Therapeutic Possibilities in the Autosomal Recessive Limb-Girdle Muscular Dystrophies

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Summary: Fourteen years ago, the first disease-causing mutation in a form of autosomal recessive limb-girdle muscular dystrophy was reported. Since then the number of genes has been extended to at least 14 and the phenotypic spectrum has been broadened. The generation of mouse models helped to improve our understanding of the pathogenesis of the disease and also served to study therapeutic possibilities. All autosomal recessive limb-girdle muscular dystrophies are rare diseases, which is one reason why there have been so very few controlled clinical trials. Other reasons are insufficient natural history data and the lack of standardized assessment criteria and validated outcome measures. Currently, therapeutic possibilities are mainly restricted to symptomatic treatment and the treatment of disease complications. On the other hand, new efforts in translational research and the development of molecular therapeutic approaches suggest that more promising clinical trials will be carried out in autosomal recessive limb-girdle muscular dystrophy in the next several years.
Key Words: Limb-girdle muscular dystrophy, sarcoglycan, calpain 3, dysferlin, dystroglycan.

INTRODUCTION

The recognition of the various different genetic entities within the group of autosomal recessive limb-girdle muscular dystrophies (LGMD2) has allowed the definition of the typical clinical features and key management implications for each of the subtypes (Table 1). The phenotypic spectrum for most of the disorders has also been well-characterized, but the systematic collection of natural history data is still incomplete. Although the different forms of LGMD2 vary somewhat in different populations, they remain individually rare, with all of the challenges inherent to rare diseases in terms of planning therapeutic trials. As a key step in the development of therapies for LGMD2, mammalian animal models have been established for many of the LGMD2 subtypes.[1] Recently, the zebra fish has also received increasing attention as a model organism to investigate genes involved in muscle development and degeneration, and as a model for muscular dystrophy.[2–4] The full characterization of the various animal models for LGMD2, and an understanding of the relevance of outcome measures in them, however, has to date received scanty attention, so that reported functional gains in these models in response to therapeutic approaches can be difficult to interpret. Nonetheless, animal models allow ready demonstration of the proof of principle of treatment strategies like gene replacement, and indeed there has been progress to early human studies for some forms of LGMD2 based on animal model experiments. Although there remain significant difficulties in envisaging the progress to large-scale gene replacement strategies, in particular the scaling up of production facilities, understanding any likely immune response and enabling systemic delivery, many of the other strategies under development for other forms of muscular dystrophy are potentially referable to LGMD2, as well. For example, the proof of principle of the mesangioblast-derived stem cell approach was initially tried in an animal model of sarcoglycanopathy,[5] although further studies have concentrated on models of dystrophinopathy. Patients with different forms of LGMD2 were involved in the human study of myostatin inhibition via a myostatin antibody (MYO029),[6] and although the results of this trial did not support further development of this agent for muscular dystrophy, other methods for downregulating myostatin are under development and again may have relatively broad applicability.

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Table 1. *The Autosomal Recessive Limb-Girdle Muscular Dystrophies and Current Key Management Issues*

