Heterogeneous pathogenesis of LGMD2: consequences for therapy

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Abstract
Autosomal recessive limb girdle muscular dystrophies (LGMD type 2) are a clinically and genetically heterogeneous group of disorders, including at least 13 different genetic entities, which are characterized by progressive involvement and wasting of proximal limb girdle muscles. All LGMD2 seem to be present worldwide, though the relative frequency of the different types is variable. The gold standard for LGMD diagnosis is the study of protein involved either by immunoblot or immunohistochemistry on the basis of clinical phenotypes, followed by a mutation study in candidate genes. Pathogenetic mechanisms are various; a different pathophysiology implies different clinical muscle involvement and might require a differential therapeutical approach.
Key words: LGMD2, phenotype, therapy.

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The diagnosis of recessive LGMD is complicated: the variability of clinical phenotype, the wide effort required in the identification of point mutations in relatively large genes, makes this target a hard task. The molecular diagnosis of LGMD type 2, which has become available only recently due to the progress of molecular genetics and the availability of antibodies for protein testing, is the necessary step to select patients for future clinical and experimental trials with drugs or gene therapy. The wide genetic heterogeneity of recessive LGMD implies a number of different pathogenetic mechanisms, which characterize each different genetic disease. Until a genetic characterization of LGMD patients is obtained, any therapeutic intervention can only be targeted to counter-act some of the general aspects of the muscular dystrophy rather than to the specific defective pathway.

LGMD2A or calpainopathy

LGMD type 2A (LGMD2A) is the most common form of LGMD in European countries, (Table 1) where it represents the 40% of LGMD and affects about 1:100,000 inhabitants (21, 37, 41, 44, 50).

LGMD2A is caused by mutations in the *CAPN3* gene, encoding for a muscle-specific member of a family of Ca^{++}-activated neutral proteases, and binds to titin (30, 43, 46, 47).

However, the search for the specific substrates of calpain-3 has been so far unsuccessful. Several interesting lines of research have investigated the pathogenesis of calpainopathy: calpain-3 could be involved in the regulation of transcription factors controlling survival genes and apoptosis (4), or in the degradation and disassembly of cytoskeletal or myofibrillar proteins (sarcomere remodeling) (28, 30, 31, 42, 48).

In calpainopathy a marked clinical heterogeneity has been observed considering the age and the phenotype at onset and the disease progression, even in patients with the same gene mutation. On this basis, according to the original clinical descriptions of LGMDs, clinical phenotype of LGMD2A can be subdivided in the pelvi-femoral form of Leyden-Moebius (with prevalent proximal muscle weakness in lower girdle) and the scapulo-humeral form of Erb (with prevalent proximal muscle weakness in upper girdle) (51); the onset may be early (< 12 years old), typical (between 12 and 30 years old) and late (> 30 years old). The loss of ambulation occurs usually 20-30 years after the onset. The variability of both the clinical phenotype and disease course may be only partly attributable to the genotype. While null type gene mutations are usually associated with absent calpain-3 protein in muscle and severe phenotype, missense type mutations (which account for about 70% of mutations in this gene) are associated with an extreme unpredictability of their effect at both the protein and the phenotype levels (20, 41, 45), suggesting that additional and still unknown genetic or environmental factors may be playing a role in modulating the phenotype.

***Table 1**: Autosomal recessive limb girdle muscular dystrophies*

TYPE	LOCUS	PROTEIN	PROTEIN FUNCTION
LGMD2A	15q15.1	calpain-3	Protease
LGMD2B	2p13	dysferlin	Membrane repair
LGMD2C	13q12	γ-Sarcoglycan	SG complex
LGMD2D	17q21	α-sarcoglycan	SG complex
LGMD2E	4q12	β-sarcoglycan	SG complex
LGMD2F	5q33	δ-sarcoglycan	SG complex
LGMD2G	17q11-12	telethonin	Cytoskeleton organization
LGMD2H	9q31-33	TRIM-32	Ubiquitin ligase
LGMD2I	19q13.3	FKRP	Glycosyl Transferase
LGMD2J	2q31	titin	Structural protein
LGMD2K	9q34.1	POMT1	Glycosyl Transferase
LGMD2L	9q31	fukutin	Glycosyl Transferase
LGMD2M	11p13-p12	?	?

