Limb–girdle muscular dystrophies

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Purpose of review

The aim of this review is to provide an up-to-date analysis of current knowledge about limb–girdle muscular dystrophies (LGMDs).

Recent findings

Over the last few years, new and interesting studies have been published on LGMD. New LGMD genes have been discovered and the clinical and genetic heterogeneity in this group of muscular dystrophies has been further enlarged by the description of new forms of LGMD. Several studies have demonstrated involvement of genes causing posttranslational modifications of α-dystroglycan in the pathogenesis of autosomal recessive LGMD. This has highlighted an important overlap in pathogenesis between LGMD and congenital muscular dystrophies, prompting further research. Moreover, new pathogenic mechanisms and pathways are emerging for LGMD, in particular calpainopathies, dysferlinopathies and titinopathies. Such new findings may suggest novel therapeutic approaches and future clinical trials.

Summary

The increased understanding of the genes and pathogenic mechanism of the LGMDs will improve diagnostic processes and prognostic accuracy, and promote therapeutic strategies. European and global LGMD patient registries will increase current knowledge on natural history and facilitate translational research.

Keywords

dystrophin–glycoprotein complex, glycosylation, limb–girdle muscular dystrophy, pathogenesis

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Introduction

Limb–girdle muscular dystrophies (LGMDs) are characterized by wide genetic and clinical heterogeneity. The classical grouping of the LGMDs into autosomal dominant-LGMD (AD-LGMD or LGMD1) and autosomal recessive-LGMD (AR-LGMD or LGMD2) forms is being complemented by a classification based on the involved proteins and the underlying genetic defects [1] (Table 1).

The important pathogenic role of genes that do not encode integral components of the dystrophin–glycoprotein complex (DGC) continues to emerge [2]. In particular, several papers have demonstrated a more prominent involvement of α-dystroglycan glycosyltransferases in the AR-LGMD.

Among the genetic causes of the autosomal dominant LGMDs, pure limb–girdle weakness appears to be a rather rare phenotype, whereas there is increasing awareness of presentations with distal myopathy or myofibrillar myopathy. Despite much progress over the last few years, the genetic cause of many cases of LGMD remains obscure and the classification of LGMDs is an ongoing process.

Here, we review current research on LGMDs and attempt to provide current knowledge on the genetic basis and pathogenic mechanism of the AR-LGMD (LGMD2).

Diagnosis

Considering the wide clinical and genetic variability of LGMDs, achieving a precise diagnosis, especially in sporadic patients, might be difficult and requires a comprehensive clinical and laboratory approach. Taking into account the geographical and ethnic origins of patients is helpful in the differential diagnosis, as the relative local frequency of the different forms of LGMDs varies considerably [3•,4•,5••,6•], as exemplified by the north–south gradient across Europe in the frequency of limb–girdle muscular dystrophy type 2I (LGMD2I).

Clinical assessment continues to represent the first step for directing further investigations. Over the last few

Table 1 Molecular classification and clinical features of autosomal recessive-limb–girdle muscular dystrophy

