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REVIEW

The Limb–Girdle Muscular Dystrophies: Is Treatment on the Horizon?

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Abstract

There has been an ever-expanding list of the Limb–Girdle Muscular Dystrophies (LGMD). There are currently 8 subtypes of autosomal dominant (AD) and 26 subtypes of autosomal recessive (AR) LGMD. Despite continued research efforts to conquer this group of genetic neuromuscular disease, patients continue to be treated symptomatically with the aim of prevention or addressing complications. Mouse models have been helpful in clarifying disease pathogenesis as well as strategizing pathways for treatment. Discoveries in translational research as well as molecular therapeutic approaches have kept clinicians optimistic that more promising clinical trials will lead the way to finding the cure for these devastating disorders. It is well known that the challenge for these rare diseases is the ability to assemble adequate numbers of patients for a clinically meaningful trial, but current efforts in developing patient registries have been encouraging. Natural history studies will be essential in establishing and interpreting the appropriate outcome measures for clinical trials. Nevertheless, animal studies continue to be key in providing proof of concept that will be necessary in moving research along. This review will briefly discuss each type of LGMD, highlighting their distinguishing features, then focus on research efforts that have been published in the literature for the past few years, many of which are still in the preclinical trial stage.

Keywords Limb–girdle muscular dystrophy · Calpain · Dysferlin · Sarcoglycan · Dystroglycan

Introduction

Limb–girdle muscular dystrophy refers to a heterogeneous group of noncongenital, genetic muscle disorders with variable age of onset, primarily causing weakness and wasting of the proximal limb (the hip/shoulder girdle) musculature. Although the name denotes the typical skeletal muscle involvement, many of the subtypes of LGMD may have associated cardiac findings. There is general sparing of bulbar muscle, although exceptions may occur. LGMD is caused by pathogenic genetic variants causing abnormal protein synthesis in the various parts of the muscle fiber including the nucleus, sarcoplasm, sarcomere, sarcolemma, and extracellular matrix. With the advancement of next-generation molecular sequencing, it has been shown that the same genetic variant may demonstrate a wide spectrum of symptoms with variable phenotypic presentations including initial distal limb involvement. Understanding the resultant defect from the genetic variant, either structural or functional, may pave the way for treatment strategies for these disorders [1–3].

The limb–girdle muscular dystrophies have historically been classified into 2 main categories based on the inheritance pattern: autosomal dominant (AD-LGMD1) and autosomal recessive (AR-LGMD2) types [4]. Under each type, a letter of the alphabet is added chronologically, each time a new chromosomal locus is reported. As of 2018, there are 8 subtypes of LGMD type 1 and 26 subtypes of LGMD type 2. X-linked disorders that present with a limb–girdle pattern of weakness include the Emery–Dreifuss muscular dystrophies and the dystrophinopathies (Duchenne muscular dystrophy, Becker muscular dystrophy, and manifesting carriers of dystrophinopathies) and are not traditionally listed in the category of LGMD.

There is considerable variability within the LGMDs, with respect to age of onset, from early childhood to adulthood,

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severity of disease, and rate of progression among the different subtypes and even within the same family.

Serum creatine kinase (CK) levels vary from normal to greatly elevated, even within the same subtype. Electromyography (EMG) shows myopathic features with low amplitude short duration motor units with an early recruitment pattern in weak muscles but findings may be subtle in mild cases. Routine muscle biopsy often shows nonspecific myopathic features. Classic dystrophic changes include degeneration/regeneration of muscle, variability in fiber size, various levels of fibrosis, and adipose tissue infiltration. To date, immunohistochemistry on muscle tissue is available for dystrophinopathies, sarcoglycanopathies, calpainopathies, dysferlinopathies, dystroglycanopathies, caveolinopathies, lamininopathies, type VI collagenopathies, emerin, desmin, myotilin, and utrophin [5, 6].

Molecular testing, including sequence analysis and copy number variants, may offer less invasive confirmation of the different subtypes of LGMD, although molecular testing will not preclude the necessity for muscle biopsy in all patients.

Traditional genetic, biochemical, and histopathological examinations yield diagnoses in approximately 30–40% of LGMD cases; targeted sequence capture has similar yields. Reddy et al. report exome sequencing has improved the diagnostic yield to the 40–45% range likely due in part to the use of trios and family studies [7]. Testing at-risk family members may also assist with medical management and provide information for reproductive decision-making.

This article will briefly review the different types of LGMD by presenting them in a tabulated form, AD-LGMD (Table 1) and AR-LGMD (Table 2), specifying the specific gene, the age of onset, and the distinguishing phenotypic features, and then focus on some of the research efforts that have been published in the literature for the past few years. Many of these studies are still in the preclinical stage.

Epidemiology

The estimated incidence of LGMD is 1 to 6 in 100,000 [2], but is likely an underestimate. The prevalence for all forms of LGMD is 1:14,500 to 1:123,000 [9] with carrier frequency of 1:150 to 1:211. Founder variants have been observed in particular populations [10]. In England, Mexico, and Turkey, LGMD is considered the second most common muscular dystrophy after dystrophinopathies, with prevalence of up to 1:14,500 and a carrier frequency of up to 1:150 [11]. Calpainopathies (LGMD2A) occur in 15–40% of LGMD and are most prevalent in Eastern Europe, the Netherlands, northern England, Czech Republic, Spain, Italy, and Brazil. Sarcoglycanopathies, dysferlinopathies, and fukutin-related protein (FKRP) occur in 15–20% of cases. The prevalence of primary sarcoglycanopathies is estimated at ~ 1:178,000. In Turkey and North Africa, as well as in Brazil, sarcoglycanopathies (LGMD 2C-2F) have a high relative proportion [11–13]). LGMD2C (γ-sarcoglycanopathy) is more common in Roman and Tunisian populations. The worldwide incidence and prevalence of dysferlinopathy are unknown. Its estimated frequency is 5–35% of LGMD cases. It is more prevalent in Asians and in regions surrounding the Mediterranean Sea and represents up to 30% of LGMD2 cases in the Middle East and India [13]. LGMD2I (FRKP) is prevalent in England, Denmark, the Netherlands, and Northern Europe with a rate of 0.43 per 100,000 [12]. Anoctaminopathies are more common in Northern Europe and seen in about 25% of patients with LGMD in the UK. Most of the other specific LGMD disorders are rare.

