LIMB-GIRDLE MUSCULAR DYSTROPHIES TYPE 2A AND 2B: CLINICAL AND RADIOLOGICAL ASPECTS

Borsato Carlo[1], Padoan Roberta[1], Stramare Roberto[2], Fanin Marina[1], Angelini Corrado[1]

[1] *Department of Neurosciences, University of Padua, Padua, Italy*
[2] *Department of Radiology, University of Padua, Padua, Italy*

Abstract

Objectives: The aim of this study was to investigate the pathologic changes, evaluated by MRI, which involve the muscles of patients affected by calpainopathy (LGMD2A) and dysferlinopathy (LGMD2B), and evaluate their correlation with muscle strength and muscle performance.

Methods: 18 patients affected by LGMD2B and 10 patients affected by LGMD2A have been evaluated clinically and by MRI imaging. We also tested, in our patients, the muscle strength of 11 upper and lower limbs muscle groups using MRC scale. Their muscular performance has been evaluated using the GSGCA scale (Gait, Stairs, Gowers, Chair, Arms functional tests). We have studied the skeletal muscles of upper and lower limbs by using MRI imaging (T1, T2, STIR sequences). The fibro-fatty replacement has been evaluated on T1 sequences by using a specific score. Myoedema has been evaluated on STIR sequences by using a new oedema score.

Results: We observed an inverse linear correlation between muscle strength (MRC scale) and functional muscular performance (GSGCA scale) in patients with LGMD2A and 2B. We, also, found a significant inverse linear correlation between the degree of fibro-fatty changes, on MRI, and the strength of the same muscles tested with MRC scale. A direct linear correlation was found between the degree of fibro-fatty changes, on MRI, and the functional muscular performance, (GSGCA scale). The presence of myoedema was detected in upper and lower limbs of LGMD2B and was described for the first time, also, in LGMD2A patients. These findings have been evaluated on STIR sequences and, in both muscle disorders, a peculiar pattern of myoedema distribution was found: the anterior compartment of lower limb was more involved than the posterior compartment in both dystrophies, the leg more than the thigh for LGMD2B, in equivalent extent in thigh and leg for LGMD2A.

Conclusions: MRI imaging is useful to study LGMD, because the quantification of fibro-fatty replacement and myoedema, associated with the clinical examination resulted of prognostic value in disease progression.

Keywords: LGMD2A, LGMD2B, MRI, Muscle Imaging, Myoedema.

Basic Appl Myol 16 (1): 17 - 25, 2006

Introduction

The limb-girdle muscular dystrophies (LGMDs) are an heterogeneous group of genetically determined progressive disorders of skeletal muscle with a primary or predominant involvement of the pelvic or shoulder-girdle musculature[15,27,36,37].

The muscle pathology is characterized by necrotic and regenerating fibres, increase in fibre size variation, fibre splitting and centrally located myonuclei. Chronic cycles of degeneration and regeneration of muscle fibres result in replacement of muscle with fatty and fibrous tissue[18,27,35].

They are, clinically, characterised by symmetrical wasting and weakness of the pelvic, scapular, and trunk muscles. The symptoms usually begin during the first two decades of life, with gradual worsening of the disease, often resulting in loss of walking ability 10 to 20 years after onset[15,27,36]. However, the clinical course is characterized by great variability, ranging from severe forms with onset in the first decade and rapid progression to milder forms with later onset and slower course[37].

The diagnostic criteria include raised serum creatine kinase activity, myopathic electromyography, muscle biopsy with features ranging from mildly myopathic to overt dystrophic changes and absence or reduction of a protein, involved in a specific form of LGMDs, on western blotting[3,4].

At least 17 genes, 7 autosomal dominant (AD) and 10 autosomal recessive (AR), responsible for LGMDs, have been mapped. The AD forms are relatively rare and represent less than 10% of all LGMDs, whereas the AR forms constitute 90% of all LGMDs[27].

LGMD2A, whose locus has been mapped to chromosome 15q15.1, is considered to be the most frequent type of recessive LGMDs, including about 30% of all AR forms[13,19,25,36].

CAPN3 gene encodes for calpain-3 (originally named p94), the muscle-specific member of a family of Ca^{2+}-activated neutral proteases, characterized by 3 exclusive sequence inserts (NS, IS1, IS2); domain I has regulatory role, domain II is the proteolytic module, domain III has a C2-domain like Ca^{2+}-binding function[23,25].

On the basis of age-onset, LGMD2A patients can be subdivided in three different clinical phenotypes: 1) "early onset LGMD" with onset of muscle weakness occurring in the pelvic girdle before the age of 12 years; 2) "LGMD" with onset of weakness in the pelvic-femoral girdle (the classical Leyden-Mobius type) or in the shoulder girdle (Erb phenotype with scapular winging) between the age of 13 and 29 years; 3) "late onset LGMD" with onset of weakness in the pelvic girdle at more than 30 years of age[19]. At onset, presence of hypertrophy of calves and joint contractures, especially at the ankles tendon, which cause the characteristic "tiptoe walk", is very frequent. These patients have difficulty in standing on their heels. Hyperlordosis and waddling gait are also, in a later stage, present[19,27,36,37].

LGMD2B is the second more frequent form of recessive LGMD (accounting for about 20% of all AR forms) caused by mutations in the dysferlin gene (DYSF) on chromosome 2p13[12,14,18,27,36].

Dysferlin is a 237-kDa protein expressed predominantly in skeletal muscle which localizes to the plasma membrane of muscle fibre, but it does not associate with the dystrophin–glycoprotein complex. Dysferlin has a single transmembrane domain at its C-terminus and six C2 domains along the length of the cytoplasmic domain.