Disease	Protein	Gene	Relative prevalence/ founder mutations	Creatine kinase levels[a]	Age of onset	Respiratory involvement	Cardiac involvement	Clinical clues
LGMD2A	Calpain3	*CAPN3*	One of the most common forms of AR-LGMD worldwide; founder mutations in Basques (2362_2363delinsTCATCT) and in eastern Europeans (550delA)	Normal–50×	1st–2nd decade (2–40 years)	–	–	Preferential involvement of posterior thigh muscles; ankle contractures; scapular winging
LGMD2B	Dysferlin	*DYSF*	More common in southern than northern Europe; founder mutations in several populations	10–100×	2nd–3rd decade (10–73 years)	+/–	–	Distal weakness and wasting; muscle pain and/or swelling; good athletic performance in childhood; inflammatory cells in muscle biopsy
LGMD2C	γ-Sarcoglycan	*SGCG*	Present worldwide; founder mutations in North Africans (521delT) and Gypsies (848G>A)	10–100×	1st decade (3–20 years)	+	+	Calf hypertrophy; scapular winging
LGMD2D	α-Sarcoglycan	*SGCA*	Present worldwide; most frequent sarcoglycan form in all populations; common mutation (229C>T), especially in northern Europe	10–100×	1st decade (3–40 years)	+	Rare	Calf hypertrophy; scapular winging
LGMD2E	β-Sarcoglycan	*SGCB*	Common in northern and southern Indiana Amish	10–100×	1st decade (3–20 years)	+	+	Calf hypertrophy; scapular winging
LGMD2F	δ-Sarcoglycan	*SGCD*	Rare all over the world; common mutation (del656C) in African–Brazilian	10–100×	1st decade (3–20 years)	+	+	Calf hypertrophy
LGMD2G	Telethonin	*TCAP*	Rarely reported outside Brazil	Normal–30×	2nd decade (9–15 years)	–	+/–	Calf hypertrophy or hypotrophy; distal leg weakness
LGMD2H	TRIM32	*TRIM32*	Only recently reported outside Hutterite population of Canada	Normal–20×	2nd decade (1–44 years)	–	+/–	Possible mild facial weakness; small vacuoles in muscle fibres
LGMD2I	Fukutin-related protein	*FKRP*	Relatively frequent in northern Europe; founder mutation in northern Europeans (826C>A)	10–100×	1st–2nd decade (1–40 years)	+	+	Calf hypertrophy; myoglobinuria; muscle pain
LGMD2J	Titin	*TTN*	Reported only in Finland	Normal–25×	1st decade (5–20 years)	–	–	Distal weakness described; proximal–distal myopathy with associated cardiomyopathy recently described
LGMD2K	*O*-Mannosyl transferase-1	*POMT1*	Few reported LGMD cases (Turkish and English families)	10–50×	Birth–6 years	+	–	Microcephaly and cognitive impairment; muscle hypertrophy (thigh and calf)
LGMD2L[b]	Fukutin	*FKTN*	Few reported LGMD cases	5–100×	<1 years	?	–	Motor function deterioration during infections
LGMD2M[b]	*O*-Mannose β-1, 2-*N*-acetylglucosaminyl transferase	*POMGn1*	Only one reported LGMD case	20–60×	12 years	?	?	Rapidly progressive
LGMD2N[b]	*O*-Mannosyl transferases-2	*POMT2*	Few reported LGMD cases	15×	<2 years	?	–/?	Calf hypertrophy; possible cognitive impairment
LGMD?[b]	?	*11p13*	Reported in French Canadian families	Normal–30×	3rd decade (11–50 years)	?	–	Quadriceps atrophy

LGMD, limb–girdle muscular dystrophy.
[a] In UI/l.
[b] No international agreement has been reached for the nomenclature of this LGMD.

years, muscle MRI has been increasingly applied to determine distinct patterns of muscle involvement. This approach appears to be a promising advance in the differential diagnosis of neuromuscular conditions in general and of LGMD in particular, and in directing appropriate, especially genetic, investigations [7••,8,9].

The muscle biopsy still represents an important and economic step in the diagnostic process. However, protein deficiency documented by immunohistochemistry in muscle may be secondary and in most patients a definite diagnosis can be obtained by genetic analysis only [10••].

Limb–girdle muscular dystrophy type 2A (calpainopathy)

Limb–girdle muscular dystrophy type 2A (LGMD2A), due to mutations in the calpain3 gene (*CAPN3*), is probably the most frequent form of LGMD, although geographic differences have been reported [3•,4•,5••,6•]. Currently, research in calpainopathies is focusing on improving diagnostic approaches and clarifying the calpain3 function in muscle fibres.

Diagnosis of LGMD2A is complicated by phenotypic variability, lack of precise protein analysis, and absence of mutational hot spots in the *CAPN3* gene [11••]. MRI studies complement clinical examination and direct molecular studies. In fact, MRI images have confirmed the clinical observation that in LGMD2A there is striking and early involvement of adductors, semimembranosus, and vastus intermedius muscles in the thigh, with relative sparing of the vastus lateralis, sartorius and gracilis [9].

