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Limb-girdle muscular dystrophies: An update

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Abstract

Limb-girdle muscular dystrophies (LGMDs) are a heterogenous group of genetically driven muscle disorders, which share the two common features of progressive, predominantly proximal girdle skeletal muscle involvement and dystrophic changes on pathology. This is a rapidly expanding landscape in neurology both in relation to diagnosis as well as evolving targeted treatment options. Thirty-one LGMD subtypes are described to date; five of autosomal dominant inheritance and 26 of autosomal recessive transmission. This article describes their pathogenesis, clinical presentation, diagnosis and recent therapeutic advances.

KEYWORDS

Calpainopathy, dysferlinopathy, gene therapy, LGMD, sarcoglycanopathy

INTRODUCTION

Limb-girdle muscular dystrophies (LGMDs) are a heterogenous group of muscular dystrophies sharing the two common features of progressive, predominantly proximal girdle skeletal muscle involvement and muscle dystrophy. They are rarer in comparison to the common dystrophies such as Duchenne muscular dystrophy (DMD), Becker muscular dystrophy (BMD) and myotonic dystrophy. They have a worldwide prevalence of 1.6- 2.3 per 100,000 population[1] accounting for 9.1% of all muscular dystrophies.[2,3] Except for a few case reports and case series, prevalence data for Sri Lanka and South Asia are lacking.[4]

The disease has been recognized since the late 18th century and defined by Walton and Nattrass in 1954.[5] The intriguing fact is that since the discovery of its first pathogenic gene in 1991, an ever-growing number of genetic mutations have been identified as causing the LGMD phenotype.[6] Up to now 31 LGMD subtypes have been described; five of autosomal dominant inheritance and 26 of autosomal recessive transmission (Table 1).

According to the newest classification from the 229th workshop of the European Neuromuscular Centre (ENMC) in 2017, LGMDs are named by inheritance (dominant- D or recessive- R), a serial number according to the order of discovery, followed by the name of the dysfunctional protein. Eight entities were estranged from the LGMD group due to non-conformity to the currently agreed disease definition; three myofibrillar myopathies, Emery-Dreifuss muscular dystrophy (due to their distal limb predominance), *PINCH2*-related myopathy, *TOR1AIP1*-related myopathy, rippling muscle disease and Pompe disease.[7] However, the olden 1995 nomenclature is still being quoted along with the newer version to improve clarity.

Pathogenesis

This is a genetically mediated disease. Most of the genetic mutations are also causative of congenital muscular dystrophy, the more virulent form, with relatively milder varieties manifesting as LGMDs. For a given single subtype of LGMD, the responsible gene locus will have hundreds of different

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possible mutations-missense, nonsense, splice site mutations, small deletions etc., which result in a spectrum of severity.

Many of such genetic variants are yet to be classified as benign or pathogenic, such that only 50-68% of LGMD phenotype patients carry a diagnosis even after whole genome sequencing.[8,9] As mitochondrial uncoupling too plays a key role in disease pathogenesis, the phenotypic expression gets diversified even further. Therefore, it can be concluded that there is little genotype-phenotype correlation in LGMDs, except for null mutations which will cause more severe phenotypes than missense mutations.[10,11]

As to testify for the phenotypic diversity of the disease, varied cellular targets malfunction due to these defective genes responsible for each LGMD subtype. These include proteins in the nucleus, cytoplasmic organelles (such as Golgi apparatus, endoplasmic reticulum), sarcomeres, sarcolemma and the extracellular matrix (Figure 1).[10]

Functionally, the above result in either loss of sarcolemmal integrity, sarcomere dissociation, glycosylation defects of key proteins i.e., dystroglycan or impaired myocyte repair mechanisms, which lead to mechanical signalling failure (compared to mechanical fragility seen in DMD), mitochondrial dysfunction, increased rate of muscle degeneration and apoptosis[11]. Studying these broad pathogenic pathways might help in identifying potential therapeutic targets in precision medicine and in clustering them for ease of understanding.[11]

Types of LGMD

The diagnostic approach to LGMD was previously clinico-pathological, but now is predominantly clinicogenetic. However, in resource poor settings with limited availability of genetic diagnostic facilities, the former still remains quite useful.