Dysferlinopathy includes three different clinical phenotypes with an early-adult onset: two distal forms: Miyoshi myopathy (MM), with an involvement of calf muscles and the distal anterior compartment myopathy (DACM)[24] of the leg and one proximal form, involving the pelvic or shoulder-girdle (LGMD2B)[17,18,34,37]. Upper limb girdle involvement in LGMD2B, usually, follows some years after the onset in lower limbs. In MM, the initial muscles involved may be restricted to the posterior compartment of the lower leg or may gradually spread to the proximal muscles and upper limbs. It is still unknown why mutations of the same gene can cause either proximal LGMD or distal myopathies. Many patients usually participated actively in sports during adolescence, before disease onset. The first symptoms appear during the second or third decade of life as "clumsiness when running", "fatigue when walking long distances" and "difficulty in climbing stairs". All these difficulties are related to proximal lower limb weakness, and are characteristic of patients with LGMD phenotype whereas the "incapacity to walk on tiptoes" is characteristic of distal phenotype. Calf hypertrophy and tendon contractures are rare. Hyperlordosis and waddling gait are also present. Patients can remain asymptomatic for many years with only hyperCKaemia before having first clinical signs of disease[27,36].

Skeletal muscles imaging is one of the most interesting applications of MRI. Among all of the modern techniques applied to the study of pathological muscles, those mostly suggested are echography, CT scan, and just recently, MRI[21,22,26,29-33]. In comparison to the other two methodologies, MRI has a greater sensitivity and a better contrast resolution; in comparison to CT scan, it does not show artefacts, due to the bone cortex and to the use of non-ionizing radiation. Various myopathies may be responsible for the muscular alterations. Changes in signal intensity have been described in various muscle pathologies like muscular dystrophies and inflammatory myopathies[21,26,29-33]. The characteristic distribution of such changes and their severity of evolution could be useful in the diagnosis and follow-up of patients with muscle diseases. The advantage of MRI is that it allows to demonstrate the variations of relaxation time induced by the quantity of intra or extracellular water in three sequences (T1, T2, STIR). The changes in the time duration of T1 and T2 reflect alterations of intra-extracellular spaces[30,32]. The increase of the extracellular liquid compartment, relative to myoedema, causes variations in the relaxation time. Extracellular water indeed has a relaxation time of T2, longer than that of the intracellular one, so that even small variations in the percentage of the liquid content of the two compartments can produce large variation in the signal intensity. The increase in signal intensity due to extracellular oedema is clear with techniques that suppress adipose tissue. STIR are sequences of inversion recovery that produce a suppression of fat signal so that STIR sequences have great sensitivity to show changes in muscle-signal intensity due to inflammatory causes[26,33]. The appearance of fat replacement in the muscle suggests an irreversible alteration: the shortening of Tl, due to the increase of fat concentration in the tissue, produces an increase of the signal intensity in T1-weighted sequences so that these sequences have greater sensitivity to dystrophic changes. Further, it is useful to monitor severity of muscle wasting and for accurate biopsy targeting[22,26].

The aims of this study are to investigate the pathologic changes, evaluated by MRI, which involve the muscles of patients affected by LGMD2A and LGMD2B, and correlate muscle strength and muscular performance of the same patients. We plan to verify if MRI can show particular patterns of fibro-fatty and myoedema distribution and to show the different clinical progression of the two muscular diseases.

Materials and Methods

Patient selection criteria

In this retrospective study, we examined patients who received LGMD2A or LGMD2B diagnosis on the basis of following criteria: increased creatine kinase level, myopathic electromyography, muscle biopsy histopathology consistent with a dystrophic or myopathic proc-

ess, reduced or absent Calpain-3 or Dysferlin protein on western blotting and gene mutation detection in molecular analysis.

Clinical and radiological study of selected patients

18 patients affected by dysferlinopathy (14 males, 4 females, mean age 33 years) and 10 patients affected by calpainopathy (8 males, 2 females, mean age 32 years) have been evaluated clinically and by MRI imaging.

We assessed the muscle strength in 11 muscle groups of upper and lower limbs (upper limbs: shoulder abduction, elbow flexion and extension; lower limbs: hip flexion, extension, abduction and adduction, knee flexion and extension, ankle flexion and extension) by using MRC (Medical Research Council) scale. Muscle strength was graded using a scale of 0-5. Grade 5: normal strength. Grade 4: slight weakness to moderate weakness. Grade 3: muscle can move the joint against gravity but not against any added resistance. Grade 2: muscle can't move the joint against gravity but only in absence of it. Grade 1: trace of contraction, Grade 0: no visible evidence of contraction.

The muscular performance has been evaluated using the GSGCA (Gait, Stairs, Gowers, Chair, Arms) scale (Figure **1**). The score of these functional tests was, previously, validated in other muscle diseases (DMD[6], LGMD2D[5], adult glycogen storage type 2[7]). We tested the capacity to walk for 10 meters, to climb stairs (4 steps), to raise from a chair without banister and from the floor, to perform Gowers' manoeuvre and to raise upper arms over the head.