The probability of identifying *CAPN3* mutations can be facilitated by the pattern of the protein deficiency observed on immunoblotting, but it should be noted that *CAPN3* mutations have been reported with normal calpain3 band on immunoblotting [4•,11••] and abnormal enzymatic activity has been observed in some genetically confirmed LGMD2A patients despite normal protein expression [12–14]. Milic at al. [15••] developed an in-vitro calpain3 activity assay to test the protein autocatalytic and proteolytic properties and to identify protein activity abnormalities in patients with mutations in *CAPN3* but normal quantitative calpain3 expression. A secondary deficiency of calpain3 has been described in several muscular dystrophies. Moreover, *CAPN3* transcriptional analysis has been proposed as a complementary approach for the diagnosis when genomic mutation screening evidenced either no mutation or only one mutation in *CAPN3* [16•,17••].

Studies using *CAPN3* cDNA analysis suggested that genomic deletions in *CAPN3* might occur more frequently than previously suspected [17••]. A more systematic application of multiplex ligation-dependent probe amplification (MLPA) should be implemented to clarify the prevalence of *CAPN3* deletions and to address the clinical relevance of this investigation, especially given the number of patients previously reported in whom only a single *CAPN3* mutation could be detected on genomic sequencing.

Genotype–phenotype correlations have been reported [4•,11••], indicating that two *CAPN3*-null mutations usually cause a more severe phenotype, with earlier onset of muscle weakness and higher risk of becoming wheelchair dependent [18]. However, additional factors that might influence disease expression were also suggested.

Calpain3 is a muscle-specific calcium-dependent cysteine protease, which binds different proteins involved in myofibrillogenesis, in regulation of fibre elasticity and in various cell-signalling pathways [19,20]. The precise function of calpain3, the mechanism by which it is activated and its protein targets in skeletal muscle are complex and poorly understood. Several cytoskeletal components have been identified as substrates for calpain3, suggesting its involvement in regulation of cytoskeleton structure and cytoskeleton–membrane interaction [21–23]. Deregulation of sarcomere remodelling has also been indicated as a new pathogenic mechanism causing LGMD2A.

Huang *et al.* [24••] identified AHNAK, a component of the dysferlin protein complex, as a further substrate of calpain3. AHNAK appears to participate in cell membrane enlargement, cell differentiation, and membrane repair. The authors concluded that the regulatory role of calpain3 in the dysferlin protein complex may implicate a relationship between muscle membrane repair and remodelling of sarcomere and sarcolemmal cytoskeleton architecture. This may suggest a previously unrecognized role of calpain3 in muscle membrane homeostasis.

The role of calpain3 in muscle homeostasis was also suggested by the observation that the antiapoptotic inhibitory protein kappa B alpha (IκBα)/nuclear factor kappa B (NF-κB) pathway was perturbed in calpain3 deficiency [25]. Benayoun *et al.* [26••] recently demonstrated a downregulation of the NF-κB-dependent antiapoptotic factor cellular-FLICE inhibitory protein (cFLIP) in LGMD2A biopsies and suggested that *CAPN3* intervenes in the regulation of the expression of NF-κB-dependent survival genes to prevent apoptosis in skeletal muscle. This study first recognized that impairment of the antiapoptotic response in muscle was a possible pathological mechanism in muscular dystrophy.

Limb–girdle muscular dystrophy type 2B (dysferlinopathy)

The clinical spectrum of dysferlinopathies has been enlarged by the report of new clinical phenotypes,

including proximodistal forms and late-onset forms in addition to the previously described phenotypes of limb–girdle muscular dystrophy type 2B (LGMD2B) that is Miyoshi myopathy and distal anterior compartment myopathy [27•–29•].

