenario is that a patient with DM1 cannot be weaned from the ventilator after surgery. The diagnosis of DM1 may not have been established before surgery in some patients. A retrospective study of 219 patients with DM at their first operation under general anesthesia showed that 18 (8.2%) had complications, most frequently pulmonary. The risk of a perioperative pulmonary complication (PPC) was significantly associated with upper abdominal surgeries and severe muscular disability (Mathieu et al., 1997). PPC is attributable to the combination of weakness of the diaphragm, intercostal and abdominal muscles (Begin et al., 1997), and aggravation of impaired respiratory drive by the drugs used for anesthesia. Although pulmonary complications may occur with a wide variety of anesthetic drugs, the hazards have been associated mainly with the use of thiopental, suxamethonium, neostigmine, and halothane.

Preoperative and postoperative administration of sedatives and narcotics, such as benzodiazepines, barbiturates, and opiates, may cause acute respiratory failure. Use of midazolam and propofol for minor procedures and endotracheal intubation may be followed by unexpectedly prolonged hypoventilation and difficulties in weaning off mechanical ventilation, although these agents can be used safely with controlled dosing (Nishi et al., 2004; Morimoto et al., 2005). The risk of pulmonary aspiration is increased in patients with DM1 owing to abnormal pharyngoesophageal functions. Careful selection of anesthetic drugs and doses, close monitoring during the early postoperative period, protection of the upper airway, chest physiotherapy, and incentive spirometry are recommended (Rogers and Clyburn, 2004).

Myotonia may be induced by drugs used in anesthesia, especially anticholinesterases (e.g., neostigmine), depolarizing neuromuscular blocking agents (e.g., succinylcholine), and inhalational anesthetics, as well as by hyperkalemia, hypothermia and shivering, and mechanical or electrical muscle stimulation (Mathieu et al., 1997; Boyle, 1999; Rogers and Clyburn, 2004). Localized myotonia in masseters, tongue, or pharynx may cause difficulty with endotracheal intubation. It should be remembered that blocking the neuromuscular junction does not alter myotonia.

Stress of surgery, certain anesthesia induction agents, hypoxia, and hypotension may reveal latent arrhythmias and conduction blocks, frequently in relatively young affected, but often undiagnosed, patients (Lazarus et al., 1999). Thus, patients with unrecognized mild DM1 may develop life-threatening cardiac complications. Anesthesiologists may encounter cardiac failure due to dilated cardiomyopathy, and should be aware of hypotension, which may result from vascular smooth muscle dysfunction and decreased cardiac output. A relatively small amount of intraoperative hemorrhage may cause life-threatening hypotension (Blumgart et al., 1990).

Overall, preoperative recognition of the disease, perioperative awareness of the diagnosis with expectation of possible complications, keeping the doses of anesthetic drugs to a minimum, and avoiding certain anesthetics, depolarizing neuromuscular blockers, and anticholinesterases are key to minimizing the anesthesia-related risks in patients with DM1.

Treatment of skeletal muscle problems

Although myotonia is a frequent and prominent clinical symptom, it is only infrequently troublesome enough to require drug therapy. Myotonia causes inconvenience or embarrassment, but most patients learn to cope with the symptoms. However, grip myotonia may interfere with effective use of the hands, as required in patients' occupation or hobby. For these patients, mexiletine, phenytoin, carbamazepine, procainamide, or quinine are agents that have been helpful. The teratogenic possibilities and the cardiac adverse effects of all of these agents must be borne in mind.

Although muscle weakness is a major cause of disability in DM1 and DM2, no drug therapy has shown convincing efficacy in improving the muscle strength. Testosterone has been shown to increase the muscle mass, but it failed to improve strength of muscles (Griggs et al., 1989). Physical therapy, occupational therapy, and speech therapy provide practical benefits in maximizing the ability of patients to use diseased muscles (Aldehag et al., 2005). Therapists should be aware of different life habits, especially the level of participation, and needs of patients in various stages of the disease (Gagnon et al., 2007a). It should also be noted that, although these treatments have generally failed to show sustained improvements in muscle strength (Lindeman et al., 1999; Tollback et al., 1999), recent studies have suggested that 12 weeks of low-intensity aerobic training improves maximal oxygen uptake and workload (Orngreen et al., 2005).

Pain management is an important part of treatment in myotonic dystrophies, especially in DM2. Although the pain is usually not excruciatingly severe, chronic or intermittent aches and pains, most frequently in limb muscles, can be quite disruptive. Different medications and combinations of medications work for some individuals; these medications include mexiletine, gabapentin, nonsteroidal anti-inflammatory drugs (NSAIDs), low-dose thyroid replacement, low-dose steroids, and tricyclic antidepressants. Unfortunately, most patients do not achieve satisfactory resolution of the pain with these medications. A comprehensive pain management approach may be useful.

Management of eye problems

Removing cataracts is a simple solution to restore the visual acuity in patients with severe lens opacities, although recurrent capsular opacification, capsulorhexis contracture, and transient rubeosis iridis have been reported after cataract surgery (Garrott et al., 2004; Baig and Discepola, 2007). For patients whose blepharoptosis interferes with their vision, blepharoplasty may be useful. However, risks of general anesthesia should be taken into considerations for these minor surgeries. There have been no reports of treatments for DM1 retinopathy.

Treatment of gastrointestinal problems

In patients with mild and vague gastrointestinal manifestations, routine endoscopic and ultrasonographic

evaluation may be of little value, and only highly sensitive targeted techniques, such as electrogastrography, manometry, electromyography, functional ultrasonography, and compartmental scintigraphy, may help to reveal the true extent of the involvement. Manometry is useful to detect motility disturbances with a reduced basal upper esophageal sphincter pressure in patients with DM1 (Costantini et al., 1996; Lecointe-Besancon et al., 1999; Modolell et al., 1999).

Diarrhea and constipation are common complaints of patients with DM1. Fiber supplements, stool softeners, and nonirritant laxatives are useful in the treatment of constipation. Gastroparesis, which may cause pseudo-obstruction and megacolon, may be treated with the prokinetic drug metoclopramide (Horowitz et al., 1987; Ronnblom et al., 2002). Erythromycin, a motilin agonist, is used in the short-term management (Nowak et al., 1984; McCallum and Brown, 1998; Ronnblom et al., 2002). Diarrhea may be treated with cholestyramine, which reduces bile acid malabsorption. Fecal incontinence due to anal sphincter involvement is a frequent and disturbing manifestation of DM1. Although conflicting results have been reported, procainamide may be tried, with precaution for cardiac adverse effects before resorting to surgical procedures, such as postanal repair, which may not provide sustained improvements.

Cholelithiasis (gallstones) could be a major problem in patients with DM1. Although advances in laparoscopic surgical technology have reduced surgical morbidity for patients who develop gallbladder diseases, the DM1-related anesthesia risk must be taken into consideration (Takhar et al., 2004).

Treatment of CNS problems

Cognitive impairments in children and adults with DM1 pose a major disability. However, there have been no satisfactory treatments for the cognitive impairments in DM1. Educational programs and school counseling are of foremost importance for children with cognitive impairment. In adult patients, various medications have been tried but none so far has been reported to have beneficial effects. Modafinil may improve the alertness and level of activity of patients with DM1 (Damian et al., 2001; MacDonald et al., 2002; Talbot et al., 2003; Wintzen et al., 2007), but effects of this drug on cognitive performance have not been evaluated. Anticholinesterases and memantine, which are used for treatment of Alzheimer's disease, have not been tried in patients with DM1.

Excessive daytime sleepiness (EDS) may be a multifactorial problem. Sleep apnea, hypoventilation, and hypercapnea may contribute to EDS, although the central mechanism seems to play a major role (Hilton-Jones et al., 2004). When EDS is identified in a patient with DM1, the patient's lifestyle, including alcohol, caffeine, and medication use, daytime activities, sleep environment, duration and interruptions of night sleep, daytime naps, and exposure to sunlight should be explored, and corrective measures taken. If the problem continues, sleep studies should be done. Sleep apnea and other sleep disorders treatable with assisted nocturnal ventilation should be treated with appropriate measures: for example, continuous positive airway pressure (CPAP) for obstructive sleep apnea and bilevel positive airway pressure (BiPAP) by nasal mask for nocturnal hypoventilation or central sleep apnea (Hilton-Jones et al., 2004). It should be kept in mind that sleep apnea may precipitate DM1 cardiac problems (Lazarus et al., 2007). Modafinil should be tried in patients who have normal breathing patterns during sleep or no satisfactory benefits from these treatments (MacDonald et al., 2002; Talbot et al., 2003).

For children with behavioral disturbances, which may be an associated feature of DM1, pharmacotherapy with psychological counseling may be appropriate. For example, methylphenidate and its extended release formula, and, potentially, modafinil, may be useful in children with attention-deficit/hyperactivity disorder. Antidepressants and anxiolytics may be useful when patients show signs of depression and anxiety.

Other treatments

Surgical treatments are important for some patients with myotonic dystrophy. Cataractectomy, cholecystectomy, blepharoplasty, and cardiac pacemaker/defibrillator implantation have already been discussed. Surgical correction of talipes and orofacial deformities (Engvall et al., 2007; Sjogreen et al., 2007) are useful, particularly in children when these problems cause significant functional impairments. However, these simple surgical procedures may not be so simple in patients with myotonic dystrophy because of potentially fatal anesthesiological complications involving respiratory failure. Percutaneous endoscopic gastrostomy (PEG) and tracheostomy are often needed at the late stage of DM1.

More important for most patients are general measures to avoid many needless complications and also to enhance everyday life. Regular surveillance for cardiac conduction defects, along with avoidance of surgical and anesthetic hazards, ranks highest in this respect. Sadly, many patients still never receive such management. Mildly affected individuals with little physical disability are probably at greatest risk, because the

risks are unappreciated. Conversely, necessary surgical measures, such as cataract extraction and correction of foot deformities, should not be withheld simply on the grounds of possible surgical complications.

Major mobility aids, such as wheelchairs, are not needed at all by many patients or, if they are, only at a late stage, but thorough assessment of needs for assisting devices is important to avoid loss of mobility and independence. Light plastic ankle splints to correct foot drop may make a considerable difference.

Among the more general measures of importance, awareness of the possibility of aspiration and pneumonia resulting from swallowing dysfunction ranks high, as does the need to assess respiratory function regularly, especially if hypoventilation is suspected as being a significant feature. Abdominal pain is a common complaint, and may (although not always) respond to agents giving relief in irritable bowel syndrome.

Finally, the importance of giving patients and families full details regarding their condition should not be underestimated. Informed patients with DM1 and DM2 are likely to know more about their own complaint than most of the doctors and other professionals that they may encounter. Full written information produced in an easily readable style is probably the best way of ensuring that patients avoid most predictable complications of the disease. Helpful information of this type is available at the web site of the Myotonic Dystrophy Foundation (www.myotonic.org) and the Muscular Dystrophy Association (www.mda.org) in the USA, the Myotonic Dystrophy Support Group (www.patient.co.uk/showdoc/26738834/) and Muscular Dystrophy Campaign (www.muscular-dystrophy.org) in the UK, and the Association Française contre les Myopathies (www.afm-france.org/) in France, amongst others. These issues have been reviewed extensively in a recent book *Myotonic Dystrophy: Present Management, Future Therapy* (Harper et al., 2004).

Experimental therapeutics

As discussed in the previous section, there has been increasingly convincing evidence for the *trans*-dominant RNA gain of function as the main pathogenic mechanism in DM1 and DM2. Experimental therapies using antisense oligonucleotides and ribozyme to counteract the RNA gain of function have been studied in cell culture and transgenic mice (Bell et al., 2002; Furling et al., 2003; Langlois et al., 2003, 2005; Phylactou, 2004). Impaired transport of the mutant *DMPK* RNA transcript from the nucleus to cytoplasm may play a role in the disease mechanism and may become a future therapeutic target (Kim et al., 2005; Mastroyiannopoulos et al., 2005). Better understanding of the mechanism of the repeat instability may lead to the development of treatments to reduce the repeat size in patients (Gomes-Pereira and Monckton, 2004; Hashem et al., 2004). However, much more work is needed to develop therapies that can slow down, halt, or reverse the progression of the disease.

