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Limb–Girdle and Congenital Muscular Dystrophies: Current Diagnostics, Management, and Emerging Technologies

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Abstract

The muscular dystrophies show muscle degeneration and regeneration (necrotizing myopathy) on muscle biopsy, typically associated with elevated serum creatine kinase, and muscle weakness. In 1986, the first causative gene was identified for the most prevalent and best-characterized form of muscular dystrophy, Duchenne muscular dystrophy. Over the past 25 years, the number of other genes determined to cause different subtypes has grown rapidly. This review gives a synopsis of the 45 genetically defined types of muscular dystrophies and describes the clinical, pathologic, and molecular aspects of each disease. DNA diagnosis remains the most sensitive and specific method for differential diagnosis, but molecular diagnostics can be expensive and complex (because of multiple genes at multiple testing facilities) and reimbursement may be challenging to obtain. However, emerging DNA sequencing technologies (eg, single-molecule third-generation sequencing units) promise to dramatically reduce the complexity and costs of DNA diagnostics. Treatment for nearly all forms remains supportive and is aimed at preventing complications. However, several promising approaches have entered clinical trials, providing tangible hope that quality of life will improve for many patients in the near future.

Keywords

Limb-girdle muscular dystrophy; Congenital muscular dystrophies; Diagnosis; Treatment; Muscular dystrophies; Hypotonia; Weakness; Calf hypertrophy; Joint contractures; Creatine kinase; Calpain deficiency; Dysferlinopathies; Sarcoglycanopathies; Fukutin–related protein; Telethonin; Titin; Emery Dreifuss; Myotilin; Caveolin; Muscle glycosylation disorders; POMT; LARGE; Merosin; Collagen VI

Introduction and Internet Resources

Muscular dystrophies are a group of inherited primary diseases of muscle, characterized pathologically by muscle fiber degeneration and clinically by muscle weakness. Classification of muscular dystrophies traditionally has been based on pathologic, clinical, and inheritance patterns (recessive, dominant, X-linked). Over the past 25 years, gene

mutation data have begun to dominate differential diagnostic methods (Tables 1 and 2). Molecular diagnostic techniques are now available for many of these disorders, although they may be challenging to implement in a systematic manner, and sequencing of multiple genes may be quite costly, with slow turnaround times. However, sequencing technologies are evolving quickly, and it may be possible within the next year or two to sequence all muscular dystrophy genes, or even the patient's entire genome, in a single test at low cost.

More in-depth coverage of the frequencies, clinical features, gene mutations, and genetic counseling of the limb–girdle muscular dystrophies (LGMDs) and congenital muscular dystrophies (CMDs) may be found at the National Institutes of Health–supported Gene Reviews website (LGMD: <http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=gene&part=lgmd-overview>; CMD: <http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=gene&part=cmd-overview>). In addition, the journal *Neuromuscular Disorders* periodically publishes a revised classification and is accessible at <http://www.muscle-genetable.org> and <http://194.167.35.195/>. For more information on current diagnostic tools available, both commercially and on a research basis, we recommend <http://www.genetests.org>.

Limb–Girdle Muscular Dystrophies

Limb-girdle muscular dystrophy describes a heterogeneous group of muscle disorders characterized by a predominantly proximal distribution of limb–girdle weakness. For decades, the LGMD diagnosis was an exclusionary one: when Duchenne muscular dystrophy (DMD)/Becker muscular dystrophy, facioscapulohumeral muscular dystrophy, myotonic dystrophy, metabolic myopathies, and other syndromic disorders were ruled out, the patient was assigned an LGMD diagnosis. The discovery of genetically distinct subtypes of LGMD has led to its current classification based on inheritance patterns, with the most common forms of LGMD, the autosomal recessive LGMDs, designated as LGMD2 (2A–2I; Table 1), and the autosomal dominant forms as LGMD1 with subtypes (1A–1E; Table 2). The sequence of disorder names is determined by the order of gene discovery, and the birthing order roughly corresponds to disease frequency. However, specific mutations may show a high frequency in certain populations and lead to a high disease frequency in that population (eg, LGMD2A in La Reunion Island, LGMD2C in North Africa, LGMD2I in Scandinavia and England). Many of the dominantly inherited forms are quite rare, often limited to a single extended family or very few families (so-called private mutations). Most individuals with LGMD show relative sparing of the heart and bulbar muscles, although given the great variability in presentation and gene mutations, exceptions occur.

The age at onset of symptoms in LGMD varies from early childhood to adulthood, but typically the onset is not congenital. In general, dominant forms tend to present after the second decade of life. Except for a few cases with rapid progression, the course usually is slowly progressive. The different LGMDs are first grouped into inheritance patterns, in which *LGMD1* refers to dominantly inherited forms (although the lamin A/C forms have recurring sporadic mutations, leading to isolated patients with dominant mutations) and *LGMD2* refers to recessive cases (typically, isolated patients).

Recessive LGMD (LGMD2 Series)

LGMD2A, or calpain 3 deficiency, is considered the most common form of recessive LGMD [1], with about 10% of US LGMD patients having this underlying gene defect [2]. The calpain 3 gene is a muscle-specific protease that appears important for muscle remodeling. Three clinically distinct phenotypes are recognized: a pelvifemoral form, the most common; the scapulohumeral form; and a very mild form manifested only by hyperCKemia. In general, the pattern is more atrophic, with significant involvement of the periscapular muscles, biceps, gluteus maximus, adductors, and hamstrings. Severe contractures develop early [3]. DNA testing is the preferred method of patient diagnosis, as biochemical testing is not particularly specific or sensitive.