Based on the immunohistochemical analysis of the defective proteins on muscle biopsies, LGMDs were historically subdivided into the following broad categories (The relevent genetic diagnoses are given within brackets).

- Calpainopathy (LGMDR1, LGMDD4)
- Dysferlinopathy (LGMDR2
- Sarcoglycanopathy (LGMDR3, LGMDR4, LGMDR5, LGMGR6)
- Dystroglycanopathy (LGMDR6, 9, 11, 13-16, 18-20)
- Others

  Anoctaminopathy (LGMDR12)

  Titinopathy (LGMD10)

  Muscle collagenopathies (LGMDR22, LGMDD5) etc.

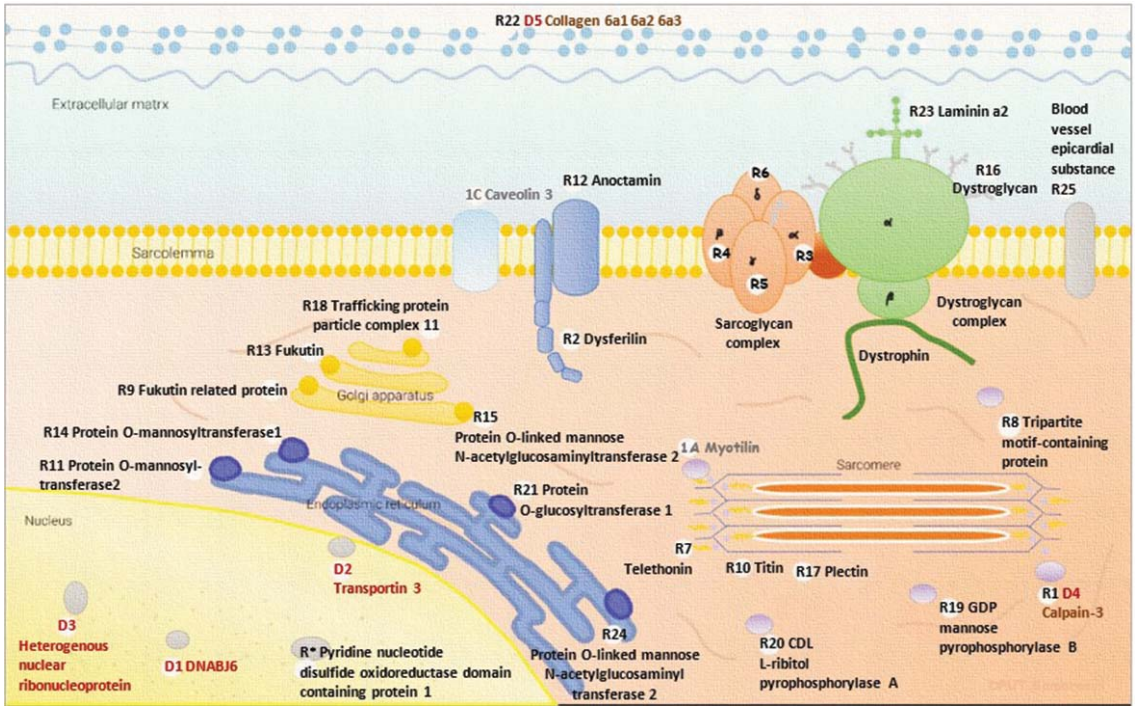

FIGURE 1 **Schematic diagram of subcellular localization of pathogenic proteins in LGMDs (Protein targets for dominant LGMDs are labelled in red and recessive forms in black).** (Credits: Adopted from Protein J. 2021; 40(4):466-488. Georganopoulou DG et al. CC BY 4.0, with permission from Spinger Nature [10]).

These include both autosomal recessive and autosomal dominant LGMD subtypes. As a general notion, autosomal recessive forms manifest earlier with greater severity and poorer prognosis compared to autosomal dominant forms. Unfortunately, around 90% of LGMD identified to date are autosomal recessive.[12]

A complete summary of clinical characteristics with relative frequency of each LGMD subtype is expanded in Table 1[13]. Among the different phenotypes some distinguishing features exist, enabling physicians to clinically narrow down the diagnosis to a possible subtype which will direct the performance of targeted genetic analysis in a resource limited setting.

Accurate diagnosis will enable patient counselling, prognostication, and direction towards therapeutic options as discussed in the latter half of this article.

