Limb girdle muscular dystrophies: update on genetic diagnosis and therapeutic approaches

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Purpose of review

This review is an up-to-date analysis of the genetic diagnosis and therapeutic strategies for limb girdle muscular dystrophies (LGMDs).

Recent findings

LGMDs are an example of both clinical and genetic heterogeneity. Clinically, by the description of non-LGMD phenotypes associated with LGMD genes and of LGMD phenotypes associated with originally non-LGMD disease genes; and genetically, by the description of new LGMD genes that further increase the diagnostic complexity. Moreover, new powerful approaches for DNA analysis, such as exome sequencing, promise to revolutionize the field of heterogeneous genetic diseases, also providing information about the true penetrance of LGMD mutations. The recent inputs on novel pathogenic mechanisms and pathways in LGMD will suggest novel therapeutic approaches and future clinical trials. In addition, therapeutic approaches of gene and cell delivery into animal models show promising results that will be translated into clinical trials.

Summary

The genetic diagnosis of LGMD from the present home-made algorithms will move toward high-throughput diagnostic strategies based on next-generation sequencing (NGS) technologies. As therapy, new powerful drug approaches based on recent pathogenetic findings will be pushed to clinical trials. In addition, novel more efficient and safer viral vectors for gene delivery will be proposed.

Keywords

diagnosis, genetic testing, limb girdle muscular dystrophy, therapy

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Introduction

The term limb girdle muscular dystrophy (LGMD) broadly defines a progressive weakness that begins from the proximal limb muscles, due to a genetic defect that is distinct from the X-linked dystrophinopathy. The disease is not congenital, with the age at onset of symptoms varying from early childhood to late adulthood [1]. The progression of muscle wasting is usually symmetric, with a variability among individuals and genetic subtypes. Before considering the diagnosis of LGMD [2], other conditions need to be excluded, such as facioscapulohumeral muscular dystrophy, dystrophinopathies, myotonic dystrophy, and metabolic myopathies. The milder the symptoms are, the more difficult is the diagnosis. MRI may be helpful to characterize the severity and pattern of muscle involvement [3•].

Muscle biopsy shows a diffuse variation in fiber size, necrosis, regeneration, and fibrosis, but the degree of these factors is variable and does not parallel the clinical severity. On the basis of the histological features alone, there is little, if any, possibility of diagnosing an LGMD or a specific LGMD form, but it is possible to discriminate LGMD from inflammatory myopathy, myofibrillar myopathy, or neurogenic atrophy.

Classification

The primary distinction is between the autosomal dominant (LGMD1, Table 1) and the autosomal recessive forms (LGMD2, Table 2), with a progressive alphabetical letter indicating the order of gene mapping [4]. There are, however, about one third of LGMD patients without any genetic classification. According to the disease mechanisms, the LGMDs may be grouped as follows: dystrophin–dystroglycan complex defects LGMD2CDEFIKMNOP; membrane defects LGMD1C, LGMD2BL; enzymatic LGMD2AH; sarcomeric LGMD1A, LGMD2GJ; and nuclear lamina LGMD1B.

LGMD1

Eight LGMD1 loci have so far been identified, but the heterogeneity is expected to be greater (Table 1). The LGMD1 forms have an adult-onset and are milder,

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Table 1 Autosomal dominant limb girdle muscular dystrophy

		Gene				Clinical phenotype				
Disease	Locus	Name	Exons no	Protein	Animal model	Typical onset[a]	Progression	Cardiomyopathy[b]	sCK[c]	Allelic disorders (OMIM #)[a]
LGMD1A	5q31.2	TTID	10	Myotilin	$Myo^{-/-}$	Adulthood	Slow	Not observed	3–4X	MFM (609200) Spheroid body myopathy (182920)
LGMD1B	1q21	LMNA	12	Lamin A/C	$Lmna^{-/-}$	Variable (4–38y)	Slow	Often observed	1–6X	CMT2B1 (605588) CMD1A (115200) EDMD2 (181350) HGPS (176670) FPLD2 (151660) MADA (248370)
LGMD1C	3p25.3	CAV3	2	Caveolin 3	$Cav3^{-/-}$	Childhood	Slow/moderate	Frequent	10X	CMH (192600) LQT9 (611818) RMD (606072) HyperCKemia, idiopathic (123320)
LGMD1D	6q22					Adulthood	Slow	Not observed	1–3X	
LGMD1E	7q36					Adulthood	Slow	Often	2–4X	
LGMD1F	7q31.1					Variable (1–58y)	Quite rapid	Not observed		
LGMD1G	4p21					Adulthood	Slow	Not observed	1–9X	
LGMD1H	3p23–p25					Variable (10–50y)	Slow	Not observed	1–10X	