LGMD2B is caused by mutations in the dysferlin gene, coding for a protein involved in membrane repair [4, 5]. Muscle involvement in dysferlinopathies (LGMD2B) may show a proximal limb-girdle distribution, a predominantly distal distribution with anterior tibial involvement, or a mixed distribution, even with identical mutations [6, 7]. Early weakness and atrophy of the gastrocnemius and soleus muscles (Miyoshi myopathy), which may lead to the inability to walk on the toes and to very high creatine phosphokinase (CPK) levels (at least early on), are suggestive of a dysferlinopathy diagnosis. Dysferlin deficiency may be misdiagnosed as polymyositis, as some patients present with a subacute inflammatory myopathy. Failure of steroid efficacy in a patient carrying an inflammatory myopathy diagnosis should trigger consideration of a dysferlinopathy. The onset typically is in the late teens or early 20s, and patients may be quite athletic before the subacute onset. The enigmatic role of the immune system in this disease is an increasing focus of research [8, 9].

LGMD2C, LGMD2D, LGMD2E, and LGMD2F are all the result of mutations in one of the four sarcoglycan protein genes (α , β , γ , δ) [10-14]. The sarcoglycan complex stabilizes the association of dystrophin with dystroglycan and the extracellular matrix. The integrity of this complex contributes to the stability of the plasma membrane cytoskeleton. Sarcoglycanopathies mimic dystrophinopathies, often with an early age of presentation and the presence of muscle hypertrophy, although cognitive deficit is rare.

LGMD2G is caused by mutations in the gene coding for telethonin, a component of the myofibrillar Z-line of the sarcomere. The disorder is relatively mild and has been reported in only five families to date. Early footdrop may indicate a telethoninopathy (LGMD2G).

LGMD2I is a common form of LGMD in northern Europe, and about 10% of LGMD patients in the United States have this diagnosis [15]. The disease is a mild variant of CMD1C (Table 2); both LGMD2I and CMD1C are the result of mutations of fukutin-related protein (FKRP). Clinical features include proximal muscle weakness and atrophy, calf muscle hypertrophy, increased incidence of cardiomyopathy, and elevated plasma levels of creatine kinase (CK) [16]. Caucasian patients with an LGMD phenotype should be evaluated for the common FKRP gene mutation, as a substantial number of these patients may have this form of LGMD.

Titin (TTN) is a sarcomeric protein connecting the Z-disc with the M-line and is associated with striated muscle development and structure and cell signaling. Tibial muscular

dystrophy results from mutations of the TTN gene (LGMD2J), and patients have a particularly early onset with a more severe phenotype [17].

Dominant LGMD

The clinical presentation of autosomal dominant LGMDs is more heterogeneous, with some families showing syndromic features beyond muscle symptoms. The most telling feature of the LGMD1 series of disorders is the dominant inheritance pattern in families (vertical transmission from affected parent to affected child). However, lamin A/C mutations, in particular, often are new mutations, whereby a child has the dominant mutation but neither parent carries it. Lamin A/C mutation-carrying patients often have heart conduction block (Emery-Dreifuss muscular dystrophy [EDMD]), some may have peripheral neuropathy, whereas others have symptoms limited to skeletal muscle weakness and wasting (Table 1) [18].

Cardiac involvement also is a common feature of LGMD1A (myotilinopathy), LGMD1B, LGMD1C (caveolinopathy), LGMD1D, and LGMD1E. In LGMD1C (caveolinopathy), asymptomatic elevation of CPK, myalgias and cramps, rippling muscle disease, distal myopathy, and prolonged QT syndrome appear to be more common than in the classic LGMD presentation [19]. A nasal quality of speech and slow progression is common in LGMD1A (myotilinopathy) [20].

Of the dominantly inherited group, the most common and most clinically variable form is caused by mutations in the nuclear envelope protein lamin A/C. Depending on the specific mutation, diagnoses may include LGMD1B, dilated cardiomyopathy, EDMD, CMD with rigid spine, autosomal recessive Charcot-Marie-Tooth neuropathy type 2A, familial partial lipodystrophy, mandibuloacral dysplasia, and premature aging, as well as a lethal phenotype. Recent studies have shown that lamin A/C EDMD mutations show abnormalities in muscle cell regeneration, and this molecular pathology is shared with the X-linked recessive EDMD form of muscular dystrophy (emerin) [21, 22].

Diagnosis

Achieving a precise diagnosis of a particular type of LGMD often is challenging. In certain specific types of LGMD, there may be clinical clues, but there is substantial overlap in clinical picture and laboratory measures among the different forms. CK usually is modestly elevated but may be very high in sarcoglycanopathies, dysferlinopathy, and caveolinopathy. Electromyography (EMG) typically shows myopathic changes with small polyphasic potentials; muscle biopsy reveals dystrophic changes with degeneration and regeneration of muscle fibers, fiber splitting, internal nuclei, fibrosis, and moth-eaten and whorled fibers. Sporadic LGMD cases with modest CK elevation may be clinically indistinguishable from spinal muscular atrophy, type III (Kugelberg-Welander). In this setting, EMG is particularly useful for differentiating a neurogenic from a myopathic process. Compared with the dominantly inherited LGMD1 group, most autosomal recessive LGMDs (designated LGMD2) have an earlier onset, rapid progression, and relatively high CK values.

The differential diagnosis of the different types of LGMD typically requires DNA analysis. A DNA-based molecular diagnosis often (but not always) will provide a definitive

molecular diagnosis into one of the specific gene-based etiologies. Carrying out a DNA-based molecular diagnosis may be a complex and expensive journey, and reimbursement for expensive molecular tests may be challenging. As such, it is important to consider the utility of a DNA-based diagnosis, particularly in this age of clinical effectiveness research in which there is an effort to balance costs with the medical relevance to patients and their families.

If the design of a therapeutic regimen depends on knowledge of the gene defect, then a strong rationale exists for pursuing a molecular diagnosis. Unfortunately, there are few available therapeutic approaches for any type of muscular dystrophy (discussed in more detail later), and therapeutic and clinical care rarely requires a DNA-based molecular diagnosis. The molecular diagnosis may be important for genetic counseling of the patient and/or the family. However, many parents of recessive LGMD patients are beyond childbearing by the time a molecular diagnosis may be defined, and the risk to the patient's future children is minimal (he or she is unlikely to find another carrier as a mate). Thus, in practice, molecular diagnosis for either therapeutics or genetic counseling only rarely is useful. For dominant LGMDs, the recurrence risk to both the parents and affected patients is 50%; in certain cases, molecular diagnostics may permit prenatal diagnosis, but the rarity of specific gene mutations makes this challenging in practice.

