The Limb-Girdle Muscular Dystrophies

By Matthew P. Wicklund, MD, FAAN

REVIEW ARTICLE

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ABSTRACT

PURPOSE OF REVIEW: **As a group, the limb-girdle muscular dystrophies (LGMDs) are the fourth most prevalent genetic muscle disease, yet they are still not well known or understood. This article defines and describes LGMDs, delineates a diagnostic strategy, and discusses treatment of the LGMDs.**

RECENT FINDINGS: **In 2018, the definition of the LGMDs was further refined, and a new nomenclature was proposed. Diagnosis of the LGMDs was long guided by the distinctive clinical characteristics of each particular subtype but now integrates use of genetics—with next-generation sequencing panels, exomes, and full genome analysis—early in the diagnostic assessment. Appreciation of the phenotypic diversity of each LGMD subtype continues to expand. This emphasizes the need for precision genetic diagnostics to better understand each subtype and formulate appropriate management for individual patients. Of significant relevance, the explosion of research into therapeutic options accentuates the need for accurate diagnosis, comprehensive disease characterization, and description of the natural histories of the LGMDs to move the field forward and to mitigate disease impact on patients with LGMD.**

SUMMARY: **The LGMDs are genetic muscle diseases that superficially appear similar to one another but have important differences in rates of progression and concomitant comorbidities. Definitive diagnoses are crucial to guide management and treatment now and in the future. As targeted treatments emerge, it will be important for clinicians to understand the nomenclature, diagnosis, clinical manifestations, and treatments of the LGMDs.**

INTRODUCTION

Genetic muscle diseases present in many different patterns (FIGURE 4-1). However, in 1954 Walton and Nattrass[1] parsed out the limb-girdle muscular dystrophies (LGMDs) from the three most prevalent muscular dystrophies: Duchenne muscular dystrophy, myotonic dystrophy, and facioscapulohumeral muscular dystrophy. Patients with LGMD had proximally predominant weakness of the shoulder and hip girdle musculature due to muscle fiber dysfunction. The subsequent decades witnessed an explosion in our understanding of these disorders on a genetic basis. Literally hundreds of genes can lead to proximal muscle weakness including disorders of the motor neuron

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Address correspondence to Dr Matthew P. Wicklund Department of Neurology, University of Colorado School of Medicine, Mail Stop B185, Academic Office 1, 12631 E 17th Ave, Room 5223, Aurora, CO 80045, *matthew.wicklund@cuanschutz.edu*.

RELATIONSHIP DISCLOSURE:
Dr Wicklund has received personal compensation for serving on the scientific advisory boards of Myonexus Therapeutics, Inc and Sarepta Therapeutics. Dr Wicklund has served on the editorial board of *Muscle & Nerve*, has held stock or stock options for Myonexus Therapeutics, Inc, and has received research/grant support as principal investigator of studies for Acceleron Pharma, Inc; the Muscular Dystrophy Association; and Orphazyme.

UNLABELED USE OF PRODUCTS/INVESTIGATIONAL USE DISCLOSURE:
Dr Wicklund discusses the unlabeled/investigational use of cell replacement therapies, gene editing, and viral vector gene therapy for the treatment of limb-girdle muscular dystrophies.

(spinal muscular atrophy), neuromuscular junction (congenital myasthenic syndromes), and muscle (genetic myopathies inclusive of LGMDs). This article discusses the definition, nomenclature, diagnosis, epidemiology, phenotypic features, genetics, and treatments of the LGMDs.

DEFINITION

The LGMDs were originally defined as postnatal onset of progressive muscle disease, which starts in and predominantly affects the pelvic and/or shoulder girdle muscles.[2] In 2018, the European Neuromuscular Centre refined the definition of LGMD to include the following criteria[3]:

> Limb girdle muscular dystrophy is a genetically inherited condition that primarily affects skeletal muscle leading to progressive, predominantly proximal muscle weakness at presentation caused by a loss of muscle fibres. To be considered a form of limb girdle muscular dystrophy the condition must be described in at least two unrelated families with affected individuals achieving independent walking, must have an elevated serum creatine kinase activity, must demonstrate degenerative changes on muscle imaging over the course of the disease, and have dystrophic changes on muscle histology, ultimately leading to end-stage pathology for the most affected muscles.

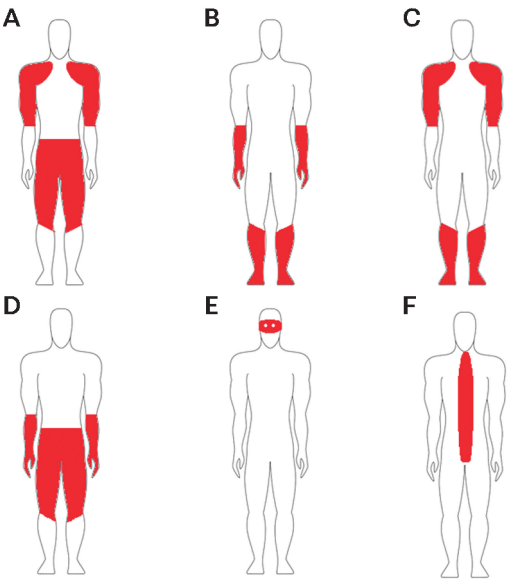

FIGURE 4-1
Patterns of muscle weakness associated with genetic muscle diseases. Genetic muscle diseases present with six different patterns. *A*, Proximal predominant or limb girdle (eg, Duchenne muscular dystrophy and limb-girdle muscular dystrophies); *B*, distal (eg, Miyoshi myopathy); *C*, scapulohumeral (eg, facioscapulohumeral muscular dystrophy); *D*, distal upper and proximal lower (eg, some cases of myotonic dystrophy type 2 and some myofibrillar myopathies); *E*, ocular/facial/bulbar (eg, oculopharyngeal muscular dystrophy); *F*, axial muscles (eg, some cases of Pompe disease).

While not all patients with LGMD will have these features continuously throughout their disease, most patients will manifest the majority of these criteria during the disease course.[4]

NOMENCLATURE

The European Neuromuscular Centre developed a classification scheme for the LGMDs in 1995.[5] Dominantly inherited LGMDs were denoted with "1," while recessively inherited LGMDs included "2." In addition to the numerical classification, a letter delineating the order of discovery of each gene's chromosomal locus was also included. Thus, calpainopathies, caused by recessive mutations in *CAPN3*, were referred to as LGMD2A since the chromosomal

locus for *CAPN3* was the first recessively inherited LGMD delineated. Likewise, LGMD1D would stand for the LGMD due to mutations in *DNAJB6* since the chromosomal locus for *DNAJB6* was the fourth discovered autosomal dominantly inherited LGMD.

In 2018, the European Neuromuscular Centre reconvened and set forth a new nomenclature in an attempt to better delineate the LGMDs from other genetic muscle diseases (TABLE 4-1 and TABLE 4-2).[3]

DIAGNOSIS

The diagnostic process for the LGMDs has changed. Where once diagnosis proceeded in an orderly manner through history, examination, laboratory testing, electrodiagnostic studies, muscle imaging, and muscle biopsy to finally arrive at genetic testing,[6] now genetic testing often can move toward the front of this list of investigations. The speed of genetic testing has increased by roughly an order of magnitude each year from 2008 to 2018, and the cost of genetic testing per gene was cut in half annually during the same timeframe; therefore, at the time of this publication, the cost for a clinical genome is less than $2000 (less than $0.10 per gene). For this reason, and due to the increased diagnostic yield of next-generation sequencing panels and exomes,[7,8] broad genetic testing should be performed once clinical suspicion for a genetic muscle disease emerges. Ancillary testing such as EMG, laboratory investigations, imaging, biopsy, biomarker analysis, and RNA sequencing can be used to clarify undiagnosed cases and variants of undetermined significance.