[a] Indicates the age of onset of the majority of patients reported; early childhood has been defined as a period of life between 0–8 years of age; late childhood 9–12 years; adolescence 13–17 years; young adulthood, 18–35 years, etc.

[b] Also indicates mild signs of cardiac involvement.

[c] Indicates the range of serum creatine kinase (sCK) levels that is observed in about 80% of patients.

[d] Only indicates allelic disorders that have been included in the Online Mendelian Inheritance in Man (OMIM) with the indicated number.

Key points

- LGMDs are highly heterogeneous and a large number of additional genes may be associated with this broad definition.
- Milder the symptoms are, more difficult is the diagnosis.
- Muscle biopsy is now required for diagnosis, but next generation sequencing will be very helpful.
- Novel therapies that have demonstrated effects in animal model will be translated into trials.

because affected parents are usually in quite good health at reproductive age. At present, only three genes are known to be involved, encoding myotilin (LGMD1A), lamin A/C (LGMD1B), and caveolin-3 (LGMD1C). Some patients with mutations in these three genes fulfill the diagnostic criteria for the LGMDs, but others show a much wider spectrum of different phenotypes [5–7]. The extreme situation is that of the lamin A/C gene mutations with a dozen different clinical conditions, including lipodystrophy, Emery–Dreifuss muscular dystrophy, cardiomyopathy, Charcot–Marie–Tooth disease, and progeroid syndromes [6]. With five loci (LGMD1D–H), no mutated gene has been identified, but the nomenclature of LGMD1D/E is not uniform [8]. The last locus (LGMD1H) on 3p23–25 has been mapped in a large pedigree from Southern Italy with onset during the fifth decade of life and a relatively benign course [9].

LGMD2A (calpain 3)

LGMD2A is caused by calpain 3 (CAPN3) mutations and represents 20–40% of cases. In most countries, LGMD2A is the most frequent LGMD [10–15]. This is due to a high heterozygote frequency (1 : 100–120), carrying a large spectrum of different CAPN3 pathogenic changes (457); some of them (17) for their frequency are included in the dbSNP database [16]. CAPN3 is a 94 kDa muscle-specific protein similar to ubiquitous calpain 1 and 2, but contains specific insertion sequences (NS, IS1, and IS2). Upon stimulation, CAPN3 both activates and deactivates itself rapidly through autolysis of the insertion sequences [17]. In the sarcomeres, CAPN3 directly binds to connectin/titin and changes its localization from the M-lines to the N2A regions as the sarcomeres extend. The mobility of calpain 3 between the M-lines and the cytosol may have a key role in physical stress, and it is compromised in muscular dystrophy when its protease activity has been lost [18•]. CAPN3 can cleave PIAS proteins and negatively regulates PIAS3 sumoylase activity [19].

CAPN3 mutations are associated with two main clinical features: LGMD with a recognizable clinical pattern of ‘calpainopathy’, characterized by atrophy, scapular