Calpainopathy (LGMDR1, LGMDD4)

This is the most common LGMD subtype described as early as 1884 and the first to be genetically mapped in an isolated population in the Reunion Island in France.[14] The defective proteolytic enzyme, calpain-3 was discovered shortly afterwards. As of 2023, only 437 pathogenic or likely pathogenic variants from a total number of 1512 variants remain identified.[15]

The age of onset is generally in the second decade (2-45 years) and is not necessarily correlated with the rate of progression. Muscle atrophy with scapular winging, toe walking due to contractures, spinal rigidity, and abdominal laxity mark the archetype patient with calpainopathy. Upper limb contractures are also common but distal involvement is not so marked as that in dysferlinopathies. Mild facial involvement and respiratory involvement is occasionally seen. Cardiac involvement is rare except for few reported cases of arrhythmias.[16] The dominant form shares the same characteristics, albeit to a milder degree.[17]

Dysferlinopathy (LGMDR2)

This is the second most common LGMD subtype. Interestingly, it shares the same DYSF gene mutation as Miyoshi muscular dystrophy which is phenotypically different.[18]

Often, the disease manifests between teenage to early adulthood (14-58 years) with core features of predominant proximal muscle weakness with subclinical distal involvement, asymmetrical muscle atrophy-even within the same muscle, leading to the interesting “bulge sign of biceps”, “calf-heads on trophy sign” and “diamond in quadriceps sign”.[19] Facial and cardiac involvement are not typical. In the initial stages serum creatine phosphokinase (CPK) level can reach 60-70 times the upper limit of normal, often mistaken with polymyositis, which may be detrimental as steroids are postulated to worsen sarcolemmal instability hastening muscle degeneration in dysferlinopathy. The CPK level tends to plummet as the disease progresses.[20]

Sarcoglycanopathies (LGMDR3 α-sarcoglycan-related, LGMDR4 β, LGMDR5 γ, LGMDR6 δ)

This group shows much heterogeneity ranging from a full blown DMD like phenotype with pseudohypertrophy of calves and macroglossia to that of isolated exercise intolerance, exercise induced myalgia or subclinical hyper-CPKaemia. Severe versions tend to manifest in childhood. Most of them have cardiac and late respiratory involvement. LGMDR3 tends to show a milder phenotype than the rest.[21]

Dystroglycanopathies (LGMDR6, 9, 11, 13-16, 18-21)

This cluster of disorders stems from under glycosylation of dystroglycan protein due to enzymatic defects located primarily on the Golgi apparatus or endoplasmic reticulum. It is difficult to draw a simplified prototype of dystroglycanopathies as they share a broad spectrum of symptoms. Most severe forms tend to manifest as congenital muscular atrophies with predominant oculo-cerebral involvement very early in life, i.e., Walker Warburg syndrome, muscle-eye-brain disease.[22] Following pursuit of assessment on follow up, these LGMDs too can demonstrate myopia, cataracts, epilepsy, and cognitive impairment in addition to myopathy.[23]

DIAGNOSIS

The definitive diagnosis comes through genetic analysis. However, 50-68% of ‘possible’ cases will remain with an open diagnosis, simply because most of the variants still have uncharted significance. Some of these subtypes have a distinctive electrodiagnostic, imaging and biopsy profile that might aid in diagnosis, in addition to the clinical features.

Serum creatine phosphokinase (CPK)

The extent of the CPK rise might be helpful to differentiate between subtypes of LGMD as depicted in Table 1. However, there is a marked inter- and intra-personal variation even within a single subtype caused by a specific genetic variant.

Electrodiagnostic studies

TABLE 1 **LGMDs with clinical, biochemical and elecrodiagnostic features, summarised according to relative incidence**