A diagnostic workup for an LGMD patient may include a muscle biopsy.

Immunohistochemistry with antibodies against α , β , γ , and δ sarcoglycans; dystrophin; dystroglycans; and merosin may offer a means for a specific biopsy diagnosis (eg, α -sarcoglycanopathy). However, none of these biochemical tests is particularly sensitive or specific, and typically these tests must be followed up with DNA mutation studies.

The lack of pathognomonic clinical signs or symptoms, the lack of sensitive and specific biochemical tests, and the complex and expensive molecular diagnostic approaches all lead to a bit of a mess, particularly when the identification of the gene mutation may have little or no impact on therapeutics or genetic counseling. That said, it is difficult for translational research to make progress toward therapeutics if patients are not characterized at the molecular level. This diagnostic dilemma may be solved in the near future. Rapidly paced developments in DNA sequencing methodologies promise to bring down costs while scaling up the amount of "genomic real estate" that can be covered in a single test. For example, the DNA sequencing technologies of 2009 involved amplification of each exon of a candidate gene (about 60 exons each for dystrophin, dysferlin, and calpain), with labor-intensive sequencing of each exon, at a cost of about \$1000 or more per gene. However, newly emerging sequencers can rapidly sequence DNA from a patient in "real time" by watching single DNA polymerases copy the patient's DNA (Fig. 1). The DNA sequencers of 2010 will enable the rapid sequencing of all muscular dystrophy genes in a single reaction, at a dramatically reduced cost. Indeed, the current trajectory of DNA sequencing technologies predicts the ability to perform whole-genome sequencing of a patient in 15 min within a hospital laboratory.

These developments should bring molecular diagnostics to the "patient bedside" with low cost, high accuracy, and short timeframes, and will in turn enable more rapid advances in novel therapeutic approaches and our understanding of disease pathophysiology.

Treatment

Treatment for the large majority of LGMD patients remains palliative and supportive. Physiotherapy to prevent joint deformities and promote walking is recommended, and these principles may be applied to other types of muscular dystrophies. A passive stretching physical therapy program should be instituted early, soon after diagnosis. The use of knee-ankle-foot orthoses at bedtime is recommended to prevent contractures. With regard to lower limb surgery, which has been studied more in the DMD population, bilateral hip and knee replacement, aponeurectomy of the iliotibial band, or Achilles tendon lengthening may prolong ambulation, but these procedures remain controversial. Surgery may prolong assisted standing ability and, in turn, prevent long-term spine deformities and back pain.

Because cardiomyopathy or conduction defects may be life threatening, monitoring of heart involvement, and clinical intervention when needed, is important. Although specific guidelines for each type of LGMD are lacking, repeat electrocardiography (ECG)/Holter monitoring and echocardiography every 2 to 5 years are advised, even for asymptomatic patients. Angiotensin-converting enzyme inhibitors and β -blockers, well described to have a beneficial effect on DMD, are recommended in the presence of left ventricular dysfunction.

With regard to the pharmacologic approach, the benefit of steroids has been reported in some types of LGMD, including LGMD2I [23], LGMD2L [24], and LGMD 2D [25]. A double-blind, placebo-controlled study of deflazacort in LGMD2B/Miyoshi myopathy is in progress (<http://clinicaltrials.gov>).

In terms of more experimental approaches, adeno-associated virus-mediated gene therapy is being pursued, although there are still many longstanding barriers, including immunologic complications, challenges of producing enough material for treatments, and difficulties in delivery to muscles throughout the body. The most promising approach in DMD is thought to be exon skipping, the only approach to show efficacy in a large animal (dog) model [26•]. However, it may be challenging to apply this approach to the LGMDs, as most genes do not seem to tolerate the in-frame deletions the dystrophin gene seems able to tolerate. Another promising approach in DMD, stop codon readthrough using the small molecule drug ataluren, is in phase 3 clinical trials for the small subset of patients showing point mutation “nonsense” mutations [27•]. If ataluren proves effective in DMD, it may be applied to the subset of LGMD patients showing stop codon mutations. This would serve as a strong rationale for molecular diagnosis of all LGMD patients, as the specific mutation would become highly relevant to therapeutic management.

Congenital Muscular Dystrophies

Infants with hypotonia and weakness at birth in whom muscle biopsies show changes consistent with muscular dystrophy are described as having congenital muscular dystrophy. A clinically similar disorder, congenital myopathy, typically is distinguished by serum CK (higher in CMD than congenital myopathy) and muscle biopsy (CMD shows evidence of degeneration/regeneration of myofibers, whereas congenital myopathy shows more of a developmental defect in myofiber formation). In most patients, particularly those with a nonsyndromic CMD type, the clinical course progresses very slowly, although in some cases

it is static and patients can acquire motor skills allowing ambulation [28, 29]. This review focuses on CMD; the reader is referred to GeneReviews and other resources for a description of the many causes of congenital myopathy.

In terms of genetic underpinnings, all CMDs are autosomal recessive, and there are two overarching groups: disorders of dystroglycan glycosylation (<http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=gene&part=cdg>) and disorders of the basal lamina (laminin α_2 and collagen IV; <http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=gene&part=cmd-overview>) (Table 1).

Serum CK concentration usually is elevated. Abnormal characteristics on muscle biopsy include extensive fibrosis, degeneration and regeneration of muscle fibers, and proliferation of fatty and connective tissue. The presence or absence of structural central nervous system abnormalities (syndromic or nonsyndromic CMD), detected by neuroimaging or at autopsy, forms the basis of CMD classification. More recently, given the advances in molecular genetics, a classification based on the protein defects has emerged. CMDs related to changes in the dystrophin-associated glycoproteins–extracellular matrix include CMD with merosin deficiency (CMD1A); collagen VI–related CMDs, including Ulrich CMD and Bethlem myopathy; CMDs with abnormal glycosylation of α -dystroglycan, including Fukuyama CMD, muscle-eye-brain disease, Walker-Warburg syndrome, CMD1C, and CMD1D; and integrin α_7 deficiency.

