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The clinical course of calpainopathy (LGMD2A) and dysferlinopathy (LGMD2B)

C. Angelini*†, L. Nardetto*†, C. Borsato*, R. Padoan*, M. Fanin*†, A. C. Nascimbeni*† and E. Tasca*†

**Department of Neurosciences, University of Padova, Padova, Italy*
†Venetian Institute of Molecular Medicine, Padova, Italy

Objective: Autosomal recessive limb girdle muscular dystrophies (LGMD type 2) are a clinically and genetically heterogeneous group of disorders, characterized by progressive involvement and wasting of limb girdle muscles. In order to describe the peculiar clinical features of LGMD2A (calpainopathy) and LGMD2B (dysferlinopathy), the most frequent forms of LGMD in European countries, we analysed and compared the phenotype and the clinical course in two relatively large groups of these patients.
Methods: We selected 22 patients with a molecular diagnosis of LGMD2A and 21 patients with LGMD2B and reported their clinical data collected from both clinical history and during periodical neuromuscular examinations: age and distribution of muscle involvement at onset, clinical functional score by the use of ten-point modified scale of Gardner–Medwin and Walton at onset and at last clinical examination, and the rate of disease progression.
Results: LGMD2A group included patients with different ages at onset (early-onset or late-onset), different phenotypes (upper girdle in Erb LGMD or lower girdle in Leyden–Moebius LGMD) and different disease progressions (rapid or slow course). LGMD2B patients differed for pattern of muscle involvement at onset (distal in Miyoshi dystrophy or proximal in Leyden–Moebius LGMD) but they had a rather homogeneous age at onset (in the second/third decade) and rate of disease progression.
Discussion: Our data show that besides the clinical differences within each group of patients, the two forms of LGMD present distinctive clinical features. The various phenotypes and courses can be attributed to specific pathogenetic mechanisms and might suggest differential therapeutic strategies.

Keywords: Calpain-3, clinical course, dysferlin, LGMD

Introduction

Autosomal recessive limb girdle muscular dystrophies (LGMD type 2) are a clinically and genetically heterogeneous group of disorders, including at least 12 different genetic entities, which are characterized by progressive involvement and wasting of limb girdle muscles.

LGMD type 2A (LGMD2A) is the most common form of LGMD in European countries, where it represents the 40% of LGMD and affects about 1 : 100,000 inhabitants[1,2]. It is the most frequently occurring neuromuscular autosomal recessive disorder after spinal muscular atrophy.

LGMD2A is caused by mutations in the CAPN3 gene, encoding for a muscle-specific member of a family of Ca^{++}-activated neutral proteases, which binds to titin[3–5]. Several interesting lines of research have suggested that calpain-3 could be involved in the regulation of transcription factors controlling survival genes and apoptosis[6], or in the degradation and disassembly of cytoskeletal or myofibrillar proteins (sarcomere remodeling)[3,7].

In calpainopathy, a marked clinical heterogeneity has been observed, even in patients with the same gene mutation. According to the original clinical descriptions of LGMDs[8], the clinical phenotype of LGMD2A can be subdivided in the pelvi-femoral form of Leyden–Moebius (with prevalent proximal muscle weakness in lower girdle) and the scapulo-humeral form of Erb (with prevalent proximal muscle weakness in upper girdle); the onset may be early (<12 years old), typical (between 12 and 30 years old) and late (>30 years old). The loss of ambulation occurs usually 20–30 years after the onset. The variability of both the clinical phenotype and the disease course may be only partly attributable to the genotype. While null type gene mutations are usually associated with absent calpain-3 protein in muscle and severe phenotype, missense type mutations (which account for ~70% of mutations in this gene) are associated with an extreme unpredictability of their effect at both the protein and the phenotype levels[2,9,10], suggesting that additional and still unknown genetic or environmental factors may be playing a role in modulating the phenotype.

Correspondence and reprint requests to: C. Angelini, Department of Neurosciences, via Giustiniani 5, 35128 Padova, Italy. [corrado.angelini@unipd.it]

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Limb girdle muscular dystrophy type 2B (LGMD2B), and the distal muscular dystrophy named Miyoshi myopathy (MM), are caused by mutations in dysferlin gene, mapped to chromosome region 2p13[11]. Dysferlin immunolocalizes to the sarcolemma similarly to dystrophin, but it does not associate with dystrophin–glycoprotein complex. The pathogenetic mechanism in dysferlinopathies is correlated to the abnormal trafficking of vesicles, which repair muscle fiber membrane[12]. The absence of dysferlin causes a failure of damaged muscle fiber repair, possibly impairing the recovery from exercise induced damage.