Potential therapeutic targets are expanded repeats in the *DMPK* and *ZNF9* genes (DNA) and their mRNAs. Drugs that can manipulate the functions of mismatch repair and other DNA repair genes might be able to direct the repeat instability toward contractions. Chemotherapeutic agents that produce double-strand breaks and other DNA damage, or inhibit DNA replication, may promote deletions of the repeats (Gomes-Pereira and Monckton, 2004; Hashem et al., 2004; Pearson et al., 2005). However, these approaches may have significant adverse effects on genomic integrity. Targeting the expanded repeats may alleviate such problems, and this may be achievable more readily if the target is RNA.

Organ-specific experimental therapeutics, which mostly target skeletal muscle, have been reviewed extensively by Moxley and colleagues (2004). The drugs reviewed include testosterone, recombinant human growth hormone (rhGH), insulin-like growth factor-1 (IGF-1), dehydroxyepiandrosterone sulfate (DHEA-S), troglitazone, tricyclic antidepressants, antioxidant free-radical scavengers (vitamin E and selenium), and creatine monophosphate, which were intended to improve muscle mass and strength; tocainide, phenytoin, carbamazepine, procainamide, disopyramide, nifedipine, dantrolene sodium, acetazolamide, taurine, and mexiletine for myotonia; and modafinil for hypersomnia. None of the drugs showed definitive efficacy in improving the muscle strength, although rhGH and testosterone increased muscle mass, and tricyclic antidepressants improved some muscle strength. Intravenous administrations of DHEA-S showed encouraging results in an open trial (Sugino et al., 1998), and subcutaneous injections of IGF-1 improved muscle mass and strength in a small number of patients who received IGF-1 in a dose greater than 70 μg/kg (Vlachopapadopoulou et al., 1995). Further studies are needed to confirm these results. Tocainide, phenytoin, carbamazepine, procainamide, disopyramide, and acetazolamide improved myotonia, taurine stabilized membrane excitability, nifedipine gave subjective improvements, and dantrolene sodium had no effect. However, potential adverse effects on cardiac conduction and arrhythmias limit the usefulness of many of these medications. Mexiletine also showed beneficial effects on myotonia without cardiac adverse events (R.T. Moxley, personal communication). Modafinil reduced somnolence and mood, and is already used in practice.

In anticipation of upcoming new therapeutic trials, outcome measures for muscle and other phenotypes

of DM1 and DM2 are being developed (reviewed in Moxley et al., 2004). In addition to those reviewed by Moxley and colleagues, the 6-minute walk test (Kierkegaard and Tollback, 2007), computerized hand grip myometry (Logigian et al., 2005; Moxley et al., 2007), and electrical bite force, ankle dorsiflexion, and pinch grip dynamometry (Whittaker et al., 2006; Guimaraes et al., 2007) have been used for assessment of muscle strength or function in patients with DM1. As fatigue is a prominent functional problem in DM1 (Kalkman et al., 2006), the Daytime Sleepiness Scale, Chalder Fatigue Scale, and Krupp's Fatigue Severity Scale (Laberge et al., 2005) have been validated in patients with DM1.

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Chapter 16

Distal muscular dystrophies

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INTRODUCTION

Distal muscular dystrophies are a group of inherited primary muscle disorders showing progressive weakness and atrophy preferentially in the hands, forearm, lower legs. or feet. Extensive progress in understanding the molecular genetic background has changed the classification and extended the list of confirmed entities (Tables 16.1 and 16.2). At the same time it is evident that some defects underlying distal dystrophies may also cause other clinical phenotypes, whereas other genes are associated only with distal phenotypes.

In the clinical diagnostic setting the clinician has to rely on clinical features and results of laboratory investigations for diagnosis and selection of eventual genes to be tested. The list of genes associated with muscular dystrophy is growing fast and not all can be tested in any dystrophic patient. Thus, the clinical classification of distal dystrophy still serves practical needs by limiting the range of known genes to consider.

The first family described as having a distal myopathy and later also verified by molecular genetics to be a genuine distal muscle disease was reported in 1943 (Milhorat and Wolff, 1943). Six patients in a dominant family with 12 affected males had distal leg weakness with onset in early adulthood. Later studies in this family showed desmin accumulations in muscle biopsy and a mutation in the desmin gene was proven to be the cause (Sjöberg et al., 1999). The first distal myopathy defined by molecular genetic linkage, however, was the autosomal dominant early-onset distal myopathy reported by Laing et al. (1995). One well-known distal dystrophy still remains without a known gene defect: Welander distal myopathy (WDM), described originally in 72 Swedish pedigrees with the term myopathia distalis tarda hereditaria (Welander, 1951). The genes responsible for distal phenotypes seem preferentially to involve sarcomeric proteins, compared with the sarcolemmal protein defects associated with proximal muscular dystrophies (Figure 16.1).

In addition to the disorders listed in Tables 16.1–16.3, a number of less well established distal syndromes exist in the literature in which a retrospective classification is difficult (Murone et al., 1963; Mehrotra et al., 1964; Huhn, 1966; Mamoli and Scarlato, 1969; Cabella and Candelero, 1970; Miller et al., 1979). Distal weakness and atrophy may also be the presenting symptom and sign in dystrophies characterized by other major findings, such as facioscapulohumeral muscular dystrophy (FSHD) and myotonic dystrophy type 1. These disorders are mentioned only briefly in this chapter. The distal presentation of those dystrophies that are mainly covered in other chapters, such as dysferlinopathy, GNE-mutated disease, and myofibrillar myopathies, are discussed briefly in this chapter.

WELANDER DISEASE

In the late 1940s the Swedish neurologist Welander collected a large amount of data involving 249 patients and published details of the disease, myopathia distalis tarda hereditaria, in her thesis (Welander, 1951). First symptoms were reduced finger extension and clumsiness in precise movements, beginning in the thumb or index fingers after the age of 40 years (Figure 16.2A,B). Distal leg involvement usually started later, with inability to stand on the heels or development of a steppage gait. In a minority, symptoms started in the lower limbs. Slow progression consisted of weakness and wasting of small muscles of the hands and both anterior and posterior compartments of lower legs. Involvement of distal flexors developed later in the disease process. These clinical observations were subsequently verified by muscle imaging (Mahjneh et al., 2004). Proximal limb muscle

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Table 16.1

Distal dystrophies with known gene defect

Type	OMIM # or reference	Onset		CK	Muscle pathology	Genetics		
		Age (years)	Early symptoms			Inheritance	Gene	Locus
Desminopathy	601419	Variable	Anterior lower leg, scapular; cardiomyopathy	Variable	Dystrophic, rimmed vacuoles, myofibrillar	AD	*Desmin*	2q35
Distal dysferlinopathy	254130	15–30	Posterior lower leg, calf	10–100×	Dystrophic	AR	*Dysferlin*	2p13
Miyoshi myopathy								
GNE-mutated DMRV	605820	15–30	Anterior lower leg	1–5×	Rimmed vacuoles	AR	*GNE*	9p1–q1
Nonaka myopathy								
Tibial muscular dystrophy	600334	>35	Anterior lower leg	1–4×	Dystrophic, rimmed vacuoles	AD	*Titin*	2q31
Udd myopathy								
Distal myosinopathy	160500	1–25	Anterior lower leg	1–8×	Type 1 fiber atrophy in TA muscle, (no) vacuoles	AD	*MYH7*	14q
Laing myopathy								
ZASPopathy	Markesbery et al. (1974)	>40	Anterior lower leg	1–3×	Large vacuoles, sarcoplasmic dark masses, myofibrillar	AD	*ZASP*	10q
Markesbery–Griggs disease								
Distal myotilinopathy	Penisson-Besnier et al. (2006)	50–60	Posterior lower leg	1–2×	Nonrimmed vacuoles, dark sarcoplasmic masses, myofibrillar	AD	*Myotilin*	5q31
Distal nebulinopathy	Wallgren-Pettersson et al. (2007)	1–18	Anterior lower leg	1–3×	No nemaline rods on light microscopy, no rimmed vacuoles	AR	*Nebulin*	2q21
Vocal cord and pharyngeal distal myopathy (VCPDM)	606070	35–60	Anterior lower legs, hands, dysphonia	1–3×	Rimmed vacuoles	AD	*Matrin3*	5q31.2

AD, autosomal dominant' AR, autosomal recessive; CK, creatine kinase; DMRV, distal myopathy with rimmed vacuoles; OMIM, Online Mendelian Inheritance in Man; TA, transversus abdominis.

Table 16.2

Distal dystrophies without known gene defect

Type	OMIM # or reference	Onset		CK	Muscle pathology	Genetics	
		Age (years)	Early symptoms			Inheritance	Locus
Welander distal myopathy (WDM)	604454	>40	Hands, finger extensors	1–4×	Dystrophic, rimmed vacuoles	AD	2p13
Distal myopathy with pes cavus and areflexia	601846	15–50	Anterior and posterior lower leg, dysphonia and dysphagia	2–6×	Dystrophic, rimmed vacuoles	AD	19p13
Adult-onset distal myopathy	Felice et al. (1999)	20–40	Foot drop and mild proximal weakness	2–6×	Nonspecific mild changes in proximal muscle	AD	2, 9, 14 excluded
Early adult-onset distal myopathy	Williams et al. (2005)	18–40	Posterior–lateral lower leg	1–2×	Myopathic – dystrophic	AD	12 genetic loci excluded
Variable-onset distal myopathy	Sumner et al. (1971)	15–50	Forearm and/or lower leg		Nonspecific	AD	
Adult-onset distal myopathy (*MPD3*)	Mahjneh et al. (2003)	>30	Hands or anterior lower leg	1–4×	Dystrophic, rimmed vacuoles and eosinophilic inclusions	AD	Both 8p-q and 12q are linked
Distal myopathy with respiratory failure	607569	32–75	Anterior lower leg in some, and proximal in some	1–2×	Dystrophic, rimmed vacuoles and eosinophilic inclusions, and amyloid and desmin	AD	2, 9, 14 excluded

AD, autosomal dominant; CK, creatine kinase.

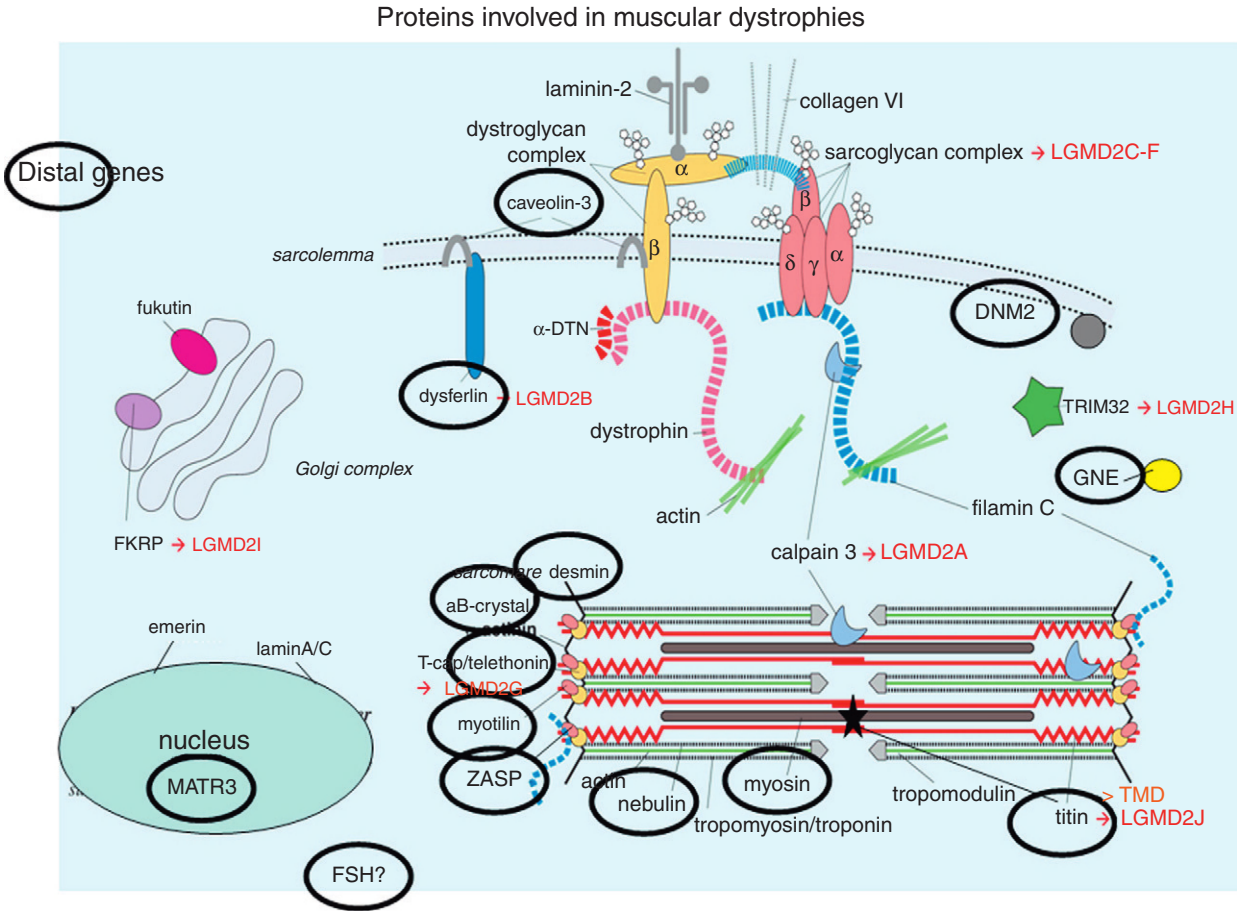

Figure 16.1. Schematic figure showing the subcellular locations of proteins associated with muscular dystrophies with the distal dystrophy genes encircled.