The clinical differential diagnosis for people with a limb-girdle pattern of weakness is broad. Important diagnoses that masquerade as LGMDs include dystrophinopathies (Duchenne and Becker muscular dystrophies along with manifesting female carriers), facioscapulohumeral muscular dystrophy (especially with minimal or no facial weakness), Emery-Dreifuss muscular dystrophies, Pompe disease, congenital myasthenic syndromes (especially those with onset after infancy), and even some of the hereditary motor neuropathies (those with a proximal predominance).

Some special situations deserve comment related to the diagnosis of LGMD. Patients previously followed for the diagnosis of myositis, especially if poorly responsive to immunomodulatory therapy, should undergo LGMD genetic testing.[9] Additionally, patients with negative panel, exome or genome LGMD genetic analysis should be tested for autoantibodies recognizing 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (even in patients without exposure to statin drugs and even in children).[10,11] Finally, patients presenting with rhabdomyolysis, especially if their creatine kinase (CK) levels remain elevated, should be evaluated for genetic muscle diseases, including the LGMDs.[12]

EPIDEMIOLOGY

Individually, distinct LGMD subtypes are relatively uncommon; however, as a group, the minimum prevalence of LGMDs likely resides between 2.27 per 100,000 and 10 per 100,000.[13] In the United States, calpainopathies are the most prevalent of the different LGMDs, followed by LGMDs with mutations in the genes for dysferlin, collagen VI, the sarcoglycans, anoctamin 5, and fukutin-related protein (FKRP) (FIGURE 4-2).[14]

KEY POINTS

- Hundreds of genes can lead to proximal muscle weakness including disorders of the motor neuron (spinal muscular atrophy), neuromuscular junction (congenital myasthenic syndromes), and muscle (genetic myopathies inclusive of limb-girdle muscular dystrophies).
- Limb-girdle muscular dystrophy is defined as a genetically inherited condition primarily affecting skeletal muscle that leads to progressive, predominantly proximal muscle weakness in individuals who have achieved independent walking and who have elevated creatine kinase levels. Degenerative changes are demonstrated on muscle imaging, and dystrophic changes are demonstrated on muscle histology in the most affected muscles.
- In 2018, the European Neuromuscular Centre set forth a new nomenclature to better delineate the limb-girdle muscular dystrophies from other genetic muscle diseases.
- The diagnostic process for the limb-girdle muscular dystrophies has changed, as now broad genetic testing should be performed once a clinical suspicion is present for a genetic muscle disease.

COMMON LIMB-GIRDLE MUSCULAR DYSTROPHIES

Although more than 100 genes can present with a phenotype of proximally predominant weakness, as noted previously, several LGMD subtypes are the most prevalent in most populations studied.

Limb-Girdle Muscular Dystrophy Type 2A (LGMD R1 Calpain 3–Related)

Two pathogenic variants in *CAPN3* lead to autosomal recessive calpainopathy (LGMD2A). Additionally, inheriting certain single pathogenic variants in *CAPN3* can cause an autosomal dominantly inherited calpainopathy (LGMD1I).[15] Calpain 3 is a calcium-dependent cysteine protease, and it participates in assembly and remodeling of the sarcomere, regulation of calcium outflow from the sarcoplasmic reticulum, and sarcolemmal repair (FIGURE 4-3).[16] Calpainopathies are the most common LGMD subtype, except in some Northern European countries and perhaps in Asia.[17,18]

In the recessively inherited LGMD2A, onset of weakness begins between 5 and 20 years of age in 75% of cases.[6] With autosomal dominant disease, onset occurs

TABLE 4-1 Nomenclature, Genes, and Protein Products of Limb-Girdle Muscular Dystrophies

New Nomenclature	Old Nomenclature[a]	Gene	Protein Product
Autosomal dominant			
LGMD D1	LGMD1D	*DNAJB6*	DnaJ heat shock protein family (Hsp40) member B6
LGMD D2	LGMD1F	*TNPO3*	Transportin 3
LGMD D3	LGMD1G	*HNRNPDL*	Heterogeneous nuclear ribonucleoprotein D like protein
LGMD D4	LGMD1I	*CAPN3*	Calpain 3
LGMD D5		*COL6A1*	Collagen type VI alpha 1 chain
Autosomal recessive			
LGMD R1	LGMD2A	*CAPN3*	Calpain 3
LGMD R2	LGMD2B	*DYSF*	Dysferlin
LGMD R3	LGMD2D	*SGCA*	Sarcoglycan alpha
LGMD R4	LGMD2E	*SGCB*	Sarcoglycan beta
LGMD R5	LGMD2C	*SGCG*	Sarcoglycan gamma
LGMD R6	LGMD2F	*SGCD*	Sarcoglycan delta
LGMD R7	LGMD2G	*TCAP*	Telethonin
LGMD R8	LGMD2H	*TRIM32*	Tripartite motif-containing 32
LGMD R9	LGMD2I	*FKRP*	Fukutin-related protein
LGMD R10	LGMD2J	*TTN*	Titin

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on average 2 decades later and the severity of weakness is milder. Otherwise, recessively and dominantly inherited calpainopathies display similar features. Strength in the lower extremities follows a relatively distinct pattern with knee flexors, hip extensors, and hip adductors weaker than their paired antagonist muscles across the joint, the knee extensors, hip flexors, and hip abductors, respectively. The medial gastrocnemius muscle often shows early and preferential fatty replacement on MRI, and the scapulae wing in approximately 20% of cases. Joint contractures are common and can affect the ankles, hips, knees, and elbows in more than half of patients with LGMD2A. Weakness is predominantly symmetric, and women tend to be less severely affected than men. Patients with two null mutations develop more weakness than those with missense mutations. Loss of independent ambulation often occurs 2 decades after disease onset (**CASE 4-1**), but symptomatic pulmonary and cardiac involvement is distinctly uncommon.[19]

CK levels are approximately 2000 U/L to 6000 U/L but range from normal late in disease to elevated as much as 110 times the normal level around the time

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New Nomenclature	Old Nomenclature[a]	Gene	Protein Product
LGMD R11	LGMD2K	*POMT1*	Protein O-mannosyltransferase 1
LGMD R12	LGMD2L	*ANO5*	Anoctamin 5
LGMD R13	LGMD2M	*FCMD*	Fukutin
LGMD R14	LGMD2N	*POMT2*	O-mannosyltransferase 2
LGMD R15	LGMD2O	*POMGnT1*	Protein O-linked mannose *N*-acetylglucosaminyltranferase 1 (beta 1,2-)
LGMD R16	LGMD2P	*DAG1*	Dystroglycan 1
LGMD R17	LGMD2Q	*PLEC1*	Plectin
LGMD R18	LGMD2S	*TRAPPC11*	Trafficking protein particle complex 11
LGMD R19	LGMD2T	*GMPPB*	GDP-mannose pyrophosphorylase B
LGMD R20	LGMD2U	*CRPPA*	CDP-L-ribitol pyrophosphorylase A
LGMD R21	LGMD2Z	*POGLUT1*	Protein O-glucosyltransferase 1
LGMD R22		*COL6A1/2/3*	Collagen VI subunits A1, A2, or A3
LGMD R23		*LAMA2*	Laminin subunit alpha 2
LGMD R24		*POMGNT2*	Protein O-linked mannose *N*-acetylglucosaminyltransferase 2 (beta 1,4-)

LGMD = limb-girdle muscular dystrophy.
[a] Blank entries indicate diseases that were not classified as LGMDs under the old nomenclature.

of symptom onset. Muscle biopsies often show lobulated fibers on nicotinamide adenine dinucleotide (NADH)-stained sections (FIGURE 4-4). Occasional biopsies reveal eosinophilic infiltrates.