In many patients with LGMD2B, the initial symptoms occur abruptly after age 20, sometimes following a regular or heavy exercise, which damages especially the bi-articular muscles. The clinician should discourage these patients from performing heavy exercise, which might exacerbate muscle breakdown. Creatine kinase is usually markedly elevated at presentation (often 20–150 times or even more the normal range)[13]. The anterior distal leg muscles and the distal arm muscles are relatively spared even in the later stages of the disease and in contrast to calpainopathy, scapular involvement is mild or absent at onset[14]. Other important clinical clues are the early inability to walk on tiptoe and the early involvement of the medial gastrocnemius muscle. Despite the clinical features of LGMD2B and MM are quite different, both phenotypes can be detected among patients belonging to the same family, thus sharing the same mutations[12]. This clinical heterogeneity might be attributed to additional epigenetic factors resulting in variable dysferlin expression. Gene expression profiling has been recently used to investigate modifier genes in dysferlinopathy[15]. Several studies reported a prominent inflammatory response in dysferlinopathy patients, but the origin of this feature and its role in the development of muscle pathology are still under investigation. Patients treated with steroids or immunosuppressant drugs do not show benefit on the long term; therefore, it seems advisable to try alternative drugs, since this muscular dystrophy develops in relation to a defective sarcolemmal repair.

Materials and methods

Selection criteria of patients

We selected 22 patients affected with LGMD2A and 21 cases with LGMD2B, attending the Neuromuscular Center of Padova. The molecular diagnosis of LGMD2A and LGMD2B was obtained following the identification of calpain-3 or dysferlin protein defect in muscle biopsy[16] and subsequent characterization of mutations in the CAPN3 or DYSF genes, respectively[2,9,17].

Neuromuscular clinical evaluation

Retrospective analysis of the clinical records collected at onset, at the time of biopsy and during the subsequent outpatient examinations was used to assess the following data:

- age at onset: early (<12 years), typical (between age 12 and 30 years), late (>age 30 years);
- clinical phenotype: Leyden–Moebius LGMD (early lower girdle involvement), Erb LGMD (early upper girdle involvement) and MM (early distal muscle involvement);
- functional clinical grade: using the ten-graded scale of Walton and Gardner–Medwin modified as follows for proximal myopathy (LGMD): grade 0=hyperCKemia, all activities normal; grade 1=normal gait, unable to run freely; grade 2=waddling gait, fatigability in lower limbs; grade 3=overt muscle weakness, climbing stairs with rails; grade 4=difficulty rise from the floor, presence of Gowers' sign; grade 5=unable to rise from the floor; grade 6=unable to climb stairs; grade 7=unable to rise from a chair; grade 8=unable to walk unassisted; grade 9=unable to eat, drink or sit without assistance. We used the scale of Walton and Gardner–Medwin modified as follows for distal myopathy (MM): grade 0=hyperCKemia, all activities normal; grade 1=normal gait, unable to run freely, myalgia or muscle hypotrophy with rigidity; grade 2=difficulty walking on tiptoes, waddling gait, fatigability in lower limbs; grade 3=stepping gait, overt muscle weakness, climbing stairs with rails; grade 4=difficulty rise from the floor, presence of Gowers' sign; grade 5=unable to rise from the floor; grade 6=unable to climb stairs; grade 7=unable to rise from a chair; grade 8=unable to walk unassisted; grade 9=unable to eat, drink or sit without assistance;
- disease progression or course was calculated from data obtained in two or three different clinical examinations and graded as rapid (worsening$\geq$3 grades of the functional clinical scale in 6 years) or slow (worsening<3 grades in 6 years). Since the rate of progression may vary during the lifespan of patients, we graded as rapid the course of patients who had either worsening$\geq$3 grades of the functional scale in at least one period of 6 years, or loss of ambulation (functional grade$\geq$8) before 35 years of age.

Statistical analysis

The analysis of variance by *t*-test was used to compare the mean values of each numerical parameter in the different groups of LGMD patients. We considered $p<0{\cdot}05$ to be significant.