Table 16.3

Distal phenotypes in other dystrophies

	Onset				Genetics		
Type	Age (years)	Early symptoms	CK	Muscle pathology	Inheritance	Gene	Locus
VCP-mutated dystrophy	Adult	Anterior lower legs	1–4×	Rimmed vacuoles	AD	*VCP*	9p13–p12
Myofibrillar myopathy (*CRYAB*)	Adult	Distal leg and hands; cardiomyopathy		Granulofilamentous desmin aggregates	AD	*αB-crystallin*	11q22–23
Desmin-related with sarcoplasmic bodies (Edström et al., 1980)	40	Hands, thenar, finger flexors	1–3×	Sarcoplasmic bodies	AD		
Oculopharyngeal distal myopathy (OPDM)	>40 in AD, <40 in AR	Lower leg and hands; extraocular	1–5×	Rimmed vacuoles	AD and AR		
Distal onset in telethoninopathy	Early	Lower leg, anterior	3–10×	Rimmed vacuoles	AR	*Telethonin*	17q12
Caveolinopathy	Early	Hands	3–10×	Reduced caveolin-3	AD	*CAV3*	3p25
Dynaminopathy	Variable	Anterior lower legs	1–3×	Central nuclei	AD	*DNM2*	19p13.2

AD, autosomal dominant; AR, autosomal recessive; CK, creatine kinase.

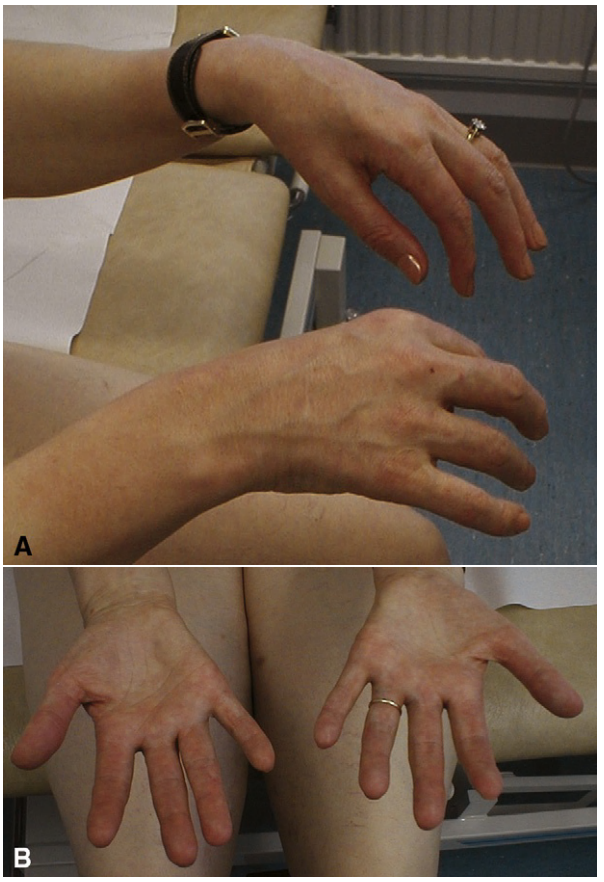

Figure 16.2. Welander disease. (**A, B**) The hands of a 47-year-old woman with symptoms of hand and finger weakness for 4 years. Early signs of mild small hand muscle (thenar and hypothenar) atrophy are visible, as well as reduced finger extension, particularly of the index fingers.

weakness was observed in 14% of the patients. At later disease stages, ankle tendon reflexes were lost. Many patients report coldness of the hands and feet. Clinically, only a mild decrease in vibration sense at an older age can be observed. The age of onset varies between 20 and 77 years (mean 47 years), with a male-to-female ratio of 1.5 : 1. The disease shows an autosomal dominant inheritance with slightly reduced penetrance.

In her thesis, Welander (1951) proposed a mild neurogenic component in WDM. Later studies have reported abnormalities on sural nerve histology (Borg et al., 1987, 1989). Studies in middle-aged adults with early symptoms of WDM revealed distal temperature sensory disturbances. Neither abnormalities of sensory nerve conduction velocities nor any neurogenic findings in eight anterior tibial muscle biopsies have been observed (Borg et al., 1991).

Epidemiology

No precise studies on the epidemiology of WDM have been performed. Estimates based on patient numbers at different regional neuromuscular centers indicate a prevalence about 5–8 per 10^5 population in Sweden (L. Edström, personal communication). Recent data have emerged from Finland where WDM has been identified in tens of families (von Tell et al., 2002). Other reports on Scandinavian cases are rare. Duemler (1962) described a Swedish patient, living in the USA, with clinical and electromyographic findings similar to those of WDM. Dahlgaard (1960) reported a 72-year-old Danish woman with a 10–15-year progressive course of distal extremity weakness.

Molecular genetics

Well-studied WDM families were used for linkage studies, and a locus on chromosome 2p13 was established with a LOD (logarithm of odds) score greater than 17 (Åhlberg et al., 1999). The WDM locus is just outside the *dysferlin* locus (von Tell et al., 2003). All known genes in the linked region of interest have been sequenced without finding the causative gene (von Tell, 2004). All patients with WDM in Sweden and Finland carry the same haplotype at the 2p13 locus, indicating a single founder mutation in the Scandinavian patients with WDM (Åhlberg et al., 1999; von Tell et al., 2002). No families with another defined genetic background have so far been reported.

Clinically, more than 90% presented with the "typical" form, in which the disease followed a dominant inheritance trait, progressed slowly, and remained distal to the elbows and knees (Welander, 1951). In 4% of patients the clinical phenotype was "grossly atypical" (9 of 249), with early involvement of proximal limb muscles and long flexors of the fingers and feet, a much more rapid disease evolution leading to severe disability (Welander, 1957). Because in one family the parents of these patients were affected with the typical disease, they were thought to represent a homozygous manifestation of the abnormal gene (Welander, 1957). At the time of molecular genetic studies these original "homozygotes" were no longer available for analysis. However, all their offspring were heterozygotes, supporting the original hypothesis, and one patient in one of the families included in the linkage studies was homozygous for the linked haplotype, confirming the more severe dystrophy phenotype compared with typical WDM (Åhlberg et al., 1999).

Laboratory investigation, imaging and muscle pathology

In patients with WDM, serum creatine kinase (CK) levels may be normal or show an up to 3-fold increase (Edström, 1975). Electromyographic (EMG) studies show brief, small, and polyphasic motor unit potentials, and in severely affected muscles a decreased number of motor unit potentials. Spontaneous

electrical activity at rest with fibrillation potentials and repetitive discharges have been reported (Edström, 1975). Computed tomography (CT) and magnetic resonance imaging (MRI) show fatty degenerative changes of the affected muscles, both anterior and posterior compartment soleus and gastrocnemius muscles in the leg (Åhlberg et al., 1994, Mahjneh et al., 2004).

In the original description, the findings in 55 biopsy specimens were reported from muscles with variable involvement (Welander, 1951). Early changes were increased variation in fiber size, central nuclei, and increased connective tissue. Marked alterations consisted of split fibers, vacuolation of fibers, fatty deposition, and macrophage infiltration. At the end stage there was replacement by connective and fatty tissue, with only a few muscle fibers remaining. Later morphological studies reported an increase in centralized nuclei, often in chains, and type 1 fiber atrophy. In advanced stages, the differentiation of type 1 and 2 fibers was blurred, and ring fibers and sarcoplasmic masses were common (Edström, 1975). In a later series of patients with WDM, rimmed vacuolar pathology as well as angulated fibers were seen (Borg et al., 1987). In 1994, the same investigators reported mild to severe changes in tibial anterior and soleus muscles, whereas the findings in vastus lateralis biopsies were considered normal. Rimmed vacuoles were found in atrophic fibers of both fiber types in affected muscles. By and large, no definite fiber-type grouping was encountered (Åhlberg et al., 1994).

Ultrastructurally, tibialis anterior specimens revealed autophagic vacuoles with dense bodies, myelin figures, and glycogen (Borg et al., 1987). In addition, 15–18-nm tubulofilamentous inclusions were observed on electron microscopy (EM) in WDM (Borg et al., 1991).

Autopsy findings in three patients, symptomatic for 9–16 years, included normal proximal arm muscles, mild myopathic changes in thigh, calf, and forearm muscles, and advanced myopathic changes in distal muscles. Spinal cord, ventral roots, and peripheral nerves were normal (Welander, 1951).

Management

There is no specific treatment for WDM. Ankle–foot orthoses are of benefit for foot drop.

TIBIAL MUSCULAR DYSTROPHY (TMD) – TITINOPATHY (UDD MYOPATHY)

In 1993, a new dominant distal dystrophy was reported in 66 Finnish patients (Udd et al., 1993). Onset of symptoms with reduced ankle dorsiflexion occurred after the age of 35 years, selectively involving the tibialis anterior and, in advanced stages, the long toe extensor muscles (Figure 16.3A,B). For many years the weakness could be very asymmetrical and progression was slow. After age 70 years, mild to moderate proximal leg muscle weakness occurred in a minority of the patients. Patients rarely become wheelchair-bound, even at advanced age. An important sign for the clinician is the sparing of extensor digitorum brevis muscles as a distinction from neurogenic foot drop. In exceptional cases only hand muscles were

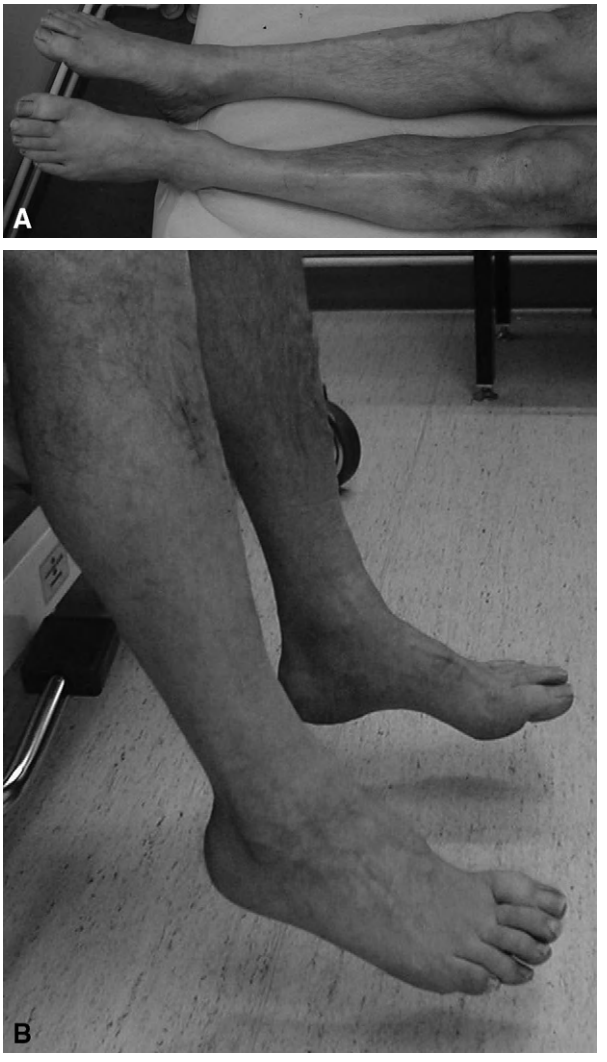

Figure 16.3. Tibial muscular dystrophy (TMD, titinopathy, Udd myopathy). (**A, B**) A 52-year-old man with reduced ankle dorsiflexion for 12 years. Mild foot drop, atrophy of the tibialis anterior, and subsequent prominence of the ventral edge of the tibial bone (**A**). Attempts to lift the feet makes the toes move upwards with the contraction of extensor digitorum brevis muscles and no significant ankle dorsiflexion (**B**).