	2017 classification	1995 classification	Childhood onset	Juvenile onset	Adult onset	Tip toe walking	Distal muscle weakness	Scapular winging	Calf hypertrophy	Calf hypotrophy	Myalgia, cramps	Myoglobinuria	Scoliosis	Joint contractures	Early loss of ambulation	Intellectual disability/ seizures	Macroglossia	Dysarthria	Dysphagia	Mild facial weakness	Respiratory involvement	Cardiac involvement	Inflammatory cell infiltrates	Serum creatine kinase		Edx	Gene symbol	Defective protein
LGMD	MFM	1A			●		●							●				●		●	●	●		●		M/N	MYOT	Myotilin
LGMD	EDMD	1B	●	●	●	●	●							●								●		●		M	LMNA	Lamin
LGMD	RMD	1C	●	●	●	●	●		●		●	●		●								●		●		M	CAV3	Caveolin-3
LGMD	D1	1D	●		●		●											●	●	●		●		●		M	DNAJB6	DNAJ/HSP40
LGMD	MFM	1E	●	●			●												●		●	●		●		M/N	DES	Desmin
LGMD	D2	1F	●	●	●		●			●			●	●					●	●		•		●		M	TPNO3	Transportin-3
LGMD	D3	1G			●		●				●			●								•		●		M	HNRPDL	Heterogeneous
LGMD	D5	1H		●	●				●												•			●		M	COL6A1-3	Collagen 6a1-3
LGMD	D4	1I									●										●	•		●		M	Calpain-3	Calpain-3
LGMD	R1	2A	●	●	●	●	●	●	•		●	●	●	●	●							●	●	●	●	M	CAPN3	Calpain-3
LGMD	R2	2B		●	●	•	●			●	●	●	●	●	●							●	●	●		M	DYSF	Dysferlin
LGMD	R5	2C	●	●	●	●		●	●				●	●	●		●				●	●		●	●	M	SGCG	Gamma-sarcoglycan
LGMD	R3	2D	●	●	●	●		●	●			●	●	●	●		●				●	●		●	●	M	SGCA	Alpha-sarcoglycan
LGMD	R4	2E	●	●		●		●	●				●	●	●		●				●	●		●	●	M	SGCB	Beta-sarcoglycan
LGMD	R6	2F	●			●		●	●				●	●	●		●			●	●	●		●	●	M	SGCD	Delta-sarcoglycan
LGMD	R7	2G		●			●		●	●				●								●		●		M	TCAP	Telethonin, titin-cap
LGMD	R8	2H	●		●		●	●	●		●									●		●		●		M/N	TRIM32	Tripartite-motif TRIM-32
LGMD	R9	2I	●	●	●	●		●	●		●	●		●	●	●	●			●	●	●		●		M	FKRP	Fukutin-related-protein
LGMD	R10	2J	●	●										●								●		●		M	TTN	Titin
LGMD	R11	2K	●											●		●						●		●		M	POMT1	Protein-mannosyl-transferase-1
LGMD	R12	2L		●	●		●		●	●	●	●		●					●	●	•	●		●		M	ANO5	Anoctamin-5
LGMD	R13	2M	●	●	●				●					●		●						●	●	●		M	FKTN	Fukutin
LGMD	R14	2N	●	●	●					●				●		●						●		●		M	POMT2	Protein-mannosyl-transferase-2
LGMD	R15	2O		●	●											●						●		●		M	POMGnT1	Protein-mannose-acetylglucosminyl-transferase-1
LGMD	R16	2P		●	●											●				●		●		●		M	DAG1	Dystrophin-associated-glycoprotein
LGMD	R17	2Q	●				●								●						•	•		●		M	PLEC1	Plectin-1
LGMD	MFM	2R			●		●													●	●	●		●		M/N	DES	Desmin
LGMD	R18	2S	●								●			●		●						•		●		M	TRAPPC11	Transport-protein-particle-complex-11
LGMD	R19	2T	●	●	●						●	●		●		●						●		●		M	GMPPB	GDP-mannose-pyro-phosphorylase-B
LGMD	R20	2U	●											●		●						●		●		M	ISPD	Isoprenoid synthase
LGMD	POMPE	2V		●	●		●														●	•		●		M	GAA	Acid alpha-glucosidase
LGMD	PINCH	2W	●														●					●		●		M	LIMS2	Lim senescent cell antigen-like domain-2
LGMD	R25	2X			●																	●		●		M	BVES	Blood vessel epicardial substance
LGMD	TRM	2Y	●										●	●								●		●		M	TOR1AIP1	Torsin-1A interacting protein-1
LGMD	R21	2Z						●														•		●		M	POGLUT1	Protein-O-glucosyltransferase
LGMD	R22	-	●	●			●	●					●	●										●		M	COL6A1-3	Collagen 6a1-3
LGMD	R23	-	●	●												●								●		N/M	LAMA2	LamininA2
LGMD	R24	-	●						●							●								●		M	POMGNT2	Protein-mannose-acetylglucosminyl-transferase-2
LGMD	R,	-	●	●	●		●			●								●	●	●	●	●		●		M	PYROXD1	Pyridine nucleotide disulfide oxidoreductase domain-containing protein 1