The syndromic CMDs have two main characteristic features: 1) they involve multiple organ systems, resulting in severe brain malformations and developmental delay, and 2) from a biochemical and molecular standpoint, they are caused by genetic defects that disrupt posttranslational modification of α -dystroglycan and other currently unknown proteins [30, 31•]. α -Dystroglycan is an integral component of the dystrophin–glycoprotein complex, which is involved in stabilizing the muscle cell during contraction and relaxation. Disruption of α -dystroglycan may occur because of errors in glycosylation (Fukuyama CMD) or O-mannosylation (Walker-Warburg syndrome and muscle-eye-brain disease). Involvement of the brain, retina, or cochlea is a disorder of development, in which dystroglycan plays a critical role in the patterning and development of the central nervous system, including neuronal migration, organization of synapses, and assembly of the basement membrane [32]. Other forms unrelated to the dystrophin–dystroglycan–extracellular matrix complex, such as rigid spine syndrome with mutation in the selenoprotein and lamin A/C deficiency, also are included in this classification, although the details of the molecular mechanism of action are not clearly understood (Table 1). Despite the rapid advances in the genetics of CMD reported over the past 8 years, there are still clinical forms in which the gene and protein defect are unknown (Table 1).

Diagnosis

The diagnosis of a specific subtype of CMD relies on a detailed medical history, physical examination, family history, neuroimaging, evaluation of serum CK concentration, and muscle biopsy for histologic examination and immunostaining. CK usually is elevated and EMG findings show myopathic features; an associated neuropathy is detected in most patients with merosin-deficient CMD [33]. Muscle biopsy usually shows dystrophic

changes, and muscle immunohistochemical examination with antimerosin, anticollagen type VI, and anti- α -dystroglycan antibodies usually reveals an absence of the respective protein in the sarcolemma of the muscle fibers. Brain and ocular abnormalities should be excluded by MRI and eye examination. At this time, commercial DNA tests are available for most of the known CMD forms, with the exception of integrin α_7 deficiency, for which a test is available only on a research basis.

Treatment

No definitive treatment is available for these disorders. As for other muscular dystrophies, management and prevention of complications are the rule. Management includes physical therapy and stretching exercises to promote mobility and prevent contractures, use of mechanical assistive devices to help ambulation and mobility, monitoring and surgical intervention for orthopedic complications, and monitoring of respiratory function. Some individuals benefit from assisted cough, noninvasive ventilation, or mechanical ventilation via tracheostomy [34].

Based on a better understanding of the pathophysiology and molecular basis of the different types of CMD, very active basic and translational research is in progress. More specifically, in Ulrich CMD, which involves a deficiency in collagen type VI, a mitochondrial defect linked to dysregulation of the mitochondrial permeability transition pore (PTP) opening was demonstrated in the Col6a1^{-/-} null mouse and later replicated in Ulrich CMD muscle cells in culture, providing justification for a clinical trial using cyclosporine, an inhibitor of PTP opening [35].

Emery-Dreifuss Muscular Dystrophy

Clinical Aspects

EDMD affects muscles in a humeroperoneal distribution, and cardiac muscle often is involved. Muscle weakness and wasting typically start in the upper extremities, with symmetric involvement of biceps and triceps and relative preservation of the deltoid muscles. Later, distal leg weakness with atrophy of the peroneal muscles is noted. Face, thigh, and hand weakness is less common and may occur late. Contractures at the elbows and ankles are an early feature, often associated with toe walking, and may be the first manifestations of the disease. Contractures on the posterior aspect of the neck and lower spine also are seen. Cardiac involvement consists of a cardiomyopathy, with atrioventricular (AV) block and often atrial paralysis. ECG may show varying degrees of AV block, small T waves, and atrial arrhythmias. Age at onset, severity, and progression of muscle and cardiac involvement demonstrate both inter- and intrafamilial variability. Clinical variability ranges from early onset with severe presentation in childhood to later adult onset with slow progression. Cardiomyopathy may lead to sudden death in approximately 50% of affected individuals, usually early in adult life.

Diagnosis

Serum CK typically is low relative to the other dystrophies, rarely above a few hundred units per liter. Although EMG usually displays myopathic features, it also may reveal

evidence of denervation, and recent studies suggest defects at the neuromuscular junction [36]. Muscle biopsy usually shows mild myopathic changes with internal nuclei, variation in fiber size, focal connective tissue proliferation, and occasional necrotic fibers. ECG may show varying degrees of AV block, small T waves, and atrial arrhythmias.

X-linked (EDMD1) [37], autosomal dominant (EDMD2), and recessive EDMD (EDMD3) are caused by mutations in genes coding for proteins of the nuclear envelope [38]. DNA testing for EDMD1 affecting emerin (Xq28) and for EDMD2 and EDMD3 due to mutations in the lamin A/C gene (*LMNA*; 1q21.2) is commercially available. Among rare patients showing clinical overlap with EDMD, mutations have been seen in the synaptic nuclear envelope protein 1 gene (*SYNE1*) in three patients in three families (EDMD4) and in the synaptic nuclear envelope protein 2 gene (*SYNE2*) in two patients in two families (EDMD5), also termed nesprin-1 and nesprin-2, respectively [39]. Inheritance was autosomal dominant or sporadic.

The differential diagnosis includes rigid spine syndrome, which in addition to elbow and ankle contractures, usually is associated with very limited flexion of the spine, relatively mild and slowly progressive myopathy, and absence of cardiomyopathy. Conversely, severe respiratory involvement is unusual in EDMD [40].