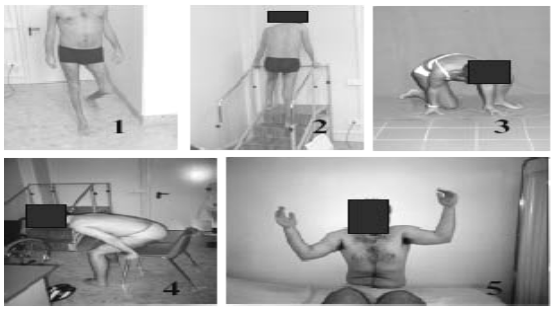

*Figure **1**. GSGCA functional scale. 1: Gait; 2: Stairs; 3: Gowers; 4: Chair; 5: Arms. Each movement has been evaluated by a score. When the score raises, the muscular performance becomes worse.*

This functional scale is able to quantify the disability of our patients with a 5-32 score: when the score raises, the patient's disability progressively increases too.

The clinical course of both diseases has been evaluated with 9 grades of the modified Gardner-Medwin-Walton as previously described[18]: grade 0 = hyperCKemia, all activities normal; grade 1 = normal gait, unable to run freely, myalgia; grade 2 = incapacity to walk on tiptoes, waddling gait; grade 3 = evident muscular weakness, steppage and climbing stairs with banister; grade 4 = difficulty to rise from the floor, Gowers' sign; grade 5 = incapacity to rise from the floor; grade 6 = incapacity to climb stairs; grade 7 = incapacity to rise from a chair; grade 8 = unable to walk unassisted; grade 9 = unable to eat, drink or sit without assistance.

The skeletal muscles of upper and lower limbs have been studied using MRI imaging (T1, T2, STIR weighted spin echo sequences) on a 1.0-tesla MRI system. Non-contrast images were obtained from right shoulder, right elbow, pelvis, bilateral thighs and legs. We obtained 12 slices from each site (12 slices for 5 anatomic sites). Slices were 10 mm thick and the gap between slices varied from 10 mm to 50 mm dependent on the site and on the size of patient.

One patient affected by LGMD2B was excluded from MRI examination because was pregnant.

The fibro-fatty replacement has been evaluated on T1 sequences by using Mercuri score[30-32], which correlates directly the dystrophic aspects with a 4 grade scale of increasing signal intensity (Table **1**). Myoedema has been evaluated on STIR sequences by using a specific new oedema score, properly created for this study to quantify this morphologic aspect (Table **2**). This new scale with 3 grades evaluates the extracellular liquid amount, expressed as intensity of muscle inflammation and as myoedema's distribution in inter-fascicular or intra-fascicular muscular spaces.

*Table **1** Mercuri score, MRI, T1 sequences.*

Stage	MRI muscle appearance
0	Normal appearance
1	Early moth-eaten appearance, with scattered small areas of increased signal
2a	Late moth-eaten appearance, with numerous discrete areas of increased signal with beginning confluence, comprising less than 30% of the volume of the individual muscle
2b	Late moth-eaten appearance, with numerous discrete areas of increased signal with beginning confluence, comprising 30-60% of the volume of the individual muscle
3	Washed-out appearance, fuzzy appearance due to confluent areas of increased signal
4	End-stage appearance, muscle replaced, increased density connective tissue and fat with only a rim of fascia and neurovasc. structures distinguishable

*Table **2** Myoedema score, MRI, STIR sequences.*

Stage	MRI muscle appearance
0	Myoedema absent
1	Slight, inter-fascicular myoedema
2a	Slight, intra-fascicular, segmental myoedema of individual muscle
2b	Slight, intra-fascicular, global myoedema of individual muscle
3a	Moderate, intra-fascicular, segmental myoedema of individual muscle
3b	Moderate, intra-fascicular, global myoedema of individual muscle

RESULTS

Clinical-functional aspects in LGMD2A

We examined 10 patients (8 males and 2 females). Two patients had consanguineous parents. The mean age is 32 years, ranging from 17 to 60 years. At the time of this clinical-radiological study, 4 patients had LGMD phenotype (the classical Leyden-Mobius type) with age onset between 13 and 29 years; 1 patient Erb phenotype with scapular winging, with age onset between 13 and 29 years (25 years old); 4 patients had an "early onset LGMD" phenotype with onset before the age of 12 years and 1 patient had "late onset LGMD" with onset after 30 years (54 years old). The GSGCA functional scale showed the following data: 1 patient had evident pelvic-girdle weakness with difficulty in climbing stairs, 5 patients had difficulty in rising from the floor, 2 patients were unable to raise from the floor and 2 patients were unable to raise from a chair. Two patients had ankle tendon retractions, 4 patients scapular winging and 2 calves hypertrophy.

Clinical progression of disease has been evaluated on the basis of modified Gardner-Medwin-Walton scale. Only one patient was excluded from the clinical scale, but not from the other imaging studies because his age was too different (65 years old) from the other patients' age, whose mean age was 32. In LGMD2A patients, the mean age-onset was 13.7 years and the mean age of loss of ambulation was 26 years. When we correlated the age of onset with the corresponding grade of functional scale (grade 0, patient asymptomatic or hyperCKemia), we observed that the progression's time to the last grade (grade 8, loss of ambulation) of disease was, on average, 12 years. Global muscular strength (evaluation of 11 movements with MRC scale) has been compared with disability score (GSGCA scale). There was an inverse linear correlation between muscle strength and functional muscular performance (GSGCA disability scale): Pearson Index (r)=-0.78; p<0.005 (Figure **2a**).