MFM- myofibrillar myopathy; EDMF Emery Dreifuss muscular dystrophy; RMD rippling muscle disease; Edx elecrodiagnostic studies, M myositis pattern N neuropathic pattern; •absent, relative frequencies depicted by the size of mark ●●● Creatine kinase <10x, 10-50, >50x ULN

The electromyogram is the most sensitive investigation, displaying myopathic changes even in 89% patients with asymptomatic hyper-CPKaemia, which is the mildest phenotype.[24] Demyelinating peripheral polyneuropathy can be seen in LGMDR8 and LGMDR23.[13,25] Myofibrillar myopathies (previously LGMD1A and LGMD1E) are characterized by myotonic and pseudomyotonic discharges. Pompe disease (previously LGMD2V) too shows myotonic activity and fibrillation potentials chiefly in paraspinal muscles.[26]

Muscle magnetic resonance imaging (MRI)

MRI is useful to discern the pattern of muscle involvement which might give a clue as to the possible subtype as illustrated in Figure 2. Nevertheless, the sensitivity and specificity have been low, 40% and 58% respectively, with good positive and negative predictive values (77% and 79%) according to a study done on 118 patients.[27]

The interesting MRI appearance noted on T1 weighted images in collagenopathies (LGMDD5, LGMDR22) is due to differential fat infiltration of the muscle which includes “tigroid appearance”, “target sign” and “sandwich sign” (Figure 3).[28]

Histopathology

Basic histopathology will often offer a blanket diagnosis of muscular dystrophy, which features degeneration (muscle fibre necrosis, phagocytosis, hyaline fibres, apoptosis); regeneration (muscle fibre splitting, large nuclei per fibre, central nuclei, ring fibres, and lobulated fibres); compensation (muscle fibre hypertrophy), and repair with fibrofatty tissue. This will give rise to an increase in fibre size variability.[29]

However, techniques such as immunohistochemistry and/or Western blot are necessary to distinguish one from another, by demonstrating the absent or deficient protein i.e., dystrophin, emerin, sarcoglycans etc. These need to be done on fresh frozen specimens. Sensitivity and specificity vary according to the subtype, 53% and 85% for calpainopathy versus 100% sensitivity and specificity for dysferlinopathy as shown in a few studies.[30,31] Inflammatory changes are seen in 25% of

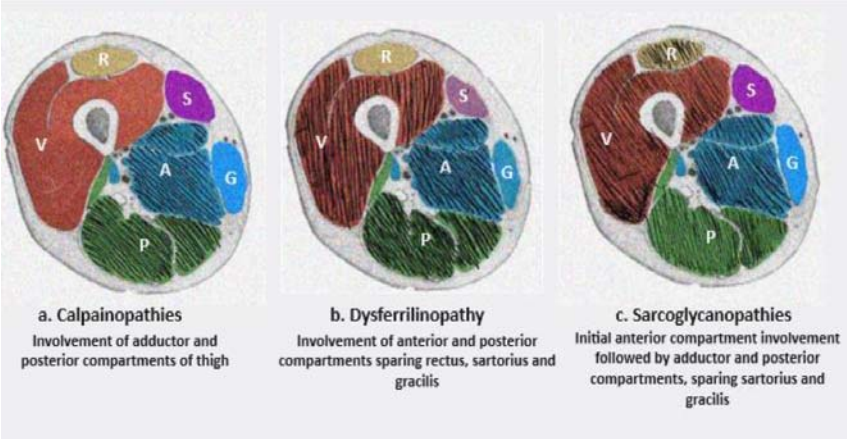

FIGURE 2 **Illustration comparing differential muscle involvement in main three LGMD subgroups. (R - Rectus femoris, V - Vastus group, S - Satorius, G - Gracilis, A - Adductor magnus, P - Posterior compartment; commonly affected muscles are shaded).**

