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Common recessive limb girdle muscular dystrophies differential diagnosis: why and how?

Diagnóstico diferencial das distrofias musculares cintura-membros recessivas comuns: como e por quê?

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ABSTRACT

Limb girdle muscular dystrophies are heterogeneous autosomal hereditary neuromuscular disorders. They produce dystrophic changes on muscle biopsy and they are associated with mutations in several genes involved in muscular structure and function. Detailed clinical, laboratorial, imaging, diagnostic flowchart, photographs, tables, and illustrated diagrams are presented for the differential diagnosis of common autosomal recessive limb girdle muscular dystrophy subtypes diagnosed nowadays at one reference center in Brazil. Preoperative image studies guide muscle biopsy site selection. Muscle involvement image pattern differs depending on the limb girdle muscular dystrophy subtype. Muscle involvement is conspicuous at the posterior thigh in calpainopathy and fukutin-related proteinopathy; anterior thigh in sarcoglycanopathy; whole thigh in dysferlinopathy, and telethoninopathy. The precise differential diagnosis of limb girdle muscular dystrophies is important for genetic counseling, prognostic orientation, cardiac and respiratory management. Besides that, it may probably, in the future, provide specific genetic therapies for each subtype.

Keywords: muscular dystrophies, ultrasonography, biopsy, magnetic resonance imaging, neuromuscular diseases.

RESUMO

As distrofias musculares progressivas cintura-membros são desordens neuromusculares hereditárias autossômicas heterogêneas. Elas produzem alterações distróficas à biópsia muscular e estão associadas a mutações em diversos genes envolvidos na estrutura e função muscular. Fluxograma diagnóstico, fotos, tabelas e diagramas ilustrados dos aspectos clínicos, laboratoriais e de imagem são apresentados para o diagnóstico diferencial de distrofias musculares cintura-membros autossômicas recessivas comuns, diagnosticadas atualmente em um centro de referência no Brasil. Exames de imagem pré-operatórios direcionam o local da biópsia muscular. O padrão de envolvimento muscular difere de acordo com o subtipo de distrofia muscular cintura-membros. A substituição fibroadiposa do tecido muscular é mais acentuada no compartimento posterior da coxa na calpainopatia e proteinopatia relacionada à fukutina; anterior da coxa na sarcoglicanopatia; difusa na coxa na disferlinopatia e teletoninopatia. O diagnóstico diferencial preciso das distrofias musculares cintura-membros é importante para o aconselhamento genético, orientação prognóstica, tratamento cardíaco e respiratório. Além disso poderá, no futuro, provavelmente, propiciar terapias gênicas específicas para cada subtipo.

Palavras-chave: distrofias musculares, ultrassonografia, biópsia, imagem por ressonância magnética, doenças neuromusculares.

The limb girdle muscular dystrophies are a varied group of hereditary neuromuscular disorders. They receive this denomination due to their predominant pelvic and scapular muscle weakness, typically sparing distal and facial muscles[1,2,3,4]. They are usually characterized by progressive course, symptoms beginning in childhood or adult age, and dominant or recessive autosomal inheritance.

The common muscle biopsy morphologic substrate to various types of muscular dystrophies is the "dystrophic

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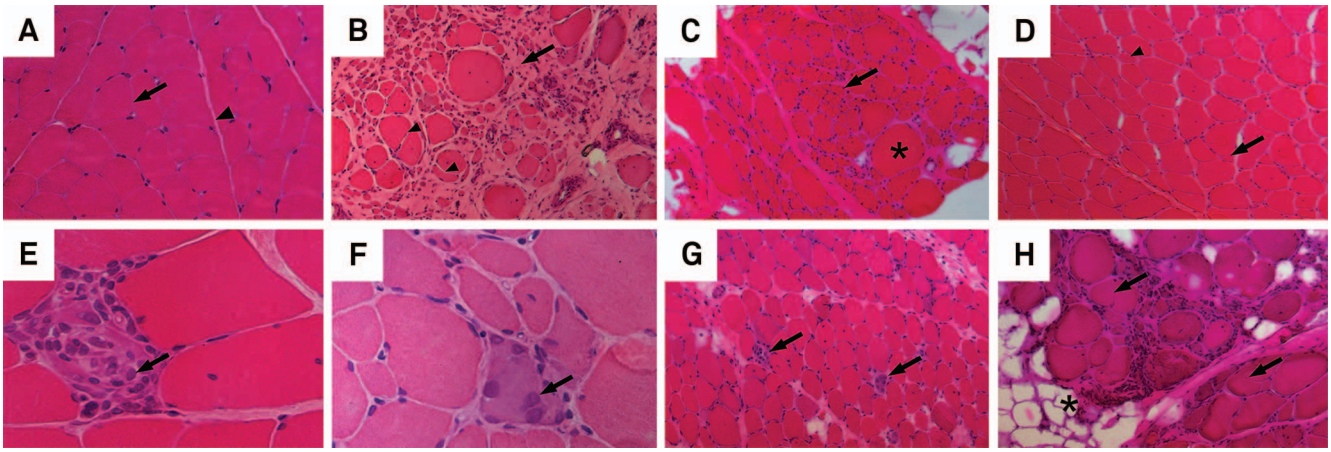

Figure 1. Muscle biopsy morphologic patterns. Normal muscle biopsy: thin perimysial (arrow head) and endomysial (arrow) connective tissue (HE 200x) (A). Dystrophic muscle biopsies (B,C,D,E,F,G,H). Endomysial fibrosis (arrow), fiber splitting (arrow heads) of a LGMD2A patient (HE 100x) (B). Fiber hypertrophy (*), groups of atrophic fibers (arrow) of a LGMD2A patient (HE 100x) (C). Isolated atrophic fibers (arrow head), hypertrophy with fiber splitting (arrow) (D). Necrosis and phagocytosis (arrow) of a LGMD2A patient (D: HE 100x; E: HE 400x) (E). Fiber regeneration (arrow) of a LGMD2I patient (HE 200x) (F). Necrosis, phagocytosis, and regeneration foci (arrows) of a dysferlin-negative patient (reaction not shown) (HE 100x) (G). Hypertrophy with fiber splitting (arrows) and muscle tissue fat replacement (*) of a dysferlin-negative patient (HE 100x) (H).

pattern" (Figure 1). Normal muscle biopsy is characterized by thin perimysial and almost imperceptible endomysial connective tissue, regular fiber size caliber, peripheral nuclei, and deep eosinophilic sarcoplasmic stain (Figure 1A). Dystrophic abnormalities are characterized by architectural disorder, pronounced variation in fiber caliber, atrophy, hypertrophy, necrosis, phagocytosis, regeneration, nuclear internalization, that progress, in late phases, to fat and fibrous replacement of the muscular tissue[4,5,6,7] (Figures 1B, 1C, 1D, 1E, 1F, 1G, 1H). Additional morphologic features and immunohistochemical evaluation of muscle frozen sections may provide either clues to limb girdle muscular dystrophy diagnosis or present peculiar findings for each subtype (Figures 2 and 3).

Limb girdle muscular dystrophies are known by the acronym "LGMD" (Limb Girdle Muscular Dystrophy). LGMD are classified by inheritance pattern as "LGMD1" for autosomal dominant and "LGMD2" for autosomal recessive disorders. They are subsequently classified with letters (LGMD1A, LGMD1B, etc.), in alphabetical order, in accordance to the chronological discovery of the correspondent mutated genes loci (Table 1)[8,9]. An updated classification table is published every year[8] (freely available at the URL: http://www.musclegenetable.fr). Until the

