How to go about diagnosing and managing the limb-girdle muscular dystrophies

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The increasing knowledge about limb girdle muscular dystrophy (LGMD) has clarified many aspects of this extensive group of neuromuscular conditions and has moreover proven their wide clinical and genetic heterogeneity. For these reasons, achieving a precise diagnosis of a particular type of LGMD may be still difficult and requires a comprehensive approach, which includes epidemiology, medical history, clinical examination, laboratory and genetic tests. All of the LGMDs are individually rare and their population frequency is highly variable. The prevalence of the different forms of LGMD in different populations has to be considered for the differential diagnosis. Some characteristic clinical features may help to distinguish subtypes of LGMD however protein analysis on muscle biopsy and genetic testing still represent the gold standard in the diagnosis of these muscular dystrophies. Reaching a precise diagnosis in all patients is important to allow genetic counseling to be properly applied and to direct appropriate medical management with a potential positive impact on the length and quality of life. Moreover, new specific therapeutic approaches, including limited local gene therapy, have been emerging over the last few years and require a precise genetic definition of the conditions. This article will concentrate on the diagnostic process by which these disorders can be defined and the implications of making these diagnoses.

Key words: Diagnosis, limb girdle muscular dystrophies

Introduction

Trying to reach a precise diagnosis in a patient with a suspected limb girdle muscular dystrophy (LGMD) is traditionally regarded as difficult. Although the increasing knowledge about the genetic basis of the different diseases in this group has made the group seem rather complicated, it also allows us the possibility of reaching a proper diagnosis. The diagnostic process needs to take into account the clinical history and examination of the patient, as well as a combination of possible specialized analysis of the muscle biopsy and DNA.

A possible diagnosis of LGMD should be considered in a patient with muscular dystrophy having predominant involvement of the “limb-girdle” (pelvic and shoulder) musculature. However, these clinical findings are not unique to the LGMDs and other neuromuscular disorders such as Becker muscular dystrophy, late onset spinal muscular atrophy, myotonic dystrophy Type 2, Bethlem myopathy and Pompe’s disease may all show overlapping clinical features and rarer disorders such as congenital myasthenic syndromes may also need to be considered in the differential diagnosis. On the other hand, for many of the genes involved in LGMDs, a broad heterogeneity of clinical presentation and muscle involvement has emerged. This is particularly true for some of the dominant disorders in the LGMD classification, such as LGMD1A where myotilin mutations are associated also with the disorders known as the myofibrillar myopathies,[1-2] LGMD1B where lamin A/C mutations can also cause a wide range of diseases[3-4] and LGMD1C where the clinical presentation includes hyperCKaemia, rippling muscle disease, myalgia and distal myopathy.[5-6] This means that there needs to be a high level of suspicion in this group of disorders if the correct diagnosis is going to be reached.

The molecular basis of the diseases is also highly heterogeneous. The process of gene identification in LGMDs has involved a combination of linkage and candidate gene analysis, resulting in a gene and protein-based classification which includes three known genes causing autosomal dominant LGMDs[7] and 14 known genes causing autosomal recessive LGMDs[8,9] [Table 1].