Limb-Girdle Muscular Dystrophy Type 2B (LGMD R2 Dysferlin-Related)

Two pathogenic variants in *DYSF*, the gene encoding dysferlin, lead to LGMD2B and also to Miyoshi myopathy, a distal myopathy with greatest involvement of the posterior calves. Dysferlin is thought to play roles in muscle membrane repair, homeostasis, and signal transduction along with myogenesis and microtubule function. In the United States, dysferlinopathies are the second most prevalent LGMD subtype. However, in Asia, LGMD2B may be the most common subtype.[17,18] Age of onset is quite broad, from infancy through the eighth decade, but weakness begins between 15 and 30 years of age in most cases.[20] At onset, leg muscles exhibit the greatest weakness, but this lower extremity weakness can be proximal, distal, or both. Hip extensors, hip adductors, knee extensors, and ankle plantar flexors are most affected, with

TABLE 4-2 **Genes, Protein Products, and Old Nomenclature of Other Myopathies With Limb-Girdle Muscular Dystrophy Phenotype**

Gene	Protein Product	Old Nomenclature[a]
Autosomal dominant		
MYOT	Myotilin	LGMD1A
LMNA	Lamin A/C	LGMD1B
CAV3	Caveolin 3	LGMD1C
DES	Desmin	LGMD1E
RYR1	Ryanodine receptor 1	
VCP	Valosin containing protein	
Autosomal recessive		
DES	Desmin	LGMD2R
GAA	Acid alpha-glucosidase	LGMD2V
LIMS2	LIM zinc finger domain containing 2	LGMD2W
BVES	Blood vessel epicardial substance	LGMD2X
TOR1AIP1	Torsin 1A interacting protein 1	LGMD2Y
RYR1	Ryanodine receptor 1	
X-linked		
DMD	Dystrophin	
FHL1	Four and a half LIM domains 1	

LGMD = limb-girdle muscular dystrophy.
[a] Blank entries indicate diseases that were not classified as LGMDs under the old nomenclature.

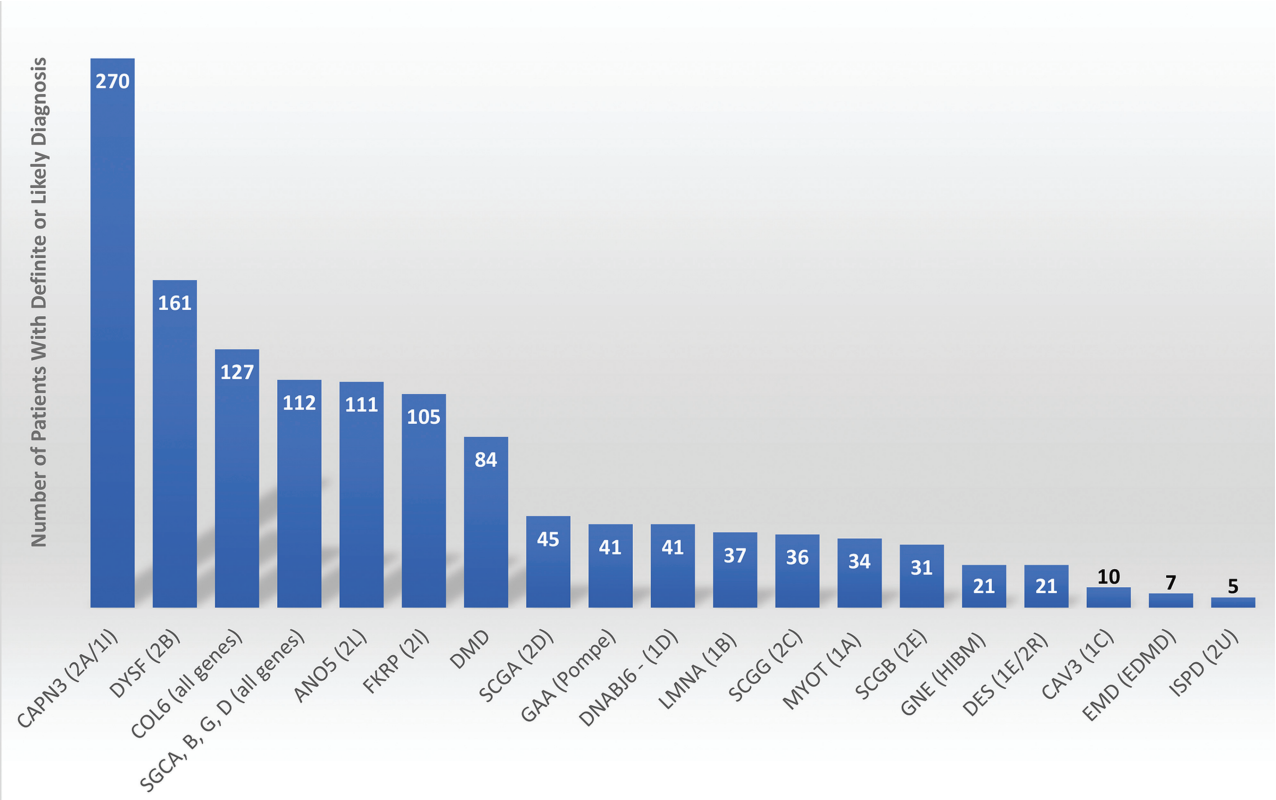

FIGURE 4-2

Relative prevalence of the limb-girdle muscular dystrophy subtypes in the United States. A total of 1,259 patients from 4,656 genetic testing samples (27%) obtained a confirmed genetic diagnosis for their limb-girdle muscular dystrophy through these genetic testing programs (sponsored through the Muscular Dystrophy Association and the Jain Foundation).[14]

relative preservation of hip abductors and hip flexors. Of note, if someone with LGMD cannot stand on his or her toes within the first few years of disease onset, strong consideration should be given to LGMD2B (and LGMD2L). Rarely, patients with LGMD2B have calf hypertrophy at onset, but calf atrophy and weakness are more common. Interestingly, prior to onset of symptoms, a disproportionate number of patients with LGMD2B were better athletically than their peers, with roughly one in five competing at the regional or national level (CASE 4-2).[21] However, it has now been reported that exercise in the teenage years is associated with earlier onset of weakness in patients with LGMD2B.[22] Cardiac involvement is virtually nonexistent. Infrequent clinically significant respiratory insufficiency is seen only late in disease or in cases with early onset and severe weakness.