Forms of LGMD2 (OMIM)	Gene Locus	Protein	Medical Implications: Current Management
LGMD2A (#253600)	15q15	Calpain-3	Contracture management, rarely require respiratory support
LGMD2B (#253601)	2p13	Dysferlin	Ankle foot orthoses, rarely require respiratory support
LGMD2C (#253700)	13q12	γ-Sarcoglycan	Contracture management, scoliosis management, risk of respiratory disease and cardiomyopathy
LGMD2D (#608099)	17q12	α-Sarcoglycan	Contracture management, scoliosis management, risk of respiratory disease and cardiomyopathy
LGMD2E (#604286)	4q12	β-Sarcoglycan	Contracture management, scoliosis management, risk of respiratory disease and cardiomyopathy
LGMD2F (#601287)	5q33	δ-Sarcoglycan	Contracture management, scoliosis management, risk of respiratory disease and cardiomyopathy
LGMD2G (#601954)	17q12	Telethonin	Not reported outside Brazil
LGMD2H (#254110)	9q31	TRIM32	Not reported outside Canadian Hutterites
LGMD2I (#607155)	19q13	FKRP	Contracture management, scoliosis management in childhood forms, risk of respiratory failure and cardiomyopathy
LGMD2J (#608807)	2q31	Titin	Not reported outside Finland
LGMD2K (#609308)	9q34	POMT1	Rare: may require contracture management, scoliosis management in childhood forms, risk of respiratory failure and cardiomyopathy
LGMD2L (%611307)	11p13	?	Described in a group of Canadian families only
LGMD2M (#611588)	9q31	Fukutin	Rare: may require contracture management, scoliosis management in childhood forms, risk of respiratory failure and cardiomyopathy
LGMD2N (*607439)	14q24	POMT2	Rare: may require contracture management, scoliosis management in childhood forms, risk of respiratory failure and cardiomyopathy

OMIM = Online Mendelian Inheritance in Man; FKRP = fukutin-related protein; POMT1 = protein O-mannosyltransferase 1; POMT2 = protein O-mannosyltransferase 2; POMGnT1 = protein O-mannose beta-1,2-N-acetylglucosaminyltransferase; TRIM32 = tripartite motif-containing protein-32.

The application of antisense oligonucleotide-mediated exon skipping is likely to be limited in the LGMD2 genes as their protein products lack the "modular" structure of dystrophin and truncated proteins will not be functional. Premature stop codon suppression, used as a novel therapeutic approach in Duchenne muscular dystrophy,[7] will also be limited by the fact that for the majority of LGMD2, the disease-associated mutations are predominantly missense rather than nonsense (see http://www.dmd.nl/).

Assembling adequate numbers of patients in any of these diagnostic categories for meaningful clinical trials will be a further challenge, although potentially aided by the development of patient registries (see http://www.treat-nmd.eu/healthcare/patient-registries/rare/). More systematic natural history studies for many of these conditions will also be necessary to establish and interpret the appropriate outcome measures for human trials.

SYMPTOMATIC TREATMENT STRATEGIES IN LGMD2

Currently the care of patients with LGMD2 mainly involves symptomatic treatment strategies and supportive measures, as well as the treatment of disease complications. In LGMD2 progressive skeletal muscle weakness and wasting will also affect respiratory function, joint mobility, and posture. Symptoms and complications that can be associated with these impairments are myalgia and athralgia, contractures and scoliosis, signs of hypoventilation, and an increased susceptibility to chest infections. Proper treatment of muscle pain is important because pain has an impact on mobility, enjoyment of life, and recreational activities, and thereby is very importantly to quality of life.[8] Myoglobinuria has also been reported in patients with different forms of LGMD2,[9–11] and it is essential that patients and parents are aware of the possibility of myoglobinuria and the need to increase oral fluid intake and seek medical attention should it occur. Beside skeletal muscle involvement, several forms of LGMD2 also show cardiac and smooth-muscle involvement, which can result in cardiomyopathy and associated cardiac complications, as well as in gastrointestinal complaints. Because of possible cardiac and respiratory involvement, patients with LGMD2 also have an increased risk when undergoing general anesthesia. The different potential complications illustrate that the care of LGMD2 patients requires an interdisciplinary approach involving geneticists, neurologists, neuropediatricians, orthopedic surgeons, cardiologists, anaesthetists, gastroenterologists, and respiratory physicians.

These medical specialists should work closely together with physiotherapists, occupational therapists, and experts from palliative care teams.

To accurately time symptomatic treatments, it is important that patients be seen on a regular basis. Beside the clinical examination, patients should be tested for their respiration (e.g., forced vital capacity, peak cough flow) and in some forms of LGMD2, also their cardiac, function (e.g., electrocardiography and echocardiography). Guidelines for the frequency of these examinations and for the treatments they entail have now been developed for some of the diseases.[12] Chest infections should be treated promptly with antibiotics and the demonstration of night-time hypoventilation by overnight pulse oximetry should lead to noninvasive nocturnal ventilation. The identification of impaired cardiac function will often result in angiotensin-converting enzyme inhibitor and/or β-blocker treatment.