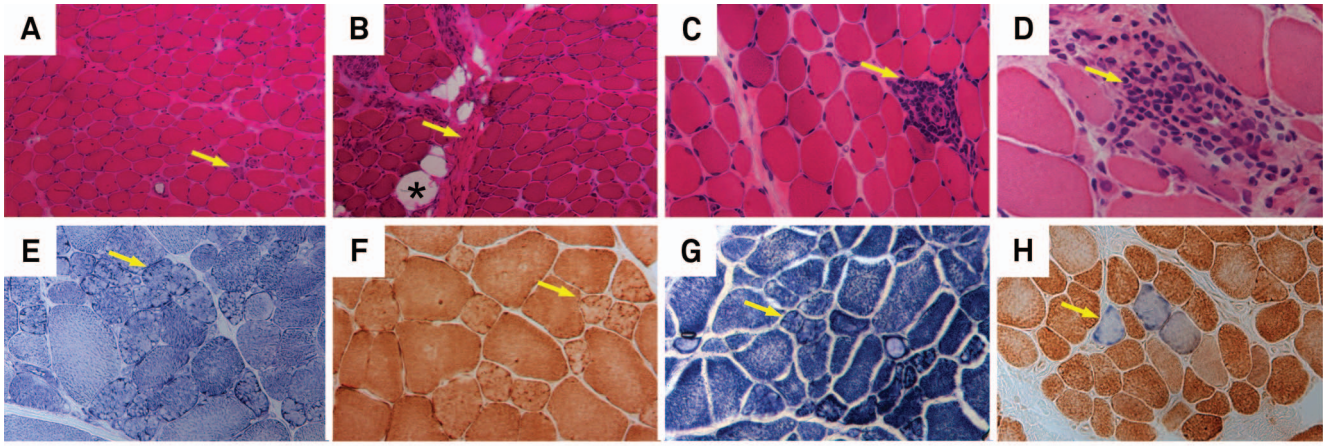

Figure 2. Additional morphologic findings of limb girdle muscular dystrophy muscle biopsies. A and B: Proximal (A), and distal (B) extremities of the same biceps brachialis medium third muscle biopsy sample (B). The proximal extremity is relatively preserved with regenerating fibers (arrow) (A). The distal extremity demonstrates fibrous (arrow) and fat (*) tissue of a dysferlin-negative patient (B). Perivascular lymphocytic infiltrate (arrow) of a dysferlin-negative patient (C). Perivascular lymphocytic infiltrate (arrow) of a LGMD2I patient (HE 400x) (D). Lobulated fibers (arrow) of a LGMD2A patient (NADH 200x) (E). Lobulated fibers (arrow) of a LGMD2G patient (combined COX-SDH 200x) (F). Lobulated fibers (arrow) of a LGMD2C patient, submitted to muscle biopsy 25 years after first symptoms (SDH 200x) (G). COX negative fibers (arrow - blue fibers) of a LGMD2A patient (combined COX-SDH reaction, 200x) (H).

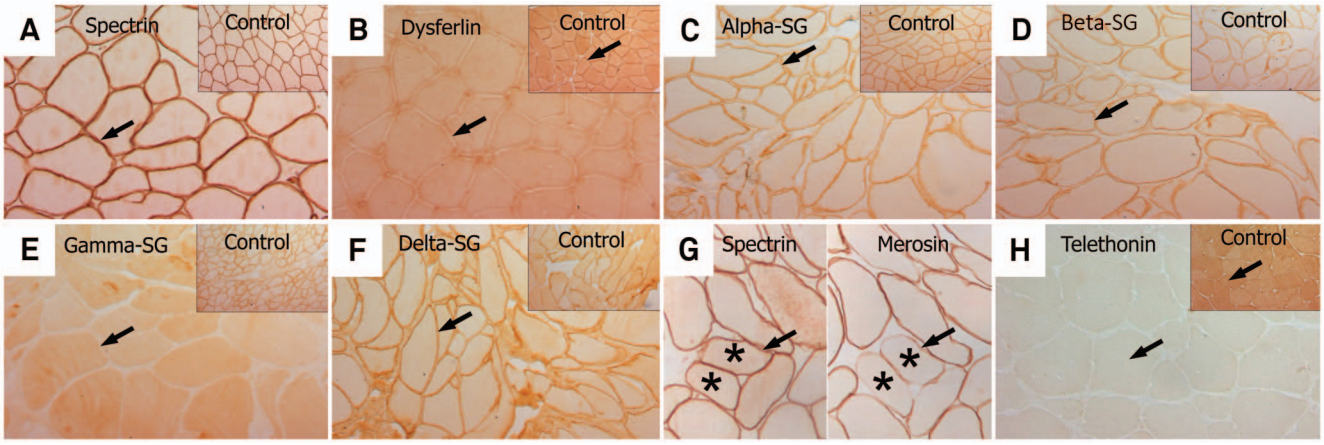

Figure 3. Immunohistochemical findings (immunoperoxidase). A and B: Sarcolemmal integrity (arrow) (A), and dysferlin deficiency of a LGMD2B patient (arrow) (B) (insets are normal controls). Anti-spectrin RBC2/3D5 200x (A). Anti-dysferlin Ham1/7B6 200x (B). C, D, E, and F: gamma-sarcoglycan deficiency on muscle sarcolemma (arrow on E) of a LGMD2C patient. Anti-alpha-sarcoglycan (Adhalin Ad1/20A6) 200x (C). Anti-beta-sarcoglycan (BSarc/5B1) 200x (D). Anti-gamma-sarcoglycan (35DAG/21B5) 200x (E). Anti-delta-sarcoglycan (DSarc3/12C1) 200x) (insets are normal controls) (F). Spectrin (left arrow) and merosin (right arrow) serial frozen sections of the same fibers (*) with focal merosin deficiency of a LGMD2I patient (G). (G - left side: anti-spectrin (RBC2/3D5) 200x; G - right side: anti-merosin laminin alpha 2 chain (Mer3/22B2) 200x). Sarcomeric telethonin deficiency (arrow) of a LGMD2G patient (inset is normal control) (antitelethonin antibody G-11 sc-25327) (H).

publication of this article, 27 LGMD subtypes have been classified, and at least five additional entities are candidates for classification (Table 1). From these 27 LGMD subtypes, 19 are autosomal recessive (more than 90% of the patients) and eight are autosomal dominant (less than 10% of the patients) (Table 1).

Table 1. Classification of autosomal dominant (LGMD1) and autosomal recessive (LGMD2) limb girdle muscular dystrophies[8,61].

Disease	Gene	Locus	Gene product
LGMD1A	*MYOT*	5q31	myotilin
LGMD1B	*LMNA*	1q22	lamin A/C
LGMD1C	*CAV3*	3p25	caveolin-3
LGMD1D*	*DNAJB6*	7q36.2	HSP-40 homologue subfamily B, number 6
LGMD1E*	*DES*	2q35	desmin
LGMD1F	*TNPO3*	7q32	transportin 3
LGMD1G	*HNRPDL*	4q21	heterogeneous nuclear ribonucleoprotein D-like
LGMD1H	-	3p25.1-p23	-
LGMD2A	*CAPN3*	15q15.1	calpain-3
LGMD2B	*DYSF*	2p13	dysferlin
LGMD2C	*SGCG*	13q12	gamma-sarcoglycan
LGMD2D	*SGCA*	17q12-q21.33	alpha-sarcoglycan
LGMD2E	*SGCB*	4q12	beta-sarcoglycan
LGMD2F	*SGCD*	5q33	delta-sarcoglycan
LGMD2G	*TCAP*	17q12	telethonin (titin-cap)
LGMD2H	*TRIM32*	9q31.2	tripartite motif-containing 32
LGMD2I	*FKRP*	19q13.3	fukutin related protein
LGMD2J	*TTN*	2q31	titin
LGMD2K	*POMT1*	9q34	protein O-mannosyltransferase 1
LGMD2L	*ANO5*	11p14.3	anoctamin 5
LGMD2M	*FKTN*	9q31-q33	fukutin
LGMD2N	*POMT2*	14q24	protein O-mannosyltransferase 2
LGMD2O	*POMGNT1*	1p34	protein O-linked mannose beta 1,2-N-acetylglucosaminyl-transferase 1
LGMD2Q	*PLEC1*	8q24	plectin 1f
LGMD2R	*DES*	2q35	desmin
LGMD2S	*TRAPPC11*	4q35.1	trafficking protein particle complex 11
LGMD2T	*GMPPB*	3p21.31	GDP-mannose pyrophosphorylase B

"-" not reported. *literature nomenclature controversy[4,8,9,56,57]. Desmin (*DES*) is associated with both LGMD1E and LGMD2R[8]. Additional candidate genes for World Muscle Society consensus classification nomenclature as LGMD2P, LGMD2U, LGMD2V, LGMD2W, and LGMD2X include: *DAG1* (3p21), *DPM3* (1q22), *ISPD* (7p21.2), *GAA* (17q25.3), and *LIMS2* (2q14)[8,61,62].