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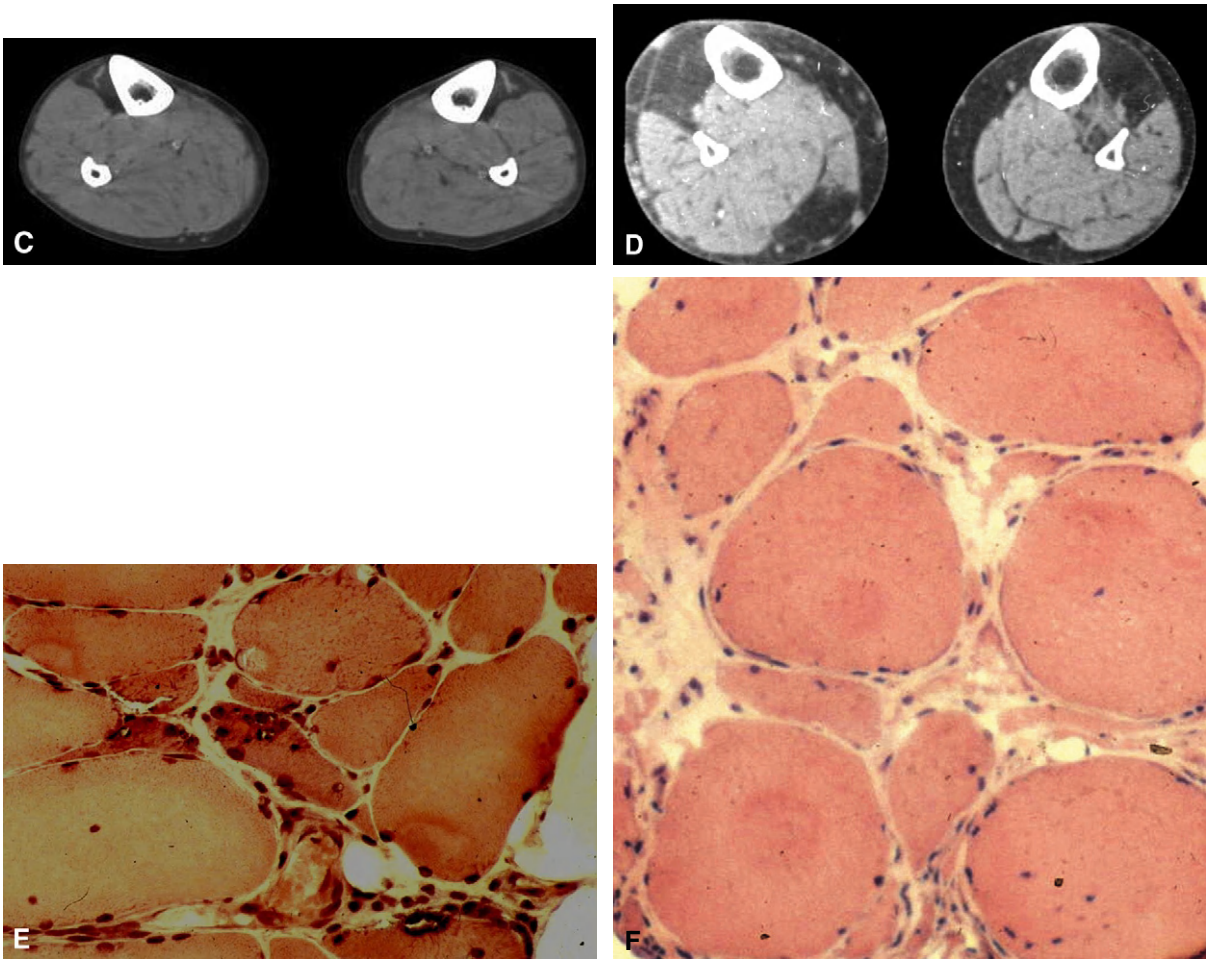

Figure 16.3 – Cont'd (**C, D**) Computed tomogram of the lower leg muscles in a 42-year-old patient with TMD showing fatty degenerative lesions in the anterior tibial muscle, while all other muscles are intact (**C**). Magnetic resonance imaging of the lower legs in a 70-year-old patient with TMD reveals more extensive dystrophic replacement of muscle tissue in all anterior compartment muscles and also in medial gastrocnemius (**D**). (**E, F**) Light microscopic muscle biopsy findings in the tibialis anterior muscle of a 44-year-old patient with TMD showing mild dystrophic changes and a few rimmed vacuolated fibers (**E**). In a muscle biopsy from gastrocnemius, a muscle not usually affected clinically, dystrophic changes may be present without rimmed vacuolar change (**F**).

affected in TMD. A later study of 207 mutations confirmed considerable variation of the phenotype in 9% of patients (Udd et al., 2005). The uncommon phenotypes included: onset of weakness and atrophy in proximal leg muscles, involvement of upper limb muscles, onset of generalized weakness in childhood, persistent asymmetrical and focal atrophies, and mild bulbar and facial weakness. All of these additional signs of muscle involvement were found in combinations with typical lesions in tibialis anterior muscle, except for one patient with proximal leg muscle involvement and another with posterior calf muscle involvement who presented with no anterior compartment involvement of the lower leg muscles (Udd et al., 2005).

Epidemiology

Studies have shown that the prevalence in Finland is more than 10 per 100 000 population (Udd, 2007). TMD is the most common muscle disease in Finland, and patients are found all over the country. However, the origins of the patients' families are usually located in the west coast region of middle Finland or in the Savo–Karelia area of eastern Finland (Udd et al., 1993). Descendants of Finnish immigrants with TMD have also been identified in Sweden, Norway, Germany, and Canada. TMD families with no ancestry in Finland have been diagnosed in several other European populations in France, Belgium, and Spain (de Seze et al., 1998; van den Bergh et al., 2003; Udd et al., 2005; Hackman et al., 2008).

Molecular genetics

Linkage of TMD to a new myopathy locus on chromosome 2q31, based on the Finnish families, was established in 1998 (Haravuori et al., 1998b). All studied dominant families presenting with a TMD phenotype carried the same haplotype, indicating an ancient founder mutation. Patients with TMD abroad and with known ancestry in Finland also carried the Finnish TMD haplotype. Genetic complexity existed in one large consanguineous pedigree in which, besides a large number of patients with TMD, eight members presented a totally different limb-girdle muscular dystrophy (LGMD) phenotype. These patients were all thought to be homozygotes for the dominant TMD gene. Five of the eight belonged to one nuclear family in two generations. These five patients had childhood onset of proximal weakness and were wheelchair-bound after 20–30 years. Molecular analysis confirmed homozygosity for the TMD haplotype in these five patients (Haravuori et al., 1998a). The other three of the eight patients showed juvenile to early-adult onset of disease and belonged to two different other nuclear families of the large pedigree. Molecular analysis in these three patients excluded a TMD-related disorder (Haravuori et al., 1998a).

Within the linked locus 2q31 there was one large muscle gene, titin (Haravuori et al., 2001). Sequencing through the large 100-kilobase coding region of the gene revealed mutations in the last exon, Mex6 (Hackman et al., 2002). The mutated part encodes for the C-terminus of the M-line segment of titin protein. In Finnish patients with TMD the founder mutation (FINmaj) was a complex 11-base pair deletion–insertion mutation changing four consecutive amino acids inframe in the last immunoglobulin-like domain, M10, of titin protein (see Figure 16.1) (Hackman et al., 2002). Two unrelated French families carry a point mutation, changing a lysine to proline in the same last Mex6 exon (Hackman et al., 2002; Udd, 2007). Later, a third mutation in the same last exon was found in a Belgian TMD family (van den Bergh et al., 2003), and recently other new mutations, in the last and second to last exons, have been identified in Spanish and other French TMD families (Hackman et al., 2008). Diagnostic DNA testing in new unrelated TMD patients is currently performed by sequencing the last three exons.

Homozygosity for the Finnish FINmaj mutation was confirmed in all four available patients with the LGMD phenotype with childhood onset previously identified with homozygosity for the linked 2q31 haplotype. As the phenotype in the homozygotes is transmitted in a recessive fashion, it has been denominated LGMD2J (Udd et al., 2005).

Laboratory investigation, imaging, and muscle pathology

Serum CK levels are slightly raised in patients with TMD, ranging from normal to 4–5 times the upper normal limit (Udd et al., 1993). EMG studies show low-amplitude, short-duration motor unit potentials on moderate activity in affected muscles (Udd et al., 1991a). In the anterior compartment of lower legs increased insertional activity, frequent fibrillation potentials, and occasional high-frequency and complex repetitive discharges at rest may be found. Some polyphasic potentials may be recorded in clinically unaffected muscles of the upper limbs (Udd et al., 1993).

The highly selective involvement of individual muscles in TMD can be assessed accurately by CT and MRI. Changes of fatty degeneration in tibialis anterior muscles appear at the time of clinical weakness (Udd et al., 1991b). In TMD, the evolution of selective muscle involvement over time is very distinct. After 10–15 years of ankle dorsiflexion weakness, lesions appear in the long toe extensor muscles together with increasing foot drop. At the same time changes occur in hamstring, gluteus minimus, and tensor fasciae latae muscles (Figure 16.3C,D) (Udd et al., 1991b). Initially, the involvement may be asymmetrical. Other focal lesions may be present, such as in the soleus or gastrocnemius medialis muscles. However, such calf lesions rarely affect the gait (Udd et al., 1991b).

Muscle biopsy findings in TMD depend on the muscle specimen sampled: in affected anterior compartment muscles of the leg, myopathic alterations, including rimmed vacuolar change, variation of fiber size, thin atrophic fibers, central nuclei, internal structural changes in myofibers, endomysial fibrosis, and, in the end-stage muscle, advanced dystrophy with fatty replacement. Necrotic fibers undergoing phagocytosis are rare in TMD. Both major fiber types are equally involved in the pathological process, without neurogenic findings. Acid phosphatase activity was increased in many, and ubiquitin was expressed in other rimmed vacuoles, which were usually not lined by sarcolemmal membrane proteins (Figure 16.3E,F). In contrast to sporadic inclusion body myositis, vacuolated fibers in TMD were negative for Congo red stain and with immunohistochemistry for β-amyloid and amyloid precursor protein. SMI-31, tested with an antibody cross-reacting with hyperphosphorylated tau protein, was negative in most rimmed vacuoles but showed cytoplasmic expression in some apparently normal muscle fibers.

EM revealed, overall, a well-preserved sarcomere structure, even in the homozygote LGMD2J mutants.

Rimmed vacuolated fibers contain focal cytoplasmic and sarcomeric degradation products and rare tubulofilamentous inclusions (Udd et al., 1993). These focal myofibrillar degradation regions contain numerous small vesicles compatible with lysosomal components, whereas the whole rimmed vacuole is not membrane bound.