Intra- and interfamilial variability is significant, and the interrelationship of type of mutations, muscle protein expression, and age at onset has been suggested to play a role [4•], although specific genotype–phenotype correlations have not been identified thus far.

Heart involvement is not common in LGMD2B although dysferlin is expressed in cardiomyocytes. A dilated cardiomyopathy has been recently described in animal models of dysferlinopathies under conditions of mechanical stress, but further work is needed to demonstrate a correlation with human disease [30•,31•].

The function of dysferlin in skeletal muscle is still under investigation. The pathogenesis of LGMD2B is attributed to impaired calcium-mediated muscle-membrane repair, rather than to increased susceptibility to muscle-membrane damage [5••].

Dysferlin localizes to the T-tubules in human skeletal muscle [32••,33]. Recent studies [5••,34•] have suggested that dysferlin-containing vesicles are transported to the T-tubules and sarcolemmal membrane in response to changes in calcium concentration that occur as a result of membrane damage.

In addition, dysferlin deficiency delays myoblast fusion or maturation *in vitro* [35], suggesting that dysferlin may also contribute to muscle differentiation or regeneration. The finding of an interaction between dysferlin and AHNAK bolsters this hypothesis and suggests that these proteins share a role in membrane fusion events during regeneration and membrane repair [32••].

Other interesting studies have investigated the role of muscle inflammation in the pathogenesis of dysferlinopathies. Because monocytes normally express dysferlin, Nagaraju *et al.* [36••] hypothesized that monocyte/macrophage dysfunction in dysferlin-deficient patients might contribute to disease onset and progression by initiating, exacerbating and perpetuating the underlying myofibre-specific dystrophic process. Finally, a recent study [37•] reported sarcolemmal and interstitial amyloid deposits in the muscle biopsies of some LGMD2B patients.

Limb–girdle muscular dystrophy type 2C→F (sarcoglycanopathies)

Although the relative frequency of mutations in the different sarcoglycan genes varies from population to population, α- and γ-sarcoglycanopathies appear to be more common than β- and δ-sarcoglycanopathies [38]. Most of the mutations in one of the sarcoglycan genes destabilize the whole sarcoglycan complex (SGC) at the plasma membrane, resulting in an inability to counteract the mechanical stress generated by contractile activity [39]. The predictive value of protein analysis in determining which sarcoglycan gene is involved is still controversial. A possible association between γ-sarcoglycan deficiency at the muscle biopsy and gene mutations has been reported [4•] but correct diagnosis still requires genetic confirmation and analysis of several sarcoglycan genes may be necessary. Gouveia *et al.* [40•] described a genetically confirmed δ-sarcoglycanopathy with preserved expression of all sarcoglycans except for δ-sarcoglycan. They hypothesized that the partial retention of the SGC might account for the milder clinical course. A systemic evaluation of a large cohort of sarcoglycanopathies may be useful to clarify this issue and evaluate its clinical relevance.

Sarcoglycans form a transmembrane glycoprotein subcomplex within the dystrophin-associated glycoproteins (DAG) that is linked to α- and β-dystroglycan and sarcospan and provides a mechano-signalling connection from the cytoskeleton to the extracellular matrix [41].

Expression of α-sarcoglycan is thought to be limited to striated muscle, whereas β-, γ- and δ-sarcoglycans are also expressed in smooth muscle in association with ε- and ζ-sarcoglycans. This different pattern of expression may explain why the heart is so rarely involved in LGMD2D compared with other sarcoglycanopathies. Hjermind *et al.* [42•] suggested a different role of the SGC εβγδ versus εβγδ in humans on the basis of the absence of signs and symptoms of muscle disease in patients with myoclonus–dystonia due to mutations in the ε-sarcoglycan gene. The recent finding of α-sarcoglycan expression in smooth muscle [43•] needs confirmation and clarification of its functional role.