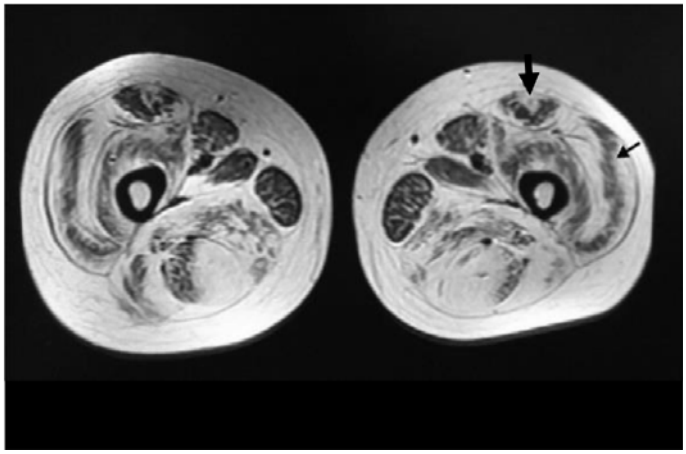

FIGURE 3 . **T1 MRI image of proximal thigh muscles demonstrating characteristic tigroid appearance, target (big black arrow) and sandwich (small black arrow) signs due to fat infiltration in dystrophic muscles[28].**

Credits: Adopted from Chin Med J (Engl) 2016;129(15):1811-1816. Fu J, et al. copyright BY-NC-SA 3.0.[28].

dysferlinopathies which might be mistaken for polymyositis. Pompe disease will show Periodic acid-Schiff positive deposits which will aid in differentiation.[32]

Genetic analysis

a. Sanger sequencing

This is useful to analyse a smaller number of genes after clinically and histopathologically narrowing down the potential candidate gene. However as clinical phenotypes can be so variable in LGMDs, this has been rendered less valuable in initial screening. Conversely, this will be quite useful in family screening, prenatal screening, segregation analysis and confirmation of a variant, where the pathogenic gene is already identified in the proband.

b. Next generation sequencing (NGS)/ Massively parallel sequencing

NGS can sequence the whole exome in a cost-effective manner. Therefore, it is commonly used for proband screening in a heterogeneous condition like LGMD, where many exons need to be sequenced to catch the culprit gene. It also has the ability of detecting novel mutations. The mutation detection rate had increased from 35% to 46% using this over Sanger sequencing.[32,33] However, this will fail to detect intronic mutations, as well as large duplications and deletions which involve entire exons.

Once a pathogenic variant is identified, there is no need for any further investigation. However, the AAN guideline suggests reaching a clinical diagnosis first, since many of the variants are yet to be determined as pathogenic or not.[34]

Genotype-phenotype correlations too are challenging due to the variable gene expression leading to different clinical courses or entirely different diseases even with identical mutations i.e., Miyoshi myopathy vs LGMDR2.

Differential diagnoses

- Dystrophinopathies – Clinical presentation of some sarcoglycanopathies and dystroglycanopathies (LGMDR9) can mimic DMD/ BMD, such that in some studies 17% of previously diagnosed LGMDs turned out to be dystrophinopathies.[35]
- Facioscapulohumeral muscular dystrophy – Some of the LGMDs have facial involvement and scapular winging and might get mistaken for FSHD. Eight percent of LGMD turn out to be FSHD on genetic analysis.[36]
- Inflammatory myopathies – Specially in genetically negative cases, immunopanels for autoimmune myopathy should be considered even when the biopsies might not be that of a typical myositis. Some patients with exact LGMD phenotype and biopsy findings were found to be anti HMG-CoA reductase (HMGCR) antibody positive with good response to immunotherapy.[37]
- Metabolic myopathies (Pompe disease (Glycogen storage disease II)/ Glycogen storage disease type XV) – Although some cases might be clinically indistinguishable, histology followed by biochemical analysis will help in the correct diagnosis.

Management options

There is power in a closed diagnosis. Although definitive therapies are still in their early stages, there is much to be done to support the patients and to stop unnecessary costly ventures trying to seek remedies. Over the past decade there has been a huge momentum in therapeutic research into the field encouraged by success in spinal muscular atrophy and DMD.