There is currently a lack of randomized controlled trials for LGMD2 and a lack of new treatment strategies, such as gene therapy, therapies that target post-transcriptional control processes or cell-based therapies are not yet established for LGMD2 and might prove more difficult than for the dystrophinopathies.

The following discussion will focus on the better understood and the more prevalent forms of LGMD2–2A (calpainopathy), LGMD2B (dysferlinopathy), LGMD2C–2F (the sarcoglycanopathies), and the forms of LGMD2 associated with secondary reduction in alpha-dystroglycan (LGMD2I, 2K, 2M, and 2N).

CALPAINOPATHY (LGMD2A)

The diagnosis of calpainopathy relies on the detection of mutations in the calpain 3 gene, supported in most cases by the finding of reduced or absent calpain 3 on immunoblotting of a muscle sample.[13] LGMD2A can present at any age from infancy (or later with toe walking, which can be seen as an early presentation) to late adulthood, although the majority of cases present between 8 and 15 years with difficulties climbing stairs or running.[13–22]

Contractures are present in a subset of patients and typically involve the Achilles tendon, elbow, and neck. In identifying the key clinical determinants that can distinguish calpainopathy from other forms of LGMD2, the presence of preserved respiratory function, contractures especially of the Achilles tendons, and scapular winging have been shown to be particularly discriminatory,[13] as is the absence of cardiac involvement and a posterior pattern of proximal leg muscle involvement (e.g., hamstrings are more affected than quadriceps).

Progression in LGMD2A is variable, but always progressive. Typically patients lose independent ambulation between 11 and 28 years after onset of the disease. The contractures, which are relatively frequently present in LGMD2A, especially at the Achilles tendons, require physiotherapy input and may need surgery depending on how they respond to stretching and splinting. Occasionally, scapular fixation may be required for particularly problematic scapular winging. Respiratory and cardiac problems are not common. Life expectancy in most cases will be normal.

Both calpain 3 knockout mice and transgenic mice with mutations of specific domains show some dystrophic changes and the proof of principle of replacing absent calpain 3 with adeno-associated virus–-mediated gene transfer has been demonstrated, prompting the planning of local human injection trials.[23–26]

DYSFERLINOPATHY (LGMD2B)

The diagnosis of dysferlinopathy relies on the demonstration of mutations in the dysferlin gene, which are frequently associated with absence of dysferlin in the muscle biopsy. There are a number of clinical features of dysferlinopathy that are almost pathognomonic of the diagnosis. For the vast majority of patients, onset of symptoms takes place in the late teens or early 20s, often with normal or even outstanding sporting ability before that age. The initial symptoms may be difficulty standing on tiptoe (Miyoshi distal myopathy presentation), foot drop (distal myopathy with anterior tibial presentation), proximal muscle weakness (“LGMD2B” presentation), or a mixture of proximal and distal weakness. Involvement of the shoulder girdle is a much later event. LGMD2B is frequently misdiagnosed as polymyositis, due to the apparently sudden nature of the onset and frequent presence of inflammatory cells in the muscle biopsy. Whether steroid medication is at all effective in patients with LGMD2B is currently being investigated in a controlled clinical trial (Walter et al., personal communication). Progression of the disease is somewhat variable. For some patients there appears to be a relatively rapid progression of disease and early confinement to a wheelchair. In others, the disease may be much more slowly progressive. Complications of cardiac impairment are not reported as frequent clinical complications, although suggestions of cardiomyopathy in animal models of dysferlinopathy have prompted the reporting of occasional cases of dysferlinopathy with cardiac disease[27,28] of uncertain significance. From published series it does not appear that respiratory failure is a common early feature of the disease, with respiratory complications coming, if at all, at a late stage with severe muscle involvement, long after the patient has become confined to a wheelchair. A very mild phenotype indeed with presentation in the 70s has recently been reported, indicating that dysferlin mutations may be compatible with only minor symptoms.[17,28–34] Where long-term follow-up

has been reported in dysferlinopathy, the disease appears to be compatible with a normal life expectancy.