Genes or gene products from 21 autosomal dominant and recessive limb girdle muscular dystrophy subtypes are represented in Figure 4. Myotilin (LGMD1A), DNAJB6 (LGMD1D[8]), and TRIM32 (LGMD2H) are located on the Z-disk of the sarcomere; myotilin and DNAJB6 are involved in protein aggregation[4] and TRIM32 function is still unknown[4]. *LMNA* is the gene that codifies lamins A/C (LGMD1B), nuclear lamina associated proteins that provide structural support to the nuclear envelope. Caveolin-3 (LGMD1C) is a sarcolemma associated protein, component of the caveola[4] (small invaginations of the plasma membrane). Desmin (LGMD1E and LGMD2R) is an intermediate filament with protein aggregation function[4]. Plectin (LGMD2Q) is a cytolinker, associated with desmin[4]. Titin (LGMD2J) is a giant sarcomeric protein that spans from Z-disk to M-line; titin acts as an adjustable molecular spring during muscle contraction and it is essential for sarcomere assembly. *POMT1* (LGMD2K), *POMT2* (LGMD2N), *POMGnT1* (LGMD2O), and *FKTN* (LGMD2M) are genes that codify putative glycosyltransferases[4] located in the endoplasmic reticulum (ER) (*POMT1* and *POMT2*) and Golgi (*POMGnT1* and *FKTN*) that are involved in the glycosylation of proteins of the extracellular matrix; these glycosyltransferases are important for the cytoskeleton-extracellular matrix link[4].

The frequency of limb girdle muscular dystrophies varies worldwide[10,11]. The most common limb girdle muscular dystrophy subtypes reported nowadays in Brazil are calpainopathy (LGMD2A) 32%, sarcoglycanopathy (LGMD2C, LGMD2D, LGMD2E, LGMD2F) 32%, dysferlinopathy (LGMD2B) 22%, fukutin related proteinopathy or *FKRPathy* (LGMD2I) 11%, and telethoninopathy (LGMD2G) 3%[10,11]. This review will focus on the differential diagnosis of these eight limb girdle muscular dystrophy subtypes (Figure 4).

LGMD2A is caused by mutations in the calpain gene that codifies calpain, a proteolytic calcium activated enzyme, that in its inactive form, lies on titin and participates in sarcomere repair and maintenance. LGMD2C-F (LGMD2C, LGMD2D, LGMD2E, LGMD2F) are caused by mutations in four genes that codify the structural proteins gamma, alpha, beta and delta-sarcoglycans, that are members of the dystrophin associated glycoprotein complex and probably act as muscle membrane stabilizers during muscle contraction. LGMD2B is caused by mutations in the dysferlin gene that codifies dysferlin, a protein involved in vesicle-membrane fusion in order to repair membrane microlesions. LGMD2I is caused by mutations in the *FKRP* gene, that codifies fukutin related protein, a glycosyl transferase located in the Golgi complex and involved with glycosylation of diverse proteins such as alpha-dystroglycan and merosin (alpha 2 laminin) probably related to membrane stabilization. LGMD2G is caused by mutations in the *TCAP* (telethonin) gene, that codifies the protein telethonin that binds to titin, promoting sarcomere stabilization, during contraction, probably involved in sarcomere regulation and development (Figure 4).

The molecular diagnosis of a specific limb girdle muscular dystrophy subtype may be achieved in about 75% of the patients[2].

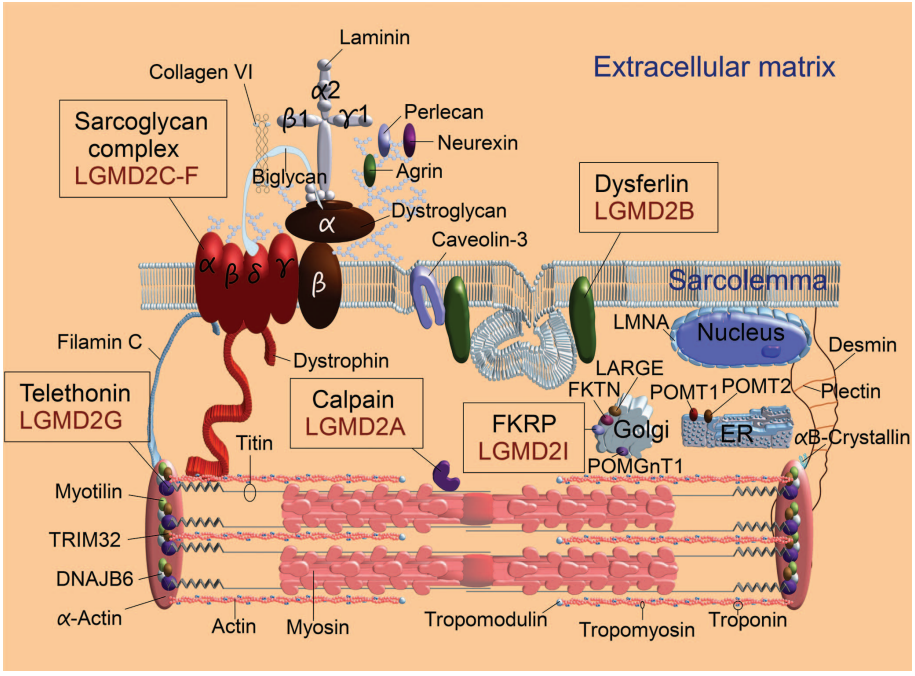

Figure 4. Molecular diagram of some proteins involved with limb girdle muscular dystrophies (LGMD). The most common LGMD subtypes in Brazil are highlighted in red type, inside rectangles: calpain (LGMD2A), dysferlin (LGMD2B), sarcoglycan complex (LGMD2C, LGMD2D, LGMD2E, LGMD2F), telethonin (LGMD2G), and fukutin-related protein (LGMD2I).

HOW DO WE KNOW IF OUR PATIENTS HAVE A LIMB GIRDLE MUSCULAR DYSTROPHY?

It is important to be sure of the limb girdle muscular dystrophy diagnosis in advance of subclassifying the disease. Therefore it is imperative to exclude both common and potentially treatable neuromuscular disorders (Figure 5).

The differential diagnosis of limb girdle muscular dystrophies is performed through an integrated multiprofessional approach considering personal and familial history, physical examination with detailed manual muscle testing, laboratorial, neurophysiological, and imaginological findings (Figure 5).

There are some neuromuscular disorders that are relatively common (compared to limb girdle muscular dystrophies), that may be suspected from clinical findings. Among these common disorders are dystrophinopathy, facioscapulohumeral muscular dystrophy, myotonic dystrophy (types 1 and 2), and spinal muscular atrophy[12]. Molecular studies usually confirm the diagnosis of these disorders[12].

Even though dystrophinopathies are X-linked inherited disorders, they may be suspected in any patient with proximal weakness and increased serum creatine kinase levels. This is due to the high prevalence of dystrophinopathy in men and symptomatic women carriers compared to limb girdle muscular dystrophies[12].

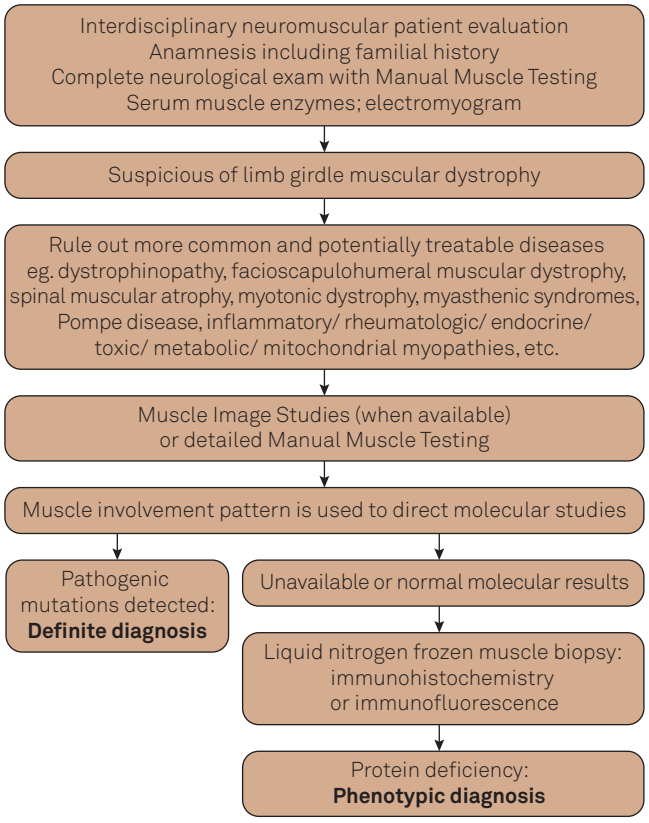

Figure 5. Limb girdle muscular dystrophy proposed diagnostic flowchart.

Facioscapulohumeral muscular dystrophy may present subtle facial weakness and clinical resemblance to limb girdle muscular dystrophy, demanding a high clinical suspicion level[13]. Asymmetric scapular weakness associated with distal lower limb weakness, pronounced lower abdominal weakness, and lumbar hyperlordosis are suggestive of facioscapulohumeral muscular dystrophy in the differential diagnosis with limb girdle muscular dystrophy[13]. The diagnostic confirmation may be done, in the vast majority of the cases, with the detection of EcoRI and EcoRI/BlnI (with p13E-11 probe) restriction fragments between 10 and 35 Kb[13,14]. The deletion of D4Z4 units in 4q35 impairs DNA methylation and alters the expression of the *DUX4* gene[13,14].