The genes and proteins causing the diseases in these groups show a huge range of localization across the muscle fiber, from sarcolemma to nuclear envelope, with functions ranging from structural to enzymatic.
<table>
<thead>
<tr>
<th>Type of LGMD</th>
<th>Gene location</th>
<th>Gene symbol</th>
<th>Protein</th>
<th>Protein localization/ function (if known)</th>
<th>Muscle biopsy Morphology</th>
<th>IHC</th>
<th>WB</th>
<th>Secondary protein deficits</th>
</tr>
</thead>
<tbody>
<tr>
<td>LGMD1A</td>
<td>5q31</td>
<td>TTID</td>
<td>Myotilin</td>
<td>Sarcomere</td>
<td>Myopathic or dystrophic, rimmed vacuoles, desmin accumulation</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LGMD1B</td>
<td>1q21.1</td>
<td>LMNA</td>
<td>Lamin A/C</td>
<td>Nuclear envelope</td>
<td>Usually dystrophic</td>
<td>Normal</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LGMD1C</td>
<td>3p25</td>
<td>CAV3</td>
<td>Caveolin3</td>
<td>Plasma membrane, caveolae</td>
<td>Myopathic or dystrophic</td>
<td>↓</td>
<td>↓</td>
<td>(dysferlin)</td>
</tr>
<tr>
<td>LGMD2A</td>
<td>15q15.1-q21.1</td>
<td>CAPN3</td>
<td>Calpain3</td>
<td>Cytoplasmic; proteolytic enzyme</td>
<td>Dystrophic, may be lobulated fibers, Dystrophic, may be inflammatory</td>
<td>↓</td>
<td>↓</td>
<td>-</td>
</tr>
<tr>
<td>LGMD2B</td>
<td>2p13.3-p13.1</td>
<td>DYSF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LGMD2C</td>
<td>13q12</td>
<td>SGCG</td>
<td>γ-sarcoglycan</td>
<td>Part of DGC in plasma membrane</td>
<td>Dystrophic</td>
<td>↓</td>
<td>↓</td>
<td>Other sarcoglycans, dystrophin</td>
</tr>
<tr>
<td>LGMD2D</td>
<td>17q12-q21.33</td>
<td>SGCA</td>
<td>α-sarcoglycan</td>
<td>Part of DGC in plasma membrane</td>
<td>Dystrophic</td>
<td>↓</td>
<td>↓</td>
<td>Other sarcoglycans, dystrophin</td>
</tr>
<tr>
<td>LGMD2E</td>
<td>4q12</td>
<td>SGCB</td>
<td>β-sarcoglycan</td>
<td>Part of DGC in plasma membrane</td>
<td>Dystrophic</td>
<td>↓</td>
<td>↓</td>
<td>Other sarcoglycans, dystrophin</td>
</tr>
<tr>
<td>LGMD2F</td>
<td>5q33</td>
<td>SGCD</td>
<td>δ-sarcoglycan</td>
<td>Part of DGC in plasma membrane</td>
<td>Dystrophic</td>
<td>↓</td>
<td>↓</td>
<td>Other sarcoglycans, dystrophin</td>
</tr>
<tr>
<td>LGMD2G</td>
<td>17q12</td>
<td>TCAP</td>
<td>Telethonin</td>
<td>Sarcomere</td>
<td>Dystrophic, rimmed vacuoles</td>
<td>↓</td>
<td>↓</td>
<td>-</td>
</tr>
<tr>
<td>LGMD2H</td>
<td>9q31-q34</td>
<td>TRIM32</td>
<td>Ubiquitin ligase</td>
<td>Dystrophic</td>
<td>↓ α-DG</td>
<td>↓ α-DG</td>
<td>Lamin α.2</td>
<td></td>
</tr>
<tr>
<td>LGMD2I</td>
<td>19q13.3</td>
<td>FKRP</td>
<td>Fukutin-related Protein substrate α-dystroglycan</td>
<td>Dystrophic</td>
<td>↓ α-DG</td>
<td>↓ α-DG</td>
<td>Calpain-3</td>
<td></td>
</tr>
<tr>
<td>LGMD2J</td>
<td>2q24.3</td>
<td>TTN</td>
<td>Titin</td>
<td>Component of sarcomer</td>
<td>Dystrophic</td>
<td>↓-Normal</td>
<td>↓-Normal</td>
<td>Calpain-3</td>
</tr>
<tr>
<td>LGMD2K</td>
<td>9q34.1</td>
<td>POMT1</td>
<td>O-Mannosyltransferase-1 Glycosyltransferase</td>
<td>Dystrophic</td>
<td>↓ α-DG</td>
<td>↓ α-DG</td>
<td>Lamin α.2</td>
<td></td>
</tr>
<tr>
<td>LGMD2L*</td>
<td>9q31</td>
<td>FKTN</td>
<td>Fukutin</td>
<td>Glycosyltransferase</td>
<td>Dystrophic</td>
<td>↓ α-DG</td>
<td>↓ α-DG</td>
<td>Lamin α.2</td>
</tr>
<tr>
<td>LGMD2M*</td>
<td>1p34-p33</td>
<td>POMGnT1</td>
<td>O-mannose beta-1,2-N-acetylgalactosaminyl transferase Glycosyltransferase</td>
<td>Dystrophic</td>
<td>↓ α-DG</td>
<td>↓ α-DG</td>
<td>Lamin α.2</td>
<td></td>
</tr>
<tr>
<td>LGMD2N*</td>
<td>14q24</td>
<td>POMT2</td>
<td>O-Mannosyl transferase-2 Glycosyltransferase</td>
<td>Dystrophic</td>
<td>↓ α-DG</td>
<td>↓ α-DG</td>
<td>Lamin α.2</td>
<td></td>
</tr>
<tr>
<td>LGMD2P*</td>
<td>11p13-12</td>
<td>?</td>
<td>?</td>
<td>Glycosyltransferase</td>
<td>Dystrophic</td>
<td>↓ α-DG</td>
<td>↓ α-DG</td>
<td>Lamin α.2</td>
</tr>
</tbody>
</table>