Treatment

Treatment is limited to prevention of contractures and control of the cardiac conduction block and cardiomyopathy. Cardiac impairment often is the life-limiting feature of the illness; therefore, the cardiac status of the patient should be investigated, even in those who are asymptomatic. Annual cardiac assessment and monitoring of respiratory function are recommended. Installation of a cardiac pacemaker is mandatory to prevent sudden death in patients with evidence of AV block. For patients with decreased left ventricular function or atrial arrhythmias, use of antithromboembolic drugs is recommended to prevent secondary complications. Heart transplantation has had successful results [41]. Cardiac evaluation also is recommended for relatives at risk for autosomal dominant EDMD and female carriers of X-linked EDMD.

Inhibition of ERK (extracellular signal-regulated kinase) activation in the development of cardiomyopathy currently is being investigated as a therapeutic option to prevent or delay heart failure in humans with EDMD and related disorders caused by mutations in *LMNA* [42].

Conclusions

Despite advances in diagnosis, the LGMD and CMD forms of muscular dystrophy remain a diagnostic challenge and therapeutic options are limited. It is hoped that recent advances in experimental therapeutics for DMD (eg, exon skipping, stop codon readthrough) may be applied to these less common muscular dystrophies. With the longer survival of patients with LGMD and CMD as a result of improvements in standard of care, there likely will be an increased caseload and, consequently, a need for a more proactive approach in patient management, including medical rehabilitation research. The basis of current muscular

dystrophy management is multidisciplinary, involving nutritionists, physiatrists and physical therapists, and experts in orthopedics and cardiorespiratory management. Prevention of infections by assuring adequate immunization also is mandatory. Genetic counseling for patients and families may be important, allowing a better understanding of the genetic condition along with family planning through genetic risk assessment and the possibility of a prenatal diagnosis. Lastly, social and emotional support to reduce the social isolation commonly felt by patients, especially teenagers, with muscular dystrophies should be part of these patients' management.

A “game-changing” development in the diagnosis of LGMD and CMD is the emerging DNA sequencing technologies that will allow large-scale sequencing of all muscular dystrophy genes in a single test at a low cost and rapid turnaround (Fig. 1). Bringing large-scale, low-cost DNA sequencing to the clinic not only will define the diagnosis with less invasive tests, but also will lead to much larger groups of molecularly defined patients who can enroll in clinical trials. Clinical trials with promising new treatment strategies can be conducted successfully only if researchers and health care providers reach a consensus on standards of diagnosis and care, and if patients can be recruited rapidly across national boundaries. Fortunately, such efforts have been supported by the Centers for Disease Control and Prevention in the United States, and publications are now available [43•, 44•]. Critical to the anticipated therapeutic advances is the establishment of neuromuscular clinical trial networks that can rapidly enroll well-characterized patients in multisite, standardized clinical trials. Fortunately, sensitive and reliable clinical trial end points are emerging [45, 46], and several clinical trial networks have been established, including the Cooperative International Neuromuscular Research Group (<http://www.cinrgresearch.org>), TREAT-NMD (<http://www.treat-nmd.eu>), and the Muscle Study Group (<http://www.urmc.rochester.edu/msg/>).

References

Papers of particular interest, published recently, have been highlighted as:

• Of importance

•• Of major importance

1. Richard I, Broux O, Allamand V, et al. Mutations in the proteolytic enzyme calpain 3 cause limb-girdle muscular dystrophy type 2A. *Cell*. 1995; 81(1):27–40. [PubMed: 7720071]
2. Chou FL, Angelini C, Daentl D, et al. Calpain III mutation analysis of a heterogeneous limb-girdle muscular dystrophy population. *Neurology*. 1999; 52(5):1015–1020. [PubMed: 10102422]
3. Richard I, Roudaut C, Saenz A, et al. Calpainopathy—a survey of mutations and polymorphisms. *Am J Hum Genet*. 1999; 64(6):1524–1540. [PubMed: 10330340]
4. Liu J, Aoki M, Illa I, et al. Dysferlin, a novel skeletal muscle gene, is mutated in Miyoshi myopathy and limb girdle muscular dystrophy. *Nat Genet*. 1998; 20(1):31–36. [PubMed: 9731526]
5. Bashir R, Britton S, Strachan T, et al. A gene related to *Caenorhabditis elegans* spermatogenesis factor fer-1 is mutated in limb-girdle muscular dystrophy type 2B. *Nat Genet*. 1998; 20(1):37–42. [PubMed: 9731527]
6. Saito H, Suzuki N, Ishiguro H, et al. Distal anterior compartment myopathy with early ankle contractures. *Muscle Nerve*. 2007; 36(4):525–527. [PubMed: 17614318]