Patients with myotonic dystrophy type 1 may present subtle myotonia and it may be necessary to search for tenar muscle percussion myotonia[15]. Myotonic dystrophy type 2 presents clinical myotonia in less than half of the patients; myotonia may be absent even at neurophysiological investigation[15]. Few patients with myotonic dystrophy type 2 present cataracts[15]. Facial and jaw weakness, temporal atrophy, and ptosis are common in myotonic dystrophy type 1 patients. These findings may be absent in myotonic dystrophy type 2 patients, that may present predominant proximal weakness and clinical resemblance to limb girdle muscular dystrophy[15]. Distal weakness (flexor digitorum profundus), disabling myalgic pain, and prominent tremors, with normal or slightly elevated serum creatine kinase levels, may be the clues to myotonic dystrophy type 2, in the differential diagnosis with limb girdle muscular dystrophy[15].

Spinal muscular atrophy type 3 patients may achieve independent ambulation and present proximal weakness after childhood[16]. Spinal muscular atrophy type 4 symptoms start after 18 years old with mild clinical course[16]. Muscle weakness is usually symmetric, more proximal than distal, and worse in lower than in upper limbs[16]. Tongue fasciculations and extremity tremor, as well as neurophysiological investigation, with neurogenic motor unit potentials, are very useful in the differential diagnosis with limb girdle muscular dystrophy. Molecular confirmation is possible in most patients with the detection of exon 7 and 8 *SMN1* gene deletion in subtypes 1, 2 and 3[16].

Potentially treatable neuromuscular disorders should be excluded from the differential diagnosis of limb girdle muscular dystrophy (Figure 5). These include myasthenia gravis; congenital myasthenic syndromes; glycogen-storage disease type 2 (Pompe disease); inflammatory (associated or not with rheumatologic diseases), endocrinological, toxic, metabolic, and mitochondrial myopathies, etc.

Myasthenia gravis and congenital myasthenic syndromes may be investigated in any patient with muscular weakness and ptosis that fluctuates along the day, affects active muscles and improves with rest[17]. Therefore, it is important to be aware that there may be only slight weakness at the time of

physical examination[17]. Some fatiguing maneuvers may increase the chance to detect fatigue (such as sustained upgaze for 60 seconds, sustained abduction of the arms for 120 seconds, and sustained elevation of the legs in supine position for 90 seconds, among others[17]). Neurophysiological examination with repetitive nerve stimulation is a valuable diagnostic tool in both myasthenia gravis and congenital myasthenic syndromes[17]. Serum anti-acetylcholine receptor antibodies and anti-Musk antibodies may confirm myasthenia gravis diagnosis.

Glycogen-storage disease type 2 (Pompe disease) may present prominent clinical resemblance to limb girdle muscular dystrophy, with predominant proximal weakness and autosomal recessive inheritance[18]. Some peculiar clinical characteristics may suggest Pompe disease diagnosis such as respiratory insufficiency and increased tongue volume[18]. Sometimes, respiratory insufficiency may manifest exclusively as increased susceptibility to respiratory infections, matinal headache, and daily somnolence, due to nocturnal hypoxia[19]. Diagnostic confirmation may be done through alpha-glucosidase enzyme activity assays in both dried blood spots (DBS) and peripheral lymphocytes. Enzyme replacement therapy is available in Brazil and many parts of the world.

The differential diagnosis between limb girdle muscular dystrophy and inflammatory myopathies may be sometimes very difficult[20]. Both subacute rapid onset and negative familial history suggest inflammatory myopathy[20]. Image studies may be regarded with caution as hyperintensities observed on magnetic resonance STIR images may indicate both inflammation in myositis or they may precede fatty degeneration in muscular dystrophies[20]. Even in these cases, image studies are a valuable tool to choose the most adequate muscle biopsy site (STIR hyperintensities on magnetic resonance). Cryostat muscle sections may reveal either CD8 positive lymphocyte invasion of non-necrotic muscle fibers in polymyositis or perifascicular atrophy and membrane attack complex deposition in capillaries in dermatomyositis[21]. It is important to be aware that the absence of classic signs of polymyositis or dermatomyositis does not exclude the diagnosis of inflammatory myopathy. Immune-mediated necrotizing myopathy may present necrotic muscle fibers with sparse inflammatory infiltrate, morphologically mimicking muscular dystrophy[20,21]. Serologic investigation should include the search for viral infections such as HTLV-1, HIV, HBV, and HCV, as well as autoantibodies for the differential diagnosis.

Toxic myopathies, associated with drugs, may be investigated in any patient without previous history of neuromuscular disorder that develops myalgia, fatigue, weakness or myoglobinuria[22]. Toxic myopathies may be induced by statin anticholesterol drugs; antirheumatic, anti-inflammatory, immunosuppressive drugs; nucleoside analogues; L-tryptophan contaminated products, etc[22]. In Brazil, the most common causes of toxic myopathies, in a neuromuscular reference center, were corticosteroids, propoxyphene, neuroleptics, zidovudine, and hypokalemiant diuretics[23].

Serum creatine kinase and aldolase levels are often increased in limb girdle muscular dystrophies and are a valuable diagnostic tool[4] (Figure 5). Patients with congenital and mitochondrial myopathies usually present normal creatine kinase levels, and frequent ptosis. It is important to consider that limb girdle muscular dystrophy patients may present transaminase increase related to the muscular disease and not to any liver damage.

Neurophysiological exams reveal myopathic motor unit potentials in almost all limb girdle muscular dystrophy patients (Figure 5). In some dystrophinopathy, facioscapulohumeral, and myotonic dystrophy patients, with typical clinical presentation, diagnostic molecular studies may be ordered by experienced clinicians at the first clinical examination. In other patients, electroneuromyography may be very useful, demonstrating peculiar findings that may suggest specific disorders. Some examples are asymmetric muscular involvement in facioscapulohumeral muscular dystrophy, paraspinal involvement in Pompe disease, myotonic discharges in myotonic dystrophy / myotonic myopathies, distal involvement in hereditary distal myopathies, and finger flexor weakness in inclusion body myositis, among others.

When personal and familial history, physical exam, neurophysiological studies and serum muscle enzymes point to the diagnosis of limb girdle muscular dystrophy, image studies may reveal particular muscular involvement patterns[24] (Figures 6 and 7). Preoperative image studies may guide muscle biopsy site selection and increase specimen adequacy rate (Figure 8). When prominent muscle involvement (fibrous and fat replacement) is observed on magnetic resonance image or computed tomography, muscle ultrasound may locate the exact area of preserved muscle, suitable for histochemical and immunohistochemical studies (Figure 8).

Muscle ultrasound findings are different in normal and dystrophic muscle. In subjects without muscular dystrophy, dark areas represent normal echogenicity of normal muscle tissue, while high echo intensity lines correspond either to normal connective tissue (epimysium and perimysium) or muscle-bone interface (Figures 8A, 8B, 8C). In subjects with muscular dystrophy, structures within and surrounding the dystrophic muscle are difficult to distinguish (Figures 8D, 8E). Image studies performed on a 15 years old female patient with limb girdle muscular dystrophy, 7 years after her first symptoms, disclosed heterogeneous vastus lateralis involvement (Figure 8D). Her right thigh muscle ultrasound demonstrated both inadequate and adequate sites for muscle biopsy (Figure 8E). Areas of increased echogenicity