*No international agreement has been reached for the nomenclature of these LGMDs (ref. 35-36-37-Jarry J, 2007, see references) α-DG: alpha dystroglycan®
Despite several comprehensive studies developed over the last few years, the genetic etiology of many cases of LGMD is not yet known.

Considering the wide clinical and genetic variability of LGMDs, achieving a precise diagnosis, especially in sporadic patients, may be difficult and requires a comprehensive clinical and laboratory approach. The definition of a precise diagnosis is increasingly allowing directed management for these diseases by the ability to predict specific complications such as those of the cardiac or respiratory systems.[10]

Proper genetic counseling can only be offered if the precise mode of inheritance is known which often depends on a genetic diagnosis and prenatal diagnosis can only be provided if the underlying molecular defect for the condition is known. Finally, in the future a precise genetic diagnosis will be the starting point for specific gene and protein-based therapies.

This article will concentrate on the diagnostic process by which these disorders can be defined and the implications of making these diagnoses.

**History and Examination**

Limb girdle muscular dystrophy (LGMD) was first designated as a disease group to address a set of patients with predominantly proximal muscle weakness and a dystrophic pattern on muscle biopsy. It separated them from the more classically recognized “Duchenne/ Becker” and facioscapulohumeral muscular dystrophies, both of which are much more common than LGMDs in most populations, and for which relatively straightforward diagnostic tests now exist. The key hallmarks of LGMD remain the marked weakness of the pelvic and shoulder girdle muscles; however, limb girdle muscle weakness is a primary problem in a large number of patients with very variable underlying muscular conditions. Moreover, the clinical course of LGMD ranges from severe forms with early onset and rapid progression to milder forms with late onset.

As the different disease entities within the group have become better defined based on their distinct genetic basis, accompanying clinical features may help to distinguish subtypes of LGMD.[11] Particular features which need to be paid attention to include muscle hypertrophy or atrophy, scapular winging, muscle rippling and contractures. While the history will in many cases be a relatively nonspecific story of progressive proximal weakness, with onset at any age, there are some clues in the history of onset which can be very suggestive of a particular diagnosis. Onset, distribution of muscle weakness and wasting and other characteristic clinical features can also help in the differential diagnosis of the different forms of LGMD [Table 2].

**LGMD2A**

The clinical examination and a careful observation of the pattern of muscle weakness may be very helpful in recognizing this particular type of LGMD. In particular, a striking and early involvement of the posterior thigh muscles (adductors, semimembranosus and vastus intermedius muscles) with relative sparing of the vastus lateralis, sartorius and gracilis[15] appears to be almost stereotypic of calpainopathy. The presence of early contractures, scapular winging and preserved respiratory function is in contradiction to most other types of LGMD and can be very helpful in pinpointing the diagnosis.[16] Patients typically lose independent ambulation between 11 and 28 years after onset of the condition however disease progression in LGMD2A can be widely variable.[17]

**LGMD2B**

The clinical phenotype of dysferlinopathies is widely variable ranging from typical LGMD2B to Miyoshi Myopathy (MM) and distal anterior compartment myopathy (DMAT).[18] Over the last year other clinical presentations associated with mutations in the DYSF gene have been described, including an intermediate form with proximal and distal weakness from onset and patients with rigid spine and leg muscle stiffness with onset in the sixth decade.[19-21] Involvement of the shoulder girdle is usually a later event, although local biceps atrophy may be present at an early stage of the disease. Around 10% of LGMD2B patients experiences muscle pain and swelling, especially in the calves.