Two naturally occurring mouse models of dysferlinopathy have been identified and a knockout mouse has also been generated.[1,35–37] The large size of the dysferlin gene makes the application of gene replacement strategies relatively problematic, unless shortened forms of the protein can be shown to be at least partially functional, as has been demonstrated for dystrophin (Krahn et al., personal communication).

SARCOGLYCAN-DEFICIENT LGMD2C–2F

The demonstration of a mutation in one of the sarcoglycan genes is necessary to confirm the diagnosis of the subtypes within this group of muscular dystrophies. On muscle biopsy analysis, the primary protein involved may be hard to predict, as all or most of the proteins of the dystrophin-glycoprotein complex are typically reduced, irrespective of the primary problem. In some cases, a reduction in dystrophin and alpha and/or beta dystroglycan may also be present, further confusing the diagnosis.

Given that the sarcoglycans belong to the same protein complex as dystrophin, it is probably not a surprise that their phenotypes overlap with dystrophinopathy and that the spectrum of severity seen in sarcoglycanopathy reflects the spectrum of dystrophinopathy as well, with the more severe end of the spectrum being equivalent to Duchenne muscular dystrophy and the milder to the Becker phenotypes. It does not appear that there are significant clinical distinguishing features between the different sarcoglycanopathies. There is a bias toward childhood[38] rather than adult onset, although both may be seen. Most patients with sarcoglycanopathy will present between 6 and 8 years of age. The disease course is always progressive, and respiratory and cardiac involvement may be seen with increasing severity of the disease; however, they are only infrequently seen early in the disease course. Cardiac involvement may be more prevalent with beta and delta sarcoglycanopathy (LGMD2E and 2F) but has been reported in association with mutations in alpha and gamma sarcoglycanopathy (LGMD2D and 2C) as well.[33,39–49]

The management principles for sarcoglycanopathies mirror dystrophinopathy. Attention to strength and joint range of movement necessitates physiotherapy input and, if necessary, orthopedic intervention. Assessment of respiratory function will identify the correct timing for nocturnal ventilatory support, and cardiac evaluation is necessary to identify patients requiring treatment for cardiomyopathy.[50] Scoliosis may be a problem in the more severely affected patients and may necessitate spinal surgery. Although glucocorticosteroids are now accepted as the gold standard for improving strength in Duchenne muscular dystrophy,[51] reaching clear evidence that it is also of benefit in sarcoglycanopathy is difficult due to the small patient numbers and the heterogeneity of the condition. Anecdotal stories of benefit in individual patients suggests that a trial of steroids in patients fully informed about the possibility of side effects and the uncertainty of benefit might be indicated in controlled conditions.

Animal models for the four sarcoglycan-deficient LGMDs have been generated and used to test new treatment strategies. There are both sarcoglycan null mouse models, as well as a mouse model with a disease-specific missense mutation.[52–57] The sarcoglycan null mice and the delta sarcoglycan-deficient cardiomyopathic hamsters (BIO14.6, BIO53.58, CHF147, TO-2) are also well-established animal models for sarcoglycan-deficient cardiomyopathy. The spectrum of therapeutic approaches that has been used to treat the dilated cardiomyopathy in the hamster models is very broad and reaches from classical pharmacotherapy approaches[58,59] to gene replacement therapy,[60–62] cellular therapies,[63–65] and recombinant growth factor therapies.[66–69] Despite numerous treatment studies in the cardiomyopathic hamster models over the past 40 years, many of which have been shown to be effective, no controlled trial to treat cardiomyopathy in patients with LGMD2C-F has been conducted so far. One reason for this lack of trials is the limited number of patients, but results from treatment studies in animal models have also illustrated that conclusions can not always easily be extrapolated from mice (hamsters) to humans and vice versa. Mice homozygous for the H77C-encoding allele, the most prevalent cause of LGMD2D, did not develop muscular dystrophy.[56,57] The studies suggest that the mutant protein that causes LGMD2D in humans is fully functional in the mouse muscle. Despite the lack of a histological phenotype, the mouse models have helped to gain insight into the molecular mechanisms leading to LGMD2D, caused by the most common mutation in the α-sarcoglycan gene, and to test new therapeutic approaches. Bartoli et al.[57] were able to pharmacologically rescue the R77C protein from retention in the secretory pathway by blocking the protein quality control using proteasome or mannosidase I inhibitors in living mice without evidence of toxicity of the treatment. A similar approach was chosen by Gastaldello et al.[70] using a heterologous cell system. They also showed that after transfection with disease-causing α-sarcoglycan mutants, proteasome inhibition reduced the degradation of the sarcoglycan complex and facilitated the assembly and targeting of the sarcoglycan complex to the plasma membrane.