There are currently 26 defined subtypes of the AR-LGMD2 (LGMD2A-Z). These recessive forms are more common than the autosomal dominant types [1–3, 9]. Several unique subtypes of LGMD have recently been identified [3] while other subtypes, reclassified.

Strategies for Treatment

Since Straub and Bushby's [14] article on the therapeutic possibilities in AR-LGMD published in this journal a decade ago, the treatment of LGMD has not changed. Other than the availability of enzyme replacement therapy for Pompe disease, now categorized as LGMD2V, we continue to treat patients symptomatically and try to prevent/address complications, despite continued translational research efforts. Cell culture assays and animal models serve to clarify pathogenesis and continue to be the key in strategizing pathways for treatment. Molecular therapeutic approaches have demonstrated promising new treatment potentials in the forms of exon skipping and gene therapy. It is essential that we have a clear understanding of the phenotype and natural history of the disease, the existence of clinically relevant outcome measures, standard of care guidelines, up-to-date patient registries, and ideally, biomarkers that can help assess disease severity or drug response. All these determinants will be necessary for future success in clinical trials.

This review will focus on research efforts that have been published in the literature for the past few years. Although most are still in the preclinical trial stage, they are essential in providing proof of concept necessary in moving research along. Although certain subtypes of the autosomal recessive LGMD have received more attention than others, they should be considered as starting points, as similar strategies can be considered once the technology becomes successful. Many challenges lie ahead in translating promising therapeutic strategies into effective and accessible treatments but clinicians are optimistic that this is on the horizon.

Table 1 Autosomal dominant limb–girdle muscular dystrophy

LGMD subtype	Gene	Onset	Phenotypes
LGMD 1A	MYOT Myotilin	18–35 years	• Weakness of the scapular–humeral–pelvic muscles • Distal leg weakness, occasional arm weakness • Cardiomyopathy may be seen • Distinct feature: nasal dysarthria and hypophonia • CK: normal or elevated (up to 9× normal) • Muscle biopsy: rimmed vacuoles often seen, occasional nemaline rod-like inclusions may have features of myofibrillar myopathy
LGMD 1B	LMNA Lamin A/C	5–25 years	• Weakness in scapular and pelvic girdle muscles—usually early in the second decade of life • Contractures affecting posture and gait—can precede muscle weakness • Dilated cardiomyopathy and conduction system defects associated with high risk of cardiac sudden death, may precede muscle weakness • CK: normal or mildly elevated (< 5× upper limit of normal) • Muscle biopsy: usually nonspecific; may have dystrophic features Note: LMNA mutations can present with variable phenotypes including LGMD, AD-EDMD, DCM (dilated cardiomyopathy), heart–hand syndrome, Dunnegan lipodystrophy, mandibuloacral dysplasia, restrictive dermopathy, axonal neuropathy (CMT2B1), and metabolic syndrome. 3 myopathic phenotypes of LMNA: LGMD1B, EDMD2, and LMNA-CMD
LGMD 1C	CAV3 Caveolin 3	1st decade–late adulthood	• 6 distinct phenotypes: ○ Proximal weakness or exertional myalgias associated with calf hypertrophy ○ Rippling muscle disease ○ Distal myopathy ○ Asymptomatic hyperCKemia ○ Familial hypertrophic cardiomyopathy without skeletal muscle weakness ○ Muscle pain, exercise intolerance and rhabdomyolysis [8] • Intra-familial variability and rate of progression • Cardiac involvement is common • CK: elevated (450–5000) • Muscle biopsy: generally normal but may include variability in fiber size, muscle degenerating/regenerating fibers with an increased number of central nuclei, and a mild increase in connective tissue
LGMD 1D	DNAJB6 DNAJ/HSP40 homolog, subfamily B, member 6	2nd decade–upper middle age	• Adult-onset: slowly progressive proximal weakness, ambulation to 60s • Childhood onset: (2 presentations) ○ Distal involvement of muscles ○ Loss of ambulation in early adulthood and respiratory involvement • CK: normal or elevated • Muscle biopsy: myopathic or dystrophic pattern. Rimmed vacuolar pathology (predominant with disease progression) myofibrillar aggregates
LGMD 1E	DES Desmin	Classic AD form: Puberty–50 years AR cases reported earlier	• Distal myopathy (usual presentation) • Generalized myopathy • Proximal weakness • Scapuloperoneal syndrome • Associated dilated cardiomyopathy and cardiac conduction defects • Dysphagia is common • Respiratory insufficiency in 25% of patients • Severe generalized myopathy • CK: normal or slightly elevated • Muscle biopsy: (EM) abundant accumulation of desmin- immunoreactive deposits and granulofilamentous material
LGMD 1F	TNPO3 Transportin 3	Infancy–late adulthood	• Adult onset: ○ Weakness of pelvic and shoulder girdle muscles ○ Variable rate of disease progression and severity ○ Generally benign course

Table 1 (continued)

LGMD subtype	Gene	Onset	Phenotypes
			• Childhood or juvenile onset: ○ Some with severe myopathy ○ Wheelchair dependency ○ Respiratory insufficiency ○ Suggestion of genetic anticipation with earlier onset in subsequent generations • Muscle biopsy: rimmed vacuoles and filamentous inclusions
LGMD 1G	HNRNPDL Heterogeneous nuclear ribonucleo-protein D-like	Adult	• Variable proximal weakness, lower > upper limbs • Slowly progressive • Distinctive feature: limitation of finger and toe flexion • Muscle biopsy: predominantly myopathic, rimmed vacuoles
LGMD 1H	Mapped to chr3p23-p25.1	2nd–5th decade	• Slowly progressive weakness • Decreased reflexes • Proximal muscle atrophy • Calf hypertrophy ± muscle weakness • Occasionally high CK and serum lactate levels • Muscle biopsy: compatible with muscular dystrophy associated with subsarcolemmal accumulation of mitochondria and the presence of multiple mitochondrial DNA deletions in muscle