Results

Calpainopathy

In this study, we analysed the clinical phenotype of 22 patients with LGMD2A (*Table 1*). The age at onset of clinical symptoms (functional grade 1) ranged from 3 to 28 years (average $14{\cdot}3 \pm 6{\cdot}9$ years); 11

patients had early onset (muscle weakness occurred <12 years), and 11 patients had juvenile or adult onset. In most patients (19/22), the clinical phenotype at onset was the pelvi-femoral form of Leyden–Moebius, with muscle weakness initially involving the lower girdle muscle, and three patients presented at onset the scapulo-humeral form of Erb (initial involvement of upper girdle). Patients with Erb phenotype always presented a relatively late-onset (on average 22·6 years). Two affected cousins included in the study (*Table 1*) showed an evident discrepancy in the phenotype (one had the Erb phenotype and the other had the Leyden–Moebius LGMD phenotype), despite they shared the same calpain-3 gene mutations and genetic background.

The degree of muscle involvement at last examination, graded using the functional clinical score, was variable: most patients (13/22) had a moderate degree of clinical severity (grades 4–5) and nine were severely affected (grades 7–9) at mean age of 32·2 years (onset on average at 10·1 years). Six patients had lost independent ambulation (grade 8) during the clinical follow-up, on average at age 35 years.

The analysis of disease course, evaluated from the whole data obtained in different clinical examinations, indicated that LGMD2A patients can be subdivided into two groups (*Figure 1*): one including 14 cases with rapid disease course and early or juvenile age at onset (in 12 cases before the age of 20), and another group of eight patients with slow course and juvenile or adult onset.

The main steps of the disease (age at onset, degree of muscle involvement by functional scale, and rate of progression) were not strictly correlated in all patients (*Table 1*). Indeed, while the early age at onset was associated in most cases (9/11) with severe muscle involvement in adulthood and rapid disease course, in few cases (2/11), the early onset was associated with a slow progression and moderate muscle involvement in adult life. Furthermore, though in few patients, the rate of clinical progression differed in various periods of life, it was usually more rapid in the first decade of disease and then had a slower progression with relative preservation of the main motor functions (*Figure 1*).

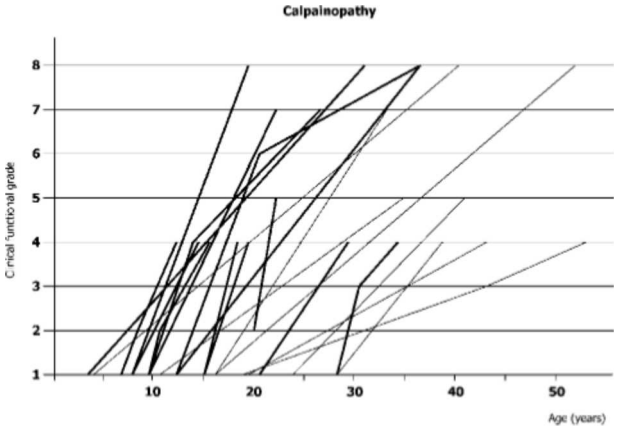

Figure 1 Graphic representation of the rate of disease progression in 22 patients with LGMD2A, evaluated using the modified ten-graded scale of Walton and Gardner–Medwin (clinical functional scale) from the onset of the disease to the last clinical examination. LGMD2A patients can be subdivided into two groups: one with rapid disease course (thick lines) and early or juvenile age at onset, and another group with slow course (thin lines) with juvenile or adult age at onset

Dysferlinopathy

In this study, we analysed the clinical phenotype of 21 patients with LGMD2B (*Table 2*). The age at onset