7. Nguyen K, Bassez G, Bernard R, et al. Dysferlin mutations in LGMD2B, Miyoshi myopathy, and atypical dysferlinopathies. *Hum Mutat.* 2005; 26(2):165. [PubMed: 16010686]
8. Nagaraju K, Rawat R, Veszelszky E, et al. Dysferlin deficiency enhances monocyte phagocytosis: a model for the inflammatory onset of limb-girdle muscular dystrophy 2B. *Am J Pathol.* 2008; 172(3):774–785. [PubMed: 18276788]
9. Kesari A, Fukuda M, Knoblach S, et al. Dysferlin deficiency shows compensatory induction of Rab27A/Slp2a that may contribute to inflammatory onset. *Am J Pathol.* 2008; 173(5):1476–1487. [PubMed: 18832576]
10. Roberds SL, Leturcq F, Allamand V, et al. Missense mutations in the adhalin gene linked to autosomal recessive muscular dystrophy. *Cell.* 1994; 78(4):625–633. [PubMed: 8069911]
11. Bönnemann CG, Modi R, Noguchi S, et al. Beta-sarcoglycan (A3b) mutations cause autosomal recessive muscular dystrophy with loss of the sarcoglycan complex. *Nat Genet.* 1995; 11(3):266–273. [PubMed: 7581449]
12. Lim LE, Duclos F, Broux O, et al. Beta-sarcoglycan: characterization and role in limb-girdle muscular dystrophy linked to 4q12. *Nat Genet.* 1995; 11(3):257–265. [PubMed: 7581448]
13. Noguchi S, McNally EM, Ben Othmane K, et al. Mutations in the dystrophin-associated protein gamma-sarcoglycan in chromosome 13 muscular dystrophy. *Science.* 1995; 270(5237):819–822. [PubMed: 7481775]
14. Nigro V, de Sá Moreira E, Piluso G, et al. Autosomal recessive limb-girdle muscular dystrophy, LGMD2F, is caused by a mutation in the delta-sarcoglycan gene. *Nat Genet.* 1996; 14(2):195–198. [PubMed: 8841194]
15. Brockington M, Yuva Y, Prandini P, et al. Mutations in the fukutin-related protein gene (FKRP) identify limb girdle muscular dystrophy 2I as a milder allelic variant of congenital muscular dystrophy MDC1C. *Hum Mol Genet.* 2001; 10(25):2851–2859. [PubMed: 11741828]
16. Mercuri E, Brockington M, Straub V, et al. Phenotypic spectrum associated with mutations in the fukutin-related protein gene. *Ann Neurol.* 2003; 53(4):537–542. [PubMed: 12666124]
17. Hackman P, Marchand S, Sarparanta J, et al. Tibial muscular dystrophy is a titinopathy caused by mutations in TTN, the gene encoding the giant skeletal-muscle protein titin. *Am J Hum Genet.* 2002; 71(3):492–500. [PubMed: 12145747]
18. Benedetti S, Menditto I, Degano M, et al. Phenotypic clustering of lamin A/C mutations in neuromuscular patients. *Neurology.* 2007; 69(12):1285–1292. [PubMed: 17377071]
19. Traverso M, Gazzero E, Assereto S, et al. Caveolin-3 T78M and T78K missense mutations lead to different phenotypes in vivo and in vitro. *Lab Invest.* 2008; 88(3):275–283. [PubMed: 18253147]
20. Hauser MA, Horrigan S, Salmikangas P, et al. Myotilin is mutated in limb girdle muscular dystrophy 1A. *Hum Mol Genet.* 2000; 9(14):2141–2147. [PubMed: 10958653]
21. Bakay M, Wang Z, Melcon G, et al. Nuclear envelope dystrophies show a transcriptional fingerprint suggesting disruption of Rb-MyoD pathways in muscle regeneration. *Brain.* 2006; 129:996–1013. [PubMed: 16478798]
22. Melcon G, Kozlov S, Cutler DA, et al. Loss of emerin at the nuclear envelope disrupts the Rb1/E2F and MyoD pathways during muscle regeneration. *Hum Mol Genet.* 2006; 15(4):637–651. [PubMed: 16403804]
23. Darin N, Krossmark A, Ahlander A, et al. Inflammation and response to steroid treatment in limb-girdle muscular dystrophy 2I. *Eur J Paediatr Neurol.* 2007; 11(6):353–357. [PubMed: 17446099]
24. Godfrey C, Escolar D, Brockington M, et al. Fukutin gene mutations in steroid-responsive limb girdle muscular dystrophy. *Ann Neurol.* 2006; 60(5):603–610. [PubMed: 17044012]
25. Angelini C, Fanin M, Menegazzo, et al. Homozygous alphasarcoglycan mutation in two siblings: one asymptomatic and one steroid-responsive mild limb-girdle muscular dystrophy patient. *Muscle Nerve.* 1998; 21(6):769–775. [PubMed: 9585331]
- 26••. Yokota T, Lu QL, Partridge T, et al. Efficacy of systemic morpholino exon-skipping in Duchenne dystrophy dogs. *Ann Neurol.* 2009; 65(6):667–676. [PubMed: 19288467] This is the first report of dystrophin-restored expression in a dog model of DMD using morpholinos and delineates the rationale for its use in DMD patients.