A history of normal childhood activities or even outstanding sporting ability until a relatively sudden onset in the late teens of muscle difficulties, often
<table>
<thead>
<tr>
<th>Type of LGMD</th>
<th>Gene product</th>
<th>Relative prevalence/founder mutations</th>
<th>Other phenotypes</th>
<th>Age at onset</th>
<th>Key features from history</th>
</tr>
</thead>
<tbody>
<tr>
<td>LGMD1A</td>
<td>Myotilin</td>
<td>Common mutation in all populations</td>
<td>Myofibrillar myopathy</td>
<td>Adulthood</td>
<td>May be dysarthria, tight TAs, swallowing difficulties. Distal weakness</td>
</tr>
<tr>
<td>LGMD1B</td>
<td>Lamin A/C</td>
<td>Present worldwide: private mutations usual</td>
<td>Dilated cardiomyopathy, CMT, progeria, mandibuloacral dysplasia, lipodystrophy, overlapping phenotypes</td>
<td>Any age</td>
<td>May be family history of sudden death</td>
</tr>
<tr>
<td>LGMD1C</td>
<td>Caveolin3</td>
<td>Present worldwide: private mutations usual</td>
<td>Rippling muscle disease, dilated cardiomyopathy (rare)</td>
<td>Any age</td>
<td>Myalgia, muscle rippling</td>
</tr>
<tr>
<td>LGMD2A</td>
<td>Calpain3</td>
<td>One of the most common forms of AR-LGMD worldwide. Founder mutations in Basques and in Eastern Europeans</td>
<td>MM, DMAT, proximal-distal phenotype from onset</td>
<td>1st – 2nd decade (2-40 years)</td>
<td>May present with toe walking</td>
</tr>
<tr>
<td>LGMD2B</td>
<td>Dysferlin</td>
<td>More common in Southern than in Northern Europe</td>
<td></td>
<td>2nd – 3rd decade (10-73 years)</td>
<td>Inability to walk on tiptoe. Calf pain and swelling</td>
</tr>
<tr>
<td>LGMD2C -&gt; 2F</td>
<td>γ-, α-, β-, δ- sarcoglycan</td>
<td>Present worldwide. Founder mutations in North Africans and Gypsies</td>
<td></td>
<td>1st - 2nd decade (3-20 years)</td>
<td>May present in Duchenne/ Becker like way</td>
</tr>
<tr>
<td>LGMD2G</td>
<td>Telethonin</td>
<td>Rarely reported outside Brazil</td>
<td>AD-dilated and hypertrophic cardiomyopathy</td>
<td>2nd decade</td>
<td>Distal weakness and wasting may be present</td>
</tr>
<tr>
<td>LGMD2H</td>
<td>TRIM32</td>
<td>Only recently reported outside Hutterite population of Canada</td>
<td>Sarcotubular myopathy, Bardet Bied syndrome</td>
<td>2nd decade</td>
<td>Possible mild facial weakness</td>
</tr>
<tr>
<td>LGMD2I</td>
<td>FKRP</td>
<td>Relatively frequent in Northern Europe. Founder mutation in Northern Europeans</td>
<td>Congenital muscular dystrophy MDC1C and intermediate phenotype</td>
<td>1st – 2nd decade (1-40 yrs)</td>
<td>Clinically may resemble Duchenne/ Becker MD</td>
</tr>
<tr>
<td>LGMD2J</td>
<td>Titin</td>
<td>Reported only in Finland</td>
<td>Heterozygotes have distal myopathy. Other mutations associated with cardiomyopathy; early onset myopathy with severely progressive cardiomyopathy</td>
<td>1st decade</td>
<td>History of distal weakness in heterozygotes</td>
</tr>
<tr>
<td>LGMD2K</td>
<td>POMT1</td>
<td>A few reported LGMD cases (Turkish and English families)</td>
<td>Walker Warburg syndrome and intermediate phenotypes</td>
<td>Birth – 6 years</td>
<td>May present early with global delay, upper limb weakness may be worse than lower limb weakness. Muscle hypertrophy may be present</td>
</tr>
<tr>
<td>LGMD2L*</td>
<td>Fukutin</td>
<td>A few reported LGMD cases</td>
<td>Fukuyama Congenital Muscular Dystrophy; Walker Warburg syndrome and intermediate phenotypes; dilated cardiomyopathy 1X</td>
<td>&lt; 1 year</td>
<td>May present early with global delay</td>
</tr>
<tr>
<td>LGMD2M*</td>
<td>POMGnT1</td>
<td>Only one reported LGMD case</td>
<td>Muscle-eye-brain disease</td>
<td>12 years</td>
<td>May present early with global delay</td>
</tr>
<tr>
<td>LGMD2N*</td>
<td>POMT2</td>
<td>A few reported LGMD cases</td>
<td>Walker Warburg syndrome and intermediate phenotypes</td>
<td>&lt; 2 years</td>
<td>Calf hypertrophy</td>
</tr>
<tr>
<td>LGMD2?*</td>
<td>?</td>
<td>Reported in French Canadian families</td>
<td></td>
<td>3rd decade</td>
<td>Quadriiceps atrophy may be observed</td>
</tr>
</tbody>
</table>
### Key features from examination