Supportive care and secondary prevention

This is a multidisciplinary care package headed by the neurologist involving cardiologists, pulmonologists, psychiatrists, orthopaedic specialists, nutritionists, physiotherapists, occupational therapists, orthotists, and speech therapists. A great service can be done for the patients simply by focusing on preserving mobility and reducing discomfort. A detailed description on this can be found in the American Academy of Neurology (AAN) guidelines.[34]

Targeted therapies

a. Drug therapies

Although steroids are used there is little evidence of significant effect. An open label trial done on a cohort of LGMD patients (n=19) in India using weekly prednisolone therapy had shown a significant improvement of gait speed and grip strength without any toxic effects.[38] However, a similar study (n=20) done in USA failed to show benefit.[39] A randomized clinical trial (n=25) done on Deflazacort use in patients with dysferlinopathy showed harmful effects such as reduced muscle strength and significantly more side effects which included further worsening of the incidence of osteoporosis.

Other drugs which have been used without supportive trial evidence include rituximab, ubiquitin-proteasome inhibitors i.e., bortezomib, givinostat, coenzyme Q and lisinopril.[40-42]

b. Molecular genetic therapies

Most of these are still in the preclinical stage or phase 1 and 2 of clinical trials. These are some of the methods explored.

- Stem cell therapy
- Vector driven gene delivery
- Gene editing using CRISPR technology
- Exon skipping – Antisense oligonucleotides are used to skip the mutated gene segment leading to a functional protein.
- RNA interference

Most of the research that has shown positive outcomes (Table 2), has used vector driven gene therapy, the same mechanism used by that of Elevidys (delandistrogene moxeparvovec), which was recently approved by the FDA for the treatment of DMD.[48]

Adenoviral vectors are preferred to deliver the functional copies of the patient's defective genes, due to their high myotropism and high cloning capacity. Alternations in genome are done to reduce the immunogenicity. However, they might need periodic boosting since they do not integrate into the host genome.[49] Favourable histological and clinical endpoints have resulted in many studies getting approval for stage 2 and 3 clinical studies. Advanced degenerative changes have shown resistance to transfection, thus highlighting the value of an early diagnosis in a future where gene therapy will become the norm.[46,50]

TABLE 2 Recent therapeutic trials targeting LGMD demonstrating positive outcomes

Phase	Method	Molecular target	Institute	Outcome
Pre-clinical	Calpain-3 expressing vector on a murine model	Calpain-3	Université d Evry Val d Essonne, France	Halting progression of cardiomyopathy and improving skeletal muscle strength.[43]
Pre-clinical	Dual adeno-associated virus vectors transferring *DYSF* gene on a murine model	Dysferlin	The Research Institute at Nationwide Children's Hospital, Columbus, USA	Production of full length functional dysferlin[44].
Pre-clinical	Adeno viral vector containing a human *SGCB* transgene on a murine modal	Sarcoglycan- β	The Ohio State University, Columbus, USA	A 98.1% transgene expression across all muscles with reverse in histopathology A 85.5% reduction in serum creatine kinase levels A 94.4% rise in diaphragm force A 48.1% reduction in kyphoscoliosis, 57% rise in overall ambulation.[45]
Phase 2	Adeno viral vector containing a human *SGCB* transgene	Sarcoglycan- β	The Ohio State University, Columbus, USA	Full sarcoglycan complex restored – in all subjects. Muscle fibre size increased in the 3-month subject.[46]
Phase 2	Ribitol- an oral drug which increases glycosylation of alpha-dystroglycan	α-Dystroglycan	Institute of Myology, Paris	A 43% increase in glycosylated ? α-dystroglycan A 70% decrease in mean CPK at 3-months An increase in the walking speed over 10 meters at 3 and 6 months.[47]

CONCLUSION

Limb-girdle muscular dystrophies are a rare cohort of genetically driven disorders displaying a continuum of clinical features. The main obstacle for research into the disease is the scarcity which is somewhat overcome by recent collective efforts across continents to maintain patient registries such as The Lantern Project and TREAT NMD.[51,52] There is a definite call for future collaborations from this part of the world as well.

Most of the studies found success by expressing the deficient protein or a substitute using adenovirus mediated gene transfer on murine models.[43-45] Moreover, these studies demonstrated improvement in functional outcomes. This encouraging success has already led to its approval for human trials.[46, 47] With renewed insight, more precise diagnostic tools, therapeutic leads and support of the pharmaceutical industry, there is much hope on the horizon for patients with LGMDs.

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