Table 1 Clinical data of LGMD2A patients

Patient no., gender	Clinical phenotype at onset (years)	Age at onset (years)	Clinical grade and age at first exam (years)	Clinical grade and age at intermediate exam (years)	Clinical grade and age at last exam (years)	Disease progression
1, M	e.o. LGMD	3	Grade 1, 3	–	Grade 8, 31	Rapid
2, M	e.o. LGMD	4	Grade 1, 4	Grade 8, 40	Grade 8, 50	Slow
3, M	e.o. LGMD	7	Grade 1, 7	Grade 2, 8	Grade 4, 12	Rapid
4, M	e.o. LGMD	8	Grade 1, 8	Grade 5, 15	Grade 8, 18	Rapid
5, F	e.o. LGMD	8	Grade 1, 8	–	Grade 4, 13	Rapid
6, M	e.o. LGMD	10	Grade 1, 10	Grade 2, 11	Grade 4, 16	Rapid
7, F	e.o. LGMD	10	Grade 1, 10	Grade 4, 12	Grade 7, 26	Rapid
8, M	e.o. LGMD	10	Grade 1, 10	–	Grade 7, 21	Rapid
9, M	e.o. LGMD	11	Grade 1, 11	Grade 3, 22	Grade 5, 35	Slow
10, F	e.o. LGMD	12	Grade 1, 12	Grade 6, 19	Grade 8, 35	Rapid
11, F	e.o. LGMD	12	Grade 1, 12	Grade 8, 35	Grade 9, 49	Rapid
12, F	LGMD	15	Grade 1, 15	Grade 4, 19	Grade 4, 23	Rapid
13, M	LGMD	15	Grade 1, 15	Grade 4, 17	Grade 4, 30	Rapid
14, M	LGMD	16	Grade 1, 16	Grade 8, 52	Grade 8, 56	Slow
15, M*	LGMD	16	Grade 1, 16	–	Grade 7, 32	Slow
16, M	LGMD	18	Grade 2, 20	Grade 5, 21	Grade 5, 26	Rapid
17, M	LGMD	19	Grade 1, 19	–	Grade 4, 38	Slow
18, M*	Erb	19	Grade 1, 19	Grade 3, 43	Grade 4, 52	Slow
19, M	Erb	21	Grade 1, 21	Grade 4, 27	Grade 4, 31	Rapid
20, F	LGMD	24	Grade 1, 24	Grade 5, 40	Grade 5, 46	Slow
21, F	LGMD	28	Grade 1, 28	–	Grade 4, 38	Slow
22, F	Erb	28	Grade 1, 28	Grade 3, 30	Grade 4, 34	Rapid

*Relatives.

of clinical symptoms ranged from 10 to 33 years (average: 18·9 ± 5·9 years); only three patients had early onset (muscle weakness occurred <12 years), and 18 patients had juvenile or adult onset. In most patients (16/21, 76%), the clinical phenotype at onset was the distal myopathy of Miyoshi type (MM), with muscle weakness initially involving the distal compartment of lower girdle, and five patients presented at onset an involvement of proximal lower girdle muscles (LGMD). Patients presenting with Leyden–Moebius LGMD phenotype had a significant earlier onset (13·8 years) when compared to the patients with MM phenotype (20·5 years, $p<0·024$). While most (4/5) of dysferlinopathy patients with LGMD phenotype had a slow progression, the only patients who had lost the ambulation had the MM phenotype.

Two of the four groups of siblings included in the study (*Table 2*) showed one member affected with MM and the other affected with LGMD, with evident discrepancy in the phenotype despite they shared the same dysferlin gene mutations and genetic background.

The degree of muscle involvement at last clinical examination was variable: eight patients had a moderate degree of clinical severity (grades 4–6) at average age of 28·5 years (with onset at age 21·3 years) and ten patients had a severe muscle involvement (grades 7–9) at average age 34·6 years (with onset at age 18·1 years). Three patients had lost independent ambulation (grade 8), in average at age 32 years.

The analysis of the rate of disease progression, calculated from the whole data obtained in different clinical examinations, indicated that LGMD2B patients had a rather homogeneous disease course, which was rapid in most cases (12/21) (*Figure 2*).

Comparison of LGMD2A and LGMD2B

The age at onset was significantly different in the two disorders: 14·3 years in LGMD2A versus 18·9 years in LGMD2B ($p=0·024$).