<table>
<thead>
<tr>
<th>Key features from examination</th>
<th>Complications</th>
<th>Specific surveillance and treatment</th>
<th>Creatine kinase levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>May be distal muscle involvement</td>
<td>Cardiomyopathy arrhythmia, respiratory failure</td>
<td>Cardiac echo, ECG +/- Holter, FVC in sitting and lying, Treatment with pacing, respiratory support</td>
<td>Normal -10X</td>
</tr>
<tr>
<td>Joint contractures, rigid spine</td>
<td>Arrhythmia, subsequent cardiomyopathy, respiratory failure</td>
<td>Cardiac echo, ECG +/- Holter, FVC in sitting and lying, High risk of ventricular arrhythmia after pacing so implantable defibrillator indicated</td>
<td>Normal -20X</td>
</tr>
<tr>
<td>PIRCs, rippling, muscle hypertrophy, normal sporting ability in childhood</td>
<td>Not reported with LGMD1C phenotype</td>
<td>Contracture management</td>
<td>3-10X</td>
</tr>
<tr>
<td>Scapular winging, contractures, muscle atrophy</td>
<td>Not frequent, Retained respiratory function can be a distinguishing feature of LGMD2A</td>
<td>4-10X</td>
<td></td>
</tr>
<tr>
<td>Usually calf wasting, biceps wasting, shoulder girdle weakness usually later than proximal/distal lower limb weakness</td>
<td>Not frequent. Cardiac impairment very rarely reported</td>
<td>10-100X</td>
<td></td>
</tr>
<tr>
<td>May be muscle hypertrophy, cardiomyopathy and scapular winging</td>
<td>Cardiac echo and ECG respiratory failure after confinement to wheelchair</td>
<td>on annual basis, FVC particularly after loss of ambulation, pulse oximetry. Medical management of cardiomyopathy, nocturnal respiratory support</td>
<td>10-100X</td>
</tr>
<tr>
<td>May be distal weakness</td>
<td>Cardiac involvement was observed in one family</td>
<td>Normal -30X</td>
<td></td>
</tr>
<tr>
<td>Facial weakness can be observed</td>
<td>Not reported</td>
<td>Normal -20X</td>
<td></td>
</tr>
<tr>
<td>Muscle hypertrophy</td>
<td>Cardiomyopathy and respiratory failure (diaphragmatic) possible even while still ambulant</td>
<td>Cardiac echo and ECG on annual basis, FVC in sitting and lying at all ages, pulse oximetry. Medical management of cardiomyopathy, nocturnal respiratory support</td>
<td>10-100X</td>
</tr>
<tr>
<td>Late respiratory complications</td>
<td></td>
<td>Normal -25X</td>
<td></td>
</tr>
</tbody>
</table>

### Cognitive impairment, microcephaly

Full spectrum of complications possibly not yet appreciated

### Motor function deterioration during infections

Full spectrum of complications possibly not yet appreciated

### Rapidly progressive

Full spectrum of complications possibly not yet appreciated

### May be cognitive impairment

Full spectrum of complications possibly not yet appreciated
with early inability to stand on tiptoe, and sometimes with sudden onset of calf pain and swelling seems to be almost pathognomonic for the diagnosis. This group of patients in particular may be misdiagnosed as myositis, which appears to be non-responsive to steroids.