- 27•. Welch EM, Barton ER, Zhuo J, et al. PTC124 targets genetic disorders caused by nonsense mutations. *Nature*. 2007; 447(7140):87–91. [PubMed: 17450125] This article explains the rationale for the use of PTC124 to increase the expression of dystrophin in patients with DMD.
28. Reed UC. Congenital muscular dystrophy. Part I: a review of phenotypical and diagnostic aspects. *Arq Neuropsiquiatr*. 2009; 67(1):144–168. [PubMed: 19330236]
29. Reed UC. Congenital muscular dystrophy. Part II: a review of pathogenesis and therapeutic perspectives. *Arq Neuropsiquiatr*. 2009; 67(2A):343–362. [PubMed: 19547838]
30. Grewal PK, Hewitt JE. Glycosylation defects: a new mechanism for muscular dystrophy? *Hum Mol Genet*. 2003; 12(2):R259–R264. [PubMed: 12925572]
- 31••. Muntoni F, Torelli S, Brockington M. Muscular dystrophies due to glycosylation defects. *Neurotherapeutics*. 2008; 5(4):627–632. [PubMed: 19019316] This article suggests that the overexpression of LARGE might be a potential therapeutic strategy in glycosylation defects.
32. Cohn RD. Dystroglycan: important player in skeletal muscle and beyond. *Neuromuscul Disord*. 2005; 15(3):207–217. [PubMed: 15725582]
33. Quijano-Roy S, Renault F, Romero N, et al. EMG and nerve conduction studies in children with congenital muscular dystrophy. *Muscle Nerve*. 2004; 29(2):292–299. [PubMed: 14755496]
34. Wallgren-Pettersson C, Bushby K, Mellies U, et al. 117th ENMC workshop: ventilatory support in congenital neuromuscular disorders—congenital myopathies, congenital muscular dystrophies, congenital myotonic dystrophy and SMA (II) 4-6 April 2003, Naarden, The Netherlands. *Neuromuscul Disord*. 2004; 14(1):56–69. [PubMed: 14659414]
35. Hicks D, Lampe AK, Laval SH, et al. Cyclosporine A treatment for Ullrich congenital muscular dystrophy: a cellular study of mitochondrial dysfunction and its rescue. *Brain*. 2009; 132(1):147–155. [PubMed: 19015158]
36. Méjat A, Decostre V, Li J, et al. Lamin A/C-mediated neuromuscular junction defects in Emery-Dreifuss muscular dystrophy. *J Cell Biol*. 2009; 184(1):31–44. [PubMed: 19124654]
37. Bione S, D'Adamo P, Maestrini E, et al. Identification of a novel X-linked gene responsible for Emery-Dreifuss muscular dystrophy. *Nat Genet*. 1994; 8(4):323–327. [PubMed: 7894480]
38. Bonne G, Capeau J, De Visser M, et al. 82nd ENMC international workshop, 5th international Emery-Dreifuss muscular dystrophy (EDMD) workshop, 1st Workshop of the MYO-CLUSTER project EUROMEN (European muscle envelope nucleopathies), 15-16 September 2000, Naarden, The Netherlands. *Neuromuscul Disord*. 2002; 12(2):187–194. [PubMed: 11738362]
39. Ferreira A, Quijano-Roy S, Pichereau C, et al. Mutations of the selenoprotein N gene, which is implicated in rigid spine muscular dystrophy, cause the classical phenotype of multiminicore disease: reassessing the nosology of early-onset myopathies. *Am J Hum Genet*. 2002; 71(4):739–749. [PubMed: 12192640]
40. Bonne G, Yaou RB, Bérout C, et al. 108th ENMC International Workshop, 3rd Workshop of the MYO-CLUSTER project: EUROMEN, 7th International Emery-Dreifuss Muscular Dystrophy (EDMD) Workshop, 13-15 September 2002, Naarden, The Netherlands. *Neuromuscul Disord*. 2003; 13(6):508–515. [PubMed: 12899879]
41. Dell'Amore A, Botta L, Martin Suarez S, et al. Heart transplantation in patients with Emery-Dreifuss muscular dystrophy: case reports. *Transplant Proc*. 2007; 39(10):3538–3540. [PubMed: 18089432]
42. Muchir A, Shan J, Bonne G, et al. Inhibition of extracellular signal-regulated kinase signaling to prevent cardiomyopathy caused by mutation in the gene encoding A-type lamins. *Hum Mol Genet*. 2009; 18(2):241–247. [PubMed: 18927124]
- 43••. Bushby K, Finkel R, Birnkrant DJ, et al. Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and pharmacological and psychosocial management. *Lancet Neurol*. 2010; 9(1):77–93. [PubMed: 19945913] This article clearly delineates the current multidisciplinary approach to DMD, which may be extended to other types of progressive muscular dystrophy.
- 44••. Bushby K, Finkel R, Birnkrant DJ, et al. Diagnosis and management of Duchenne muscular dystrophy, part 2: implementation of multidisciplinary care. *Lancet Neurol*. 2010; 9(2):177–189. [PubMed: 19945914] This article clearly delineates the current multidisciplinary approach to DMD, which may be extended to other types of progressive muscular dystrophy.

45. Mayhew JE, Florence JM, Mayhew TP, et al. Reliable surrogate outcome measures in multicenter clinical trials of Duchenne muscular dystrophy. *Muscle Nerve*. 2007; 35(1):36–42. [PubMed: 16969838]
46. Mercuri E, Mayhew A, Muntoni F, et al. TREAT-NMD Neuromuscular Network. Towards harmonisation of outcome measures for DMD and SMA within TREAT-NMD; report of three expert workshops: TREAT-NMD/ENMC workshop on outcome measures, 12th-13th May 2007, Naarden, The Netherlands; TREAT-NMD workshop on outcome measures in experimental trials for DMD, 30th June-1st July 2007, Naarden, The Netherlands; conjoint Institute of Myology TREAT-NMD meeting on physical activity monitoring in neuromuscular disorders, 11th July 2007, Paris, France. *Neuromuscul Disord*. 2008; 18(11):894–903. [PubMed: 18818076]

Fig. 1.

Newly emerging single-molecule DNA sequencers. The Pacific Biosciences single molecule sequencer (<http://www.pacificbiosciences.com>). A glass slide is constructed with 160,000 picowells, in which each “reaction chamber” holds only a single DNA polymerase. The DNA polymerase at the bottom of the well is loaded with patient genomic DNA, and the polymerase begins to create a new strand (copy; replicate) the patient DNA using fluorescent substrates from the solution above the metal cladding. The detector below the cladding detects only the single fluorescent molecule being added to the growing strand (in this case, a yellow G nucleotide is being added to the preexisting red A nucleotide). The next addition of a base will cleave the yellow color from the G and then detect only the color of the next nucleotide added. In this manner, a real-time analysis of the growing chain is recorded, providing the DNA sequence of the patient. This process is done for 160,000 different wells on the same slide and is much more rapid than existing technologies. The current version of the machines can read 3 bp/s; however, the speed is anticipated to increase to 50 bp/s within a few years. Thus, all muscular dystrophy genes could be sequenced in parallel, very quickly, and at low cost. *ATP* adenosine triphosphate, *CTP* cytidine triphosphate, *GTP* guanosine triphosphate, *TTP* thymidine triphosphate

Table 1

Clinical features and cellular defects in autosomal recessive muscular dystrophies