Molecular pathogenesis

Titin makes the third filament system in the sarcomere and is the third most abundant muscle protein after myosin and actin. Well-known ligands of titin are calpain-3 and telethonin. Before the titin mutations were known, secondary calpain-3 deficiency was observed, particularly in the muscle of patients with homozygous LGMD2J (Haravuori et al., 2001). LGMD2A is caused by primary calpain-3 defects. Interaction sites for calpain-3 in titin include the N2A line of I-band titin, Z-disk domains, and the second to last is 7 domain of C-terminal titin in the region of the known TMD/LGMD2J mutations. Previous studies in LGMD2A showed apoptotic myonuclei (Richard et al., 1995). Clusters of apoptotic myonuclei were also detected in TMD/LGMD2J muscle with a secondary calpain-3 defect, suggesting similarities in molecular pathology. RNA studies and immunohistochemistry using antibodies for different portions of the protein show that mutant titin is transcribed, translated, and incorporated in the sarcomere. However, when C-terminal antibodies that recognize the third to last domain are applied, no signal can be obtained in homozygous muscle, indicating that the C-terminus is either cleaved off in the mutant protein or massively conformationally changed, even 200 amino acids upstream of the mutations. Titin C-terminus is rich in epitopes for signaling and contains a catalytic kinase domain interacting with several signaling molecules (Hackman et al., 2003, Lange et al., 2005).

Management

Causal treatment is not available. Molded polypropylene orthoses are used for patients with foot drop. Surgical transposition of the tibialis posterior tendon has been employed in a few middle-aged patients with severe foot drop. This has provided some functional benefit and the need for orthoses could be reversed.

ZASP DISTAL DYSTROPHY – MARKESBERY–GRIGGS DISEASE

One of the classical textbook families with late-onset autosomal dominant distal myopathy was first described in 1974 (Markesbery et al., 1974). Distal leg muscle weakness started between 43 and 51 years of age, with later involvement of the intrinsic hand and wrist extensor muscles, and eventually of the proximal limb and trunk muscles. Weakness remained limited to the distal leg muscles over 15 years in one patient. No involvement of facial, bulbar, or respiratory muscles was observed. There was slow progression and the lifespan was not shortened. Late in senescence, loss of ambulation occurred and one patient developed clinical cardiomyopathy.

Molecular genetics

As soon as the linkage assignment of TMD was known, the titin locus was tested using the identical 2q31 markers for genotyping this family. The first results suggested the family had linkage to the same locus with full segregation of a 2q31 haplotype with the affected individuals and a LOD score of 1.5, well in line with the calculated maximum for the family (Haravuori et al., 1998b). Sequencing the titin gene did not provide mutations responsible for the disease. At the same time a few *ZASP* mutations were reported in a subset of patients with myofibrillar myopathy (Selcen and Engel, 2005). The fact that muscle pathology findings in this family were more similar to those of myofibrillar myopathy (MFM) than TMD was an additional reason for a candidate gene approach. Sequencing of *ZASP* identified the previously reported p.523C>G mutation in the *ZASP* gene in all patients in the family (Griggs et al., 2007).

Laboratory investigation, imaging, and muscle pathology

Serum CK levels are slightly raised in patients with ZASP distal dystrophy (Markesbery et al., 1974). Low-amplitude, short-duration motor unit potentials on moderate activity were recorded by EMG. Increased insertional activity, frequent fibrillation potentials, and occasional high-frequency and complex repetitive discharges at rest were obtained in many patients (Markesbery et al., 1974).

Muscle imaging provides a different pattern of muscle involvement compared with that in TMD. Early subclinical changes of fatty degeneration appeared first in soleus muscle before any lesions were observed in the anterior compartment. At late stages all lower leg muscles were involved, including lateral peroneal and deep flexor muscles, but with considerably fewer changes in the proximal leg muscles.

Besides more specific myofibrillar changes, muscle biopsy shows general myopathic–dystrophic alterations, variation of fiber size, central nuclei, structural changes in the myofibers, endomysial fibrosis, and fatty replacement of muscle (Markesbery et al., 1974). Phagocytotic necrotic fibers and rimmed vacuolated

fibers are abundant, together with single or multiple nonrimmed vacuoles without glycogen or lipids. With Gomori trichrome stain, cryostat sections show focal blue–red accumulation of homogeneous, granular material (Figure 16.4A). Neurogenic changes were not observed and both major fiber types were equally involved in the pathological changes. Immunohistochemistry confirmed myotilin, αB-crystallin, and desmin accumulations in the abnormal fibers, with much less abnormality of ZASP itself (Figure 16.4B). Western blotting indicated no change in the amount or stability of the mutant ZASP protein (Griggs et al., 2007).

EM revealed alterations in almost all portions of the muscle fiber (Markesbery et al., 1977). Dilatation and vacuolization of the sarcoplasmic reticulum, and streaming and disruption of the Z-disk were early changes. Frequent findings were myofibrillar disorganization, disruption, and fragmentation, as well as widening of the intermyofibrillar spaces. Z-disk material was found in clumps without the periodicity of nemaline rods. Glycogen granules, lipofuscin bodies, tiny vacuoles, degenerated myofilaments, Z-disk fragments, and myeloid figures were common. These observations from the 1970s are compatible with what was later reported as myofibrillar myopathy. Some vacuoles are membrane-bound and contain osmiophilic vesicles, granular membrane structures, myeloid figures, and other products of cytoplasmic degeneration, fitting the morphological criteria of autophagic vacuoles. As these are frequently located at the fiber periphery and sometimes covered only by the basement membrane, they may undergo exocytosis. Other vacuoles are relatively empty.

Two patients from the original family underwent autopsy (Markesbery et al., 1974). One patient with moderately advanced clinical disease had mild myopathic changes in trunk muscles, intermediate changes in proximal limb muscles, and end-stage alterations in

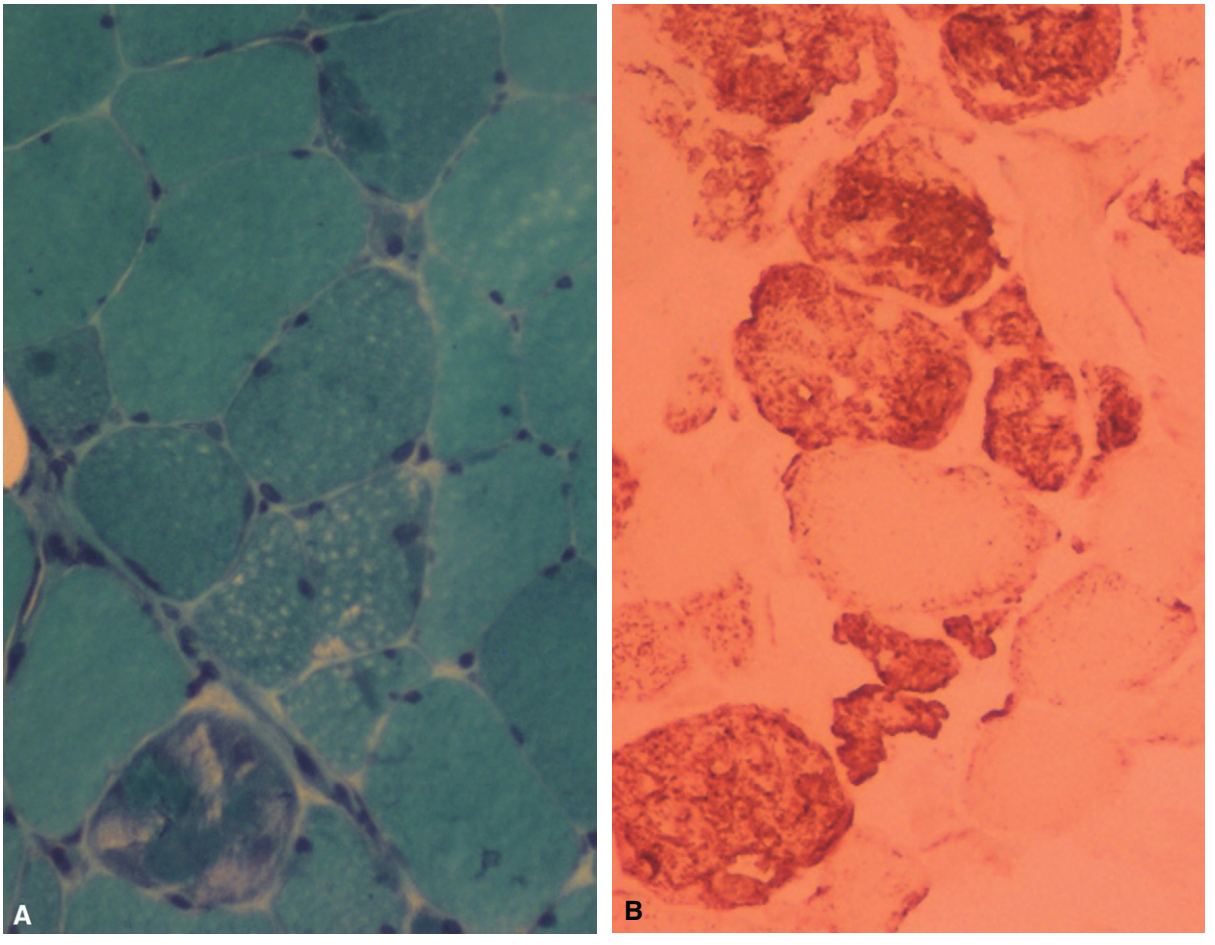

Figure 16.4. ZASP distal dystrophy (Markesbery–Griggs disease). (**A**) Light microscopic muscle biopsy findings in a patient with zaspopathy showing darker, homogeneous, granular material in the cytoplasm with Gomori trichrome. (**B**) Immunohistochemistry of the same biopsy shows increased focal desmin accumulation in abnormal fibers.

distal muscles. This patient had pre-existing diabetes mellitus, which may have explained mild degenerative changes in peripheral nerves and spinal roots. There was also clinical evidence of a cardiomyopathy with cardiomegaly, congestive heart failure, and intractable tachyarrhythmias requiring pacemaker implantation. At autopsy, the heart showed diffuse interstitial fibrosis and diffuse degenerative changes consistent with cardiomyopathy. The brother of the patient died at a more advanced clinical stage of the disease. Autopsy findings of the skeletal muscles were similar, although more pronounced. No pathology was found in his spinal cord or brain.

Molecular pathogenesis

ZASP (Z-disc alternatively spliced PDZ domain-containing protein, *LDB3* gene product) is a component of the Z-disk. Interactions are known with α-actinin (see Figure 16.1) (Selcen and Engel, 2005), with ALP and FATZ proteins (Huang et al., 2003; Klaavuniemi et al., 2006), and were also shown with nebulette and protein kinase C (te Velthuis et al., 2007). ZASP is a member of the PDZ/LIM family of proteins that, among others, are involved in actin dynamics (te Velthuis et al., 2007). Most mutations causing distal dystrophy are located in exon 6. This region of the protein is important for skeletal muscle-specific isoforms and contains a conserved ZM domain needed for α-actinin binding (Klaavuniemi et al., 2006). By immunohistochemistry the myofibrillar protein aggregations in patients' muscle samples show more abnormality with myotilin, αB-crystallin, and desmin than ZASP itself (Griggs et al., 2007). ZASP may thus provide a ruler function for the location of these other Z-disk components, and may have to do with the fact that ZASP knockout mice show a severe phenotype whereas myotilin knockout mice have a milder phenotype (Zhou et al., 2001).

Management

Similar to the ankle weakness leading to tripping in WDM and TMD, patients with ZASP distal dystrophy can be helped by molded polypropylene orthoses. However, ZASP mutations may also cause potential cardiomyopathy, which may need monitoring – at least in the later stages of the disease.

DISTAL MYOTILINOPATHY

Very late onset of ankle weakness around the age 60 years, segregating as an autosomal dominant trait, was reported in a French family (Penisson-Besnier et al., 1998). Posterior calf muscles were more affected than the anterior lower leg muscles, and within 10 years the disease progressed to proximal and upper limb muscles with subsequent walking difficulties. Cardiomyopathy was not observed in these patients.