Limb–girdle muscular dystrophy type 2G (telethoninopathy)

To date, limb–girdle muscular dystrophy type 2G (LGMD2G) has been described only in four Brazilian families, one of which had Italian origin [44]. Heart involvement was observed in one of these families. Heterozygous mutations with low penetrance in the gene (*TCAP*) encoding for telethonin/titin-cap were reported in some patients affected by inherited dilated and hypertrophic cardiomyopathies [45,46]. A role of telethonin in autosomal dominant cardiomyopathy was suggested.

Further studies are needed to clarify the pathogenic role of these heterozygous mutations and their possible relevance in patients with LGMD2G.

TCAP is thought to be one of the titin-interacting Z-disk proteins involved in the regulation and development of normal sarcomeric structure [47]. The mechanism whereby TCAP deficiency results in a dystrophic phenotype is still unclear. Markert *et al.* [48•] observed a marked decrease in the expression of the myogenic regulatory factors (MRFs), including myogenic differentiation (MyoD) and myogenin, in cultured *TCAP* knockdown skeletal muscle cells. This new result indicates a possible regulatory role of TCAP in myoblast proliferation and differentiation during muscle growth.

Limb–girdle muscular dystrophy type 2H (TRIM32 deficiency)

Saccone *et al.* [49••] recently reported three novel putative mutations in the *TRIM32* gene in three Italian and one Croatian unrelated patients. This paper is the first description of limb–girdle muscular dystrophy type 2H (LGMD2H) in a non-Hutterite population, reinforcing the role of TRIM32 in the pathogenesis of LGMD.

Although the mutations identified in European cases differ from the Hutterite founder mutation, all of them cluster at the NHL domain of the protein. By testing *TRIM32* and its mutants, the authors demonstrated that *TRIM32* mutants had lost their ability to self-interact when the interaction between TRIM32 and the ubiquitin-conjugating enzyme E2N was weakened [49••,50]. They suggested that loss of a specific interaction property might be responsible for the dystrophic changes in LGMD2H patients.

Dystroglycanopathies

Defects in the glycosylation of α-dystroglycan are classically associated with congenital muscular dystrophies (CMDs). It is now becoming clear that the phenotypic spectrum of disorders associated with mutations in the six known glycosyltransferase genes is significantly wider than initially suspected and includes LGMDs without brain or eye involvement [51].

In this context, the fukutin-related protein (*FKRP*) gene is most commonly involved, accounting for one of the most common AR-LGMD in northern Europe [52,53]. Over the last few years, mutations in most of the six known or putative glycosyltransferase genes have been associated with milder LGMD phenotypes.

A possible hierarchical involvement of muscle and brain depending on individual gene mutations has been hypothesized, with more frequent central nervous system (CNS) impairment in patients with *POMT1* and *POMT2* mutations than in patients with *FKRP* and fukutin gene mutations [54••].

The existence of intermediate phenotypes between LGMDs and CMDs and the evidence of either structural or functional CNS involvement in some of these new forms of LGMD, further complicate the classification of these new phenotypes and genetic entities.

Fukutin-related protein

The wide clinical variability associated with *FKRP* mutations has been expanded by the description of a 21-year-old patient who showed severe early-onset dilated cardiomyopathy without symptoms of muscle weakness [55•]. The mechanisms whereby *FKRP* mutations cause variation in clinical presentation are not clearly understood, but phenotypic severity appears to correlate with the levels of α-dystroglycan hypoglycosylation in muscles [56••]. Patients with two null-*FKRP* alleles have not been reported, suggesting that the complete lack of *FKRP* results in embryonic lethality.

Homozygosity for the common missense mutation (L276I) is frequently associated with a milder clinical phenotype. Moreover, mild LGMD phenotypes in patients compound heterozygous for a nonsense mutation and the common mutation suggest that one copy of the common mutant *FKRP* allele is sufficient to protect the individual from severe muscle damage [56••].