Table 2 Autosomal recessive limb girdle muscular dystrophy

		Gene				Clinical phenotype				
Disease	Locus	Name	Exons no.	Protein	Animal model	Typical onset[a]	Progression	Cardiomyopathy[b]	sCK [c]	Allelic disorders (OMIM #)[d]
LGMD2A	15q15	CAPN3	24	Calpain 3	Capn3$^{-/-}$; Capn3$^{CS/CS}$	Adolescence	Moderate/rapid	Rarely observed	3–20X	
LGMD2B	2p13.2	DYSF	56	Dysferlin	SJL/J; Dysf$^{-/-}$	Young adulthood	Slow	Possible	5–40X	MM (254130) DMAT (606768)
LGMD2C	13q12	SGCG	8	γ-Sarcoglycan	Sgcg$^{-/-}$	Early childhood	Rapid	Often severe, rare in 2D	10–70X	
LGMD2D	17q21.33	SGCA	10	α-Sarcoglycan	Sgca$^{-/-}$	Early childhood	Rapid	Often severe, rare in 2D	10–70X	
LGMD2E	4q12	SGCB	6	β-Sarcoglycan	Sgcb$^{-/-}$	Early childhood	Rapid	Often severe, rare in 2D	10–70X	
LGMD2F	5q33	SGCD	9	δ-Sarcoglycan	BIO14.6; Sgcd$^{-/-}$	Early childhood	Rapid	Often severe, rare in 2D	10–70X	CMD1L (606685)
LGMD2G	17q12	TCAP	2	Telethonin	Tcap KO	Adolescence	Slow	Yes	10X	CMD1N (607487)
LGMD2H	9q33.1	TRIM32	2	Tripartite motif containing 32	Trim32$^{-/-}$	Adulthood	Slow	Not observed	5–15X	BBS (209900) STM (602290)
LGMD2I	19q13.3	FKRP	4	Fukutin related protein	FKRP-neo-P448L E310del FKRP^{Tyr307Asn}	Late childhood	Moderate	Yes	10–20X	MDC1C (606612) MEB (253280) WWS (236670)
LGMD2J	2q24.3	TTN	312	Titin	Mdm MCKcre^{+} Ti MEx1+2$^{lox/lox}$	Young adulthood	Severe	Not observed	10–40X	HMERF (603689) EOMFC (617105) CMD1G (604145) CMH9 (188840 TMD (600334)
LGMD2K	9q34.1	POMT1	20	Protein-O-mannosyltransferase 1	Pomt1$^{-/-}$	Childhood	Slow	Not observed	10–40X	WWS (236670)
LGMD2L	11p13-p12	ANO5	22	Anoctamin 5		Variable (young to late adulthood)	Slow	Not observed	1–15X	
LGMD2M	9q31	FKTN	11	Fukutin	Fukutin null	Early childhood	Moderate	Sometimes	10–70X	CMD1X (611615) FCMD (253800) WWS (236670)
LGMD2N	14q24	POMT2	21	Protein-O-mannosyltransferase 2		Early childhood	Slow	Rare	5–15X	WWS (236670)
LGMD2O	1p34.1	POMGnT1	22	Protein O-linked mannose beta1, 2-N-acetylglucosaminyltransferase	POMGnT1$^{-/-}$	Late childhood	Moderate	Not observed	2–10X	MEB (253280) WWS (236670)
LGMD2P	3p21	DAG1	3	Dystroglycan	Dag1null	Early childhood	Moderate	Not observed	20X	

[a] Indicates the age of onset of the majority of patients reported; early childhood has been defined as a period of life from 0–8 years of age; late childhood 9–12 years; adolescence 13–17 years; young adulthood, 18–35 years, etc.

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winging, weakness of the hip adductors, involvement of the posterior thigh muscles, and joint contractures [20]; a spectrum of variable phenotypes, often misdiagnosed [13,21], ranging from a common asymptomatic hyperCKemia to inflammatory disorders of muscle with eosinophilic infiltrates [22] or Becker muscular dystrophy (BMD)-like phenotypes. Eosinophils can be found in LGMD2A [23], but also in LGMD2C [24].