Evaluation of patients with LGMD2B customarily reveals markedly elevated CK levels (mean of 5000 U/L with a range from normal to >30,000 U/L). Muscle MRI of the legs reveals early involvement of the medial gastrocnemius in the calves, the hamstrings in the thighs, and the paraspinal muscles. MRI of the legs in patients with dysferlinopathy with LGMD or Miyoshi myopathy demonstrates similar patterns of muscle involvement as the disease progresses despite predominantly proximal and distal weakness, respectively. In patients with LGMD2B, muscle biopsies reveal dystrophic changes later in the disease.

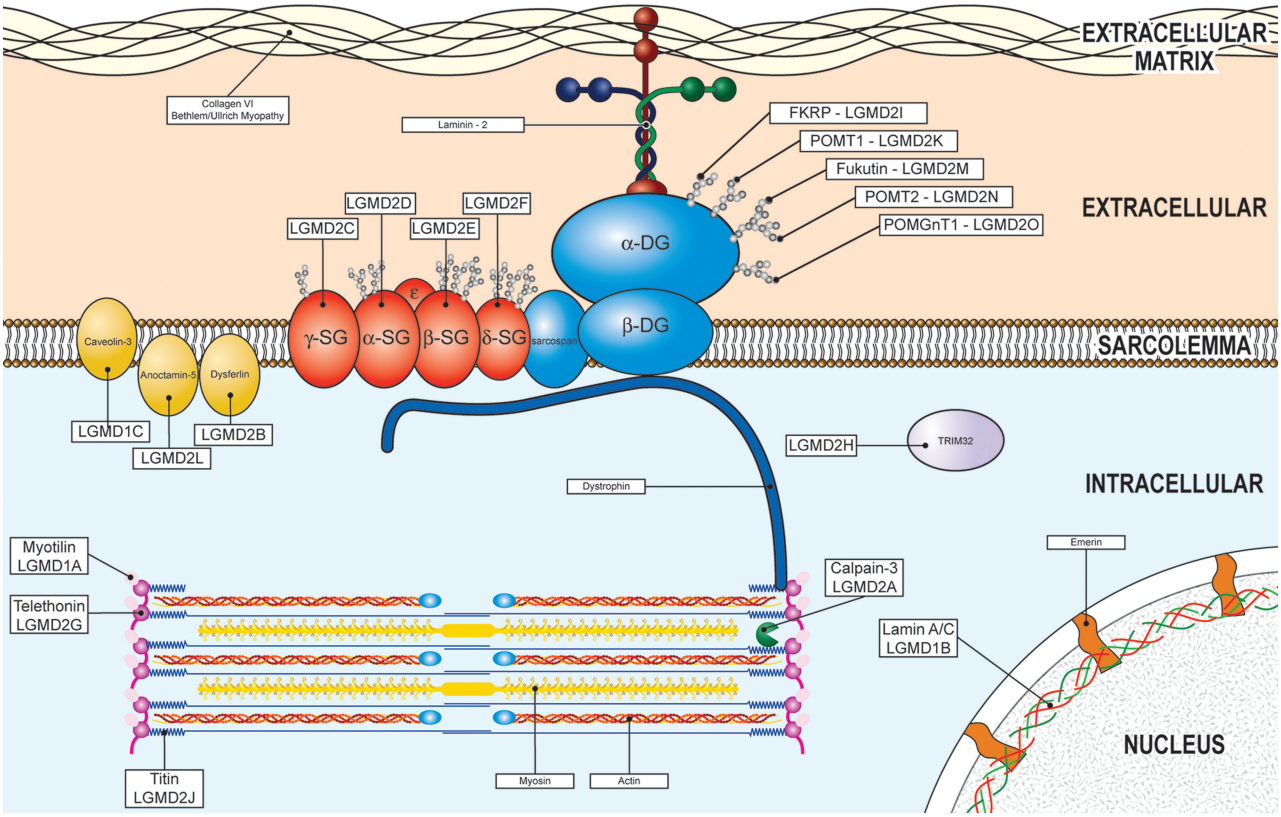

FIGURE 4-3

Schematic of the limb-girdle muscular dystrophies and their localizations in muscle fibers.
FKRP = fukutin-related protein; LGMD = limb-girdle muscular dystrophy; POMT = protein-O-mannosyltransferase; DG = dystroglycan; POMGnT1 = protein O-linked mannose *N*-acetylglucosaminyltransferase 1 (beta 1,2-); SG = sarcoglycan; TRIM32 = tripartite motif-containing 32. Reprinted with permission from Aminoff MJ, Daroff RB.[16] © 2014 Elsevier.

However, earlier in the disease, LGMD2B muscle biopsies can contain prominent endomysial, perimysial, and perivascular inflammatory infiltrates, suggesting polymyositis. In this author's experience, out of more than 50 patients with LGMD2B, nearly half were initially misdiagnosed with polymyositis and received treatment with immune therapies (without benefit) for as long as a decade. Treatment with deflazacort in patients with LGMD2B provided no benefit in a randomized, placebo-controlled clinical trial.[24] Dysferlin immunostaining may be absent, diminished, and sometimes normal or even increased in the sarcoplasm. However, reduced dysferlin immunostaining also occurs in biopsies from some other LGMDs, such as calpainopathies (LGMD2A). Absent dysferlin on Western blot is highly specific for disease, but some dysferlinopathy patients' Western blots can also manifest relatively normal levels of dysferlin. Thus, genetic testing remains the definitive diagnostic modality.

Limb-Girdle Muscular Dystrophy Types 2C, 2D, 2E, and 2F (LGMD R3, R4, R5 and R6 Sarcoglycan–Related)

The four sarcoglycan proteins (α-sarcoglycan, β-sarcoglycan, γ-sarcoglycan, and δ-sarcoglycan) form a tetrameric structure stabilizing the linkage of dystrophin

up through α-dystroglycan and β-dystroglycan as a key component in the dystrophin glycoprotein complex (FIGURE 4-3). The overall prevalence of the four sarcoglycanopathies is approximately 0.5 to 1.0 per 100,000. However, in selected populations, such as Amish communities in the United States or the Romani of Eastern Europe, the prevalence may be much higher. The relative prevalence of the four sarcoglycanopathies combined is 5% to 10% of the LGMDs, with LGMD2F (δ-sarcoglycanopathy) being the least common.

CASE 4-1

A 51-year-old woman with a long history of progressive weakness presented for neurologic follow-up for her muscular dystrophy. She had begun to walk on her toes occasionally at 5 years of age. By 9 years of age, she was toe walking constantly and had difficulty running and keeping up with her peers athletically. At 12 years of age, her creatine kinase (CK) level was noted to be 4281 U/L, and a subsequent EMG revealed "low-voltage, highly polyphasic motor units" with a small percentage of narrow motor units "with durations ranging from 2 to 4 milliseconds," and "an increase in the number of motor units relative to the degree of contraction." She was thus diagnosed with limb-girdle muscular dystrophy (LGMD) at 12 years of age. Also at 12 years of age, because of her significant tip-toe gait with heels 6 inches off the floor bilaterally, she underwent a lateral gastrocnemius muscle biopsy, which failed to reveal diagnostic features. The following year, she had heel cord lengthening surgery. By high school, she had difficulty traversing stairs, and in her early thirties she had begun using a power wheelchair full time. In her fifties, she experienced moderate respiratory insufficiency requiring nocturnal bilevel positive airway pressure, but she had no breathing difficulties during the daytime. She had not had any cardiac symptoms or abnormalities on cardiac testing.