Calpainopathy (LGMD2A)

Bartoli et al. (2006) [16] evaluated the safety and efficacy of adeno-associated virus (AAV)-mediated calpain 3 gene transfer in a mouse model of LGMD2A using rAAV2/1 pseudotyped vectors and muscle-specific promoters to avoid calpain 3 cell toxicity more than a decade ago. They reported efficient and stable transgene expression in muscle with restoration of the proteolytic activity without evident toxicity, plus calpain 3 was correctly targeted to the sarcomere. Improvement of the histological features and therapeutic efficacy at the physiological levels, including correction of atrophy and full rescue of the contractile force deficits, was demonstrated [15]. This provided proof of concept that AAV-mediated calpain 3 gene transfer is feasible as a therapeutic approach.

In 2013, Roudaut et al. reported the association of a lethal cardiac toxicity in calpain 3-deficient mice given systemic injection of calpain 3-expressing AAV vector. They attributed this to an unregulated proteolytic activity of overexpressed calpain 3 in the heart that would lead to "lysis of the transduced fibers and formation of fibrotic scar." They developed new AAV vectors with skeletal muscle-specific promoters that include the calpain 3 (CAPN3) promoter itself and introduced microRNA (miRNA)-208a target sequences in the 3′-untranslated region of CAPN3 transgene in the cassette. This miRNA-regulated cassette has the ability to suppress calpain 3 expression of cardiac-specific miRNA-208a, which is not modified by calpain 3 deficiency. Thus, the expression of the transgene is restricted to the skeletal muscles leading to reduction of toxicity of the CAPN3 transfer to the heart as well as other organs like the liver, the primary organ transduced following intravenous injection of AAV vectors. This study by Roudaut et al. shows that cardiac toxicity from CAPN3 transgene expression can be successfully suppressed. Moreover, its expression in the skeletal muscles was sufficient to revert the myopathological signs of calpain 3 deficiency [16].

Insulin-like growth factor-1 (IGF-1), which mediates many of the actions of growth hormone (GH), has been shown to improve muscle function and prevent muscle degeneration in dystrophic mouse models [17]. In 2017, Phram et al. reported their experience using daily low-dose injections with recombinant human growth hormone (somatropine) to stabilize or improve muscle strength and walking capability in a patient with dominantly inherited calpainopathy. Somatropine was injected daily over a period of 4.5 years, except for a 6-month pause. Repeated muscle dynamometry tests demonstrated improvement in strength and 6-minute walk tests (6MWT) stabilized during the initial 18-month treatment period, but deteriorated in the 6 months off treatment with improvement to pretrial levels when treatment was resumed. They conclude that supplementation with physiological doses of somatropine may be beneficial and safe for patients with calpainopathy [18]. Further randomized placebo-controlled trial needs to be undertaken.

Dysferlinopathies (LGMD2B)

The anti-inflammatory pathway has been the focus of many clinical trials in muscular dystrophies. Steroids have played an important role with proven efficacy in the treatment of Duchenne muscular dystrophy (DMD) and are considered

Table 2 Autosomal recessive limb–girdle muscular dystrophy

LGMD subtype	Gene	Onset	Phenotype
LGMD2A	CAPN3 Calpain 3	2–53 years	• 3 phenotypes: ○ Pelvic–femoral LGMD or Leyden–Möbius ■ BMD-like phenotype in 2/3 ■ DMD-like phenotype in 10% ○ Scapulo-humeral LGMD (Erb) phenotype ○ HyperCKemia (6%) • Early contractures of elbows/calves, scoliosis • Cardiomyopathy and respiratory dysfunction is rare • CK levels range from 4000 to >20,000
LGMD2B	DYSF Dysferlin	15–30 year	• 2 phenotypes: ○ Limb–girdle syndrome (LGMD2B) ■ Onset in adolescence or young adulthood ■ Early weakness and atrophy of the pelvic and shoulder girdle muscles ■ Slow progression ■ No respiratory and cardiac muscle involvement ○ Distal phenotype (Miyoshi myopathy) ■ Onset in young adulthood ■ Marked distal gastrocnemius–soleus weakness and atrophy with progression to the thigh/gluteal muscles ■ Forearms may be mildly atrophic with decreased grip ■ Small hand muscles are spared • CK: as high as 40,000 • Muscle biopsy: endomysial/perimysial infiltrates in up to 50%
LGMD 2C	SGCG Gamma-sarcoglycan		• Complete deficiency: ○ Onset: 1–15 years of age ○ Difficulty running/walking due to proximal weakness ○ Calf hypertrophy ○ Scapular winging ○ Macroglossia ○ Lumbar hyperlordosis ○ Late contractures or scoliosis ○May have cardiac and respiratory impairment ○Wheelchair confinement by ~ 15 years old • Partial deficiency of sarcoglycans ○Onset: adolescence to young adulthood ○Cramps/exercise intolerance ○ Asymptomatic hyperCKemia • CK levels range from 1000 to 25,000 U/L • Cardiomyopathy is common
LGMD 2D	SGCA α-sarcoglycan		• The same phenotypic features as LGMD 2C • Cardiomyopathy is rare
LGMD2E	SGCB β-sarcoglycan		• The same phenotypic features as LGMD 2C • Cardiomyopathy is common
LGMD 2F	SGCD δ-sarcoglycan		• The same phenotypic features as LGMD 2C • Cardiomyopathy is common
LGMD 2G	TCAP Titin-cap	9–15 years	• All have significant proximal weakness ○ Weakness of the lower limbs ■ Difficulty running due to early quadriceps weakness ■ Footdrop from anterior tibialis weakness ○ Proximal upper limb weakness • Significant variability in the phenotype: ○ Some develop distal atrophy ○ Others have calf hypertrophy • Cardiac involvement is seen in half of the cases • Females appear to be less severely affected than males
LGMD 2H	TRIM32 Tripartite motif-	8–27 years	• Slowly progressive weakness (wheelchair late in life) ○ Early quadriceps and pelvic girdle weakness: ■ Waddling gait