Western blot analysis is currently the 'gold standard' to identify LGMD2A. Loss of all CAPN3 bands by 2C4 (exon 1) and 12A2 (exon 8) [25] antibodies is specific, but the sensitivity is incomplete, because some LGMD2A patients may retain normal amounts of nonfunctional protein [26]. CAPN3 may be reduced in amount in other LGMDs (e.g. LGMD2B [27] and 2J [28]) as a secondary effect. By immunohistochemistry, the complete absence of the 2C4 signal is 100% specific for LGMD2A [29]. Mutation detection is usually carried out by DNA analysis of all exons, but some intronic splice mutations can be overlooked [30] and heterozygous deletions [31] missed. The sensitivity is much higher by adding mRNA testing [32]. In this case also there is a problem of specificity, because many missense mutations await experimental proof of pathogenicity.

LGMD2B (dysferlin)

LGMD2B is caused by mutations in the dysferlin (DYSF) gene that is the second form in order of frequency (about 15–25%) in many geographical areas [10,15,33], but not everywhere [11]. Dysferlin is a ubiquitous 230 kDa transmembrane protein involved in calcium-mediated sarcolemma resealing [34]. Although muscle inflammation is widely recognized in dysferlinopathy and dysferlin is expressed in immune cells, the contribution of the immune system to the pathology remains obscure.

DYSF mutations are associated with heterogeneous clinical pictures ranging from severe functional disability to mild late-onset forms [35,36]. About 25% of cases are clinically misdiagnosed as having polymyositis [37]. The same mutations also cause Miyoshi myopathy (MM1) [38] and distal myopathy with anterior tibialis onset (DMAT), but mixed phenotypes are possible. This classification into separate phenotypes does not reveal true disease differences [39].

Typical features of LGMD2B are: early adult onset; high serum creatine kinase (CK), higher than in LGMD2A [40]; prominent inflammatory infiltration; slow progression; and inability to stand on tiptoes, due to the weakness of the gastrocnemius and soleus. Fifty-three percent of the patients were very active and sporty before the onset of symptoms [41] and this suggests that a nonpenetrance of DYSF mutations is possible. Regeneration seems to be attenuated [42].

Western bloy analysis is very useful and specific, when less than 20% level of dysferlin has been identified [43•], although dysferlin can be also increased [35] or secondarily reduced [27]. Genetic testing is laborious for the huge number of exons to be screened and the lack of mutational hot spots. mRNA analysis is also reliable from monocytes, albeit with some splice differences [44].

LGMD2C (gamma-sarcoglycan), LGMD2D (alpha-sarcoglycan), LGMD2E (beta-sarcoglycan), and LGMD2F (delta-sarcoglycan)

Mutations in any of the four sarcoglycan genes (sarcoglycanopathies) constitute about 10–15% of all LGMD2s [1,4,45], but 68% of the severe forms [46]. LGMD2D is the most prevalent form, but LGMD2C is common in the Maghreb and India [47] for the high allele frequency of 525delT and in gypsies for the C283Y allele [48]. The sarcoglycans are N-glycosylated transmembrane proteins that form a heterotetrameric complex linked to the dystrophin–dystroglycan complex [45].

The clinical picture of the sarcoglycanopathies is heterogeneous with both severe and mild forms that are also found in the same families [49]. In general, the disease is more severe and rapid than in the other LGMDs. The typical form has a childhood onset that resembles the intermediate forms of Duchenne/Becker muscular dystrophies usually with quadriceps muscle wasting. Cardiomyopathy may occur in all forms [50,51], rarely in LGMD2D. In animal models, the delta sarcoglycan mutations are associated with cardiomyopathy [52,53]. Restrictive lung disease and hypoventilation often require ventilatory assistance. Diagnosis may be made by Western blot or on section by immumofluorescence. LGMD2E and LGMD2F patients show most frequently the absence of the mutated and the secondary absence of nonmutated sarcoglycans, whereas LGMD2C patients may show the absence of gamma-sarcoglycan together with traces of the other nonmutated sarcoglycans. In LGMD2D cases, there is no rule. This could be explained by the presence of two other sarcoglycans (epsilon and zeta) that are nonmuscle homologues of alpha and gamma sarcoglycan. The genetic analysis is oriented to genotype common mutations or to sequences from DNA samples, the exonic regions of a specific sarcoglycan. More than in other LGMD genes, the sensitivity is lower without muscle mRNA testing and/or multiplex ligation-dependent probe amplification, because some nonobvious splice mutations are missed together with copy number mutations that are common in the gamma-sarcoglycan gene [54,55].