**LGMD2C→F (Sarcoglycanopathies)**

Before the molecular analysis, sarcoglycanopathies were labeled “severe childhood autosomal recessive muscular dystrophy” (SCARMD) to describe children who showed a Duchenne-like clinical phenotype with an autosomal recessive mode of inheritance. Similar to Duchenne and Becker muscular dystrophy, patients affected by sarcoglycanopathies frequently show calf and tongue hypertrophy. However, an important distinguishing factor from dystrophinopathies is the absence of any cognitive impairment, in all types of sarcoglycanopathies.

Alpha-sarcoglycanopathy (LGMD2D) tends to be clinically milder compared to the other sarcoglycanopathies although no pathognomonic clinical features can distinguish the different sarcoglycanopathies.

**LGMD2G**

LGMD2G or Telethoninopathy is a rare form of AR-LGMD described only in a few Brazilian families. Clinically it appears to be characterized by mildly progressive proximal weakness in the upper and lower limbs and distal muscle involvement with foot drop. Calf hypertrophy is often present and can be asymmetric.

**LGMD2H**

LGMD2H results from mutations in the TRIM32 gene and has been only recently described in non-Hutterite populations.

**Alpha-dystroglycanopathies**

Defects in the glycosylation of alpha-dystroglycan (alpha-DG) are classically associated with congenital muscular dystrophies (CMDs). However, over the last few years new LGMD phenotypes associated with mutations in the six known glycosyltransferase genes have been emerging. In this context, the most common form of LGMD due to abnormal glycosylation of alpha-DG is LGMD2I. It results from mutations in the Fukutin-related protein gene (FKRP). LGMD2I shows a high variability in clinical presentations, ranging from the severe Duchenne-like disease course to late-onset and slow progressive forms. Characteristic clinical features of this LGMD include calf and tongue hypertrophy. A response to steroid treatment (prednisolone) has been described in some patients with LGMD2I and future clinical trials may clarify this observation.

Other LGMD phenotypes have been recently shown to be associated with mutations in other known or putative glycosyltransferase genes, in particular POMT1, POMT2, fukutin and POMGnT1. The observation of such intermediate phenotypes between LGMDs and congenital muscular dystrophies, the structural or functional brain involvement in some of these new forms of LGMD and the pathogenic background complicate the classification of these new phenotypes and genetic entities.

**LGMD2J**

LGMD2J is a severe form of autosomal recessive muscular dystrophy which so far has been described only in the Finnish population. It is characterized by onset in the first to third decade with progressive weakness of all proximal muscles. Facial muscles are not involved but some patients develop late distal muscle weakness.

**Associated complication**

Muscular dystrophies are often multisystem disorders. Identification of associated complications can be helpful in the differential diagnosis of the different LGMD subtypes. Also, the recognition of a particular type of muscular dystrophy can help to assign a more precise risk of complications and to direct appropriate management and type-specific follow-up. The more frequent and severe complications in LGMDs include respiratory and heart involvement. The cardiovascular complications include the development of a progressive cardiomyopathy or arrhythmias, while respiratory complications may or may not have significant diaphragmatic weakness as a clear component of the problem. These complications are present at different frequencies within the various disorders in the LGMD group [Table 2]. Preserved respiratory and heart function is a distinguishing clinical feature of LGMD2A. Restrictive respiratory abnormalities and cardiomyopathy with reduced ejection fraction are almost constantly associated with LGMD2I and sarcoglycanopathies, with the exception of LGMD2D which is rarely complicated by heart involvement. These complications need to be sought specifically and an appropriate management of any detected problems may have a positive impact on the length and quality of life.