Current examination revealed a nonambulatory woman seated in a power wheelchair. Her cognition, cranial nerves, sensation, and coordination were normal. On motor examination, she had only minimal movement of her legs, and her arm strength was 2/5 at the shoulders, 3/5 at the elbows, and 4/5 at the wrists and in the fingers. There was mild, bilateral scapular winging.

At age 51 years, genetic testing revealed heterozygous pathogenic variants in *CAPN3* (c.1468C>T, p.Arg490Tyr & c.IVS11+1G>C, splice site mutation), the gene for calpain 3. This confirmed her diagnosis of LGMD2A.

COMMENT

This case is typical of LGMD2A: the patient's onset of symptoms occurred in elementary school, she experienced ankle contractures, her CK level was 20 times the normal level, she experienced an indolent progression of weakness with need for a power wheelchair 2 decades after disease onset, she did not experience symptomatic pulmonary dysfunction until very late in the disease course, and no cardiac involvement was present.

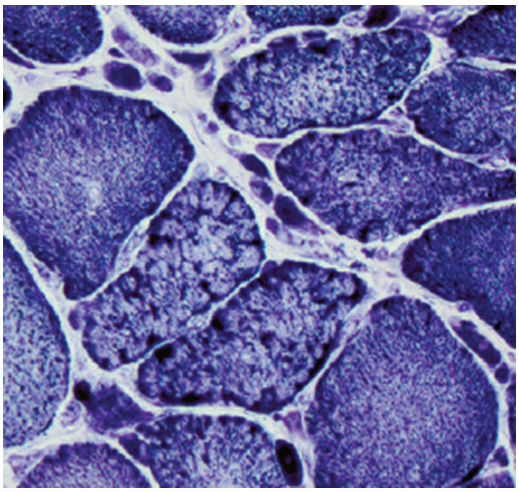

FIGURE 4-4
Lobulated fibers in a muscle biopsy from a patient with calpainopathy. Nicotinamide adenine dinucleotide (NADH) stain at 400×.

Since autosomal recessive mutations in any one of the sarcoglycans tends to lead to dysfunction and decreased quantities of the sarcoglycan complex, the clinical manifestations of all four sarcoglycanopathies are fairly similar. Disease onset ranges from infancy through adulthood but occurs in the first decade in most cases.[6] Milder cases with later onset have been reported.[25,26] Weakness begins in the proximal lower extremities and then involves the proximal upper extremities. Scapular winging, calf hypertrophy, macroglossia, ankle contractures, and scoliosis are common features in early-onset, more severe cases. Loss of ambulation occurs in the second through fourth decades, with the majority of patients wheelchair dependent in their teenage years. Dilated cardiomyopathy afflicts a minority of patients, but respiratory insufficiency necessitating nocturnal noninvasive ventilation affects many patients later in the disease course.[27] Left ventricular systolic dysfunction occurs less often in α-sarcoglycanopathies than the other sarcoglycanopathies.

In evaluation of patients with sarcoglycanopathies, CK levels are elevated 4 to 100 times the normal level. In the sarcoglycanopathies, leg MRI reveals principal involvement of lumbar paraspinal, gluteal, and thigh muscles with sparing of calf muscles until late in the disease course after loss of ambulation. In the anterior thighs, a predictable proximal to distal gradient of fatty and fibrous replacement occurs with relative sparing of the distal vasti muscles.[28] Muscle biopsies reveal dystrophic findings with diminished or absent staining for the sarcoglycans. When immunostaining for the four sarcoglycans (γ-sarcoglycan, α-sarcoglycan, β-sarcoglycan, and δ-sarcoglycan), the level of diminished staining does not predict which sarcoglycan gene is mutated. Complete absence of immunostaining correlates with earlier onset of disease, a more severe phenotype, and earlier loss of ambulation.[29]

Limb-Girdle Muscular Dystrophy Type 2I (LGMD R9 *FKRP*–Related)

Autosomal recessive mutations in *FKRP*, the gene encoding fukutin-related protein (FKRP), cause muscular dystrophy with a spectrum from congenital onset with muscle, eye, and brain involvement through asymptomatic hyperCKemia in middle age. A high relative prevalence exists in Northern European countries (approximately 1 in 50,000 persons in Denmark and Norway).[30] Patients often have concomitant cardiorespiratory dysfunction in addition to limb-girdle weakness. FKRP is one of more than 18 enzymes involved in glycosylation of α-dystroglycan, a process key to the structural integrity between the sarcolemma and the extracellular matrix (FIGURE 4-3). Disease severity depends on each patient's pathogenic variants. A common pathogenic variant

exists in Northern European populations (c.826C>A; p.L276I). In the United States, this c.826C>A mutation was present in 72% of alleles, and more than 95% of patients with LGMD2I had at least one copy of this c.826C>A pathogenic variant. Patients homozygous for the c.826C>A mutation always present with the LGMD phenotype or with milder disease. Patients heterozygous for the c.826C>A mutation have a more severe, Duchenne muscular dystrophy–like disease with onset in the first decade, loss of ambulation in the second decade, and need for ventilator support by the fourth decade.

Mean age of onset for the LGMD presentation is the second decade of life (range of 2 to more than 60 years of age). Weakness most commonly presents in the proximal lower extremities, with hip flexion and hip adduction most affected. Scapular winging occurs in less than half of cases, calf hypertrophy is commonplace, and hypertrophy also occurs in the tongue sometimes.[6] Exercise-induced muscle pain and myoglobinuria afflict as many as two-thirds and one-third of patients with LGMD2I, respectively.[31,32] Interestingly, patients with LGMD2I can experience abrupt, reversible weakness in conjunction with a febrile illness. This acute illness-associated weakness sometimes occurs in children prior to onset of weakness associated with LGMD2I, lasts for a few days to a more than a month, may occur more than once, and has an associated increase in CK levels (often more than double baseline) during the weakness.[32]

In the evaluation of LGMD2I, CK levels are nearly uniformly elevated, most often more than 10-fold. Dilated cardiomyopathy is commonly delineated through echocardiography, with myocardial fibrosis often noted on cardiac MRI. Importantly, the severity of cardiac and skeletal muscle involvement may not correlate, thus cardiac surveillance is recommended even in patients with mild weakness. Forced vital capacity is reduced in more than half of cases, with nocturnal noninvasive respiratory support required in 25% to 50% of patients. Thigh MRIs reveal abnormal signal with fatty and fibrous infiltration in the iliopsoas, adductors, and gluteus maximus with relative conservation of the anterior thigh muscles. Muscle biopsies reveal a dystrophic picture with reduced immunostaining for glycosylated α-dystroglycan. Overall, no clear correlation exists between clinical severity and muscle biopsy features in terms of the extent of histopathologic changes, level of glycosylated α-dystroglycan immunostaining, or quantity of glycosylated α-dystroglycan on immunoblots.[33]

Limb-Girdle Muscular Dystrophies Type 2L (LGMD R12 Anoctamin 5–Related)

LGMD2L is an autosomal recessive disorder resulting from mutations in *ANO5*,[34] the gene for anoctamin 5, a calcium-activated channel involved in phospholipid scrambling and important for membrane fusion and repair.[35] The relative prevalence of LGMD2L among a US cohort of 1003 patients with genetically confirmed LGMD is 7.2%.[14] However, in Northern European countries, the relative prevalence is higher, and the absolute prevalence may be as high as 2 per 100,000 in Finland, whereas in Southern Europe, LGMD2L is relatively uncommon, comprising only perhaps 2% of LGMD cases.[36] Interestingly, more males than females manifest symptoms, and the weakness in males tends to be more severe than in females.