Muscular dystrophy type	Important associated clinical features	Deficient protein/ locus	Cellular defect
LGMD2A (calpainopathy)	Proximal (normal hip extensors and adductors) scapular winging, early contractures, scoliosis	Calpain-3	Disruption of normal regulatory mechanism of proteolysis
LGMD2B (dysferlinopathy)	Calf and deltoid hypertrophy	Dysferlin	Loss of Ca ²⁺ -regulated membrane repair
Sarcoglycanopathies LGMD2C LGMD2D LGMD2E LGMD2F	Calf hypertrophy; dilated cardiomyopathy, more common in LGMD2E and 2F. Mild phenotypes may present with cramps and exercise intolerance.	γ-, α-, β-, and δ-Sarcoglycan	Maintenance of muscle membrane integrity
LGMD2G	Calf hypertrophy (50%) Cardiomyopathy (50%)	Telethonin (titin-cap)	Disruption of sarcomere stability
LGMD2H	Slow progression, very variable phenotype, lower > upper extremities	TMP-32	Transcription regulator activity
LGMD2I	Cardiomyopathy common, tongue hypertrophy, reflexes preserved until late, variable presentation	FKRP	Mannosylation of α-dystroglycan complex
LGMD2J	Non cardiomyopathy	Titin	Regulation of sarcomere contraction–relaxation
LGMD2K	Mild calf pseudohypertrophy, mental retardation, joint contractures at the ankles	POMT1	Mannosylation of α-dystroglycan complex
LGMD2L, LGMD2M, LGMD2N	Calf pseudohypertrophy, tongue hypertrophy, cardiomyopathy in some patients	LGMD2L: chromosome 11p13-p12 LGMD2M: fukutin N: ?	Unknown
CMDs			
Fukuyama CMD	CNS involvement	Fukutin	Impaired glycosylation of α-dystroglycan complex
Muscle-eye-brain disease	CNS involvement, retinal dysplasia, early cataracts	POMGNT1	Disruption of dystroglycan complex mannosylation
Walker-Warburg syndrome	CNS involvement, cobblestone complex, retinal dysplasia, coloboma, microphthalmia, seizures	POMT1 and POMT2	Disruption of dystroglycan complex mannosylation
CMD type 1D	Extensive white matter changes in CNS, developmental delay, spasticity, EMG myopathy	LARGE	Impaired glycosylation of α-dystroglycan complex
CMD with complete merosin deficiency	White matter changes (increased signal on T2); mental retardation; some cases with neuropathy, death secondary to respiratory failure	LAMA2	Impaired attachment, migration, and organization of myoblasts
CMD type 1C	Initial hypotonia; muscle hypertrophy, especially in calf; normal brain or cerebellar cysts	FKRP	Impaired glycosylation of α-dystroglycan complex
CMD with <i>ITGA7</i> mutations	Initial hypotonia; developed walking by 3–4 y	ITGA7	Impaired cell–cell and cell–matrix interaction in muscle membrane
CMD with early spine rigidity (RSS)	~3–7 years limited flexion of neck and spine, CNS minor conduction defects, nocturnal hypoventilation early	Selenoprotein N, 1	Unknown; role in myogenesis?
Ullrich CMD	Distal hyperextensibility, micrognathia,	Collagen type VI,	Disrupted stability of extracellular matrix

Muscular dystrophy type	Important associated clinical features	Deficient protein/ locus	Cellular defect
	nocturnal hypoventilation	COL6A1, COL6A2, and COL6A3	

CMD congenital muscular dystrophy, *CNS* central nervous system, *EMG* electromyographic, *LGMD* limb-girdle muscular dystrophy

Table 2

Clinical features and cellular defects in autosomal dominant and X-linked muscular dystrophies

Muscular dystrophy type	Important associated clinical features	Deficient protein/locus	Cellular/protein defect
DMD/BMD	Calf hypertrophy, cardiomyopathy	Dystrophin	Stabilization of membrane during contraction and relaxation, role in muscle fiber differentiation and postsynaptic membrane organization
X-EMD	Cardiac conduction block	Emerin	Integral protein of nuclear membrane
LGMD1A	Cardiomyopathy (50%), myalgias, dysarthria (30%)	Myotilin	Regulates sarcomeric organization
LGMD1B	Cardiomyopathy (62%)	Lamin A/C gene mutation	Role in myoblast differentiation
LGMD1C	Calf hypertrophy	Caveolin-3	Integral membrane protein, interacts with nNOS
LGMD1D	Legs > arms, dysphagia (30%)	Unknown; linkage to 7q	Unknown
LGMD1E	Dilated cardiomyopathy	Unknown; linkage to q23	Unknown
LGMD1F	Cramps	Unknown/7q32.1-32.2	Unknown
LGMD1G	Cramps	Unknown/4q21	Unknown
FSHMD	With larger deletions, congenital forms and mental retardation; hearing loss, visual loss (Coat's)	Unknown/4q35	Unknown
EDMD2	EMG: myopathy + distal denervation, contractures (rigid spine), and cardiomyopathy	Lamin A/C	Role in myoblast differentiation
EDMD4	No reported cardiac involvement	Nesprin-1	Nuclear membrane protein involved in mechanical signaling
EDMD5	No reported cardiac involvement	Nesprin-2	Nuclear membrane protein involved in mechanical signaling
OPMD	Axonal loss neuropathy	PABN1, PABP2	Regulation of mRNA production
Distal myopathies Distal tibial/Udd	Anterior leg atrophy	Titin	Assembly and functioning of striated muscles
Myofibrillar myopathies Desmin	Distal leg and forearm, cardiomyopathy	Desmin	Connects myofibrils to each other and to plasma membrane
Myotilinopathy	Soleus and gastrocnemius primarily affected	Myotilin	Contributes to stability of myofibrils and assembly of Z-disc
A-B crystallinopathy	May cause cataracts without myopathy	A-B crystallin	Maintains myosin enzymatic activity and prevents aggregation under heat-shock stress
ZASP-related	Lower legs and hands	LDB3-ZASP	Integrin assembly in functional myotendinous junctions
Myotonic dystrophy 1	Cataracts; cardiac arrhythmias; diabetes; low IQ; narcolepsy; percussion myotonia; severe neonatal form, CNS abnormalities	CTG repeats in DMPK gene	Inhibition of myosin phosphatase, which plays a role in muscle contraction/relaxation
Myotonic dystrophy 2	Usually less severe than myotonic dystrophy 1	CCTG repeats ZNF9	Interference in RNA processing?

BMD Becker muscular dystrophy, *CNS* central nervous system, *DMD* Duchenne muscular dystrophy, *EDMD* Emery-Dreifuss muscular dystrophy, *EMG* electromyography, *FSHMD* fascioscapulohumeral muscular dystrophy, *LGMD* limb-girdle muscular dystrophy, *nNOS* neuronal nitric oxide synthase, *OPMD* oculopharyngeal muscular dystrophy, *X-EMD* X-linked EDMD