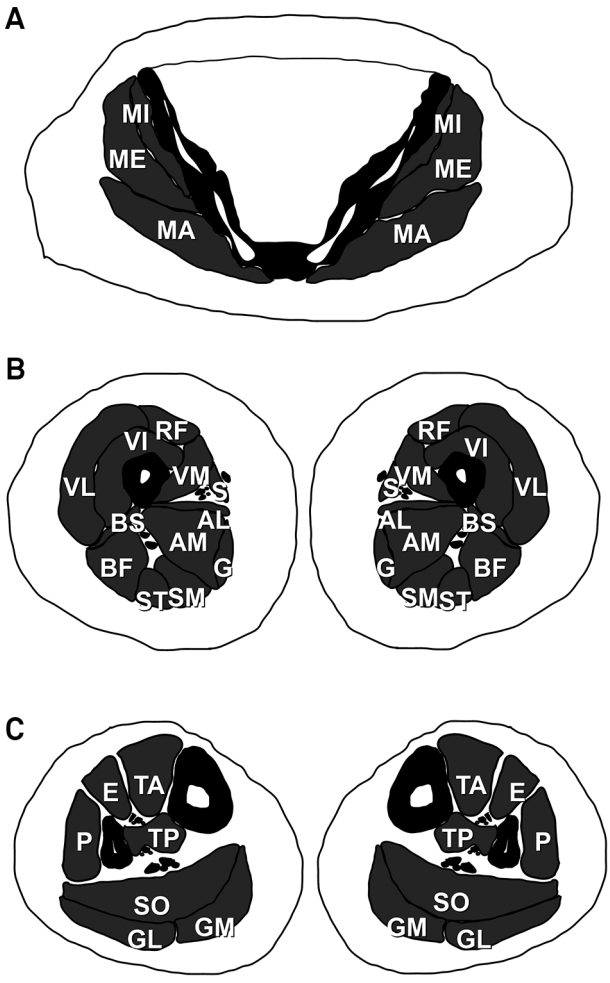

Figure 6. Schematic axial image diagram of part of the pelvis and lower limb muscles, relevant to neuromuscular disorders differential diagnosis. MA: Gluteus maximus; ME: Gluteus medius; MI: Gluteus minimus; VL: Vastus lateralis; VM: Vastus medialis; VI: Vastus intermedius; RF: Rectus femoris; S: Sartorius; G: Gracilis; AM: Adductor magnus; AL: Adductor longus; BF: Biceps femoris long head; ST: Semitendinosus; SM: Semimembranosus; BS: Biceps femoris short head; TA: Tibialis anterior; E: Extensor group (extensor digitorum longus and extensor hallucis longus); P: Peroneus group (peroneus longus and peroneus brevis); TP: Tibialis posterior; SO: Soleus; GM: Gastrocnemius medialis; GL: Gastrocnemius lateralis.

represent fibrous and fat tissue replacement in advanced muscular disease and should be avoided for biopsy. A surgical pen was used for skin site demarcation, previous to muscle biopsy. Some years after muscle biopsy, molecular investigation became available, and revealed, in this patient, a c.390 G>A (p.Try130*) homozygous exon 3 mutation in the calpain (*CAPN3*) gene, confirming the diagnosis of calpainopathy (LGMD2A).

When available, image studies should be performed prior to muscle biopsy. Careful manual muscle testing should be always performed. When image studies are unavailable, the muscle biopsy should be guided by detailed manual muscle testing[6]. Grade 3 or (preferable) grade 4 Medical Research Council (MRC) strength muscles should be selected[6]. This practice may avoid "end-stage" muscle biopsies occurrence.

Multiprofessional evaluation of clinical, laboratorial, neurophysiological, and image studies provide specific limb girdle muscular dystrophy subtype phenotypic diagnosis. Diagnostic confirmation is done according to available molecular or cryostat frozen immunohistochemical/immunofluorescence muscle biopsy studies (Figure 5).

WHY SHOULD WE PERFORM THE DIFFERENTIAL DIAGNOSIS OF SPECIFIC LIMB GIRDLE MUSCULAR DYSTROPHY SUBTYPES?

There are at least four main reasons to make specific limb girdle muscular dystrophy subtypes differential diagnosis: genetic counseling, cardiorespiratory risk evaluation, prognostic assumption, and future therapeutic possibilities. Adequate genetic counseling demands the correct identification of the specific inheritance pattern, either autosomal recessive or dominant (Table 1). Patients with sarcoglycanopathy (LGMD2C, LGMD2D, LGMD2E, and LGMD2F), telethoninopathy (LGMD2G), and fukutin related proteinopathy (LGMD2I) present increased risk of cardiac complications[1,25]. Besides that, LGMD2I patients may present early respiratory insufficiency, even while still ambulating. Patients with calpainopathy (LGMD2A) and dysferlinopathy (LGMD2B) characteristically present cardiac risk similar to the general population. Disease progression rate is usually slow in dysferlinopathy and telethoninopathy; moderate in calpainopathy and fukutin related proteinopathy, and rapid in sarcoglycanopathy[4]. Nowadays, there are many studies considering specific therapeutic possibilities according to the particular limb girdle muscular dystrophy subtype. One example is the use of lymphocyte depletion treatments for dysferlinopathy[26]. There is great hope in future genetic treatments directed to correct specific gene defects, as already tested in calpainopathy murine models[27].

HOW CAN WE MAKE THE DIFFERENTIAL DIAGNOSIS OF COMMON LIMB GIRDLE MUSCULAR DYSTROPHIES?

Calpainopathy (LGMD2A)

Calpainopathy is associated with pathogenic mutations in the calpain gene (*CAPN3)*, located in 15q15.1, that codifies the enzyme calpain. Patients with calpainopathy usually present first symptoms around 13 years old, with an onset age range from 1 to 67 years[28] (Table 2). First symptoms may start either in lower or upper limbs. In a common presentation, symptoms start almost simultaneously in lower and

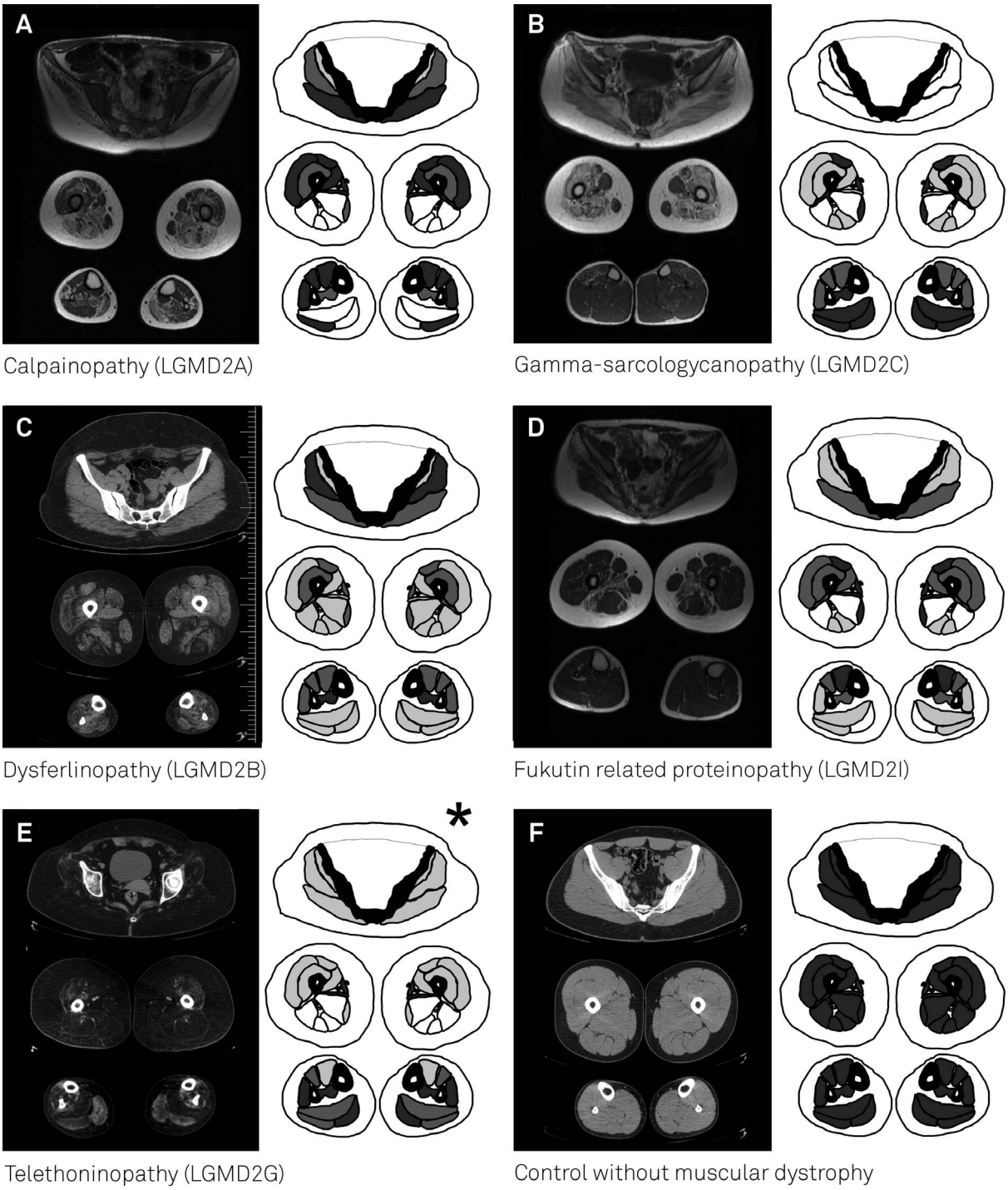

Figure 7. Image studies and their schematic diagrams. LGMD2A (A). LGMD2C (B). LGMD2B (C). LGMD2I (D). LGMD2G (E). Control computed tomography of a 32 years old male without muscular dystrophy (F). *Schematic LGMD2G diagram based on previous publications (see text).