Laboratory investigation, imaging, and muscle pathology

Serum CK levels were either normal or slightly raised. Myopathic changes together with fibrillations and complex repetitive discharges were recorded on EMG. The extensive involvement of calf muscles was clearly defined by muscle imaging showing dystrophic fatty replacement. The distal preference was confirmed by considerably less involvement of proximal leg muscles. Muscle biopsy in proximal upper limb muscles displayed a wide variety of pathological findings, including multiple large nonrimmed vacuoles, focal sarcoplasmic desmin reactive masses that stained darker on Gomori trichrome, rimmed vacuoles, and inclusion body myositis (IBM)-like cytoplasmic and nuclear filaments on EM (Penisson-Besnier et al., 1998). Myofibrillar disintegration was the main finding on EM.

Molecular genetics

Based on pathology findings compatible with MFM, a candidate gene approach was applied in this family. Sequencing the myotilin gene uncovered a previously reported mutation in exon 2, S60F, which segregated with the affected patients in the family and was absent in healthy individuals (Penisson-Besnier et al., 2006). Myotilin mutations were first reported in two families with the predominant proximal LGMD phenotype (LGMD1A) (Hauser et al., 2000). However, all later muscular dystrophies caused by myotilin mutations have been associated with predominantly distal or mixed phenotypes (Selcen and Engel, 2004; Olive et al., 2005). In addition, the pathologically defined spheroid body myopathy proved to be caused by myotilin mutations (Foroud et al., 2005). Myotilin is a component of the Z-disk of the sarcomere, where it binds to α-actinin and is important for actin dynamics (Salmikangas et al., 2003). Myotilin accumulates together with desmin and αB-crystallin in the abnormal fibers with disintegrated myofibrils (Figure 16.5), irrespective of the primary gene defect. Exactly which molecular abnormalities eventually lead to loss of myofibers due to the known mutations is not known, but so far all mutations have been located in exon 2 of the myotilin gene.

MYOSINOPATHY – LAING DISTAL MYOPATHY

The first skeletal muscle disease with a distal phenotype to be defined by molecular genetics was an early-onset, autosomal dominant form reported in an

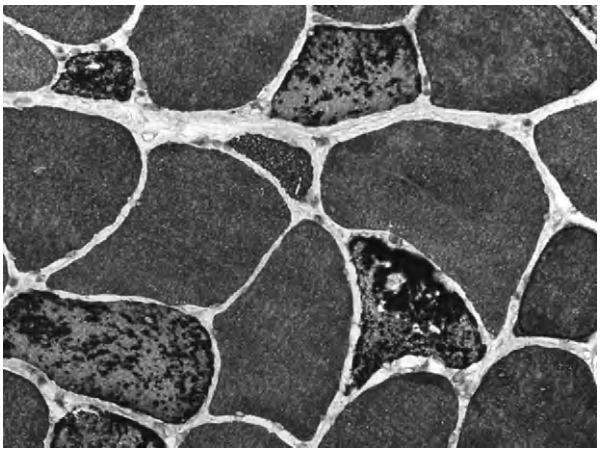

Figure 16.5. Distal myotilinopathy. Myotilin immunohistochemistry performed on a biceps muscle biopsy in a patient with myotilin S60F mutation showing abnormally located myotilin in fibers undergoing myofibril disintegration.

Australian family (Laing et al., 1995). Muscle weakness and atrophy in the anterior compartment muscles of the lower legs was already present in early childhood or developed before the age of 25 years. The distal changes were combined with characteristic severe atrophy and weakness of the sternocleidomastoid muscles. There was slow disease progression with variable involvement of finger flexors, shoulder, trunk, facial, and tongue muscles. Common findings were also scoliosis and tendon contractures, mainly in the ankles (Figure 16.6A). Severe respiratory problems have not occurred and cardiomyopathy has been described only rarely (Hedera et al., 2003).

Molecular genetics

The disease was linked to a new distal myopathy locus (*MPD1*) on chromosome 14q (Laing et al., 1995), with the mutation in slow myosin heavy chain gene *MYH7* reported later (Meredith et al., 2004). A few phenotypically similar Italian (Scoppetta et al., 1995), German (Voit et al., 2001), and US families (Hedera et al., 2003) have also been linked to the same 14q locus. Myosin genes *MYH6* and *MYH7* were positional candidates and *MYH7* proved to be the causative gene (Meredith, 2001; Meredith et al., 2004). More families have since then been identified in various populations (Zimprich et al., 2000; Lamont et al., 2006; Auer-Grumbach et al., 2007). One of the mutations, K1617del, was found in a German and an Austrian family (Zimprich et al., 2000; Meredith et al., 2004) without any haplotype sharing at the locus, suggesting nonrelated mutations. The same K1617del mutation was also identified as a *de novo* mutation in a Finnish

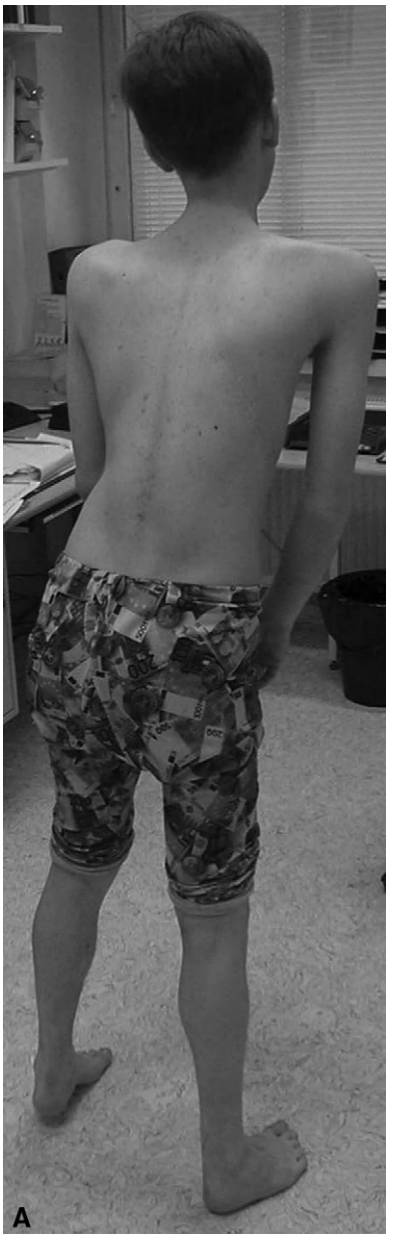

Figure 16.6. Distal myosinopathy (Laing myopathy). (**A**) The generalized reduced muscle bulk and contractures of the ankles, combined with scoliosis (operated) and neck flexor weakness, make an unusual posture. (**B**) Magnetic resonance imaging of muscle showing rather selective degenerative involvement of anterior tibial muscles compared with other lower leg muscles.

family (Lamont et al., 2006), indicating a mutational hotspot. Notably, many mutations occur *de novo*, and this disorder should thus be considered also in isolated cases (Meredith et al., 2004).

Laboratory investigation, imaging, and muscle pathology

Serum CK levels are either normal or slightly raised, and on EMG early-recruiting, short-duration, low-amplitude, and polyphasic motor unit action potentials can be recorded in most muscles studied. In severely affected muscles spontaneous activity was also found, with no sign of neuropathy (Mastaglia et al., 2002). As well as overall reduced muscle mass, highly selective degenerative changes in the anterior tibial muscles are obtained by muscle imaging (Figure 16.6B). At an advanced age, lesions will occur in medial gastrocnemius and the proximal thigh muscles. Muscle imaging can also show the early atrophy of sternocleidomastoid muscles (Lamont et al., 2006).

In the index family, muscle biopsy of proximal muscles did not show any rimmed or other vacuoles (Mastaglia and Laing, 1999). By myosin heavy chain immunohistochemistry, a very distinct pathology can be shown in the more affected anterior tibial muscle: bimodal fiber size distribution with large number of highly atrophic tiny fibers scattered and in groups, simulating group atrophy. In contrast to normal fiber-type distribution in anterior tibial muscle, practically all fibers express fast myosin. Almost all type 1 fibers expressing slow myosin belong to the highly atrophic group, and they also express fast myosin, suggesting a complete reprogramming of fiber-type specifications due to the slow myosin *MYH7* defect (Lamont et al., 2006). Later investigations showed that rimmed vacuolar pathology may occur with this disease (Lamont et al., 2006, Auer-Grumbach et al., 2007).

Molecular pathogenesis

Mutations in *MYH7* causing the distal phenotype are located in the tail region of the slow myosin hexameric molecule (Meredith et al., 2004). This location is in the region of the reported domains for interaction with myomesin and titin. Mutations in the ultimate C-terminus of slow myosin are known to cause hyaline body myopathy, with no preference for distal muscles, and mutations in the proximal neck, the head part, and different mutations in the rod domain may cause cardiomyopathy (Blair et al., 2002; Richard et al., 2003). Most mutations for the distal phenotype are targeted to lysine residues, but further mechanistic explanations for the molecular pathogenesis are lacking.

Management

Scoliosis and contractures may need surgical intervention, and neck flexor weakness may cause retroposition of the head with consequences for breathing and swallowing.

DISTAL DYSFERLINOPATHY – MIYOSHI MYOPATHY (MM)

In the late 1970s, reports from Japan and the USA described patients having a characteristic distal dystrophy with early adult onset, high CK values, and the disease occurring as a recessive/sporadic trait (Markesbery et al., 1977; Miyoshi et al., 1977). Weakness and atrophy started in the distal lower extremities, particularly in the calf muscles, resulting in difficulty climbing stairs, walking briskly, or running, or inability of patients to hop on one leg. Intrinsic foot and anterior compartment muscles were often strikingly normal early in the disease (Miyoshi et al., 1986; Barohn et al., 1991). At onset the proximal muscles were only minimally affected, as were hand muscles, and the majority of patients showed normal tendon reflexes.

Moderate to slow progression to proximal muscle involvement occurred after 10–20 years of disease duration. At later stages the different phenotypes of dysferlinopathy, MM, LGMD2B, and distal anterior onset forms tend to end up with a similar generalized weakness and muscle atrophy.

Epidemiology

Molecular diagnostics in dysferlinopathy by muscle biopsy immunohistochemistry and immunoblotting using antidysferlin antibodies, and by direct DNA genetic analysis, have identified patients with MM in many different populations (Cupler et al., 1998; Linssen et al., 1998; Argov et al., 2000; Eymard et al., 2000; McNally et al., 2000; Ren et al., 2007). The disorder seems to occur with a frequency of about 1–2 in 10^6 population.

Molecular genetics

Linkage to the chromosome 2p13 locus had been shown in 1994 in dysferlinopathy LGMD2B, and 1 year later linkage was reported in MM families (Bejaoui et al., 1995). Both disorders soon proved to be caused by mutations in the dysferlin gene (Bashir et al., 1998; Liu et al., 1998). There were also complex pedigrees where the two phenotypes of MM and LGMD2B occurred in different individuals in the same family (Illarioshkin et al., 1996, 2000; Weiler et al., 1996). It is still not clarified how the identical homozygous mutation may cause different phenotypes within the

same family. The anterior leg phenotype reported by Illa and colleagues (2001) could also occur with the regular MM phenotype within the same family. The number of different dysferlin mutations is very large and they are distributed all over the gene, seemingly with the same effect of absent protein product irrespective of mutational type. Newer methodology for mutation screening using cDNA from muscle has been described (Thierren et al., 2005).

Not all patients with MM-like disease have dysferlinopathy. Linkage to the MM locus 2p13 was excluded in four families with adult and later onset of symptoms (Linssen et al., 1998). Instead, a genome-wide screen suggested linkage to a locus on chromosome 10 (LOD 2.57) in two of these families, and linkage to both loci was excluded in one family. In two brothers of a Finnish family with MM-like disease, dysferlinopathy was excluded even when morphological studies indicated a membrane repair defect similar to that in dysferlinopathy (Jaiswal et al. 2007). The MM phenotype is apparently genetically heterogeneous. The term MM might better be reserved for the dysferlin mutated form, whereas others may be termed MM-like phenotypes, such as the above-mentioned brothers in whom mutations in anoctamin-5 were recently reported (Bolduc et al., 2010).