Clinical-functional aspects in LGMD2B

We examined 18 patients (14 males and 4 females, 3 couples of brothers including 1 couple of siblings). Two patients had consanguineous parents. The mean age was 33 years, ranging from 16 to 50 years. At the time of this clinical-radiological study, 13 patients had MM phenotype, 3 patients had classical LGMD phenotype and 2 patients had only hyperCKemia. The GSGCA functional scale showed the following data: 2 patients (12%) were asymptomatic with only hyperCKemia, 5 patients (28%) had difficulty in rising from the floor, 5 patients (28%) were unable to rise from the floor, 3 patients (17%) were unable in rising from a chair and 3 patients (17%) were not able to walk without assistance.

Clinical progression of disease has been evaluated on the basis of the modified Gardner-Medwin-Walton scale. In LGMD2B patients, the mean age-onset was 19.2 years and the mean age of loss of ambulation was 32 years. When we correlated the age of onset with the corresponding grade of functional scale, we observed that the progression's time to the last grade (grade 8, loss of ambulation) of disease was, on average, 13 years. Global muscular strength (evaluation of 11 movements with MRC scale) was compared with disability score (GSGCA scale). There was an inverse linear correlation between muscle strength and functional muscular performance (GSGCA disability scale): Pearson Index (r)=-0.93; p<0.005 (Figure **2b**).

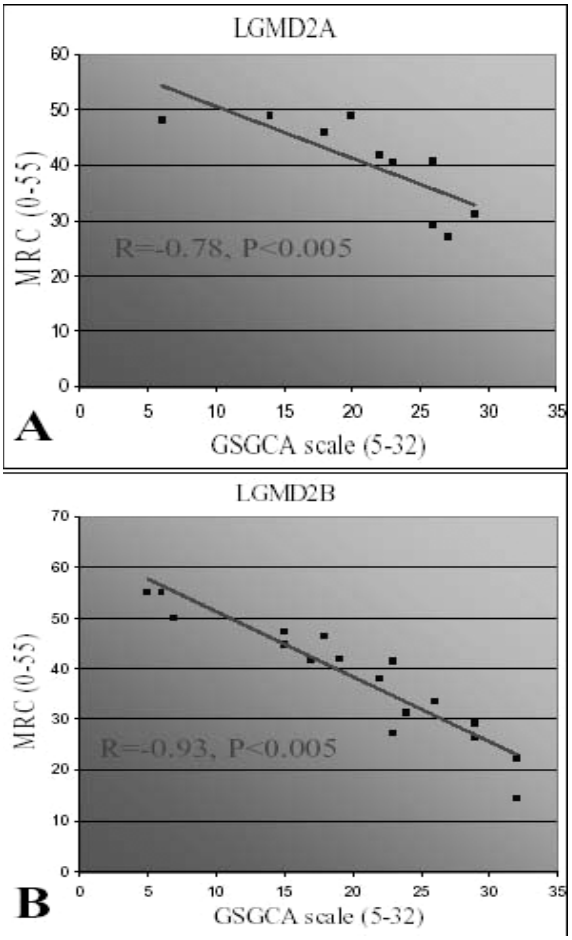

*Figure **2**. Relationship between functional muscular performance (evaluated by GSGCA scale) and muscle strength (evaluated by MRC score) in LGMD2A (**A**) and LGMD2B (**B**). In both LGMDs, there is an inverse linear correlation: when the global muscle strength decreases, the muscular performance, also, becomes worse.*

Radiological features in LGMD2A

Each patient was evaluated by MRI imaging (T1, T2, STIR sequences). The fibro-fatty changes were quantified by Mercuri score on T1 sequences. The imaging score, resulting from a global evaluation of dystrophic process (sum of each muscle score) was compared with global muscular strength (MRC scale) and disability score (GSGCA disability scale). There was an inverse linear correlation between Mercuri (T1) score and muscle strength (MRC scale): Pearson Index (r)=-0.79; p<0.005. There was a direct linear correlation between Mercuri (T1) score and disability score (GSGCA disability scale): Pearson Index (r)=0.98; p<0.001. The dis-

tribution of fibro-fatty replacement in lower limbs was, also, investigated: in 9 patients (90%) the posterior compartment of the thigh and of the leg was more involved than the anterior; the mean fibro-fatty replacement grade in the posterior compartment of thigh and leg (% respect to the entire posterior compartment of the thigh and leg) was 78.5% and 72%, respectively (Figure **3a**).

STIR sequences analysis revealed an hyper-intense signal whose meaning should be correlated to extracellular oedema (myoedema). The quantification of this inflammatory aspect (myoedema score) revealed a peculiar distribution: in 8 patients (80%) the anterior compartment of the thigh was more involved than the posterior, whereas, in the leg, this percentage decreased to 50%. The mean myoedema grade in the anterior compartment of thigh and leg (% respect to the entire anterior compartment of thigh and leg) was 35.7% and 42%, respectively (Figure **3b**).