Table 2 (continued)

LGMD subtype	Gene	Onset	Phenotype
	containing protein 32		■ Difficulty with stairs ○ Trapezius and deltoid weakness ○ Facial weakness ○ Calf muscle wasting can be seen • No scoliosis or contractures • Variable severity • Severe end of phenotype: sarcotubular myopathy (STM)
LGMD 2I	FKRP Fukutin-related protein	Infancy–4th decade of life	• Difficulty running and walking due to hip girdle weakness with distal leg and proximal arms weakness • Calf hypertrophy • Tongue hypertrophy • Lumbar lordosis • Prominent respiratory and cardiac dysfunction with dilated cardiomyopathy in about 50% • Variable in severity
LGMD 2J	TTN Titin	Childhood–late adulthood	• Variable phenotype: any phenotype is possible ○ Childhood-onset limb–girdle weakness (AR) ○ Adult-onset (AD—more common) anterior tibial weakness, upper limb weakness with posterior calf atrophy and weakness
LGMD 2K	POMT1 Protein *O*-mannosyl-transferase 1	1–3 years	• Mild proximal > distal muscle weakness ○ Increased fatigability ○ Difficulty climbing stairs and running • Cognitive limitation • Language impairment • Hypertrophy of calves and thighs • Ankle contractures • CK: 20–40× normal range
LGMD 2L	ANO5 Anoctamin 5	10-20 years	• Distal leg phenotype • Prominent asymmetric thigh atrophy • No cardiac or respiratory dysfunction • CK: 200–35,000 U/L
LGMD 2M	FKTN Fukutin	4 months–4 years	• Early-onset proximal lower > upper limb weakness, leading to difficulties climbing stairs • Hypertrophy of calves, thighs, and triceps may be present • No scoliosis or contractures Note: common cause of Fukuyama muscular dystrophy
LGMD 2N	POMT2 Protein *O*- mannosyl-transferase 2	Early onset	• Phenotype of 2 cases: ○ 5-year-old female, asymptomatic with normal intellect but neurological exam showed scapular winging, calf hypertrophy, and slowness in running and getting up ○ 18 months old with developmental delay, intellectual disability, muscle hypertrophy, and right bundle branch block
LGMD 2O	POMGNT1 Protein *O*-mannose β-1,2-N-acetyl-glucos-aminyl-transferase 1		• LGMD phenotype is more benign and rare • One case report of a female: onset at 12 years old ○ Normal cognition ○ Progressive proximal > distal muscle weakness (difficulties rising from sitting and climbing stairs) with neck, hip girdle, and shoulder abductor muscles involved ○ Hypertrophy of the calves and quadriceps ○ Wasting of the hamstring and deltoid muscles ○ Ankle contractures ○ Severe myopia Note: common cause of muscle–eye–brain disease
LGMD 2P	DAG1 Dystrophin-associated glycoprotein 1		• Case report: cognitive delay

Table 2 (continued)

LGMD subtype	Gene	Onset	Phenotype
LGMD 2Q	PLEC1 Plectin		• PLEC-associated phenotypes: ○ Epidermolysis bullosa simplex with late-onset progressive muscular dystrophy ○ Myasthenic syndrome with late-onset myopathy ○ Early childhood-onset progressive muscular dystrophy without skin involvement
LGMD 2R	DES Desmin	Early childhood–2nd decade	• Proximal muscle weakness, • Facial weakness • Respiratory muscle weakness • High arched palate • Scoliosis • Severe atrioventricular conduction defects requiring cardiac pacemaker placements
LGMD 2S	TRAPPC11 Trafficking protein particle complex, subunit 11	Early childhood	• Proximal weakness • Scapular winging • Mild myopathic facies • Global developmental delays • Infantile-onset hyperkinetic movements (dystonia/chorea) with truncal ataxia with myopathic EMG and cerebral volume loss on brain MRI • CK: as high as 10× normal
LGMD 2T	GMPPB GDP-mannose pyrophos-phorylase B	Birth–40 years	• Slow progression of proximal weakness • Early-onset cases: infantile hypotonia, intellectual disabilities, occasional seizures • Older patients: enlarged calves, rhabdomyolysis and cramps; some have cardiac involvement
LGMD 2U	ISPD Isoprenoid synthase domain-containing	Early childhood	• Hypotonia • Gait disorder • Gower's sign due to predominantly proximal weakness • Muscle hypertrophy may be present as well as cardiac involvement with left ventricular dysfunction • CK: 3–50× normal
LGMD 2V	GAA α-1,4-Glucosidase	Infantile–adolescent–adult onset	• Phenotypes of Pompe Disease: ○ Classic infantile form of cardiomyopathy and muscular hypotonia ○ Adolescent and adult patients have proximal muscle weakness, thigh adductor weakness associated with respiratory insufficiency • EMG: CRDs or myotonic discharges specifically in the thoracic paraspinal muscles • Muscle biopsy: subsarcolemmal periodic-acid Schiff (PAS)-positive inclusions • Treatment: enzyme replacement therapy available Note: 8% of unclassified LGMD
LGMD 2W	LIMS2 Lim and senescent cell antigen-like domains 2	Childhood	• Severe proximal upper and lower extremity weakness • Slow progression • Distinctive features: ○ Macroglossia ○ Triangular tongue ○ Calf hypertrophy • Cardiac involvement is seen by the 3rd decade of life with dilated cardiomyopathy • CK: up to 25× normal
LGMD 2X	BVES Blood vessel epicardial substance	Adulthood	• Slowly progressive proximal lower limb weakness • Cardiac arrhythmias may cause syncopal episodes • CK: elevated
LGMD 2Y	TOR1AIP1 Torsin A-interacting protein 1	1st–2nd decade	• Slowly progressive proximal lower limbs, followed by distal upper and lower limb muscle weakness and atrophy • Joint contractures—fingers (PIP and DIP), ankles