Laboratory investigation, imaging, and muscle pathology

Characteristically, patients with MM have very high serum CK values, in the range of 10–100-fold the upper level of normal (Miyoshi et al., 1986; Barohn et al., 1991; Cupler et al., 1998). On EMG, involved muscles show increased insertional activity, fibrillation potentials and abundant small motor unit potentials with early recruitment (Miyoshi et al., 1977, 1986; Barohn et al., 1991). The preferential involvement of different muscles during the disease process can be assessed objectively by muscle CT or MRI. The methodology can also be used for assessment of individuals at risk. In patients with dysferlinopathy who present with the anterior leg phenotype (Illa et al., 2001), imaging studies uncover marked fatty degeneration of the gastrocnemius and soleus muscles (Illa and Brown, 1999).

Nonspecific myopathic/dystrophic features with abundant necrotic fibers, regenerating fibers, abnormal variation in fiber size, and later replacement by fat and connective tissue are central findings on muscle biopsy (Barohn et al., 1991). On many occasions dysferlinopathy has been confused with polymyositis, because many biopsies contain some inflammatory infiltrates together with infiltrates of macrophages (Gallardo et al., 2002). There is one report of an autopsy study in a 68-year-old man with MM (Miyoshi et al., 1986). Dystrophic features with necrotic fibers, especially in the calves, and milder changes in limb-girdle, arm, and trunk muscles were reported. No pathology was found in brain, spinal cord, nerve roots, or peripheral nerves.

Antidysferlin antibodies applied on muscle biopsy specimens for immunohistochemistry and immunoblotting usually show severe dysferlin deficiency, and this diagnostic method is currently the gold standard for pathological assessment and diagnosis (Selcen et al., 2001). However, immunostaining of cryostat sections is less reliable. Currently available antidysferlin antibodies may show weak expression signals even at high concentration. Moreover, nonspecific cytoplasmic overexpression together with increased desmin expression can be observed in many regenerative fibers in any myopathic disorder.

Molecular pathogenesis

The main location of dysferlin in the muscle cell is the sarcolemma, with minor expression in the cytoplasm (see Figure 16.1). Dysferlin is not linked to the dystrophin-associated protein complex (Matsuda et al., 1999). By analogy to the *FER-1* gene in *Caenorhabditis elegans*, and because of the calcium-binding C2 domains, a role in membrane fusion and maintenance of the structural integrity of the plasmalemma has been proposed (Matsuda et al., 1999). EM studies of the early events in dysferlinopathy show numerous submicron-sized defects in the plasma membrane, replacement of the plasma membrane by small vesicles, frequently disintegrating small papillary projections, and numerous small subsarcolemmal vacuoles (Selcen et al., 2001). Because of these findings, a role for dysferlin in maintaining muscle fiber surface membrane integrity has been proposed. An interaction of dysferlin with caveolin-3, a skeletal muscle protein important in the formation of sarcolemmal caveolae, has been reported (Matsuda et al., 2001). Dysferlin expression is decreased in patients with caveolin-3 mutations (Matsuda et al., 2001; Tateyama et al., 2002). One function of dysferlin could then be to serve the signaling functions of caveolae (Matsuda et al., 2001). Patients with dysferlinopathy also show secondary calpain-3 defects on muscle biopsy western blotting (Anderson et al., 2000), but the interaction of calpain-3 with dysferlin has not been confirmed.

Management

Specific treatment is not available. Corticosteroids and azathioprine have not been of benefit in carefully studied cases of MM (Barohn et al., 1991).

GNE-MUTATED DISEASE – NONAKA DISTAL MYOPATHY

Another type of autosomal recessive distal dystrophy with onset in early adulthood but with weakness typically in the anterior compartment, causing foot drop, was reported as distal myopathy with rimmed vacuoles (DMRV) in Japanese patients by Nonaka and coworkers (1981). Progression to posterior compartment and proximal muscles with major disability occurred within 10–15 years after onset. Intrinsic muscles were also involved. DMRV is now considered the same disease as autosomal recessive quadriceps-sparing myopathy – hereditary inclusion body myopathy (HIBM).

Molecular genetics

DMRV and HIBM were both linked to the same locus on chromosome 9p12–13.4. Gene defects in the UDP-*N*-acetyl-glucosamine 2-epimerase/*N*-acetyl mannosamine kinase (GNE) were first identified in patients with HIBM (Eisenberg et al., 2001). Shortly afterwards, DMRV in Japanese patients was also shown to be caused by mutations in the same *GNE* gene (Tomimitsu et al., 2002). Despite many different mutations in *GNE* shown in Japanese patients with DMRV (Kayashima et al., 2002; Nishino et al., 2002), one of them, V572L, is more common and considered a founder mutation (Tomimitsu et al., 2002). Families of other ethnic origin (Asian Indian, North American, and Caribbean) are usually heterozygous for distinct missense mutations in the kinase and epimerase domains of *GNE*.

Laboratory investigation, imaging, and muscle pathology

In patients with DMRV, CK values are normal or slightly increased. On EMG, abundant small motor unit potentials and spontaneous discharges, including positive sharp waves and fibrillations, have been observed (Nonaka et al., 1981, 1985).

By definition, the main muscle biopsy findings in DMRV are abundant rimmed vacuoles in muscle fibers (Nonaka et al., 1981; Kumamoto et al., 1982). Necrotic fibers are less common. In the rimmed vacuoles, the acid phosphatase activity as an indicator of increased lysosomal activity was variable (Nonaka et al., 1981; Kumamoto et al., 1982). The nonlysosomal proteolytic activity, as measured by ubiquitin expression, was also increased (Kumamoto et al., 1982, 1994, 2000; Murakami et al., 1995). On EM, some authors have reported membrane-bound rimmed vacuoles containing membranous myeloid bodies, filamentous material, degenerating organelles, and cellular debris (Kumamoto et al., 1982; Mizusawa et al., 1987), whereas others have shown the vacuoles not to be membrane-bound (Nonaka et al., 1981). Filamentous bodies, small paracrystalline inclusions in mitochondria, and myofibrillar alterations were described in DMRV (Isaacs et al., 1988), as well as proliferation of the Golgi apparatus, T-system proliferation, and autophagosomes coalescing to form large autophagic vacuoles partially surrounded by a single membrane (Mizusawa et al., 1987). Tubulofilamentous inclusions were also observed in DMRV (Sunohara et al., 1989). All of these ultrastructural studies were performed at a time when molecular genetic diagnosis was not yet available.

Molecular pathogenesis

GNE is a bifunctional rate-limiting enzyme that catalyzes the first two steps in the biosynthesis of *N*-acetylneuraminic acid, or sialic acid. The two enzymatic activities of GNE are carried out by separate proteins in bacteria. Many biological processes depend on sialic acid modification of glycoproteins and glycolipids expressed at the cell surface, including cell adhesion and signal transduction. In DMRV, hyposialylation of proteins in affected muscles has been proposed (Nishino et al., 2005), but was not confirmed by others (Salama et al., 2005). Recently, a GNE-deficient mouse model was reported to replicate features of the disease including hyposialylation (Gagiannis et al., 2007). Immunohistochemistry results indicate that GNE protein localization and expression is not altered in patient muscle (Krause et al., 2007).

Management

No disease-specific treatment is yet available for DMRV. Ankle orthoses may be of benefit for the foot drop. Cardiac or pulmonary failure has not been reported.

DISTAL NEBULINOPATHY

Mutations in nebulin are a known cause of autosomal recessive congenital nemaline myopathy (Wallgren-Pettersson et al., 2003), with generalized weakness and muscle atrophy of variable severity. Recently, a number of patients with early-onset sporadic or recessive distal myopathy, referred as having possible TMD titinopathy, have been identified with nebulin mutations (Wallgren-Pettersson et al., 2007). Extensor muscles of fingers, hands, and feet are severely involved. The progression is very mild and patients do not experience major disability, even in late adulthood.

Molecular genetics

In the four families reported, two different missense mutations in the large nebulin gene have been identified (Wallgren-Pettersson et al., 2007). All patients were homozygous for either of the two mutations in exon 121 or exon 155 of the gene. All heterozygous carriers were healthy, even in late senescence. Molecular diagnosis of this group of patients is currently laborious because of the large size of the gene.

Laboratory investigation, imaging, and muscle pathology

Serum CK levels are either normal or slightly raised. EMG shows myopathic changes in the affected muscles. Muscle imaging reveals selective degeneration in the anterior tibial muscles (Figure 16.7), later also with lesions in other anterior compartment muscles and in the medial head of gastrocnemius. Muscle biopsy findings were confusing, with scattered and grouped atrophic fibers. The reason for the disorder not to have been identified previously as a nebulinopathy was that nemaline rods were never observed on light microscopy, despite multiple biopsies in some cases (Wallgren-Pettersson et al., 2007). Ultrastructurally, rare scattered small rod-like dense material associated with Z-disks may be observed. In other patients, nonspecific sarcomeric alterations alone, with no rod-like material, have been reported (Wallgren-Pettersson et al., 2007).

Molecular pathogenesis

A retrospective analysis of the different nebulin mutations known to date has shown that more disruptive mutations (nonsense, stop codons, deletions, etc.) are associated with congenital nemaline myopathy, whereas the mild nondisabling distal phenotype is associated with missense mutations occurring on both chromosomes.

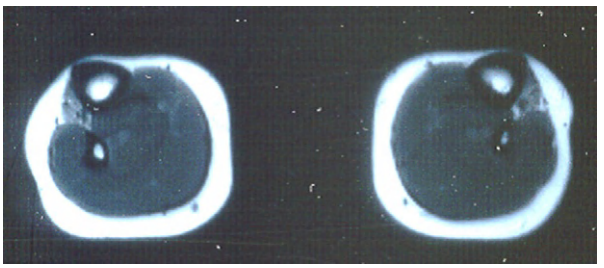

Figure 16.7. Distal nebulinopathy. Magnetic resonance imaging of muscle of the lower legs in a 24-year-old woman with early-onset distal myopathy caused by a homozygous missense mutation in nebulin, showing selective lesions in anterior tibialis muscles bilaterally.

Management

No disease-specific treatment is applicable. Orthoses may be of benefit. Unlike in nemaline myopathy, respiratory failure has not been observed, although monitoring may be good practice.

VOCAL CORD AND PHARYNGEAL DISTAL MYOPATHY (VCPDM)

In 1998, Feit and colleagues described an autosomal dominant disease characterized by distal upper and lower extremity weakness, atrophies, and symptoms of vocal cord and pharyngeal dysfunction. Onset varied from age 35 to 60 years, starting with weakness either in ankle dorsiflexors and toe extensors or in finger extensors, sometimes asymmetrically. Soon afterwards, paretic dysphonia and dysphagia were evident (Feit et al., 1998).

Molecular genetics

The disease was mapped to a locus (*MPD2*) on chromosome 5q overlapping the myotilin locus. However, myotilin mutations were carefully excluded (Garvey et al., 2006). Recently, two different mutations in *MATR3* were claimed to be responsible for the autosomal dominant disease in this and a second family (Senderek et al., 2009).

Laboratory investigation, imaging, and muscle pathology

Serum CK levels range from normal to 8-fold increased. Neurography showed mild slowing of velocities, and on EMG myopathic potentials were recorded (Feit et al., 1998). Muscle biopsy findings consisted of general myopathic–dystrophic features including rimmed vacuolated fibers.

OTHER DISTAL DYSTROPHIES – SINGLE FAMILIES

Over the years several single families with myopathic distal atrophies have been reported. Some of these apparently represent distinct entities, in particular those where association with known genetic dystrophies have been excluded by molecular genetics.

Servidei et al. (1999) reported an Italian family with 10 affected members in three generations. Weakness and atrophy started in the lower legs between the second and sixth decades of life, progressed to upper limbs and proximal muscles, and showed considerable variation in severity. Early signs were also pes cavus, lost reflexes, dysphagia, and dysphonia, with sparing of facial and extraocular muscles (Servidei et al., 1999).

A mixed pattern of both myopathic and neurogenic findings was obtained on EMG. Linkage was established on chromosome 19p13 with a LOD score of 3.03. Muscle biopsy showed dystrophic changes with rimmed vacuoles, and changes attributed to both lysosomal and nonlysosomal degradation were reported (Di Blasi et al., 2004).

Felice and colleagues (1999) reported four affected members in three generations in an autosomal dominant Polish family. Weakness in the anterior muscles of the lower legs started in adulthood, with slow progression to proximal muscles, upper limb extensors, and truncal muscles. Cataracts developed in three patients. Mildly raised serum CK levels and myopathic changes on EMG were other features. Muscle biopsy (from a proximal muscle) showed mild nonspecific changes. All loci for distal dystrophies that had been defined at the time were excluded by linkage.