**The diagnostic Process [Figure 1]**

The first step to diagnosing a particular type of LGMD is knowledge of the prior probability of seeing any one of the particular subtypes in a particular population. All of the LGMDs are individually rare and some of them have only been described in a few families. Moreover, their population frequency is highly variable and the
Figure 1: A diagnostic algorithm for achieving diagnosis in LGMD based on clinical, creatine kinase, muscle biopsy and molecular genetic testing.

CK = creatine kinase. CK levels are given as a general guide to levels of CK likely at diagnosis, but this can vary from patient to patient and with disease progression. CK level A = normal or mild elevation (<10x normal) CK level B = moderate elevation (8-40x normal) CK level C = extreme elevation (>40x normal). Other abbreviations: ADEDMD = autosomal dominant Emery Dreifuss muscular dystrophy, EDMDX = X-linked Emery Dreifuss muscular dystrophy, PIRCs: percussion-induced repetitive contractions

prevalence of the different forms of LGMD differs between populations, depending on geographical and ethnic factors. There are some problems in comparing the prevalence of the different types of LGMD as the ways used to define the different entities (for example, by protein diagnosis or by a combination of protein and DNA studies) as well as the denominators for the different studies are not uniform.

In the Caucasian population the autosomal dominant forms of LGMD seem to be responsible for around 14% of all cases but they are probably less common in other ethnicities. LGMD2A seems to be the most frequent form of LGMD worldwide, though the relative frequency of the different autosomal recessive forms in different populations is still under investigation, and comparison from country to country is complicated by the fact that different studies report their findings in different ways.

In Brazil the relative proportion of the different forms among classified patients with AR-LGMD (through DNA and/or muscle protein analysis) from 120 families was found to be 32% for LGMD2A, 22% for LGMD2B, 32% for the sarcoglycanopathy group, 3% for LGMD2G and 11% for LGMD2L. In the Italian population, looking at both dominant and recessive diagnoses, LGMD2A was the most common with 28.4%, dysferlinopathy 18.7%, sarcoglycanopathy 18.1%, LGMD2I 6.4%, LGMD1C 1.3% and undetermined diagnosis 27.1%. In the North of England population, LGMD2A represented 26.5% of the whole LGMD group, LGMD2I 19.1%, sarcoglycanopathy 11.7%, LGMD2B 5.9% and unclassified LGMD constituted 27.9% (Norwood et al., in preparation). In this population, LGMDs as a whole represented 6.15% of the total clinic population (when the most common diagnoses were myotonic dystrophy and dystrophinopathies). In Australia, calpainopathy represented 8% of a total muscular dystrophy population and dysferlinopathy 5%, while LGMD2I was less frequent (3%). By contrast, LGMD2I represents a common type of LGMD in the German and Scandinavian populations. The most common LGMDs in the United States are calpainopathies, dysferlinopathies, sarcoglycanopathies and dystroglycanopathies with a distribution of immunophenotypes of
12% calpainopathy, 18% dysferlinopathy, 15% sarcoglycanopathy, 15% dystroglycanopathy and 1.5% caveolinopathy. In the Netherlands, LGMD2A is the most frequent type of LGMD, consisting of 21% of the classified families. LGMD2B is rare, while sarcoglycanopathies account for 16% of the classified families and of 69% of these have a severe, Duchenne like, autosomal recessive LGMD phenotype. LGMD2I consists of 8% of the genetically defined cases in this population.\textsuperscript{[47]} Data from other populations are scanty given the lack in many areas of access to specialized diagnostic facilities.

None of the different types of LGMD can be distinguished on basic muscle histology or electrophysiology. Electromyography may be useful in distinguishing this myopathic group of diseases from myotonic dystrophy Type2 (PROMM, DM2) which may be clinically similar. Morphological pattern of the muscle biopsy may be crucial in distinguishing LGMDs from Pompe’s disease or other metabolic syndromes.

The serum creatine kinase (CK) is variably elevated in the different disorders and this may serve as a useful indicator to subdivide the different types of LGMD, though it is no more than a guide. As a rule of thumb, the autosomal recessive types of LGMD are typically associated with a much higher level of CK elevation than the dominant forms of LGMD, with the exception of LGMD1C. Among the recessive forms, LGMD2B, 2I and sarcoglycanopathies are usually associated with very high CK levels.