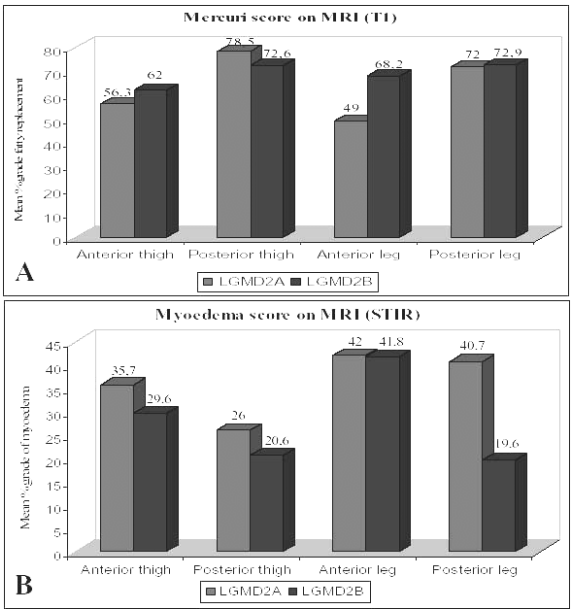

*Figure **3**. (**A**) Mean distribution of fibro-fatty changes (T1 sequences) in lower limbs of 10 patients affected by LGMD2A and 17 by LGMD2B. Note the greater mean fibro-fatty replacement grade in the posterior compartments respect to the anterior compartments in both dystrophies.*
*(**B**) Mean distribution of myoedema (STIR sequences) in the lower limbs of the same patients. Note that, in both dystrophies, distribution of myoedema involves particularly the anterior compartments of the lower limbs (opposite distribution respect to fibro-fatty replacement described in (**A**)).*

Radiological features in LGMD2B

We evaluated 17 patients by MRI imaging (T1, T2, STIR sequences). There was an inverse linear correlation between Mercuri (T1) score and muscle strength (MRC scale): Pearson Index (r)=-0.84; p<0.001. There was a direct linear correlation between Mercuri (T1) score and disability score (GSGCA disability scale): Pearson Index (r)=0.95; p<0.005. The distribution of fibro-fatty replacement in lower limbs was, also, investigated: in 15 patients (88%) the posterior compartment of the thigh and of the leg was more involved than the anterior; the mean fibro-fatty replacement grade in the posterior compartment of thigh and leg (% respect to the entire posterior compartment of the thigh and leg) was 72.6% and 72.9%, respectively (Figure **3a**).

STIR sequences analysis reveals a hyper-intense signal (myoedema), also in these patients. The quantification of this inflammatory aspect (Myoedema score) reveals a particular distribution: in 14 patients (82%) the anterior compartment of the thigh and leg is more involved than the posterior. The mean myoedema grade in the anterior compartment of thigh and leg (% respect to the entire anterior compartment of thigh and leg) is, respectively, 29.6% and 41.8% (Figure **3b**).

DISCUSSION

We have investigated a total of 28 patients, 10 affected by LGMDA (mean age 32) and 18 by LGMD2B (mean age 33).

LGMD2A is caused by defects in a protein with an enzymatic rather than structural function. Some gene mutations are expected to impair the main biochemical activities of calpain-3: autocatalytic activity, α-fodrin proteolysis, and binding to titin[25]. Due to its nuclear localization signal sequence, calpain-3 might act in the nucleus in particular conditions and might be involved in the regulation of transcription factors controlling survival genes and apoptosis. Calpain-3 deficiency would be associated with myonuclear apoptosis and a profound perturbation of the IkBα/NF-kB pathway[8,9].

In LGMD2B, also, the dystrophic process is not due to structural mechanism defects. Dysferlin presents a high degree of sequence homology with the protein Fer-1 in *Caenorhabditis elegans*: because the mutant worm has an abnormal spermatogenesis, it was argued that mutant Fer-1 might be involved in the failure of membrane fusion between the organelles and plasmalemma[1,10,11,28,35]. Later, the pathogenetic mechanism in dysferlinopathy was correlated with the abnormal trafficking of vesicles to sarcolemma, and to abnormal repair of damaged muscle fibres. The repair pathway is initiated by an influx of calcium through the site of sarcolemma's injury. This, in turn, triggers the accumulation of vesicles, which fuse with one another and then with the plasma membrane, within the injury. A 'patch' is thereby added across the wounded area, resealing the plasma membrane[10,18].

The clinical progression of the two dystrophies, evaluated by modified Gardner Medwin – Walton scale, seems to have a similar slope of progression but differs in age onset: in each step of modified Gardner Medwin – Walton clinical scale's grade, LGMD2A patients, on average, preceded LGMD2B patients of 5 years. In our LGMD2A population, the mean age-onset was 13.7, the following progression's time of disease, to reach the last grade (loss of ambulation) was 12 years. In our LGMD2B population, the mean age-onset was 19.2, the

following progression's time of disease, to reach the last grade (loss of ambulation) was 13 years.

There was an inverse linear correlation between muscle strength (evaluated by MRC scale) and functional muscular performances (evaluated by GSGCA scale) in patients with LGMD2A and 2B (Figure **2a** and **2b**). In both LGMDs, when the global muscle strength decreased, the muscular performance, also, worsened. There was, also on MRI, a strong correlation between the degree of fibro-fatty changes, evaluated by using Mercuri score on MRI (T1 sequences), and the strength of the same muscles tested with MRC scale in LGMD2A and 2B. In both diseases, when the fibro-fatty replacement became worse (rise of Mercuri score), the global muscle strength, consequently, decreased. A direct linear correlation was between the degree of fibro-fatty changes, evaluated by Mercuri score on MRI (T1 sequences), and the functional muscular performances, evaluated by GSGCA scale in LGMD2A and 2B. In both diseases, when the fibro-fatty replacement became more evident (rise of Mercuri score), the muscular performance, also, worsened.

Clinical and instrumental examinations revealed peculiar aspects for both the dystrophies: muscular MRI imaging, revealed that dystrophic process involved both proximal and distal musculature of limbs, particularly the posterior compartments, which have a more evident fibro-fatty replacement than the anterior compartments.