Table 2 (continued)

LGMD subtype	Gene	Onset	Phenotype
			• Rigid spine—cervical region • Restricted pulmonary function • May have mild cardiac involvement • CK: normal to elevated
LGMD 2Z	POGLUT1 Protein *O*-glucosyl-transferase 1	Young adult	• Slowly progressive proximal upper and lower limb muscle weakness and atrophy • Scapular winging • CK: mildly increased

part of its standard of care [19–21]. Although its mechanism of action is not entirely clear, anti-inflammatory effects as well as membrane-stabilizing effects are postulated. The dysferlin gene plays a role in calcium-mediated repair of muscle membrane, vesicle trafficking, and calcium homeostasis, as well as the expression of nonselective calcium-permeable channels that contributes to the inflammatory process. Muscle biopsies of dysferlinopathy patients have demonstrated marked inflammation [22] and are frequently mistaken for inflammatory myopathy. There are anecdotal reports that steroids are effective in dysferlinopathy. Walter et al. (2013) conducted a prospective randomized placebo-controlled cross-over clinical trial in 25 genetically confirmed patients to determine the effect of deflazacort. Rather than improvement, there was a trend toward worsening of muscle strength during the deflazacort trial period, with recovery during the wash-out period when drug was discontinued. They speculated that steroids have a negative effect on dysferlin expression or function and concluded that off label use of deflazacort is not warranted and may even harm patients with dysferlinopathy [23].

Myostatin is a member of the transforming growth factor b (TGF-b) family that arrests muscle growth. The prevention of muscle loss by means of myostatin inhibition has been an ongoing aspect of therapeutics research for neuromuscular disorders. About a decade ago, Wagner et al. conducted a safety trial of a neutralizing antibody to myostatin, MYO-029, in adult muscular dystrophies (Becker muscular dystrophy, facioscapulohumeral dystrophy, and limb–girdle muscular dystrophy). They concluded that MYO-029 had good safety and tolerability plus there was a trend toward increased muscle size in a limited number of patients but the study was not powered to look at efficacy. They recommended further evaluation of more potent myostatin inhibitors for stimulating muscle growth in muscular dystrophy [24].

Lee et al. (2015) investigated the effect of blocking the myostatin pathway in dysferlin-deficient (Dysf−/−) mice, either by transgenic expression of follistatin in skeletal muscle or by systemic administration of the soluble form of the activin type IIB receptor (ACVR2B/Fc). Follistatin can stimulate muscle growth by inhibiting additional TGF-b family members, including activin, even in myostatin-null mice. In this study, they showed that myostatin inhibition by follistatin transgene expression in Dysf−/− mice results in early improvement in histopathology, but ultimately, exacerbate muscle degeneration that is specific to mice lacking dysferlin. This was not observed in dystrophin-deficient (mdx) mice. When they injected Dysf−/− mice with ACVR2B/Fc, significant increase in muscle mass and amelioration of expected fibrotic changes was observed but some of these mice had increase in serum CK. Elevation of CK suggests the possibility of muscle damage induced by muscle hypertrophy, which may increase stress in dystrophic muscles and lead to accelerated disease progression [25]. Kornegay et al. (2016) recently reported on dystrophin-deficient dogs with reduced myostatin having unequal muscle growth and greater joint contractures [26]. Thus, the intricacies of myostatin inhibition need careful consideration.

Sarcolemmal membrane repair is another possible target for therapy. Gushchina et al. (2017) tested the ability of recombinant human MG53 (rhMG53) protein to improve membrane repair in dysferlin-deficient mouse model of LGMD2B (B6.129-*Dysf*^{tm1Kcam}/*J*). They found that intraperitoneal injection of rhMG53 into mice before acute eccentric treadmill exercise can decrease the release of intracellular enzymes from skeletal muscles and decrease the entry of immunoglobulin G and Evans blue dye into muscle fibers *in vivo*, suggesting increased integrity of the sarcolemmal membrane. This is independent of the known dysferlin-mediated Ca^{2+}-dependent pathway for sarcolemmal membrane repair [27].

Early gene therapy studies used stable, nonviral vector transfection of full-length dysferlin DNA in dysferlin-deficient myoblasts. Escobar et al. found that this resulted in stable dysferlin expression and cell survival after engraftment in dysferlin-, immune-deficient mice. The grafted muscle cells exhibited normal regenerative capabilities *in situ* [28].

Sondergaard's group developed a two-vector system (AAV.DYSF.DV), using adeno-associated viral vectors to deliver full-length dysferlin gene. Dysferlin-deficient mice and nonhuman primates (rhesus macaques) that were treated,

either by intramuscular or intravascular delivery, exhibited high levels of dysferlin expression in muscle and restoration of membrane repair ability with no apparent toxicity or systemic immune rejection [29]. This laid the foundation for preclinical safety studies.

Although gene delivery using AAV shows promising results, it has some challenges in LGMD2B therapy. One issue is the large size of the dysferlin-coding sequence, which is 6.2 kb long. AAV is able to package only genes shorter than 5 kb. Research studies are underway to find the best method to deliver the gene. There are concerns that dual vector strategies or deleted forms of dysferlin may reduce the efficiency or effectiveness of the therapy. Other concerns include vector immunogenicity, vector loss, and the challenges and expense of large-scale viral vector production. Naso et al. (2017) report gene therapy using rAAV has been demonstrated to be safe and well-tolerated in virtually every clinical setting in which it has been used. These studies, along with basic research on its biology, have revealed many facets of this vector that can be applied to future efforts. Among the critical parameters to be considered are vector design, capsid selection, desired target cell and tissue type, and route of administration. The transgene to be delivered optimized for expression, the right AAV variant with an appropriate capsid for target cell transduction and immunoreactivity profile, and the appropriate delivery approach to maximize target tissue exposure while limiting off-tissue exposure are key focal points for AAV-based therapies [30]. Colella et al. (2018) reported transient immunosuppression could be safely applied with gene transfer to avoid detrimental immune responses and to maintain long-term expression of the transgene product [31].