upper limbs[28]. Muscular weakness usually starts in the lower limbs and, in less than two years, it evolves to the upper limbs[28]. Disease progression is considered intermediate among limb girdle muscular dystrophies and ambulation is usually lost around 35 years old or in the first 20 years of evolution[3,28]. No cardiac and respiratory complications are common and life expectancy is similar to the general population[3]. There is great phenotypic variability among patients with calpainopathy, even among members of the same family with the same calpain mutation[29]. Physical exam may reveal winging scapulae and there is usually no calf increase[28]. Serum creatine kinase is generally increased by 3 to 20 fold[3,28]. Image studies usually demonstrate prominent posterior (biceps femoris, semitendinosus, semimembranosus)

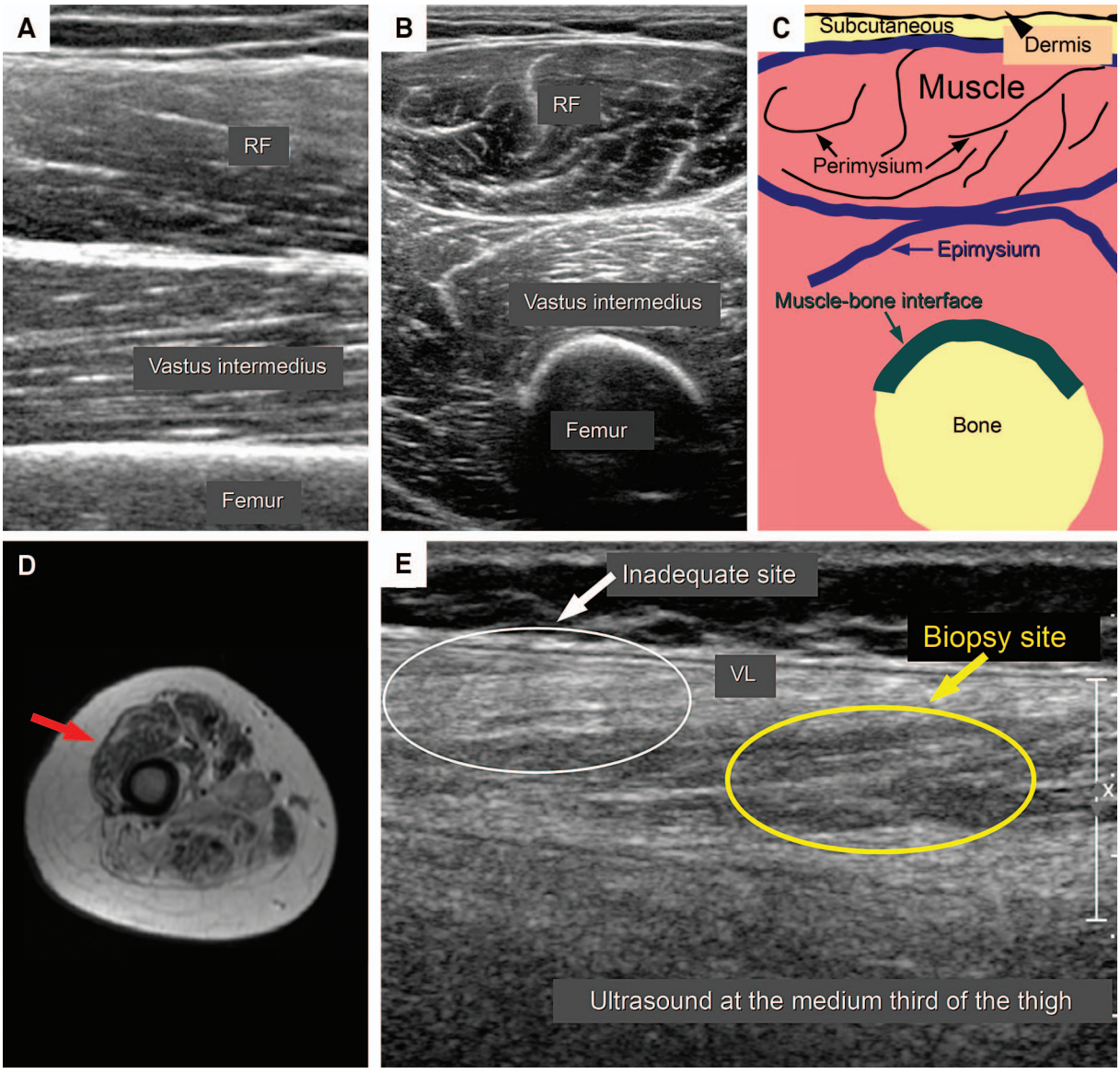

Figure 8. Preoperative image studies. Longitudinal (A). Transversal (B). Transversal schematic muscle ultrasound diagram of an adult without muscular dystrophy (C). RF: Rectus femoris. (D) Right thigh magnetic resonance image. (E) Right vastus lateralis ultrasound of a LGMD2A patient with heterogeneous muscle involvement (red arrow on D). There are inadequate (white arrow and ellipse on E) and adequate (yellow arrow and ellipse on E) muscle biopsy sites in the same muscle. VL: Vastus lateralis.

and medial thigh (adductor magnus) (Figures 7 and 8) muscles involvement. In the legs, there is soleus and medial gastrocnemius involvement[24]. Rectus femoris is usually equally affected compared to other quadriceps femoris muscles[24].

Muscle biopsy in calpainopathy usually presents variable grades of dystrophic abnormalities (Figures 1B, 1C, 1D, 1E). Severe endomysial fibrosis was the most striking abnormality of a 16 years old female patient muscle biopsy, performed 6 years after her first symptoms (Figure 1B); later molecular studies demonstrated a c.2306G>A (p.Arg769Gln) exon 22 homozygous mutation in the calpain gene. Groups of atrophic fibers were the most prominent features of a 15 years old female patient submitted to muscle biopsy 6 years after her first symptoms (Figure 1C); later molecular studies demonstrated a c.328C>T (p.Arg110) homozygous exon 2 mutation in the calpain gene. Slight variation in fiber caliber, with focal necrosis and phagocytosis (Figures 1D, 1E) were the principal changes of a 22 years old male patient submitted to muscle biopsy 8 years after his first symptoms; later molecular studies demonstrated a c.328C>T (p.Arg110) homozygous exon 2 mutation in the calpain gene. Even though the same mutation was found in the latter two patients, their muscle biopsies demonstrated distinctive morphologic abnormalities.

Lobulated or trabeculated fibers are characterized by groups of small irregular fibers with sarcoplasmic and subsarcolemal intermyofibrillar network aggregates. This is a common feature in calpainopathy, as observed on a 15 years

Table 2. Differential diagnosis of the most common autosomal recessive limb girdle muscular dystrophy subtypes reported in Brazil[10,11].

Clinical and laboratorial findings	LGMD 2A	LGMD 2C-F*	LGMD 2B	LGMD 2I	LGMD 2G
Mean onset age (years)[28,32,35,36,45,49]	13	6	19	19	12
Typical onset age range (years)[28,6,35,45,11]	8-15	6-8	17-25	2-40	9-15
Wide onset age range (years)[28,32,36,37,46,51]	1-67	1-30	1-58	2-50	1-20
Disease progression rate[3]	++	+++	+	++	+
Subacute start mimicking polymyositis[3,38,45,46,49]	-/+	-	++	-	-
Ambulation loss (age in years)[28,11,6,38,45,49]	21-40	12-16	18-58	>12	>30
Cardiomyopathy[3,28,25,1,38,45,46,11]	-	+++	-	+	+
Early respiratory abnormality[28,32,1,38,45,46,11]	-	-	-	+	-
Upper and lower limbs interval (years)[28,6,38,45,11]	<2**	**	>6**	**	**
Increased calf[1,3,28,11,32,35,45,46]	-/+	++	-/+	++	++
Winged scapula[3,28,11,32,38,45,46]	++	++	-	-/+	+
"Dystrophinopathy-like" phenotype[3,11,32,38,45,46]	-/+	++	-	++	-
Serum creatine kinase (CPK) times increase[1,51,62]	3-20x	5-40x	10-70x	10-20x	1-30x
Distal myopathy phenotype[1,35,38,11]	-	-	-/+	-	-/+
Contractures[1,45,11]	+	+	-	-	-

Onset age: age at first symptoms. 2A: LGMD2A: calpainopathy. 2C-F: LGMD2C, LGMD2D, LGMD2E, LGMD2F: sarcoglycanopathy. 2B: LGMD2B: dysferlinopathy. 2I: LGMD2I: fukutin related proteinopathy. 2G: LGMD2G: telethoninopathy. Grading: "+" slow/slight, "++" intermediate/moderate, "+++" fast/pronounced; "–/+" possible; "-" not reported. *Except LGMD2D: mean onset age at 13 years old[31] and ambulation loss at the age of 25[32]. Upper and lower limbs interval (years) = interval between first symptoms in lower and upper limbs. ** = variable.

old female patient muscle biopsy (Figure 2E), 7 years after her first symptoms; later molecular studies revealed a c.390G>A (p.Try130*) homozygous exon 3 mutation in the calpain (*CAPN3*) gene. The same patient presented COX (cytochrome c oxidase) negative fibers on muscle biopsy (Figure 2H). COX negative fibers are not pathognomonic of primary respiratory chain disorders, and they may occasionally be found as a secondary phenomenon in other neuromuscular disorders, as in this case.