Harboring pathogenic variants on both alleles in *ANO5* may lead to proximal weakness (LGMD), distal weakness (Miyoshi-like muscular dystrophy type 3), or asymptomatic hyperCKemia. Onset usually occurs in the third or fourth decades (range from 11 to 57 years of age) with weakness in the proximal lower

KEY POINTS

- Patients previously followed for the diagnosis of myositis, especially if poorly responsive to immunomodulatory therapy, should undergo limb-girdle muscular dystrophy genetic testing. Patients with limb-girdle muscular dystrophy with negative panel, exome or genome genetic analysis should be tested for autoantibodies recognizing 3-hydroxy-3-methylglutaryl coenzyme A reductase (even without exposure to statin drugs and even in children).

- Calpainopathies are the most common limb-girdle muscular dystrophy subtype, except in some Northern European countries and perhaps in Asia.

- If someone with limb-girdle muscular dystrophy cannot stand on his or her toes in the first few years after onset of disease, strong consideration should be given to limb-girdle muscular dystrophy type 2B (and type 2L).

- In the sarcoglycanopathies (limb-girdle muscular dystrophy types 2C through 2F), in leg MRIs, one often finds a predictable proximal to distal gradient of fatty and fibrous replacement in the anterior thigh, with relative sparing of the distal vasti muscles.

- Exercise-induced muscle pain affects two-thirds and myoglobinuria affects one-third of patients with limb-girdle muscular dystrophy type 2I.

extremities in LGMD2L. Similar to dysferlinopathies (LGMD2B), anecdotal evidence suggests athletic prowess prior to the onset of symptoms in LGMD2L. Weakness and atrophy preferentially afflict the quadriceps and biceps muscles, but initial quadriceps hypertrophy has been observed in some. Shoulder abduction is relatively spared compared to the biceps muscle weakness and atrophy. The majority of patients with LGMD2L remain ambulatory most of their lives but may need canes, walkers, or wheelchairs 20 to 40 years after symptom onset (CASE 4-3). Dysphagia to solids occurs in a minority of patients but is rarely problematic. Symptomatic respiratory insufficiency is not reported. However, since anoctamin 5 is also expressed in cardiac muscle, an increased number of patients with LGMD2L have ventricular premature complexes and some develop cardiomyopathies with reduced left ventricular ejection fractions.[37]

CK levels are usually elevated to around 1500 U/L to 4500 U/L (range of 200 U/L to 40,000 U/L) and tend to diminish over time. Some patients develop

CASE 4-2

A 30-year-old man presented for a follow-up neurologic visit for his 4-year history of progressive weakness. He first presented at the age of 26 years. At that time, he was a professional football player who stood 1.98 m (77 in) tall, weighed 138 kg (305 lb), and played on the defensive line. He noted he had "lost his explosiveness" off of the line of scrimmage. Additionally, he noted new-onset persistent muscle soreness, independent of vigorous physical activity. However, his examination was not just normal; it was supernormal. On motor examination, the examiner could not overpower a single muscle despite gaining a mechanical advantage and using all his weight.

At 27 years of age, he was hospitalized on several occasions for "elevated liver function tests" and recurrent episodes of muscle pain with creatine kinase (CK) levels of 11,000 U/L to 16,000 U/L (even when not working out over the off season). His history was complicated by muscle building with supplements during training (creatine, inhalers, anabolic steroids, and human growth hormone) along with use of amphetamines during games.

At 28 years of age his follow-up examination again demonstrated phenomenal strength with an inability of the examiner to overpower any muscle group. However, by 29 years of age, he had difficulty standing on his toes, and his hamstrings were weak (4+/5).

His examination at 30 years of age showed 4+/5 strength of the biceps and mild weakness of the proximal and distal muscle groups in his lower extremities, and his quadriceps had a "diamond on quadriceps" sign (ie, atrophy of the proximal and distal aspects of the quadriceps leaving a diamond-shaped appearance to the quadriceps during a partial squat).

Muscle biopsy disclosed only mild myopathic changes but was also absent of dysferlin immunostaining at the muscle membrane (FIGURE 4-5). Genetic testing revealed heterozygous pathogenic variants in *DYSF*.

myoglobinuria with exercise or have exercise intolerance prior to onset of weakness. EMGs reveal a myopathy often with sparse fibrillation potentials in more affected muscles. MRI demonstrates fatty and fibrous replacement in the medial gastrocnemius, soleus, hamstring, adductor, lumbar paraspinal, and biceps brachii muscles. Muscle biopsies may be relatively normal prior to onset of weakness but later reveal nonspecific myopathic changes such as variability in fiber size, increased internal nuclei, fiber splitting, and endomysial fibrosis. Noteworthy, amyloid deposition may be seen in the endomysium and in walls of intramuscular blood vessels in some biopsies.[38]

Bethlem Myopathy (LGMD D5 Collagen VI–Related)

Collagen VI–related myopathies include not only infantile-onset Ullrich congenital muscular dystrophy, but also the milder, later-onset Bethlem myopathy. For more information, refer to the article "Congenital Muscular

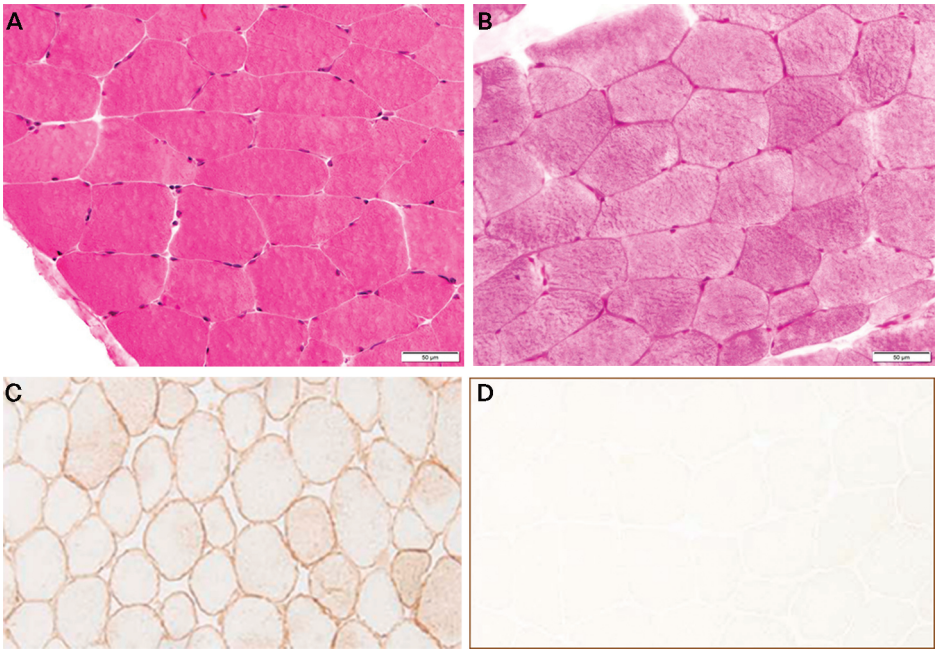

FIGURE 4-5

Muscle biopsy from a patient with dysferlinopathy. *A*, Hematoxylin and eosin (H&E) stain shows a nearly normal muscle biopsy with mild variability in fiber size; *B*, normal periodic acid-Schiff stain (decreasing the likelihood of a metabolic myopathy in this patient); *C*, normal dysferlin immunostaining in a control sample; *D*, absent dysferlin immunostaining in this patient with limb-girdle muscular dystrophy type 2B.