An abnormal pattern of protein transportation and retention of FKRP within the endoplasmic reticulum was suggested to play a role in determining a very severe congenital phenotype [57]. However, confounding results were reported in other studies [for example: [58]]. Keramaris-Vrantsis *et al.* [56••] recently confirmed the pattern of FKRP localization previously described both in cell culture and in in-vivo muscle from mouse and human carriers of *FKRP* mutations. These results support the observation that mutations associated with severe phenotypes are more likely to cause mislocalization of the mutant proteins outside the Golgi apparatus. However, there is no evidence of a direct correlation between the degree of loss of Golgi localization and severity of clinical manifestations.

Keramaris-Vrantsis *et al.* [56••] suggested that individual missense point mutations can have two independent effects on FKRP, one causing reduction or loss of its enzymatic activity, the other causing mislocalization and that the two effects could influence clinical severity.

POMT1

The absence of eye and structural brain abnormalities and the milder muscle involvement in patients with mutations in the *POMT1* gene led to the description of a new clinical phenotype, named limb–girdle muscular dystrophy type 2K (LGMD2K) [54••,59]. Onset was between birth and 6 years of age and all reported cases

showed mental retardation and microcephaly, making the distinction between CMDs and LGMDs difficult.

Fukutin

Mutations in the fukutin gene are frequent cause of muscular dystrophy in Japan but are rare in other populations. Godfrey *et al.* [60] reported three patients carrying mutations in the fukutin gene, who showed an LGMD phenotype, no functional or structural brain abnormalities, and remarkable clinical response to steroids. These cases together with the description of fukutin mutations in patients with predominant heart involvement [61] have suggested that mutations in non-Japanese populations cause milder clinical phenotype without brain involvement [54••].

POMT2

Biancheri *et al.* [62•] recently described mutations in the *POMT2* gene in a patient with LGMD, inflammatory changes in the muscle biopsy, normal intelligence and no brain abnormalities. An additional patient with similar phenotype but with mental retardation has been reported by Godfrey *et al.* [54••].

POMGnT1

Clement *et al.* [63•] recently widened the spectrum of disorders associated with mutations in *POMGnT1* to include LGMD with onset in childhood and without brain involvement. The patient carried a novel homozygous point mutation in exon 20, which had not been reported in the severe congenital form. POMGnT1 activity in the patient's fibroblasts showed altered kinetic profile, but less marked than in patients with CMD, suggesting an explanation for the relatively mild phenotype in this patient.

Limb–girdle muscular dystrophy type 2J

Limb–girdle muscular dystrophy type 2J (LGMD2J) is the severe homozygous expression of mutations in the titin (*TTN*) gene, whose heterozygous state causes milder distal myopathy (TMD), dilated cardiomyopathy type 1G, or hypertrophic cardiomyopathy type 9 [64].

Cardiac involvement is not described in LGMD2J. Recently, Carmignac *et al.* [65••] extended the clinical spectrum of titinopathies by describing a new recessive phenotype characterized by early-onset proximal–distal myopathy, progressive dilated cardiomyopathy, and rhythm disturbances. This phenotype was associated with homozygous out-of-frame *TTN* gene deletions, which have not been described in patients with LGMD2J and TMD [66].

All identified mutations in the *TTN* gene affect the last immunoglobulin domain (M10) of the protein, suggesting the importance of this domain for maintaining functional fibrils. Mutations associated with more severe phenotypes disrupt key structural features of the M10 immunoglobulin fold. Fukuzawa *et al.* [67••] observed that these mutations weaken or abrogate titin obscurin and titin Obs11 binding and lead to obscurin mislocalization. The authors suggested that interference with the interaction of these proteins at the M-band might be of pathogenic relevance for human disease.

Treatment

There are no established specific drug treatments for LGMD [68]. Different therapeutic approaches, including gene therapy, cell therapy, and pharmacological trials are currently under investigation in animal models and experimental studies [69••].

A recent clinical trial with a neutralizing antibody to myostatin, MYO-029, in adult muscular dystrophies, including different forms of LGMD2, showed good safety and tolerability. No improvement in muscle strength or function was demonstrated after 9 months of treatment, but the study was not powered to provide proof of efficacy [70••].