LGMD2G (Tcap/telethonin)

Mutations in titin cap (Tcap)/telethonin cause LGMD2G, one of the rarest forms of LGMD. Tcap provides links to the N-terminus of titin and other Z-disc proteins. Patients show adolescence-onset weakness initially affecting the proximal pelvic muscles and then the distal legs with calf hypertrophy. Recently, a patient with a homozygous nonsense mutation in the Tcap gene has been reported presenting with a congenital muscular dystrophy [56]. The Tcap gene has also been associated with cardiomyopathy [57], whereas common variants may play a role in genetic susceptibility to dilated cardiomyopathy [58]. Immunofluorescence and Western blot assays may show a telethonin deficiency. Full sequencing testing may be cost-effective in all cases, because the gene is only composed of two small exons.

LGMD2H (TRIM32)

Mutations in TRIM32 cause LGMD2H, a late-onset form that accounts for about 3% of LGMD. TRIM32 is a ubiquitous E3 ubiquitin ligase that belongs to a protein family comprising at least 70 human members sharing the tripartite motif (TRIM) [59]. The D487N mutation of TRIM32 was originally identified in the inbred population of Manitoba Hutterites [60] that may also show the more severe sarcotubular myopathy (STM) [61]. Other TRIM32 mutations were then identified in non-Hutterite LGMD2H patients [62,63]. Recently, two other LGMD2H patients have been described associated with STM morphotype [64]. In general, LGMD2H cannot be diagnosed without genetic studies. DNA sequencing of the unique coding exon is routinely performed, but in few laboratories. mRNA analysis is dispensable.

LGMD2I (FKRP), LGMD2K (POMT1), LGMD2M (fukutin), LGMD2N (POMT2), LGMD2O (POMGnT1), and LGMD2P (dystroglycan gene)

Mutations in these genes affect dystroglycan glycosylation and cause congenital muscular dystrophies, muscle–eye–brain disease or Walker–Warburg syndrome; however, some hypomorphic alleles are associated with LGMD [65,66••]. The most frequent LGMD gene in this group is FKRP that causes LGMD2I [67]. In some countries (England, Denmark, and Norway [68]), LGMD2I is more common than LGMD2A, for the high carrier frequency of the L276I allele (1 : 116), reported 377 times in the Leiden database. LGMD2I with both L276I alleles is generally milder than compound heterozygotes [69]. LGMD2I with the L276I allele is a muscular dystrophy that is clinically similar to BMD, with a late-childhood onset, calf hypertrophy, high serum CK, respiratory impairment, and cardiomyopathy that can also prevail [70,71]. A mild cognitive impairment of executive functions and visuo-spatial planning with aspecific MRI findings has been reported [72]. Myoglobinuria and myalgia following exercise may be common [73]. The principal diagnostic tool is the immunostaining of muscle that reveals a significantly reduced signal with antibodies recognizing the glycosylated epitopes of alpha dystroglycan. There is a correlation between the reduced alpha dystroglycan staining and clinical course in individuals with mutations in POMT1, POMT2, and POMGNT1, but this is not always the case in FKTN and FKRP gene mutations [74].

Recently, Hara *et al.* [75••] have reported a missense mutation in the dystroglycan gene in an LGMD patient with cognitive impairment. This substitution interferes with LARGE-dependent maturation of phosphorylated O-mannosyl glycans on α-dystroglycan affecting its binding to laminin.

LGMD2J (titin gene)

A homozygous mutation in the C terminus of titin (FINmaj 11 bp deletion/insertion) causes LGMD2J [76]. Titin is the giant sarcomeric protein that forms a continuous filament system in the myofibrils of striated muscle, with single molecules spanning from the sarcomeric Z-disc to the M-band [77]. Other ‘titinopathic’ clinical pictures are tibial muscular dystrophy (TMD, Udd myopathy) or more severe cardiac and muscular phenotypes.