COMMENT

This case illustrates key features of dysferlinopathies (limb-girdle muscular dystrophy [LGMD] type 2B): (1) athleticism appears to be more common in patients with LGMD2B; (2) CK levels may be quite high (>10,000 U/L); and (3) at some point during the course of the disease, perhaps one-fourth to one-half of patients will manifest the diamond on quadriceps sign.[23]

Dystrophy and Congenital Myopathy" by Russell J. Butterfield, MD, PhD, FAAN,[39] in this issue of *Continuum*. Mutations in the gene for collagen XII, *COL12A1*, have also been associated with limb-girdle weakness.

Clinically, the age of onset in Bethlem myopathy ranges from 2 years of age up through the seventh decade. Patients present with proximal muscle atrophy and weakness, often with truncal weakness, and frequently have flexion contractures (interphalangeal joints, wrists, elbows, and ankles) along with superimposed distal joint hyperlaxity (distal interphalangeal joints of the hands along with metacarpophalangeal joint of the thumb).[40] Around two-thirds of patients will require an ambulatory aid by the age of 60 years.[41] Most patients with Bethlem myopathy have decreased respiratory function parameters but do not usually require noninvasive ventilation, except perhaps in the second half-century of life. Forced vital capacity correlates very well with skeletal muscle function but does not correlate well with age.[42] Cardiac

CASE 4-3

A 58-year-old man presented for neuromuscular evaluation for progressively worsening weakness. At 8 years of age, the patient had a creatine kinase (CK) level drawn as a normal control in a research study only to find the CK value was 4578 U/L. Annual CK levels were obtained for surveillance and ranged from 2000 U/L to 8000 U/L. However, the patient was asymptomatic and had significant athletic prowess. In his teenage years he won the state wrestling championship, in his twenties he competed as a body builder, and in his thirties he biked up to 3000 miles annually.

At 47 years of age, he developed weakness in his left leg, with subsequent progression of asymmetric weakness involving hip girdle muscles (weakest in the quadriceps, hamstrings, and adductors) and subsequently his biceps (FIGURE 4-6).

At the time of his evaluation, he used a cane and was unable to arise from the floor. He had a history of "skipped heart beats" all his life. He was of Danish and Finnish heritage, and his parents were healthy into their early eighties (they were first cousins). His younger brother was affected similarly to the patient, and his older sister had hyperCKemia, without weakness.

On evaluation, his examination showed normal mental status, cranial nerves, sensation, and coordination. However, his strength was graded as follows (scores shown for right/left with a maximum score of 5): shoulder abductors 5/5, elbow flexors 4−/4, elbow extensors 5/5, wrist and finger muscles 5/5, hip flexors 4+/4+, knee extensors 4−/4+, ankle dorsiflexors 5/5, and ankle plantar flexors 4/4−. EMG revealed a moderate, nonirritable myopathy. A biceps muscle biopsy demonstrated end-stage muscle.

Echocardiogram was normal, but a 48-hour Holter monitor discovered more than 10,000 premature ventricular contractions per 24 hours. Pulmonary function testing was normal. Genetic testing revealed homozygous pathogenic variants in *ANO5* (c.172C>T; p.R58W) in the patient, his younger brother, and his older sister.

conduction abnormalities are described in approximately 10% of patients with Bethlem myopathy. CK levels may be normal but can also elevate up to 10-fold.

RYR1-Related Myopathy

The ryanodine receptor is located in the sarcoplasmic reticulum and mediates excitation-contraction coupling in muscle fibers through release of calcium from the sarcoplasmic reticulum to activate contraction of sarcomeric proteins in skeletal muscle.[43] Pathogenic variants in *RYR1*, the gene for the ryanodine receptor, lead to a myriad of muscle conditions including malignant hyperthermia, central core disease, multiminicore disease, nemaline myopathy, centronuclear myopathy, axial myopathy, distal myopathy, congenital myopathy, King Denborough syndrome, exercise-induced rhabdomyolysis, pyridostigmine-responsive fatigable weakness, periodic paralysis, heat

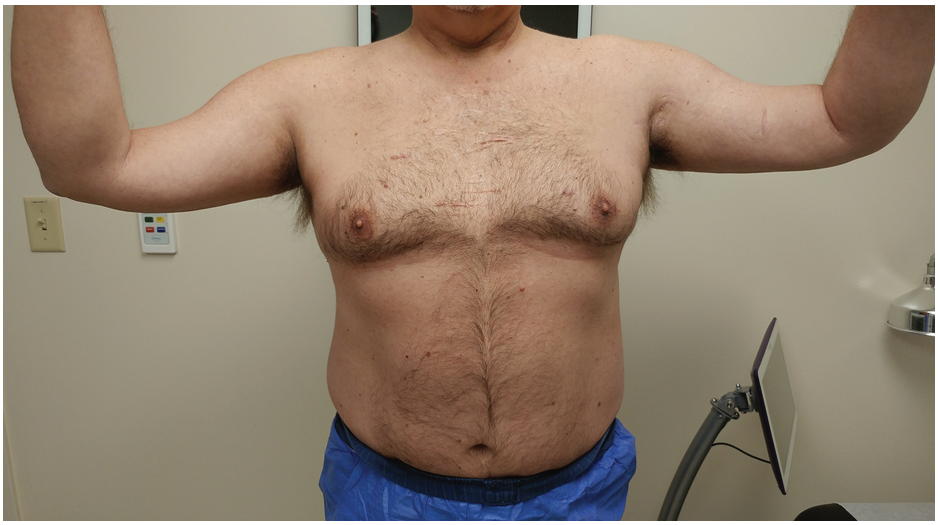

FIGURE 4-6
Biceps atrophy in a former weight lifter with limb-girdle muscular dystrophy type 2L.

COMMENT

This case highlights several features in limb-girdle muscular dystrophy type 2L: onset in middle age, predominantly Northern European ethnic background, asymmetric weakness, and more symptomatic involvement in males than females.

intolerance, and asymptomatic hyperCKemia.[44] Studies have revealed that a significant proportion of undiagnosed patients with an LGMD phenotype harbor *RYR1* pathogenic variants.[45,46] *RYR1* may be the causative gene in approximately 5% of patients with LGMD, usually in an autosomal dominant fashion.