A study by Bauer et al.[71] illustrated that steroid treatment in *Scgd* null mice deteriorated cardiac function, and dystrophin-deficient *mdx* mice also do not seem to benefit much from steroid treatment if one looks at histological changes in skeletal muscle,[72] despite the fact that

steroids are beneficial for boys with DMD[12] and possibly also for patients with sarcoglycanopathy.[73] Sarcoglycan-deficient mouse models have also been used to test the therapeutic effect of myostatin blockade.[74–77] These findings suggest that myostatin inhibition may be beneficial when applied at an early stage of the disease process, but might be less effective in advanced stages of muscular dystrophy.

The zebrafish has been found to express orthologues of many dystrophin-glycoprotein complex components,[78] and a number of recent studies have investigated the consequences of genetic mutations or targeted downregulation of expression of muscular dystrophy associated genes on muscle fiber development in the zebrafish.[79-82] Zebrafish embryos lacking delta sarcoglycan show delayed cardiac development, symptoms of somitic muscle disorganization and lack of swim bladder inflation.[83,84] The results in the delta sarcoglycan morphant shows similarity to the characteristics of human patients with LGMD2F, and suggests that zebrafish may be a good model in which to genetically analyze the sarcoglycanopathies.

LGMD2 ASSOCIATED WITH SECONDARY REDUCTION IN ALPHA DYSTROGLYCAN

The most common form of LGMD in this group is LGMD2I, which is also the most common form of LGMD in Northern Europe. In LGMD2I, a founder mutation in fukutin-related protein (FKRP [C826A]) facilitates diagnosis.[11,85,86] The other forms of LGMD due to mutations in genes causing secondary reduction in alpha dystroglycan are more commonly associated with a congenital muscular dystrophy phenotype; however, these should be pursued for cases in which a reduction of alpha-dystroglycan is detected on muscle biopsy and mutation testing in FKRP is negative.

LGMD2I is characterized by a high variability in clinical course, with a spectrum of phenotypes ranging from a Duchenne-like presentation to milder phenotypes with a slow progression, although even the milder cases may show complications of cardiomyopathy and respiratory impairment. In early onset cases there may be delayed motor milestones or hypotonia during the first year of life. The spectrum extends to milder phenotypes with even late adult onset, essentially resembling Becker muscular dystrophy. This is typically the most common presentation of LGMD2I, especially when patients are homozygous for the common C826A mutation. The muscle weakness has a pronounced predilection for axial muscles, neck flexors, and the proximal limb muscles. There may also be mild facial weakness, in particular in the very early onset cases. Muscles of the shoulder girdle may be weaker than those of the pelvic girdle, with atrophy of the pectoralis major and deltoid muscles. In contrast, there can be prominent hypertrophy of the tongue, the brachioradialis, the calves, and possibly other leg muscles. There is often prominent lordosis. In a cohort of patients from Germany, myalgia, and myoglobinuria were noted as the first symptoms in the majority of patients.[11] Rhabdomyolysis precipitated by anesthesia has also been reported.[11] Clinically significant, dilated cardiomyopathy develops in approximately half of the patients, and it is independent of the severity of the skeletal muscle weakness.[87,88] There is also an important respiratory involvement in patients with FKRP mutations, manifesting initially as a drop in forced vital capacity followed by nocturnal hypoventilation on the basis of diaphragmatic weakness. In contrast to Duchenne and Becker muscular dystrophy, it is important to note that the respiratory failure can occur while the patient is still ambulatory.[87,89–92]