Plasmid DNA has been investigated as a tool to deliver the large dysferlin protein since plasmid has no size limit, is not immunogenic, is cheaper to produce, and has the potential to integrate into the genome if an integration mechanism is provided [27]. Encouraged by the result of Wolff et al.'s investigation using plasmid DNA as a vector for carrying the large dystrophin gene to mdx mouse models and another study demonstrating that co-delivery of follistatin with the dystrophin gene can be beneficial in mdx mouse models of Duchenne muscular dystrophy [32–34], Ma et al. in 2017 studied the intravascular codelivery of dysferlin with follistatin (FST) gene into all major muscle groups of the hind limb of dysferlin knockout mice. They confirmed persistent strong dysferlin expression over the 3-month time of the study, the presence of follistatin gene, and the presence of plasmid DNA. This study showed a statistically significant reduction in Evan's blue dye permeability, a sign of sarcolemmal membrane fragility, in hamstring muscles after gene therapy with a plasmid encoding both dysferlin and follistatin. This suggests the possibility that it can be an effective approach to treating LGMD2B [35].

Gene editing is a promising new strategy used to treat genetic neuromuscular disorders. Using patient-derived induced pluripotent stem cells (iPSC), Turan et al. (2016) corrected the dysferlin nonsense mutation c.5713C>T; p.R1905X and the most common α-sarcoglycan mutation, missense c.229C>T; p.R77C. The single-stranded oligonucleotide-mediated gene editing—the CRISPR/Cas9 gene editing—system was utilized to enhance the frequency of homology-directed repair. A seamless, allele-specific correction was demonstrated at efficiencies of 0.7–1.5%. This group then carried out precise gene addition strategies for correction of the LGMD2B iPSC by integration of wild-type dysferlin cDNA into the H11 safe harbor locus on chromosome 22, using dual integrase cassette exchange (DICE) or TALEN-assisted homologous recombination for insertion precise (THRIP), thereby achieving targeting efficiencies of ~ 20%. They demonstrated the precise correction of LGMD iPSC with appropriate levels of dysferlin and correct localization by immunohistochemistry [36]. This opens the possibility of cell therapy utilizing these corrected iPSCs. Gene editing strategy is being investigated in other forms of muscular dystrophy like DMD. Ongoing challenges to gene editing include efficient gene delivery, identification and reduction of off-target interactions, and immunogenicity of genome engineering tools, delivery vectors, and other neoantigens [37].

Sarcoglycanopathies

LGMD2C Due to Mutations of the γ-Sarcoglycan Gene

Herson et al. (2012) reported one of the first gene therapy clinical trials in LGMD2C with their phase 1 study involving 9 non-ambulatory patients with del525T homozygous mutation of the γ-sarcoglycan gene and no γ-sarcoglycan immunostaining on muscle biopsy. Three escalating doses of an AAV-1 vector expressing the human γ-sarcoglycan gene under the control of the desmin promoter were used for 3 equal patient groups receiving intramuscular injections to the extensor carpi radialis muscle. No serious adverse effects occurred during 6 months of follow-up. All nine patients became AAV-1 seropositive and one developed a cytotoxic response to the AAV-1 capsid. Immunohistochemical analysis of injected muscle biopsy specimens performed 30 days later showed γ-sarcoglycan expression in all three patients who received the highest dose while real-time polymerase chain reaction detected γ-sarcoglycan messenger RNA. In one patient, γ-sarcoglycan protein was detected by Western blot. For two other patients who received the low and intermediate doses, discrete levels of γ-sarcoglycan expression were also detectable. They concluded that expression of γ-sarcoglycan protein can be induced in patients with LGMD2C by adeno-associated virus serotype 1 gene transfer, with no serious adverse effects [38].

Another strategy that has been investigated is the use of exon skipping tools similar to the ones developed for DMD

patients. Antisense oligonucleotides are designed to bypass premature stop codons targeted at the pre-mRNA level, allowing one or more exons to be omitted to restore the disrupted reading frame, thereby producing the necessary, albeit truncated protein. Eteplirsen is an exon 51-skipping drug approved by the FDA to treat Duchenne muscular dystrophy patients with exon 51 skippable mutations [39]. The potential role of exon skipping in sarcoglycanopathy is being explored but proof that truncated sarcoglycan can be functional needs to be demonstrated first. To test whether an internal, in-frame truncation of a transmembrane protein γ-sarcoglycan is functional, Gao et al. (2015) generated an internally truncated γ-sarcoglycan protein, which they called Mini-Gamma, by deleting a large portion of the extracellular domain. Mini-Gamma provided functional and pathological benefits to correct the loss of γ-sarcoglycan in a *Drosophila* model, in heterologous cell expression studies, and in transgenic mice lacking γ-sarcoglycan. They generated a cellular model of human muscle disease and showed that multiple exon skipping could be induced in RNA that encodes a mutant human γ-sarcoglycan. Since Mini-Gamma represents removal of 4 of the 7 coding exons in γ-sarcoglycan, this approach provides a viable strategy to treat the majority of patients with γ-sarcoglycan gene mutations [40].

LGMD2D Due to Mutation of α-Sarcoglycan Gene

LGMD2D is the most common form of sarcoglycanopathy [10] and one of the most extensively studied in clinical trials. Intramuscular injection of rAAV1 has been shown to restore muscle histology to normal and muscle strength increased to levels exceeding control knockout mice but not to the same degree as wild-type mice. Using a gene therapy approach similar to AAV-mini-dystrophin transfer for DMD, Mendell's group worked on gene transfer in LGMD2D with the objective of attaining long-lasting α-sarcoglycan gene expression in patients. Since full-length SG cDNA is < 2 kb and is well within the packaging capacity of rAAV, they used AAV-mediated α-sarcoglycan gene transfer controlled by a muscle-specific promoter (tMCK), which targets muscle-controlled gene expression, to improve its safety profile. A double blind placebo-controlled trial was undertaken using rAAV1.tMCK.hSGCA via intramuscular injection to the extensor digitorum brevis (EDB) muscle of three genetically confirmed subjects with the control side receiving saline. They found persistent α-sarcoglycan gene expression for 6 months in two of three LGMD2D subjects. They also noted improvements in other markers for muscle fiber transduction, like the expression of major histocompatibility complex I, increase in muscle fiber size, and restoration of the full sarcoglycan complex. The third patient, who demonstrated an early rise in neutralizing antibody titers and T cell immunity to AAV that was validated by enzyme-linked immunospot (ELISpot) on the second day after gene injection, failed this gene transfer. They concluded that long-term, sustainable gene expression of α-sarcoglycan is possible following gene transfer mediated by AAV under the control of a muscle-specific tMCK promoter and it can potentially reverse the disease [41, 42].