Clinically, patients with *RYR1*-related myopathy with an LGMD phenotype have typical proximal weakness of the hip and shoulder girdle muscles with onset from infancy through later adulthood.[47] Patients with *RYR1*-related myopathy can also have hyperlaxity of joints, some distal weakness (especially of the hands), and may have some impairment of extraocular movements. In earlier-onset disease, scoliosis and axial weakness may be present. In later-onset cases, scapular winging and camptocormia may occur. Importantly, malignant hyperthermia should be considered a risk in all patients with *RYR1*-related myopathy as some patients have undergone as many as seven anesthesia exposures prior to their first episode of malignant hyperthermia.

Although substantial overlap occurs between phenotypes, genotypes, and histologic features on muscle biopsy, certain genotypes more often present with a particular phenotype or histologic type. Muscle biopsies can reveal nonspecific myopathic features such as internal nuclei and type 1 fiber predominance. However, they do not generally reveal the dystrophic features of excessive endomysial fat and fibrous tissue, nor do they have significant degenerating fibers or regenerating fibers. Cores, minicores, and central nuclei (usually without the typical "halo") may be present on biopsies but may not be apparent until decades into disease in milder cases.[44] CK levels are often normal but may be elevated up to 10-fold. Noteworthy, the CK level may be dramatically elevated in patients presenting with rhabdomyolysis. On MRI of the thigh, the rectus femoris muscle tends to be selectively spared. Since *RYR1* is such a large gene, it remains a challenge to understand the abundant numbers of variants of undetermined significance in the context of individual patients with a limb-girdle phenotype. Likely, many modifying genes and posttranslational modifications impact how the disease manifests in distinct patients.

Limb-Girdle Muscular Dystrophy Type 1B (Lamin A/C–Related Myopathy)

Through alternate splicing, *LMNA* is the gene that encodes lamin A and lamin C, intermediate filaments of the inner membrane of the nucleus. Mutations in *LMNA* cause LGMD1B but also are responsible for numerous other, sometimes overlapping, phenotypes including autosomal dominant Emery-Dreifuss muscular dystrophy, congenital muscular dystrophy, dilated cardiomyopathy, axonal neuropathy, Dunnigan lipodystrophy, Hutchinson-Gilford progeria syndrome, restrictive dermopathy, mandibuloacral dysplasia, and others. Lamin A/C–related myopathies likely account for approximately 3% to 5% of LGMD cases.[6] Because of its autosomal dominant inheritance along with its variability in the severity and phenotypic presentation of disease, once one patient with LGMD1B is found, often many other family members are discovered, though not always with the same clinical manifestations.

Onset of disease ranges from infancy through later adult life with a mean age of 27 years.[48] Weakness predominates in the proximal leg and arm muscles with predilection for humeral muscles and sometimes calf muscles. Most patients remain ambulatory for decades. Contractures of the elbows, ankles, hips, and

neck muscles are milder and later in onset than in the Emery-Dreifuss muscular dystrophy phenotype. Scapular winging, although common in the Emery-Dreifuss muscular dystrophy phenotype, is not common in LGMD1B. Heart involvement, cardiac arrhythmias, and dilated cardiomyopathy are common from the second decade onward and may presage weakness in skeletal muscles. Of utmost importance, early cardiology consultation and proactive intervention with cardiac pacemakers, defibrillators, and transplantation mitigate complications related to malignant arrhythmias and heart failure.

On evaluation, CK levels may be normal to elevated 5 times the normal range. EMG reveals a nonspecific myopathy, although a concomitant axonal neuropathy will be seen in a minority of cases. Muscle imaging reveals fatty infiltration predominantly in the medial gastrocnemius, adductor magnus, hamstring and gluteus muscles. Muscle biopsy may reveal fiber size variability, mild dystrophic features, and occasionally desmin accumulations.

TREATMENT

Guidelines for treatment of the LGMDs have been published.[6,49] Primary management of patients with LGMDs involves rehabilitation therapies and prevention of other organ system complications. Treatment recommendations for patients with LGMD include (1) medical management through multidisciplinary neuromuscular clinics; (2) access to cardiology, pulmonary, and orthopedic evaluation and treatment; (3) involvement of physical, occupational, and speech therapy along with access to orthotic and durable medical equipment services; (4) availability of genetic testing, interpretation, and counseling; and (5) encouragement for patients to remain active and lead fulfilling lives.

It is an exciting time for research in the LGMDs as small molecule, gene replacement, gene editing, and cell replacement therapies are in various stages of development and implementation.[50] Already, just as viral vector gene therapy has been successful in infants with spinal muscular atrophy, proof of concept and efficacy for viral vector gene replacement strategies in the LGMDs have been successful in animal models for β-sarcoglycan[51] and dysferlin,[52] along with phase 1 and 2 first in human studies. Viral vector gene therapy via systemic delivery in humans is in various stages of development for a number of LGMD genes. Additionally, the promise of gene editing through CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/CRISPR associated-9)[53] and other technologies may allow for perinatal correction of disease. Some patients with late stage LGMD may not benefit from gene correction therapies because of the extensive preceding loss of muscle mass with concomitant fatty and fibrous replacement of muscle. For these patients, myoblast, satellite, and stem cell therapies hover more distantly on the horizon. However, further progress in those areas may be much closer than previously expected.[54]

CONCLUSION

The LGMDs consist of a myriad of genetic muscle diseases with the similar phenotype of predominant weakness in the hip and shoulder girdles. Diagnosis via panel testing along with exome and genome sequencing up front has significantly decreased the invasiveness, cost, and timeline associated with

KEY POINTS

- Similar to dysferlinopathies (limb-girdle muscular dystrophy type 2B), anecdotal evidence exists for athletic prowess prior to onset of symptoms in anoctaminopathies (limb-girdle muscular dystrophy type 2L).
- A significant proportion of undiagnosed patients with a limb-girdle muscular dystrophy phenotype harbor *RYR1* pathogenic variants.
- In patients with limb-girdle muscular dystrophy type 1B, early cardiology consultation and proactive intervention with cardiac pacemakers, defibrillators, and transplantation mitigate complications related to malignant arrhythmias and heart failure.
- Treatment recommendations for patients with limb-girdle muscular dystrophy include (1) medical management through multidisciplinary neuromuscular clinics; (2) access to cardiology, pulmonary, and orthopedic evaluation and treatment; (3) involvement of physical, occupational, and speech therapy along with access to orthotic and durable medical equipment services; (4) availability of genetic testing, interpretation, and counseling; and (5) encouragement for patients to remain active and lead fulfilling lives.

diagnosis. Treatments continue to transition from supportive and rehabilitative care to interventions to ameliorate or eliminate manifestations of these genetic muscle disorders.

USEFUL WEBSITES

CLINICALTRIALS.GOV
ClinicalTrials.gov has up-to-date information regarding clinical trials on limb-girdle muscular dystrophies.
clinicaltrials.gov

LGMD-INFO.ORG
This website is a source for information related to the limb-girdle muscular dystrophies and includes a listing of the limb-girdle muscular dystrophy organizations and foundations for specific genetic subtypes.
lgmd-info.org/organizations

MUSCULAR DYSTROPHY ASSOCIATION
This website provides overviews of muscular dystrophies for clinicians, descriptions of current research, and patient-centered information.
mdausa.org

NEUROMUSCULAR DISEASE CENTER
This is an exceptional website due to its breadth of information related to the epidemiologic, genetic, protein, clinical, diagnostic, and treatment information related to LGMDs and other neuromuscular disorders. The format is bulleted for easy navigation, and the site contains frequent updates.
neuromuscular.wustl.edu

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