Western blot studies may present abnormal calpain protein results[29]. Confirmatory molecular studies usually demonstrate two pathogenic mutations in the calpain gene (*CAPN3*)[28].

Calpain is a proteolytic calcium activated enzyme that, in its inactive form, lies on titin (giant protein with putative function of sarcomere stabilization during actin and myosin filaments contraction) (Figure 4). Therefore, it is believed that calpain plays an important role in sarcomere repair and maintenance[30]. The name "calpain" derives from two words "calcium" and "papain", describing its calcium activation and its homology to the protease enzymes similar to "papain" (the papaya's proteolytic enzyme)[30].

Sarcoglycanopathy (LGMD2C, LGMD2D, LGMD2E, LGMD2F)

Patients with sarcoglycanopathies present pathogenic mutations in any of the four subtypes of sarcoglycan genes, that have gene products expressed in the sarcolemma: *SGCG* (LGMD2C), *SGCA* (LGMD2D), *SGCB* (LGMD2E), and *SGCD* (LGMD2F), respectively located in 13q12, 17q12-q21.33, 4q12, and 5q33, that codify gamma, alpha, beta, and delta-sarcoglycan proteins. Symptoms onset usually occurs around 6 years old, with an age range from 1 to 30 years in all forms, except for LGMD2D, with first symptoms circa 13 years old[31,32] (Table 2).

Clinical presentation is generally similar to dystrophinopathy with early predominant proximal weakness, frequent falls, Gowers maneuver, and rapid evolution to gait loss and cardiac complications[25]. Muscle enzymes are usually 5 to more than 40 times elevated[1,3] (Table 2). Image studies usually demonstrate severe involvement of adductor magnus and biceps femoris, and moderate involvement of vastus lateralis, vastus intermedius, vastus medialis, adductor longus, semimembranosus and semitendinosus muscles[24] (Figure 7).

On the contrary to Duchenne muscular dystrophy patients, sarcoglycan patients present equal male and female frequency, winging scapula, preserved cognitive functions and early true calf hypertrophy (initial increased calf muscle volume contrary to the early calf fat replacement observed in Duchenne's pseudohypertrophy). Diagnostic confirmation is done either by multiplex PCR (polymerase chain reaction) directed to the most common mutations according to the patient geographic area[33] or *SGCG* / *SGCA* / *SGCB* / *SGCD* genes sequencing[32].

Muscle biopsy in sarcoglycanopathy patients usually shows a dystrophic pattern. On the contrary to calpainopathy and telethoninopathy, lobulated fibers are not a usual feature in sarcoglycanopathy. Even though, a 29 years old female patient, submitted to muscle biopsy 25 years after her first symptoms, presented lobulated fibers on her muscle biopsy (Figure 2G). Later molecular investigation demonstrated a c.525delT homozygous exon 6 mutation in the gamma-sarcoglycan (*SGCG*) gene. In this case, lobulated

fibers could be, perhaps, related to the long duration of her symptoms.

When molecular tests are unavailable, a phenotypic diagnosis may be rendered through immunohistochemical studies on muscle biopsy frozen sections, directed to the four gene products, using commercially available antibodies to gamma, alpha, beta and delta-sarcoglycan proteins. A 29 years old male patient, submitted to muscle biopsy 17 years after his first symptoms, presented complete gamma-sarcoglycan immunohistochemical deficiency on muscle sarcolemma (Figure 3E) with preserved expression of the other sarcoglycan proteins (Figures 3C, 3D, 3F). Later molecular studies demonstrated a c.525delT homozygous exon 6 mutation in the gamma-sarcoglycan (*SGCG*) gene.

There is no universal correlation between the sarcoglycan subtype immunohistochemical deficiency and the sarcoglycan mutated gene. A mutation in one gene may generate a secondary deficiency of the other proteins of the complex[32]. Therefore, it is not possible to precisely infer the specific sarcoglycanopathy subtype, based on the immunohistochemical finding of deficiency of one specific protein[32].

The four sarcoglycans, associated with LGMD2C-F sarcoglycanopathies, are "sarco-lemmal" "glyco-proteins", that are components of the dystrophin associated glycoprotein complex. They probably act as muscle membrane stabilizers during muscle contraction[34] (Figure 4).

Dysferlinopathy (LGMD2B)

Dysferlinopathy is caused by pathogenic mutations in the dysferlin gene (*DYSF*), located in 2p13, that codifies the protein dysferlin. First symptoms usually begin in a narrow age range around 19 years old, with exceptional cases starting from birth to 58 years old[35,36,37] (Table 2). Different from other limb girdle muscular dystrophies, subacute presentation may occur in about 25% of the patients. It may simulate both clinically and histologically inflammatory myopathies such as polymyositis[38], as observed on a muscle biopsy of a 16 years old dysferlin-negative female patient (Figure 2C).

Some dysferlinopathy patients present predominant distal weakness, others proximal and distal weakness[38]. Rare patients may present predominant anterior compartment distal weakness[39]. Besides that, there are oligo symptomatic patients with creatine kinase increase[38]. Usually, there is lower limb weakness that, after a period of about 6 years, is followed by upper limb weakness, but this interval may vary from 1 to 16 years[35]. Even though decreased calf volume is the most common clinical presentation, calf volume increase may be observed in about 28% of the patients[35].

A frequent clinical finding on physical examination is the relative deltoid muscle volume preservation, compared to biceps brachialis lower third[35]. Clinical and pathological exam revealed prominent distal biceps brachialis atrophy of a 18 years old dysferlin-negative male patient. The worse distal biceps brachialis involvement may be noticed on muscle biopsy (Figures 2A and 2B). At this time of the investigation it is necessary to remind that deltoid volume preservation may be observed in facioscapulohumeral muscular dystrophy, that has already been excluded from the differential diagnosis (Figure 5).

Muscle enzymes are usually excessively elevated (more than 10 to 70 fold reference values)[1,3] (Table 2). Image studies may demonstrate diffuse involvement of both anterior and posterior thigh compartments, with moderate involvement of the vastus lateralis, vastus medialis, adductor magnus, adductor longus, biceps femoris, semitendinosus, semimembranosus, soleus, medial and lateral gastrocnemius muscles[24] (Figure 7). When magnetic resonance imaging is performed, fat suppressed T2 and STIR weighted sequences may demonstrate hyperintensities, difficult to differentiate from inflammatory myopathies[40].

Muscle biopsy in dysferlinopathy patients usually shows a dystrophic muscle pattern and the variability in morphologic findings may be related to the duration of symptoms. Necrosis, phagocytosis, and regeneration foci were the most prominent muscle biopsy features of a 20 years old female dysferlin-negative patient, submitted to muscle biopsy 4 years after her first symptoms (Figure 1G). On the other hand, fiber caliber variation, atrophy, hypertrophy with fiber splitting were severe on a muscle biopsy of a 33 years old dysferlin-negative female patient, submitted to muscle biopsy 15 years after disease onset (Figure 1H).

Phenotypic diagnosis is usually suggested through complete or partial[38] dysferlin deficiency with commercially available antibodies on muscle biopsy, as observed on a 33 years old female patient muscle biopsy (Figures 3A and 3B). Dysferlin deficiency may also be detected through peripheral monocytes Western blot[38,41]. Genotypic diagnosis is confirmed through dysferlin gene sequencing[38,41].

Dysferlin is a protein that anchors on the sarcoplasmic membrane and it is necessary to repair membrane microlesions[42] (Figure 4). This occurs through vesicle formation and fusion with the sarcolemma[42] (Figure 4). Transmission electron microscopy in patients with dysferlinopathy demonstrates plasma membrane microlesions and subsarcolemmal vesicle accumulation[43]. The name "dysferlin" is derived from "dys-" from "dystrophy" and "fer-lin" from its homology to the "fer-1" (fertility factor 1), involved in membrane fusion during spermatogenesis[44].