LGMD 2B or dysferlinopathy

Limb-girdle muscular dystrophy type 2B (LGMD2B), and the distal muscular dystrophy named Miyoshi myopathy (MM), are caused by mutations in *dysferlin* gene, mapped to chromosome region 2p13 (34). Dysferlin immunolocalizes to the sarcolemma similarly to dystrophin, but it does not associate with dystrophin-glycoprotein complex. This protein presents a high degree of sequence homology with the protein Fer-1 in *C. elegans*. Since the mutant worm has an abnormal spermatogenesis, it was argued that mutant fer-1 may be involved in the failure of membrane fusion between the organelles and plasmalemma. By analogy, the pathogenetic mechanism in dysferlinopathies is correlated to the abnormal trafficking of vesicles, which repair muscle fiber membrane (6, 12). The absence of dysferlin causes a failure of damaged muscle fibers repair, possibly impairing the recovery from exercise induced damage. In many patients with LGMD2B the initial symptoms occur abruptly after age 20, sometimes following a regular heavy exercise, which damages especially the biarticular muscles. The clinician should discourage these patients from performing heavy exercise, which might exacerbate muscle breakdown. Creatine kinase is usually markedly elevated at presentation (often 20-150 times or even more above the normal range) (35). The anterior distal leg muscles and the distal arm muscles are relatively spared even in the later stages of the disease, and, in contrast with calpainopathy, scapular involvement is mild and absent at the onset (10). Other important clinical clues are the early inability to walk on tiptoes and the early involvement of the medial gastrocnemius muscle. Despite the clinical features of LGMD2B and MM are quite different, both phenotypes can be detected among patients belonging to the same family, thus sharing the same mutations (6). This clinical heterogeneity might be attributed to additional epigenetic factors resulting in variable dysferlin expression. Gene expression profiling has been recently used to investigate modifier genes in dysferlinopathy (11). Several studies reported a prominent inflammatory response in dysferlinopathy patients, but the origin of this feature and its role in the development of muscle pathology is still under investigation. Patients treated with steroids or immunosuppressant drugs do not show benefit on the long term, therefore it seems advisable to try alternative drugs, since this muscular dystrophy develops in relation to a defective sarcolemmal repair.

LGMD 2C – 2F or sarcoglycanopathies

The sarcoglycanopathies are due to mutations in the genes encoding for the components of the sarcoglycan (SG) complex. The SG complex, composed of 5 glycoproteins (α-, β-, γ-, δ-, ε-SG), is a member of the dystrophin-associated glycoprotein (DAG) complex localized to the sarcolemma of muscle fibers, which acts as a link between the extracellular matrix and the cytoskeleton, confers structural stability and protects the sarcolemma from mechanical stress developed during muscle contraction. This molecular complex can also participate in the transmission of various signals from the inside to the outside of the cell and vice-versa. The pathogenetic mechanism underlying sarcolemmal damage and fiber necrosis in sarcoglycanopathies is related to the fact that the deficiency in any SG subunit results in a loss of the whole SG complex, causing the disruption of the DAG complex and the inability to counteract the mechanical stress of contractile activity. One hypothesis is that SG complex masks a metalloproteinase cleavage site of the β-dystroglycan in normal muscle: the loss of SG in sarcoglycanopathies would lead to activation of the cleavage site of β-dystroglycan, causing the disruption of the link between the sarcolemma and the basement membrane. The clinical phenotype of sarcoglycanopathies ranges from a severe Duchenne-like dystrophy to a relatively mild LGMD (2, 3).

α- and γ-sarcoglycanopathy are characterized by presentation with proximal lower limb weakness, predominance of childhood onset, frequent calf hypertrophy, usually rare cardiac complications and normal intelligence. In the early-onset cases, loss of ambulation usually occurs within the second decade. β-sarcoglycanopathy is characterized by broad range of age at onset and clinical severity, while δ-sarcoglycanopathy usually presents a very severe course. The

cardiac involvement, which represents an important and sometimes life-threatening clinical complication, is often observed in association with mutations of β-SG (19) and δ-SG genes, whereas patients with mutations in the α-SG gene only rarely display cardiomyopathy. Recent studies demonstrate that β-SG and δ-SG are both expressed in striated muscle and smooth muscle of coronary arteries, suggesting that a perturbation of coronary vascular function might represent a common mechanism in the pathogenesis of the cardiomyopathy.

LGMD2G

LGMD2G results from mutation in the gene for the sarcomeric protein *telethonin* (38). Telethonin is a protein associated with the Z-disk region that seems to be involved in the reorganization of the cytoskeleton during myofibrillogenesis and in the inhibition of the secretion of myostatin, a negative regulator of skeletal muscle growth (36, 39). Patients with telethoninopathy usually present a severe involvement of the proximal muscles of the upper and lower limbs but also a marked weakness of the distal muscles of legs: tibialis anterior muscle is early affected, leading to foot drop and to difficulty with walking on the heels. CK levels are mildly elevated and muscle biopsy shows rimmed vacuoles (38).

LGMD2H

LGMD2H is a mild muscular dystrophy firstly identified in the Manitoba Hutterite community of Canada that is caused by mutations in the gene encoding TRIM32 (23). TRIM32 is a member of the tripartite motif (TRIM) family, characterized by the presence of three motifs: a RING-finger domain, a B1 box and a coiled coil domain. TRIM32 is an E3-ubiquitin ligase, which marks proteins for degradation by the proteasome pathway and contains six NHL domains that could be involved in protein-protein interactions and could confer specifity in protein selection. The missense mutation in TRIM32 identified in LGMD2H does not abolish its E3 ubiquitin ligase activity but seems to change its interaction with a yet unknown substrate (the wrong protein target or aberrant accumulation of a protein normally degraded) (14, 32, 33).