Chinnery et al. (2001) reported a British family with seven affected patients with a dominant distal phenotype and early failure of respiratory muscles. However, some patients had more proximal weakness, and some even had respiratory problems at onset (Birchall et al., 2005). First symptoms were noted between the ages of 32 and 75 years, usually as weakness of ankle dorsiflexion in patients with the distal presentation. Nasal mask ventilation was applied for early nocturnal hypoventilation. The disease progression was also variable: one patient was wheelchair-bound 7 years after onset, whereas others remained ambulatory after 20 years. The CK concentration was normal or slightly increased, and myopathic abnormalities were recorded on EMG. Muscle imaging showed a highly characteristic pattern with selective involvement of semitendinosus and rectus femoris muscles, as well as anterolateral involvement of lower leg muscles (Birchall et al., 2005). Clinically affected muscle biopsies showed dystrophic changes, rimmed vacuoles, and peculiar eosinophilic inclusions. Congophilia and desmin, β-amyloid, and phosphorylated-tau positivity were observed in the inclusions. All known distal dystrophy loci have been excluded in the family.

Another family with an autosomal dominant distal phenotype was recently identified in the Finnish population (Mahjneh et al., 2003). Weakness of the intrinsic hand muscles or asymmetrical weakness in the anterior lower leg muscles started around the age of 30 years. The disorder was difficult to distinguish from both WDM or TMD, apart from the slightly earlier onset. Rimmed vacuoles were common, with eosinophilic inclusions on muscle biopsy. Linkage studies excluded the loci for other known distal disorders and showed a significant LOD score of more than 3 for two separate loci, 8p22–q11 and 12q13–q22. Both loci segregated the chromosomes to affected and nonaffected individuals identically in the family (Haravuori et al., 2004).

Williams and coworkers (2005) reported a large Australian family with a dominantly inherited distal phenotype showing pronounced involvement of posterior calf muscles and sparing of anterior lower leg muscles. Molecular genetic studies excluded 12 different genetic loci implicated in distal phenotypes. Serum CK levels were mildly raised, and muscle imaging revealed an unusual posterolateral involvement on the legs combined with a distinct semitendinosus and rectus femoris involvement on thigh level. No rimmed vacuolar or myofibrillar myopathy features were reported on muscle biopsy.

Many other inherited late-onset distal phenotypes have been reported (Huhn, 1966; Ricker and Mertens, 1968; Tomlinson et al., 1974; Swash et al., 1988; Ishpekova and Milanov, 1997; Uesaka et al., 1997; Fardeau and Tomé, 1998). Some of these reports dealt with disorders with onset from early infancy (Magee and DeJong, 1965; Heyck et al., 1968; Van der Does de Willebois et al., 1968; Bethlem, 1980). Without any molecular genetic information it is impossible to classify these further.

DISTAL PHENOTYPE IN OTHER MYOPATHIES

The disorders described above were all reported as distal dystrophies/myopathies. However, many disorders may present with a distal phenotype but have been classified or reported under different terms (see Table 16.3).

Other myofibrillar myopathies (MFM)

MFM disorders are defined by their histopathological features. Recent progress has delineated different genes that may underlie such muscle pathology: desmin, αB-crystallin, myotilin, *ZASP*, filamin C, *SEPN*, *Bag3*, and four-and-one-half LIM domain 1 (*FHL1*) (see Chapter 11: Myofibrillar myopathies) (Selcen and Engel, 2004, 2005). Patients and families with dominant MFM frequently present with a distal phenotype (Nakano et al., 1996; Fidzianska et al., 1999; Dalakas et al., 2000; Sugawara et al., 2000). This was very obvious in the classical family later shown to have a desminopathy (Milhorat and Wolf, 1943; Horowitz and Schmalbruch, 1994; Sjöberg et al., 1999). Clinically, patients with MFM display a wide range in age of onset, patterns of muscle involvement, and progression rate. As another example, the classical Stark–Kaeser type of scapuloperoneal syndrome was shown to be caused by mutated desmin in the original family (Walter et al., 2007). Patients frequently have

cardiomyopathy and respiratory muscle weakness. Accumulations of desmin, myotilin, αB-crystallin-positive material, congophilic products of myofibrillar degradation, and ectopic cytoplasmic expression of dystrophin, gelsolin, and amyloid, and cell cycle-related proteins in muscle represent both unusual and unifying morphological features. Desmin, however, is frequently expressed in the cytoplasm of atrophic and regenerating fibers of any myopathy.

One form of desmin-related distal disorders was found in a Swedish family with adult onset of atrophy in thenar and finger flexor muscles, extending in some patients to sternocleidomastoid weakness and cardiomyopathy (Edström et al., 1980). Distinct sarcoplasmic bodies and intermediate filament accumulations containing desmin were the hallmark of muscle biopsy findings, suggestive of abnormal turnover of intermediate filaments. X-ray microanalysis of these bodies revealed an increased content of sulfur (Edström and Wroblewski, 1981). The molecular genetic background is unknown.

Distal VCP-mutated disease

Most patients with the combination of myopathy, Paget's disease, and frontotemporal dementia have a proximal phenotype and mutations in valosin-containing protein (VCP), which has a role in the ubiquitin–proteasome pathway (Song et al., 2005). One subgroup has a clearly distal phenotype with onset in the anterior lower leg muscles and atrophy of the small hand muscles, rimmed vacuolar myopathology, and late dementia, but without Paget's disease (B. Udd, unpublished data).

Telethoninopathy

Recessive LGMD2G is caused by mutations in telethonin, a relatively rare disorder identified first in a few Brazilian families (Moreira et al., 1997). Muscle biopsy pathology findings include rimmed vacuoles. At onset, telethoninopathy frequently presents with distal weakness and atrophy.

Oculopharyngodistal myopathy

Both late-onset dominant and early-adult recessive forms have been reported where the distal limb muscle atrophy was combined with prominent oculopharyngeal weakness, and in one case was associated with cardiomyopathy (Goto et al., 1977; Satoyoshi and Kinoshita, 1977; Scrimgeour and Mastaglia, 1984; Uyama et al., 1998). Recently, patients with marked facial weakness have been reported (van der Sluijs et al., 2004, Witoonpanich et al., 2004).

Caveolinopathy

LGMD1C is caused by mutations in caveolin-3, which also may cause "rippling muscle disease." Distal phenotypes with pronounced atrophy of intrinsic muscles in hands and feet have been described with caveolin-3 mutations (Tateyama et al., 2002; Sotgia et al., 2003).

Other disorders

Dynamin-2-mutated centronuclear myopathy frequently presents as a distal phenotype. Muscle imaging in patients with later onset forms have underlined the distal predominance of affected muscles (Schessl et al., 2007).

A Swedish family was reported with dominant late-onset weakness and atrophy of hand and lower leg muscles combined with cardiomyopathy, EMG myotonia, and linked to a locus on chromosome 10q (Melberg et al., 1999). Both rimmed vacuoles and nemaline rods were found on muscle biopsy in a sporadic patient with distal myopathy (Sieb et al., 1997).

Three young boys in a French family had very early bilateral foot drop and calf muscle hypertrophy (Lapresle et al., 1972). Muscle biopsy findings were suggestive of a mitochondrial myopathy. Distal weakness and atrophy in both hands and feet occurred between 5 and 15 years of age in a large Dutch family (Biemond, 1955). The disorder remained limited to distal muscles and became stationary after age 50.

Differential diagnosis

The advance in molecular genetics has changed the classification and made the differential diagnostic procedure both easier and more difficult. Compared with the situation just 10–15 years ago, there is now a number of entities with different genetic causes for distal dystrophy phenotypes. A further level of complexity is due to the fact that many of the genes known to cause other characteristic clinical presentations may in some instances cause a distal phenotype (Table 16.4).

Distal phenotypes first have to be differentiated from neurogenic disorders. The axonal form of Charcot–Marie–Tooth disease with late-onset distal weakness and more or less normal nerve conduction still causes confusion (Dyck, 1993). Clinically, distal forms of chronic spinal muscular atrophy may mimic those of the distal dystrophies. However, EMG will indicate anterior horn cell dysfunction, and muscle biopsy from an affected muscle reveals chronic neurogenic atrophy (McLeod and Prineas, 1971). In exceptional cases, the diagnosis may still remain undetermined.

Table 16.4

Other differential diagnostic possibilities

Facioscapulohumeral muscular dystrophy
Sporadic inclusion body myositis
Nemaline myopathy
Myotonic dystrophies
Scapuloperoneal syndromes
Distal spinal muscular atrophy
Focal motor neuron disease (e.g., Hirayama, Sobue)
Central core disease
Debranching enzyme deficiency
Phosphorylase β kinase deficiency
Lipid storage myopathy

Adult-onset myotonic dystrophy type 1 is associated with marked distal weakness and atrophy, but is hardly ever a differential challenge because evident myotonia will lead to DNA analysis for the $(CTG)_n$ expansion mutation.

If muscle biopsy shows rimmed vacuolar pathology combined with even minor lymphocyte infiltration, patients may easily be given an erroneous diagnosis of sporadic IBM (sIBM) (Carpenter et al., 1978). Confusingly, familial cases of otherwise typical sIBM have also been described (Baumbach et al., 1990; Neville et al., 1992). Autoaggressive inflammatory infiltrates with partial invasion, and the increased expression in all myofibers of major histocompatibility complex class 1 antigen by immunohistochemistry, helps to differentiate this entity from the distal dystrophies with rimmed vacuoles and findings of secondary lymphocyte activation. The inflammatory component of sIBM can be visualized by MRI with maximal fat suppression sequences for additional distinction. Small intracellular congophilic inclusions and increased expression of multiple proteins present in the brain in Alzheimer's disease, such as β-amyloid, β-amyloid precursor protein, tau, ubiquitin, etc., have been reported in vacuolated muscle fibers in sIBM (Griggs et al., 1995; Askanas and Engel, 1998). Both vacuolated and nonvacuolated fibers show immunoreactivity for αB-crystallin (Banwell and Engel, 2000). In GNE-mutated HIBM and DMRV, these changes have been observed to a minor extent (Askanas and Engel, 1998; Tomimitsu et al., 2002). In both TMD and WDM, such congophilic deposits or β-amyloid immunoreactivity are unusual.

Facioscapulohumeral dystrophy (FSHD) and other scapuloperoneal syndromes may present with ankle dorsiflexion weakness long before facial or shoulder weakness is evident. These are not very rare and may cause diagnostic confusion. DNA analysis is mandatory to identify "sporadic" cases of FSHD (Van der Koi 2000), and desmin needs to be considered. Of the early-onset forms, both nemaline myopathy (Brooke, 1977) and central core disease (Kratz and Brooke, 1980) may rarely show scapuloperoneal distribution of muscle involvement with predominant distal weakness. Rare cases with distal presentation have also been reported in centronuclear myopathy (Moxley et al., 1978), debrancher enzyme deficiency myopathy (glycogenosis type 3) (DiMauro et al., 1979), phosphorylase β kinase deficiency (Clemens et al., 1990), and lipid storage myopathies (Salmon et al., 1971), and secondary to nephropathic cystinosis (Vester et al., 2000).

CONCLUSIONS

1. Increasing numbers of reports suggest increasing awareness of distal phenotypes in muscular dystrophy.
2. Some disorders regularly progress eventually to involve proximal muscle, whereas others such as TMD, WDM, and distal nebulinopathy remain distal throughout the patient's lifetime.
3. Pathologically there is a gradual degeneration and loss of muscle fibers with replacement by fibrous and fatty connective tissue, similar to the proximal forms of muscular dystrophy, frequently but not always with rimmed vacuolar degradation change.
4. Strikingly, many of the genes involved in distal dystrophies code for sarcomeric proteins, although the genetic programs leading to preferential involvement of distal muscles are as yet unknown.
5. Further understanding of the downstream effects of genetic defects on protein functions and interaction pathways is needed.
6. Efficient new methods for large-scale survey of muscle genes will be needed because many of the sarcomeric genes are too large to sequence in individual patients.

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