FKRPathy or Fukutin related proteinopathy (LGMD2I)

Fukutin related proteinopathy is caused by mutations in the fukutin related protein (*FKRP)* gene, located in 19q13.3, that codifies the fukutin related protein. Symptoms usually start in a broad age range from 2 to 40

years, with a mean onset age around 19 years[45,46] (Table 2). Most patients present clinical symptoms and signs that mimic dystrophinopathy (both "Duchenne-like" and "Becker-like" cases), with predominant proximal muscle weakness and calf volume increase in about 76% of the patients[45,46]. Other muscles may present increased volume such as the brachioradialis[45,46]. Unexpected to limb girdle muscular dystrophies, about 20% of the patients with fukutin related proteinopathy may present facial weakness[45]. About 30% (15% to 46%) of the patients present cardiac complications[45,46]. Respiratory abnormalities are common and occur in about 65% of the cases, even in ambulant patients, on the contrary to most muscular dystrophies[45,46] (Table 2). It is important to remind that Pompe disease (glycogen storage disease type 2) may present respiratory insufficiency and has already been excluded from the differential diagnosis (Figure 5).

Muscle biopsy may demonstrate dystrophic pattern and secondary merosin deficiency[45] (Figures 1F and 3G). Muscle biopsy may present, in some patients, inflammatory infiltrate[63], as observed on a 11 years old female patient submitted to muscle biopsy 4 years after her first symptoms (Figure 2D); later molecular studies demonstrated two pathogenic mutations, c.826 C>A (p.Leu276Ile) and c.1384 C>T (p.Pro462Ser), in the fukutin related protein (*FKRP*) gene.

Serum creatine kinase is usually elevated. Image studies may demonstrate severe involvement of the posterior thigh muscles, mainly biceps femoris, and adductor muscles[24] (Figure 7). There is usually slight involvement of the quadriceps femoris with relative preservation of the rectus femoris[24]. Moderate involvement of the posterior leg muscles, with abnormalities of both medial and lateral gastrocnemius may be observed[24]. At this time of the investigation, molecular studies may be performed in accordance to muscle involvement pattern (Figures 5 and 7).

Fukutin related protein is located in the Golgi complex and it is involved with glycosylation of diverse proteins such as alpha-dystroglycan and merosin (alpha 2 laminin)[47] (Figure 4). Alpha-dystroglycan connects extracellular membrane proteins, such as merosin, with beta-dystroglycan that resides in the sarcolemma and is part of the dystrophin associated glycoprotein complex (Figure 4). Therefore, the putative function of the fukutin related protein is to promote the correct glycosylation of extracellular matrix proteins, essential to membrane stabilization during muscle contraction. The name "fukutin related protein" derives from its proximity to the "fukutin protein" in the Golgi complex. The name "fukutin" is an acknowledgment to Yukio Fukuyama, that described the first cases of Fukuyama congenital muscular dystrophy, associated with mutations in the fukutin (*FKTN*) gene, later related to fukutinopathy (LGMD2M)[2,3,4,48].

Telethoninopathy (LGMD2G)

Telethoninopathy is caused by mutations in the telethonin (*TCAP)* gene, located in 17q12, that codifies the protein telethonin. Symptoms usually start between 9 and 15 years old; exceptionally there may be congenital and around 20 years old onset[49,50,51] (Table 2). Ambulation loss usually occurs around the fourth decade of life[51]. Patients usually present proximal and distal muscular weakness. Early foot drop, related to tibialis anterior muscle weakness, may be the first disease presentation[51]. Cardiac abnormalities are common[11]. Image studies may demonstrate diffuse muscle involvement of the thigh (Figure 7). Severe adductor magnus, biceps femoris, semitendinosus, semimembranosus, and tibialis anterior muscles involvement may be observed, as well as moderate involvement of the vastus lateralis, vastus intermedius, rectus femoris, vastus medialis, adductor longus and gracilis muscles (Figure 7)[51,52,53]. Muscle biopsy may demonstrate dystrophic pattern with rimmed vacuoles[51]. Lobulated fibers have been commonly described on telethoninopathy patients and they were observed on the muscle biopsy of a 54 years old female patient muscle biopsy, 46 years after her first symptoms (Figure 2F); molecular investigation, on a research basis, revealed a c.157C>T (Q53X) homozygous mutation in the telethonin (*TCAP*) gene (patient previously described)[51].

A phenotypic diagnosis of telethoninopathy may be performed through immunofluorescence, Western blot or immunohistochemistry (Figure 3H), with commercially available antibodies. Diagnostic confirmation may be performed through direct sequencing of the telethonin gene.

Telethonin binds to titin and received the name of "titincap" (Figure 4). Titin is a giant elastic protein that extends from the "Z" disk to the "M" line in the sarcomere, promoting sarcomere stabilization during actin and myosin sliding. The putative function of telethonin is associated with sarcomere regulation and development mechanisms[54]. Telethonin received its name after its identification, in a cooperative brazilian-italian research, that received donations from the Italian "Telethon" ("tele" from "television" and "thon" from "marathon")[49,51].

SUMMARY OF MUSCLE INVOLVEMENT PATTERNS OF COMMON RECESSIVE LIMB GIRDLE MUSCULAR DYSTROPHIES

Image studies and schematic diagrams are very useful for the differential diagnosis of common autosomal recessive limb girdle muscular dystrophies (Figures 6 and 7)[24,40]. Magnetic resonance image of a 15 years old female patient, 7 years after her first symptoms, with homozygous c.390G>A (p.Try130*) exon 3 mutation in the calpain (*CAPN3*) gene, revealed prominent involvement of the adductor magnus,

posterior thigh, soleus and medial gastrocnemius muscles (LGMD2A) (Figure 7A). Magnetic resonance image of a 16 years old, male patient, 4 years after his first symptoms, with homozygous c.525delT (p.F175fs) exon 6 mutation in the gamma-sarcoglycan (*SGCG*) gene, showed involvement of the glutei, adductor magnus, biceps femoris, and quadriceps femoris muscles (LGMD2C)[24,40] (Figure 7B). Computed tomography image of a 23 years old female patient, 4 years after her disease onset, with complete immunohistochemical dysferlin deficiency in muscle biopsy frozen sections, presented moderate diffuse involvement of vastus lateralis, vastus medialis, adductors, posterior thigh and posterior leg muscles (LGMD2B) (Figure 7C). Magnetic resonance image of a 11 years old female patient, 4 years after her first symptoms, with two pathogenic c.826C>A (p.Leu276Ile) and c.1384C>T (p.Pro462Ser) mutations in the fukutin related protein (*FKRP*) gene, showed severe adductor magnus and biceps femoris muscles involvement, with rectus femoris signal preservation (LGMD2I)[24,40] (Figure 7D). Computed tomography image of a 54 years old female patient, 46 years since her first symptoms, with complete immunohistochemical and immunofluorescence telethonin deficiency and c.157C>T mutation in the telethonin (*TCAP*) gene (LGMD2G), disclosed severe diffuse involvement of pelvis, thigh and legs (Figure 7E). The LGMD2G schematic diagram was based on previous LGMD2G publications, with diffuse thigh and early tibialis anterior involvement[51,52,53].

CONCLUSIONS

In conclusion later studies describing the molecular mechanisms (Figure 4) involved in limb girdle muscular dystrophies will be necessary to elucidate the physiopathogenic mechanisms of these diseases[4,9,29,34,42,57,58,59]. The precise differential diagnosis of limb girdle muscular dystrophies may be achieved through an integrated clinical, laboratorial, neurophysiological and image studies approach. Immunohistochemical muscle biopsy frozen section analysis contributes to the phenotypic diagnosis of sarcoglycanopathy, dysferlinopathy, and telethoninopathy; it may reveal secondary merosin deficiency in fukutin related proteinopathy. Muscle Western blot may reveal calpain decrease in calpainopathy.

Muscle image studies are very useful to select muscle biopsy site in order to provide specimen adequacy. Besides that, careful manual muscle testing and image studies may direct confirmatory molecular studies. It is necessary to exclude most common or potentially treatable neuromuscular conditions prior to the diagnosis of limb girdle muscular dystrophy. The differential diagnosis of a specific limb girdle muscular dystrophy subtype is important for adequate genetic counseling, intervention in treatable cardiac and respiratory complications, and prognostic considerations. There is a hope that, in the future, the diagnosis of a specific limb girdle muscular dystrophy subtype may improve" quality of life, with the advent of specific new therapies.

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