The presence of myoedema was been confirmed in upper and lower limbs of patients with dysferlinopathy[16-18] and it was demonstrated, for the first time, also in calpainopathy patients. These findings were particularly evident on STIR sequences. Both muscular dystrophies showed a particular pattern of myoedema distribution: the anterior compartment of lower limb was more involved than the posterior, the leg more than the thigh. In the thighs, vastus medialis and lateralis muscles seemed to be more inflamed than other muscles of anterior and posterior compartments; in the legs, tibialis anterior, extensor digitorum longus muscles appeared to be more inflamed than other muscles of posterior calves, in both dystrophies (Figure **4**).

The fibro-fatty replacement seemed to have an opposite distribution: where the presence of myoedema was more marked (anterior compartment), the fibro-fatty replacement did not appear with the same intensity, while, in the posterior compartment, the fibro-fatty replacement was more marked than myoedema. In LGMD2A and 2B, there was an early fibro-fatty replacement of semitendinosus, semimembranosus and biceps femoris muscles (posterior thigh), then a progressive involvement of vastus medialis and lateralis muscles (anterior thigh). Fibro-fatty replacement of the leg, was more characteristic and severe in LGMD2B, particularly in distal form: in fact in Miyoshi myopathy (MM), the posterior compartment of calves was involved early (medial gastrocnemius and soleus muscles); however, in the proximal form, pelvic-shoulder girdle (LGMD) there was, also, a distal involvement. In both dystrophies, sartorius and gracilis muscles are, usually, spared even when all others muscles were completely replaced (Figure **5**).

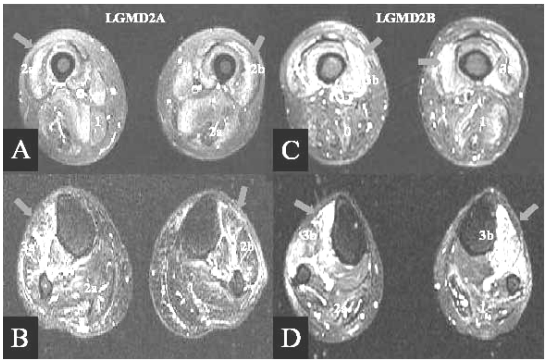

*Figure **4**. Evaluation of myoedema grade in lower limbs of LGMD2A and 2B patients, by using myoedema score on MRI (STIR sequences). Traverse slices of thigh (**A**) and leg (**B**) in patient affected by LGMD2A put in evidence (red arrow) an hyper-intense signal in the anterior compartments due to inflammatory myoedema. Traverse slices of thigh (**C**) and leg (**D**) in patient affected by LGMD2B show an hyper-intense signal (red arrow) in the anterior compartments due to inflammatory myoedema. The leg seems to be more involved than the thigh in both dystrophies.*

The over inflammatory aspects did not correlate with muscle strength or with functional muscular performance. This could be explained because the functional aspects (muscle strength and muscular performance), mainly, depend on skeletal-muscle structure's integrity which is conditioned, directly, by dystrophic changes, whereas inflammatory aspects are secondary to fibre injury. However, the inflammatory aspects (evaluated by myoedema score) could be useful to monitor the dystrophic process' activity: in the posterior compartments, at the moment of MRI scan, the fibro-fatty replacement has been almost completed, and the inflammation activity has a low grade; in the anterior compartments, the dystrophic changes have just started and not yet concluded: here the inflammation has a greater grade.

MRI imaging is useful for the study of LGMD, because the evaluation of the degree of fibro-fatty changes with the physical examination, may be a prognostic tool of the evolution of the disease.

MRI imaging, also, revealed a peculiar pattern of distribution of myoedema which represents a new finding in these diseases.

Previous immunopathological studies revealed that there is muscle inflammation and MHC I up regulation in muscular dystrophy with lack of dysferlin[16,18]. These findings suggest a relationship between myoedema (quantified on STIR sequences) and defective membrane repair: the inflammatory response secondary to necrosis was identified by the presence of macrophages[18], which, in turns, could increase the speed and distribution of the same dystrophic process, by releasing

oxidizing enzymes or lytic substances which produce fibre injury.

Recent studies revealed, also, that patients with calpainopathy have more apoptotic myonuclei on muscular biopsy than those in specimens from patients affected by other myopaties[8,9,20,23]. Muscular myoedema could have a role in disease progression also for patients with calpainopathy because inflammation might modify the apoptosis regulation[8,9], resulting in a increased dystrophic activity.

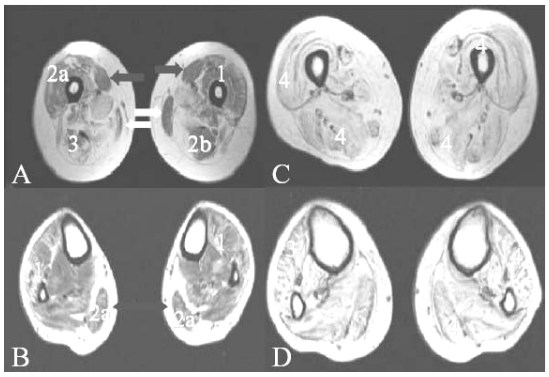

*Figure **5**. Evaluation of fibro-fatty replacement in lower limbs of LGMD2A and 2B patients, by using Mercuri score on MRI (T1 sequences). (**A**) Traverse slices of thigh in patient affected by LGMD2A. Note an hyper-intense signal in the posterior compartment due to a heavy fibro-fatty replacement. The anterior compartment is relatively spared, particularly sartorius (red arrow) and gracilis (yellow arrow) muscles. (**B**) Traverse slices of calves in patient affected by LGMD2B. This is an asymptomatic patient with only hyperCKemia. However, MRI demonstrates an early dystrophic process in the leg: note an hyper-intense signal in the posterior compartment due to a early fibro-fatty replacement of medial gastrocnemius muscles (red arrow). Traverse slices of thigh (**C**) and calves (**D**) in patient affected by LGMD2B (MM). Note a diffuse, hyper-intense signal in the anterior and posterior compartments due to a strong fibro-fatty replacement. This patient had the greatest disability grade (GSGCA scale) and was unable to ambulate.*

Concluding remarks

MRI study has an important role in follow up of the muscle diseases; it is useful in evaluating inflammatory aspects which could influence LGMDs progression, and it is an useful tool for prognosis, because we found a strong correlation between clinical and radiological data.