Early recognition of dilated cardiomyopathy and early respiratory failure is of great importance. Cardiac disease may be detected pre-clinically to allow early introduction of prophylactic treatment (i.e, "routine" management of cardiomyopathy is indicated and in some cases even cardiac transplantation may be necessary).[87,88] Respiratory impairment may manifest first as diaphragmatic weakness, so investigation of respiratory muscle strength while lying as well as sitting is mandatory. Night-time ventilatory assistance should be introduced in a timely manner. In this type of recessive LGMD, serious and life-threatening complications that are amenable to treatment may supervene at a stage when the patient is still ambulant; therefore, it is very important to establish the correct diagnosis. The phenotypic overlap with dystrophinopathy can lead to problems with genetic counseling. Because the genetic implications of the two disorders are so different, it provides a further practical reason for determining the correct diagnosis by mutation analysis. Although no trials have been conducted on glucocorticosteroid treatment in LGMD2I, there is a report of benefit,[93] which has also been seen in LGMD2K.[94] A recent study that looked at the effect of moderate-intensity endurance training showed that it is a safe method to increase exercise performance and daily function in patients with LGMD2I.[95] Exercise studies in other forms of LGMD2 have not yet been systematically performed.

Although there are several genes known to cause LGMD associated with secondary reduction in alpha-dystroglycan, the number of available animal models for this group of disorders is limited. This is due to the fact that the corresponding null mice are embryonic lethal. Nevertheless, the available animal models for the dystroglycanopathies are beginning to add to our understanding of their pathogenesis and the vital importance of dystroglycan in the development of the basement membrane. A dystroglycan and an FKRP-deficient zebrafish model have been described, which developed a range of abnormalities reminiscent of the defects observed in hu-

man dystroglycanopathies.[82,96] FKRP morphant embryos showed a spectrum of phenotypic severity involving alterations in somitic structure and muscle fiber organization. The pathological phenotype in the FKRP morphants was found to correlate with a reduction in alpha-dystroglycan glycosylation and reduced laminin binding similar to patients with LGMD2I.[95] Attempts to model LGMD2K and 2M and the allelic congenital muscular dystrophies by the generation of mice null for *fukutin* or *Pomt1* have resulted in early embryonic lethality attributed to basement membrane defects.[97,98] Moving closer to the human disease situation, however, a chimeric mouse deficient in *fukutin* escapes embryonic lethality to develop a Fukuyama congenital muscular dystrophy phenotype,[99,100] similar to that observed in the Large^{myd} mouse harbouring a deletion in the mouse *Large* gene.[101] In Large^{myd} mice, one of the few viable animal models for the dystroglycanopathies, Barresi et al.[102] found that overexpression of transduced Large ameliorated the dystrophic phenotype and induced synthesis of glycan-enriched alpha-dystroglycan with high affinity for extracellular ligands.[102] These experiments underline the importance of animal models to test new therapies for LGMD2.

CONCLUSIONS

Over the last 14 years, since the first characterization of a disease-causing mutation in a form of LGMD2,[103] we have learned a lot about this large and heterogenous group of progressive muscle diseases. The identification of the underlying genes has enabled clinicians to provide patients with specific diagnoses, to inform them about the disease prognosis, to counsel families, and to offer prenatal testing. Knowledge about the natural history of the diseases made it possible to monitor patients for disease complications and implement symptomatic treatments. The characterization of animal models and the development of pre-clinical molecular and cell-based therapies will now need to be translated into clinical applications.

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