A phase 1 double blind gene transfer therapy trial using intramuscular injection of rAAV1.tMCK.human-α-sarcoglycan was recently completed. The primary goal was to assess its safety and secondarily to determine the dose of rAAV1.tMCK.hαSG vector required to achieve a detectable level of α-sarcoglycan in the muscle of 6 subjects with LGMD2D. One extremity received the vector with the transgene and the opposite side, receiving placebo, served as control. Intravenous methylprednisolone was administered about 4 h prior to gene transfer with repeat doses on two consecutive mornings in order to diminish immune response to the vector. Safety endpoints assessed include inflammatory reaction to the vector seen on muscle biopsy, changes in hematology, serum chemistry, urinalysis, immunologic responses to rAAV1 and α-sarcoglycan, as well as reported history and observations of symptoms. Each patient had 10 to 12 follow-up visits for the next 2 years after the initial infusion. Result has not been published (see Clinicaltrials.gov).

Mendell's group has redesigned their clinical trial and now investigating gene therapy using systemic administration. There is an ongoing phase I/IIa dose escalation study to establish maximum tolerated dose (MTD) when self-complementary scAAVrh74.tMCK.hSGCA is administered intravascularly to LGMD2D (α-sarcoglycan deficient) subjects. Dose is delivered via a major lower limb artery of each leg sequentially by isolated limb perfusion (ILP). Safety monitoring as well as efficacy measures are assessed. Pre- and post-treatment muscle biopsies will be undertaken (see Clinicaltrials.gov).

Small molecules can also be synthesized to target pathway repair. The majority of missense mutations in the SGCA gene affect folding and trafficking of α-sarcoglycan protein. This defective polypeptide is recognized as such by the endoplasmic reticulum-QC so it is delivered for degradation [43]. This disrupts the membrane complex and reduces the stability of sarcolemma during muscle contraction. To target the repair of this pathway, small molecules can be used to facilitate the folding process in order to rescue the protein and reduce the disposal of defective protein [44]. CFTR correctors (correctors of the cystic fibrosis transmembrane regulator) have been developed for their ability to correct defective folding and trafficking of type II mutants of the chloride channel [45]. As proof of principle that targeting the sarcoglycan maturation process can potentially treat LGMD2D patients, Carotti et al. (2018) added CFTR correctors to myotubes from a patient with LGMD2D and found that it induced the proper relocalization of the whole sarcoglycan complex, reducing sarcolemmal fragility and rescuing the plasma membrane

[46]. Although the mechanism of action of CFTR correctors is unclear, this is the first to report that use of small molecules can lead to the functional recovery of sarcoglycan complex in human pathologic samples.

As previously mentioned, efforts at gene editing using patient-derived induced pluripotent stem cells (iPSC), to correct the most common α-sarcoglycan mutation, missense c.229C>T; p.R77C, was undertaken by Turan et al. [31].

LGMD2E Due to Mutations in the β-Sarcoglycan (SGCB) Gene

Poszgai et al. (2017) published the result of the gene therapy on SGCB knockout mice model of LGMD2E, presenting with progressive muscle weakness, respiratory failure, and cardiomyopathy. The study investigated systemic SGCB gene transfer to treat skeletal and cardiac muscle deficits, using scAAVrh.74.MHCK7.hSGCB (self-complementary AAVrh74 vector containing a codon-optimized human SGCB transgene driven by a muscle-specific promoter). This intravenous delivery to the tail vein of the SGCB mice led to 98.1% transgene expression across all muscles, accompanied by improvements in histopathology, a reduction of serum CK levels, an increase in diaphragm force production, a reduction of kyphoscoliosis of the spine, an increase in overall ambulation, and a dramatic increase in vertical rearing. There were no adverse effects noted [47]. This preclinical study is very promising and may clear the way for clinical trials using AAV-mediated gene therapy for patients with LGMD2E.

α-Dystroglycanopathies

LGMD2I Due to Mutations in the FKRP Gene

α-Dystroglycan is an integral part of the dystrophin–glycoprotein complex that ensures the proper anchoring of the muscle and basement. FKRP is a ribitol-5-phosphate transferase, which participates in the glycosylation of α-dystroglycan (α-DG). Its defect causes LGMD2I.

In 2014, Qiao et al. published the result of gene replacement therapy study in LGMD2I. They generated and characterized a new mouse model with late-onset and mild dystrophic muscle pathology that closely resembles the classic late-onset phenotype of LGMD2I patients in both skeletal and cardiac muscles. These homozygous knock-in mice ($L276I^{KI}$) harbor the common human mutation leucine 276 to isoleucine (L276I). Their group tested systemic delivery of human FKRP gene by AAV9 vector in the $L276I^{KI}$ mice, either neonatally or at 9 months of age. They found that overexpression of FKRP in both skeletal and cardiac muscles effectively restored the biochemical deficiency and normalized glycosylation of α-DG, without discernable toxicity. When mice were treated in the neonatal period, the development of dystrophic pathology was prevented and it was ameliorated when treated at the adult age. They noted that overexpression of FKRP resulted in recovery of contractile function of skeletal as well as cardiac muscles. This was the first report that systemic FKRP gene transfer can achieve body-wide FKRP gene expression and fully restore glycosylation of α-DG in the LGMD2I mouse model [48]. This suggests that gene therapy is a viable if not an excellent approach for treating FKRP deficiency and other related dystroglycanopathies.