Clinical and instrumental examinations revealed peculiar aspects for both the dystrophies: muscular study, with application of MRI imaging, revealed that dystrophic process involve both proximal and distal musculature of limbs, particularly the posterior compartments show a more evident fibro-fatty replacement than the anterior compartments. MRI imaging, also, revealed the presence of a particular pattern of distribution of myoedema which represents a new element in these diseases.

MRI imaging is useful for the study of LGMD, because the evaluation of the degree of fibro-fatty changes by using E. Mercuri score is able to give, with the physical examination, a prognostic estimation of the disease.

Furthermore, MRI study has an important role in follow up of these diseases because it is useful in evaluating these new inflammatory aspects which could influence the same LGMDs progression, and so it is useful as a prognostic predictor.

Acknowledgements

We thank all our patients for accepting to take part to this study and all people who made possible this work.

Address correspondence to:

Carlo Borsato, Dr., Department of Neurosciences, University of Padua, telephone: +393332871946,
E-mail: carloborsato@yahoo.it

Abbreviations

Limb girdle muscular dystrophy (LGMD); Autosomal dominant (AD); Autosomal recessive (AR); Limb girdle muscular dystrophy type 2A (LGMD2A); Limb girdle muscular dystrophy type 2B (LGMD2B); Miyoshi myopathy (MM); Distal anterior compartment myopathy (DACM); Computed tomography (CT); Magnetic Resonance Imaging (MRI); Short T1 recovery inversion (STIR).

References

[1] Anderson LVB, Davison K, Moss JA, Young C, Cullen MJ, Walsh J, Bashir R, Britton S, Keers S, Argov Z, Beckmann JS, Bushby KMD: Dysferlin is a plasma membrane protein and is expressed early in human development. Hum Mol Genet (1999); 8: 855-861.

[2] Anderson LVB, Davison K, Moss JA, Cullen MJ, Bushby KMD: Characterisation of monoclonal antibodies to calpain-3 and protein expression in muscle from patients with limb girdle muscular dystrophy type 2A. Am J Pathol (1998); 153: 1169-1179.

[3] Anderson LVB, Davison K, Moss JA et al: Multiplex western blotting for the analysis of muscular dystrophy proteins. Am J Pathol (1999); 154: 1017-1022.

[4] Anderson LVB: Optimised protein diagnosis in the autosomal recessive limb girdle muscular dystrophies. Neuromusc Disord (1996); 6: 443-446.

[5] Angelini C, Fanin M, Menegazzo E, Freda MP, Duggan DJ, Hoffman EP: Homozygous α-sarcoglycan mutation in two siblings: one asymptomatic and one steroid-responsive mild limb-girdle muscular dystrophy patients. Muscle Nerve (1998); 21(6): 769-775.

[6] Angelini C, Pegoraro E, Turella E, Intino MT, Pini A, Costa A: Deflazacort in Duchenne dystrophy: study of long term effect. Muscle Nerve (1994); 17(4): 386-391.

[7] Angelini C, Pegoraro E, Zambito-Marsala S, Vergani L, Nascimbeni AC, Fulizio L, Fanin M: Adult acid maltase deficiency: an open trial with albuterol and branched-chain aminoacids. Bas Appl Myol (2004); 14(2): 71-78.

[8] Baghdiguian S, Martin M, Richard I et al: Calpain-3 deficiency is associated with myonuclear apoptosis and profound perturbation of the IkBα/NF-kB pathway in LGMD type 2A. Nat Med (1999); 5: 503-511.

[9] Baghdiguian S, Richard I, Martin M, Coopman P, Beckmann JS, Mangeat P, Lefranc G: Pathophysiology of limb girdle muscular dystrophy type 2A : hypothesis and new insights into the IkBα/NF-kB survival pathway in skeletal muscle. J Mol Med (2001); 79: 254-261.

[10] Bansal D, Campbell KP: Dysferlin and the plasma membrane repair in muscular dystrophy. Trends Cell Biol (April 2004); 14: 206-213.

[11] Bashir R, Britton S, Strachan T et al: A gene related to Caenorhabditis elegans spermatogenesis factor fer-1 is mutated in limb-girdle muscular dystrophy type 2B. Nat Genet (1998); 20: 37-42.

[12] Bashir R, Keers S, Strachan T et al: Genetic and physical mapping at the limb girdle muscular dystrophy locus (LGMD2B) on chromosome 2p. Genomics (1996); 33: 46-52.

[13] Beckmann JS, Richard I, Hillaire D, Broux O, Feingold J: A gene for limb girdle muscular dystrophy maps to chromosome 15 by linkage. CR Acad Sci Paris (1991); 312: 141-148.

[14] Bejaoui K, Hirabayashi K, Hentati F et al: Linkage of Miyoshi myopathy (distal autosomal recessive muscular dystrophy) locus to chromosome 2p12-14. Neurology (1995); 45: 768-772.