LGMD2I-2K-2L or dystroglycanopathies

This group of LGMD is due to mutations in the genes encoding various glycosyl-transferases that are believed to be Golgi resident. They share the same defective glycosylation of α-dystroglycan, causing an abnormal interaction with laminin-α2 and other extracellular matrix proteins. Dystroglycanopathies are a group of disorders whose phenotype range from severe congenital muscular dystrophy to relatively mild LGMD.

LGMD2I is caused by mutation in *FKRP* gene (15) and it is a milder allelic variant of congenital muscular dystrophy type 1C (MDC1C) (9). Recent studies suggest that the severe phenotype in MDC1C is due to the ER-retention of mutant FKRP, while in LGMD2I the protein is still able to reach the Golgi complex (17, 18). The spectrum of LGMD2I phenotypes ranges from Duchenne-like forms with cardiomyopathy, to milder forms with a relatively benign course.

LGMD2K is caused by mutations in *POMT1* gene, which have originally been identified in patients with Walker-Warburg syndrome, the most severe form of MDC. Recently, a distinct LGMD phenotype, resulting from a common mutation, has been described in Turkish population (5). LGMD2K patients present LGMD, mental retardation, microcephaly, but without eye abnormalities (13).

LGMD2L is caused by mutation in fukutin gene (24) and it is considered a milder variant of Fukuyama congenital muscular dystrophy. LGMD2L is characterized by an acute and severe onset of muscle weakness, and drugs response.

LGMD2J

LGMD2J is caused by missense mutation in the titin gene and it is associated with secondary calpain-3 defects (26, 27). It has been so far reported only in the Finnish population, and when a single allele is mutated, the disease manifests itself as a dominant disorder, the Tibial Muscular Dystrophy.

Conclusions and future prospectives for treatment

Since different pathogenetic mechanisms are at play in the different types of LGMD, it is conceivable that different types of therapeutic intervention(s) should be required to slow down muscle destruction (Table 2). Nowadays the main importance of establishing an exact molecular diagnosis in individual patients with dystrophy is genetic counseling, but in the future the discovery of different pathogenetic mechanism might suggest differential therapeutic strategies. Up to now, several preclinical studies have been conducted:

Experimental gene transfer: gene replacement has been tried in sarcoglycanopathies and in calpainopathy using non viral and viral vectors (adenoviruses and AAV) (1, 7, 22). Even if several experiments have reached preclinical proof of efficiency and persistency of gene transfer in muscle tissues, for gene transfer in humans there are many difficulties to be overcome, like the host immune response against the vector or the gene (53), the necessity of performing the vector injection before the onset of the dystrophic pattern (1), and the difficulty to obtain an efficient delivery in such a large amount of tissue.

Cell therapy: several sources of stem cells or early precursor cells have been identified, and the possibility of restoring muscle function by providing cells capable of regenerating damaged tissues has been tested in sarcoglycanopathies and in dysferlinopathies (25).

***Table 2**. Pathogenesis of LGMD2: consequences for therapy*

TYPE	PATHOGENETIC MECHANISM	EXPERIMENTED THERAPEUTIC INTERVENTIONS
LGMD2A	Loss of proteolytic activity	Gene replacement/ inhibitors of myostatin
LGMD2B	Defective membrane repair	Cell therapy/ to inhibit the complement membrane attack complex
LGMD2C	Defective membrane integrity	Gene replacement/ Cell therapy/ Drugs to inhibit the proteasome
LGMD2D	Defective membrane integrity	Gene replacement/ Cell therapy / Steroids/ inhibitors of myostatin/ up regulation of other proteins/ Drugs to inhibit the proteasome
LGMD2E	Defective membrane integrity	Gene replacement/ Cell therapy/ Drugs to inhibit the proteasome
LGMD2F	Defective membrane integrity	Gene replacement/ Cell therapy/ Drugs to inhibit the proteasome
LGMD2G	Abnormal Z-line assembly	?
LGMD2H	Abnormal ubiquitin-proteasome system	?
LGMD2I	Defects in proteins glycosylation	?
LGMD2J	Abnormal sarcomeric assembly	?
LGMD2K	Defects in proteins glycosylation	?
LGMD2L	Defects in proteins glycosylation	Steroids
LGMD2M	Loss of proteolytic activity	?

Pharmacological therapies: a recent study reported the beneficial effect of steroids on LGMD2L, and there is evidence of a beneficial effect on LGMD2D (16). Other possible therapeutic approaches are focused to:

1) to counteract muscle wasting enhancing muscle mass, using inhibitors of myostatin in LGMD2A and in LGMD2D (8, 40);

2) to up-regulate of proteins that can compensate the deficiency of the mutated ones (for example ε-sarcoglycan for α-sarcoglycan (49);

3) to inhibit the down regulation of SG-complex into the proteasome or blocking Calcium with the consequent activation of ubiquitous calpains (29);

4) to inhibit the complement membrane attack complex in dysferlinopathies, where decay-accelerating factor is down-regulated (52).

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