Over the last few years muscle magnetic resonance imaging (MRI) has been increasingly applied in order to determine distinct patterns of muscle involvement and may represent a promising advance in the differential diagnosis of neuromuscular conditions and of the different forms of LGMD and in directing appropriate protein and genetic investigations.\textsuperscript{[15,48,49]}

Despite the new knowledge about the molecular basis of most of the LGMDs, a diagnostic approach exclusively based on genetic testing is not usually applicable as it is often inefficient and expensive. The muscle biopsy is unavoidable in most cases and still represents an informative and economic step in the diagnostic process. Diagnosis therefore relies on a combination of clinical assessment, specialized muscle immunohistochemical and Western Blot analysis are so far available for the AD-LGMD, with the exception of caveolin3.

**Autosomal dominant limb girdle muscular dystrophy**

In most of the autosomal dominant AD-LGMD, the muscle biopsy is uninformative and shows an unspecific pattern that can either resemble a myopathy or a muscular dystrophy. No specific antibodies for immunohistochemical and Western Blot analysis are available for the AD-LGMD, with the exception of caveolin3.

**LGMD2A**

Immunoblotting has been an accepted test required for the diagnosis of LGMD2A. However, there is variability in the quantity and function of calpain3 protein detected on immunoblots even for those patients with genetically confirmed LGMD2A. Secondary deficiencies of calpain3 have been described in several muscular dystrophies, including LGMD2B and LGMD2J. Moreover, a normal calpain3 expression on immunoblot has been observed in some patients in whom calpain3 mutations were proven\textsuperscript{[50-53]} and seems to be related to abnormal protein enzymatic activity.

**LGMD2B**

In LGMD2B, qualitative and quantitative protein analysis on muscle sections by immunostaining and Western Blot typically shows complete or partial absence of dysferlin, which seems to be predictive of mutations in the DYSF gene.\textsuperscript{[43]} There may also be a secondary reduction in the expression of calpain3 and caveolin3.\textsuperscript{[52-54]} The presence of inflammatory infiltrates is encountered at times and patients with dysferlinopathy may be misdiagnosed with polymyositis.

**Sarcoglycanopathies**

In sarcoglycanopathies the immunofluorescence analysis of muscle biopsy shows combined or more rarely isolated reduction of alpha, beta, gamma and delta sarcoglycans. A concomitant reduction of dystrophin
and in same cases of dystroglycan expression can also be found, complicating the differential diagnosis with dystrophinopathies. In most cases immunofluorescence analysis or immunoblotting do not allow a reliable specific diagnosis because primary defects in a single sarcoglycan affect the expression of other sarcoglycans. A possible association between γ-sarcoglycan deficiency at the muscle biopsy and gene mutations has been reported[148] but so far, correct diagnosis requires genetic confirmation and analysis of several sarcoglycan genes may be necessary.

**Alpha-dystroglycanopathies**

The muscle biopsy immunostaining in patients with any alpha dystroglycanopathies usually shows a reduction in the dystroglycan expression if the antibody is directed against the carbohydrate moieties of alpha dystroglycan. The abnormal glycosylation pattern can also be detected on Western Blot. However, the commonly available antibodies can give unspecific results and the interpretation of the protein analysis may be difficult. Secondary reduction of laminin-alpha-2 on immunolabeling has been reported in most of the patients with alpha dystroglycanopathies and can be helpful to recognize these LGMDs.[55,56]

**Conclusion**

Achieving a precise diagnosis in all patients is important to apply genetic counseling properly and to plan appropriate medical management. It will become increasingly important as new specific therapies are developed, and as trials of therapies in different types of LGMDs, including limited local gene therapy trials get under way. As diagnostic testing begins to be systematically applied in different countries, the epidemiology of these diseases can increasingly be understood, and a variable pattern of disease frequency will be elucidated. Therefore the future development of diagnostic testing and indeed therapeutic studies will need to take these regional differences into account. Particularly striking is the predominance of LGMD2I due to the common C826A mutation in Northern Europe,[18] compared to a relatively higher proportion of LGMD2A and LGMD2B cases in Southern Europe. A highly focused approach to LGMD diagnostics is already improving management and is going to become increasingly important in the future.

**References**