Two clinical trials are currently in progress in LGMD accordingly to the U.S. National Institute of Health (www.clinicaltrials.gov). The first is a phase I, escalation dose clinical trial aimed at assessing the safety of intramuscular administration of recombinant adeno-associated virus serotype 1 (rAAV1) – human ε-sarcoglycan gene (hαSG) vector to α-sarcoglycan-deficient individuals. In animal models, the construct was shown to initiate the production of a functional α-sarcoglycan protein, to reverse the dystrophic phenotype, and to partially increase muscle strength [71•,72••].

The second clinical trial for dysferlinopathy is being carried out by the Friedrich Bauer Institute, Ludwig-Maximilians University, Munich (www.md-net.org). This is a double-blinded placebo-controlled study designed to evaluate therapeutic efficacy and side effects of steroids (deflazacort) in LGMD2B/Miyoshi myopathy patients.

Finally, there are some recent anecdotal reports of remarkable response to steroids in α-dystroglycanopathies. Godfrey *et al.* [60] reported two patients affected by LGMD due to mutations in the fukutin gene, who showed rapid motor improvement on treatment with prednisolone. Darin *et al.* [73••] observed similar benefits in two patients with LGMD2I. These clinical observations together with the evidence of benefits from steroids in the more severe form of Duchenne muscular dystrophy suggest the importance of future randomized controlled studies to investigate the potential benefits

and side effects of corticosteroids in α-dystroglycan deficiencies.

Discussion

Over the last few years, molecular genetic characterization of LGMD has made great strides, showing that the 21 (or more) molecularly characterized forms that are at present defined as LGMD in fact not have much in common. Novel genes have been recently associated with new LGMD phenotypes and the list is likely to expand. To further complicate the spectrum of LGMD, it is also becoming evident that the same gene mutations may lead to very different clinical and pathological phenotypes.

A more comprehensive understanding of genetics and pathophysiology of LGMD will be helpful to identify future therapeutic targets and strategies. Some treatment options are being applied to limited human studies and new therapeutic strategies will become available in the near future.

Considering the low frequency of each LGMD form in single populations, adequate clinical studies and therapeutic trials will require collaboration amongst multiple neuromuscular centres. In this regard, current work in LGMD is also focusing on the development of European and global patient registries and databases (www.treatnmd.eu/registry). These represent a fundamental key to increase our resources of the natural histories of these conditions, to identify genotype–phenotype correlations and other prognostic factors, to define standards of care, and to facilitate translational research.

Acknowledgement

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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 (pp. 620–621).

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The paper describes clinical and genetic variability within the LGMDs in a large Italian cohort of patients and suggests some genotype–phenotype–protein expression correlations among in the different forms of LGMD2.

5 •• Lo HP, Cooper ST, Evesson FJ, *et al.* Limb-girdle muscular dystrophy: diagnostic evaluation, frequency and clues to pathogenesis. Neuromuscul Disord 2008; 18:34–44.
The paper reports the frequency of LGMD subtypes in a large cohort of Australian muscular dystrophy patients using protein and DNA sequence analysis and discusses diagnostic approaches for the different forms, in particular for dysferlinopathies.

6 • Van der Kooi AJ, Frankhuizen WS, Barth PG, *et al.* Limb-girdle muscular dystrophy in the Netherlands: gene defect identified in half the families. Neurology 2007; 68:2125–2128.
The paper reports the frequency of the different forms of LGMD in The Netherlands and suggests the involvement of new genes in the genetically unconfirmed LGMD2. Some genotype–phenotype correlations are suggested.

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In this paper, the authors present an in-vitro assay to detect the proteolytic activity of calpain3 in a muscle sample. This new approach appears to be more sensitive than common analysis by immunodetection in identifying patients affected by LGMD2A.

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The paper proposed cDNA analysis for CAPN3 as a complementary approach for the diagnosis of calpainopathy and indicates that calpainopathy is an uncommon cause of LGMD in the Denmark.

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