CAPN3 binds M-band titin at is7 within the region affected by the LGMD2J mutations and shows a secondary deficiency in LGMD2J muscle [28]. Interactions with titin may protect CAPN3 from autolytic activation and removal of the CAPN3 protease reverses the titin myopathology [78].

Identification of the French nonsense mutation (Q33396X) located in Mex6 seems to cause a milder phenotype than the typical FINmaj mutation [77]. Due to the huge gene size, there is limited availability of genetic tests for titin defects, based on mutation-specific genotyping.

LGMD2L

Recessive mutations in the putative calcium-activated chloride channel Anoctamin 5 (ANO5) cause proximal LGMD2L and distal MMD3 muscular dystrophies [79•,80,81]. ANO5 represents a relatively common cause of adult onset muscular dystrophy in England, for the regional prevalence of the c.191dupA mutation. Lower limb involvement is atrophic and often asymmetric, with high serum CK, the weakness is generally slowly progressive. Sequencing of all exons is necessary.

Upcoming molecular diagnoses

It is generally accepted that, after the results from a biopsy and protein testing, a specific genetic test is performed to confirm and complete the LGMD diagnosis [2]. There is, however, the prospect that next-generation sequencing (NGS)-based targeted exome sequencing [82•] will reverse this order, making affordable a universal DNA test that screens for all the neuromuscular disease genes. In this case many nonpenetrant mutations will be discovered and the interpretation of the results will be crucial. Universal tests are already in use for quantitative mutations, such as large deletions or duplications [31,43•,83], by custom comparative genomic hybridization arrays, such as the neuromuscular disorders chip or the Motor chip. Whole genome homozygosity mapping has been proposed for the mapping of consanguineous cases of LGMD2 [84,85]. Sequence analysis of mRNA is required for the diagnosis of more than 10% of mutations, as deep intronic or elusive exonic variations may disrupt the correct splicing: this requires a muscle biopsy, even if mRNA may be used from blood (only LGMD2A,B) or perioral muscle fibers (skin biopsy) [86].

Upcoming therapies

Treatment of LGMD remains palliative and supportive. Physiotherapy to prevent joint deformities and promote walking is recommended. A passive stretching physical therapy programme should be instituted early, soon after diagnosis. The use of knee–ankle–foot orthoses at bedtime is recommended to prevent contractures.

The benefit of steroids has been reported in some types of LGMD, including LGMD2D [87], LGMD2I [88], and LGMD2L [89]. A double-blind, placebo-controlled study of deflazacort in LGMD2B/Miyoshi myopathy is in progress (http://clinicaltrials.gov).

An alpha-sarcoglycan gene expression in two of three LGMD2D subjects was obtained for 6 months by adeno-associated virus-mediated (AAV) gene transfer to the extensor digitorum brevis muscle [90•,91]. Although a systemic AAV gene therapy is effective in terms of extending lifespan in animal models [92], there are still many barriers for human treatment, including immunological complications, challenges in producing sufficient material for treatments, and difficulties in delivery to muscles throughout the body.

Similarly to the molecular therapy of Duchenne muscular dystrophy, the problem with the dysferlin gene is its huge size [93]. To overcome the size limitation, exon skipping has been proposed as a method to by-pass dysferlin mutations [94] or the set up of a functional mini-dysferlin [93,95], or dual AAVs [96]. Genetic ablation of the complement factor C3 has mitigated the phenotype in dysferlin-deficient mice [97•]. Complement-mediated muscle injury may be relevant to the pathogenesis of dysferlinopathy. This suggests the complement system as a candidate for therapeutic target. Recently, it has been shown that intra-arterial injection of wild-type mesoangioblasts is able to colonize the dystrophic muscles and restore dysferlin expression in SCID/BlAJ mice [98].

Conclusion

Advances in the knowledge of LGMDs have been made and 24 different LGMDs have been so far recognized. Next generation sequencing technologies promise a revolution in diagnostics and characterization of additional LGMD genes. Novel systemic therapies that have been effective in the different animal models will be translated into clinical trials.

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Conflicts of interest

There are no conflicts of interest.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 513).

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