In the most frequently encountered FKRP mutation (L2761), the function of α-dystroglycan is affected by abnormal glycosylation. Gicquel et al. (2017) investigated gene transfer with a rAAV2/9 vector expressing FKRP in a LGMD2I knock-in mouse model presenting with skeletal muscle function impairment from 2 months of age and a moderate dystrophic pattern evident starting from 6 months of age. This gene transfer restored biochemical defects, corrected the histological abnormalities and improved the resistance to eccentric stress. However, injection of high doses of the vector induced a decrease of α-DG glycosylation and laminin binding, even in wild-type (WT) animals. Finally, intravenous injection of the rAAV-FKRP vector into a dystroglycanopathy mouse model due to Fukutin (FKTN) knockout indicated a dose-dependent toxicity. They conclude from these data that AAV-mediated transfer of FKRP shows therapeutic efficacy but requires control of FKRP gene expression [49].

FKRP gene encodes for glycosyltransferase. Its mutation is associated with abnormal glycosylation of α-dystroglycan, a secondary reduction in ribitol-5-phosphate and reduced expression of *O*-mannose-phosphate-linked glycans needed for extracellular matrix protein binding. Frattini et al. (2017) hypothesized that FKRP might circulate as an extracellular glycosyltransferase and may be able to modify distal glycan structures. To demonstrate the feasibility of transducing both dystrophic blood-derived CD133+ cells isolated from a MDC1C patient with FKRP gene alterations, and satellite cells derived from FKRP $L276I^{KI}$ mouse model, they employed a lentiviral vector expressing the wild-type human FKRP gene. They showed that FKRP-transduced cells were driven to release exosomes carrying FKRP. This circulated freely and its distribution determined the restoration within muscles tissues. There was an overall recovery of α-DG glycosylation and improved muscle strength, suggesting a systemic supply of FKRP protein acting as glycosyltransferase [50].

The overexpression of B4GALNT2 (previously GALGT2) has previously been reported to inhibit the development of muscle pathology in mouse models of Duchenne muscular dystrophy, congenital muscular dystrophy 1A, and limb-girdle muscular dystrophy 2D [51, 52]. GALGT2 overexpression induces the glycosylation of α-dystroglycan as well as the ectopic overexpression of dystrophin and laminin α2 surrogates, such as utrophin, plectin 1, laminin α5, and agrin that normally bind dystroglycan at the neuromuscular junction and

myotendinous junction both in mice and in macaques. GALGT2 overexpression has a profound impact in preventing muscle injury, including that caused by eccentric contractions, and can inhibit the development of muscle pathology in mdx mice. Recently, Thomas et al. tested whether GALGT2 overexpression affects LGMD2I-like disease, where dystroglycan is functionally impaired but is not deleted. This group tested a surrogate gene therapy, rAAVrh74.MCK, and found that GALGT2-treated FKRP P448Lneo muscles showed reduction in number of centrally nucleated myofibers, reduced muscle variability with increased size of myofiber diameters, reduced myofiber immunoglobulin G uptake and muscle wasting at 3 and 6 months after treatment. GALGT2 overexpression did not cause substantial glycosylation of α-dystroglycan or increased expression of dystrophin and laminin α2 surrogates in mature skeletal myofibers, but it increased the number of embryonic myosin-positive regenerating myofibers. These data demonstrate that GALGT2 overexpression can reduce muscle pathology in the FKRP P448Lneo mice model for limb-girdle muscular dystrophy 2I, via a different mechanism from its ability to induce surrogate gene expression [53].

Agrin is a basement membrane-specific proteoglycan that regulates the orientation of cytoskeleton proteins and acts as a link between laminin(s) and α-dystroglycan (α-DG) in skeletal muscles. Miniaturized forms of agrin (mAgrin) have been used to ameliorate disease pathology in a laminin α2 knockout mouse model of muscular dystrophy [54]. With a similar concept in mind, Vannoy et al. (2017) worked to determine if mAgrin might similarly improve pathologies associated with LGMD2I. *In vitro* studies show that mAgrin enhances laminin binding to primary myoblasts and fibroblasts from an FKRP mutant mouse model, but enhancement is abolished when there is excess of mAgrin relative to laminin. *In vivo* delivery of mAgrin via adeno-associated virus (AAV) into FKRP mutant mice did not improve its histological or functional phenotypes. They hypothesize that this failure is due to insufficient binding of mAgrin to hypoglycosylated α-DG on muscle fibers, possibly due to excess of mAgrin delivered by AAV. For this to be useful in treatment of LGMD2I, ways to modify mAgrin in order to strengthen its binding to other membrane components, including hypoglycosylated α-DG, need to be explored [55].

Myostatin inhibition has also been a focus of investigation in LGMD2I. The investigational product PF 06252616, a humanized anti myostatin monoclonal antibody that neutralizes myostatin (GDF8), is in development to preserve and/or improve muscle function in LGMD2I subjects. This study at the Hugo W. Moser Research Institute at Kennedy Krieger, Inc., has an expected completion in August 2018. It is a phase 1b/2, open-label multiple ascending dose escalation study to evaluate the safety, tolerability, efficacy, PK, and PD of PF 06252616 in ambulatory adults with LGMD2I. The study design is intended to determine the optimal safe and pharmacologically active dose of the product in LGMD2I while providing an opportunity for all subjects to receive active drug for a rare and disabling disorder.

Conclusion

Historically, the LGMDs have been considered to be untreatable neuromuscular conditions with dire prognoses. Molecular-based approaches utilizing gene transfer to replace or provide surrogate genes, small molecules for exon skipping and mutation suppression, and more recently gene editing all hold tremendous promise for advancing the science and treatment of LGMD. These novel therapeutic approaches offer the potential for significant improvement in patient morbidities, mortality, and perhaps curative intervention.

Required Author Forms Disclosure forms provided by the authors are available with the online version of this article.

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