[15] Bushby KMD, Beckmann JS: The limb girdle muscular dystophies. Proposal for a new nomenclature. Neuromusc Disord (1995); 5: 337-343.

[16] Confalonieri P, Oliva L, Andreetta F, Lorenzoni R, Dassi P, Mariani E, Mora M, Cornelio F, Mantegazza R: Muscle inflammation and MHC class I up regulation in muscular dystrophy with lack of dysferlin: an immunopathological study. J Neuroimmunol (2003); 142: 130-136.

[17] Cupler EJ, Bohlega S, Hessler R, McLean D, Stigsby B, Ahmad J: Miyoshi myopathy in Saudi Arabia: clinical, electrophysiological, histopathological and radiological features. Neuromusc Disord (1998); 8: 321-326.

[18] Fanin M, Angelini C: Muscle pathology in dysferlin deficiency. Neuropathol Appl Neurobiol (2002); 28: 461-470.

[19] Fanin M, Fulizio L, Nascimbeni AC, Spinazzi M, Piluso G, Ventriglia VM, Ruzza G, Siciliano G, Trevisan CP, Politano L, Nigro V, Angelini C: Molecular diagnosis in LGMD2A: Mutation analysis or protein testing? Hum Mutat (2004); 24: 52-62.

[20] Fanin M, Nascimbeni AC, Fulizio L, Trevisan CP, Petrusa MM, Angelini C: Loss of calpain-3 autocatalytic activity in LGMD2A patients with normal protein expression. Am J Pathol (2003); 163: 1929-1936.

[21] Franczak MB, Ulmer JL, Mark LP, Prost RW: Spin lock magnetic resonance imaging of muscle in patients with autosomal recessive limb girdle muscular dystrophy. Magn Reson Imaging (2000); 10: 73-77.

[22] Herlidou S, Rolland Y, Bansard JY, Le Rumeur E: Comparison of automated and visual texture analysis in MRI: characterization of normal and diseased skeletal muscle. Magn Reson Imaging(1999); 17: 1393-1397.

[23] Huang J, Fosberg NE: Role of calpain in skeletal muscle protein degradation. Proc Natl Acad Sci (1998); 95: 12100-12105.

[24] Illa I, Serrano-Munuera C, Gallardo E, Lasa A, Palmer J, Matsuda C, Brown RH: Distal anterior compartment myopathy: a dysferlin mutation causing new muscular dystrophy phenotype. Ann Neurol (2001); 49: 130-134.

[25] Kinbara K, Sorimachi H, Ishiura S, Suzuki K: Skeletal muscle-specific calpain, p94. Structure and Physiological function. Biochem. Pharmacol (1998); 56: 415-420.

[26] Lamminen AE: MRI of primary skeletal muscle diseases: patterns of distribution and severity of involvement. Br J Radiol (1990); 63: 946-950.

[27] Laval SH, Bushby KMD: Limb-girdle muscular dystrophies – from genetics to molecular pathology. Neuropathol Appl Neurobiol (2004); 30: 91-105.

[28] Matsuda C, Aoki M, Hayashi YK, Ho MF, Arahata K, Brown RH: Dysferlin is a surface membrane-associated protein that is absent in Miyoshi myopathy. Neurology (1999); 53: 1119-1122.

[29] McDaniel JD, Ulmer JL, Prost RW, Hamilton CA, Mark LP: Magnetization transfer imaging of skeletal muscle in autosomal recessive limb girdle muscular dystrophy. J Neuroimag (2001); 2: 80-86.

[30] Mercuri E, Cini C, Pichiecchio A, Allsop J, Counsell S, Messina S, Kinali M, Brown SC, Muntoni F: Muscle magnetic resonance imaging in patients with congenital muscular dystrophy and Ullrich phenotype. Neuromusc Disord (2003); 13: 554-558.

[31] Mercuri E, Cini C, Counsell S, Allsop J, Sewry C, Brown SC, Pepe G, Muntoni F: Muscle MRI findings in a three-generation family affected by Bethlem myopathy. Eur J Ped Neurol (2002); 6: 309-314.

[32] Mercuri E, Pichiecchio A, Counsell S, Allsop J, Cini C, Uggetti C, Bydder G: A short protocol

for muscle MRI in children with muscular dystrophies. Eur J Ped Neurol (2002); 6: 305-307.

[33] Messineo D, Cremona A, Trinci M, Francia A, Marini A: MRI in the study of distal primary myopathologies and of muscular alterations due to peripheral neuropathies: possible diagnostic capacities of MR equipment with low intensity field (0.2 T) dedicated to peripheral limbs. Magn Reson Imag (1998); 16: 731-741.

[34] Miyoshi K, Tada Y, Nakano M, Kawai H: Autosomal recessive distal muscular dystrophy as a new type of progressive muscular dystrophy: 17 cases in 8 families including an autopsied case. Brain (1986); 109: 31-54.

[35] Piccolo F, Moore SA, Ford GC, Campbell KP: Intracellular accumulation and reduced sarcolemmal expression of dysferlin in limb girdle muscular dystrophy. Ann Neurol (2000) 48: 902-912.

[36] Zatz M, De Paula F, Starling A, Vainzof M: The 10 autosomal recessive limb-girdle muscular dystrophies. Neuromusc Disord (2003); 13: 532-544.

[37] Zatz M, Vainzof M, de Paula F, Starling A: Limb-girdle muscular dystrophy: one gene with different phenotypes, one phenotype with different genes. Curr Opin Neurol (2000); 13: 511-517.