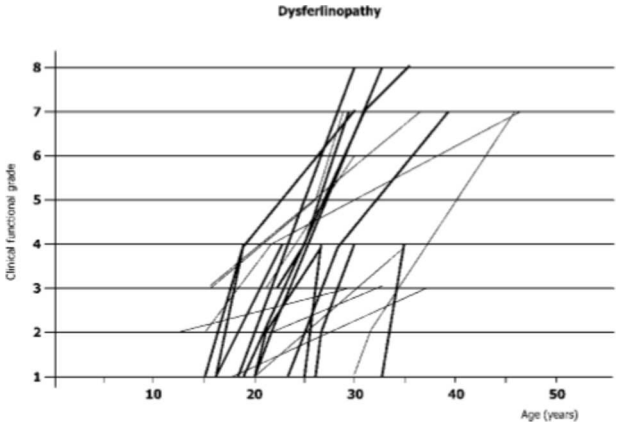

Figure 2 Graphic representation of the rate of disease progression in 21 patients with LGMD2B, evaluated using the modified ten-graded scale of Walton and Gardner–Medwin (clinical functional scale) from the onset of the disease to the last clinical examination. LGMD2B patients had a rather homogeneous age at onset (on average in adulthood) and disease course, which is in most cases with rapid progression (thick lines)

The age at loss of independent ambulation was earlier in LGMD2B (average: 32 years, three cases) than in LGMD2A (average: 35 years, six cases), but this difference was not significant because of the small number of cases that have already reached this end-point.

The clinical progression appears to evolve more rapidly and homogenously in LGMD2B than in LGMD2A.

Discussion

Strengths and limitations of the study and reference with the existing literature

In this study, we analysed and compared the data on the natural history of two relatively large groups of

Table 2 Clinical data of LGMD2B patients

Patient no., gender	Clinical phenotype at onset (years)	Age at onset (years)	Clinical grade and age at first exam (years)	Clinical grade and age at intermediate exam (years)	Clinical grade and age at last exam (years)	Disease progression
1, F#	LGMD	10	Grade 3, 15	–	Grade 7, 36	Slow
2, F*	LGMD	11	Grade 3, 15	Grade 5, 25	Grade 7, 28	Slow
3, F	MM	11	Grade 2, 12	Grade 3, 29	Grade 3, 36	Slow
4, M	MM	15	Grade 1, 15	Grade 4, 19	Grade 7, 30	Rapid
5, F#	LGMD	15	Grade 2, 15	Grade 4, 20	Grade 7, 45	Slow
6, M	MM	15	Grade 3, 20	Grade 6, 30	Grade 6, 39	Slow
7, M	LGMD	16	Grade 1, 16	–	Grade 4, 17	Rapid
8, M	MM	16	Grade 1, 16	–	Grade 4, 21	Rapid
9, F	LGMD	17	Grade 1, 17	–	Grade 3, 37	Slow
10, M	MM	17	Grade 1, 20	Grade 2, 21	Grade 3, 32	Slow
11, M	MM	18	Grade 1, 18	–	Grade 8, 30	Rapid
12, M	MM	19	Grade 1, 19	Grade 7, 31	Grade 8, 34	Rapid
13, M§	MM	19	Grade 2, 21	Grade 4, 24	Grade 8, 32	Rapid
14, M	MM	20	Grade 1, 20	–	Grade 4, 34	Slow
15, M°	MM	20	Grade 1, 20	Grade 2, 20·5	Grade 4, 26	Rapid
16, M§	MM	21	Grade 3, 21	Grade 4, 24	Grade 7, 27	Rapid
17, M#	MM	23	Grade 1, 23	Grade 4, 27	Grade 7, 39	Rapid
18, F°	MM	25	Grade 0, 16	Grade 1, 25	Grade 4, 27	Rapid
19, F	MM	26	Grade 1, 26	Grade 2, 26·5	Grade 4, 30	Rapid
20, M	MM	30	Grade 1, 30	Grade 2, 31	Grade 7, 45	Slow
21, M *	MM	33	Grade 0, 20	Grade 1, 33	Grade 4, 34	Rapid

*, °, §, #Groups of siblings. MM=Miyoshi distal myopathy.

LGMD2A and LGMD2B patients, identified by biochemical and molecular diagnosis. Previous studies independently described both typical and unusual clinical phenotypes of calpainopathy and dysferlinopathy[10,13,14,18,19], but the comparison of the clinical features in these two disorders using a homogeneous clinical protocol and the same standard scale has never been reported.

Summary of main findings

Concerning the main clinical steps of the disease (age of onset, age at loss of ambulation) and the different phenotypes at onset (Erb or Leyden–Moebius in calpainopathy, and MM or LGMD in dysferlinopathy), our LGMD2A and LGMD2B patients matched our previous and other observations[9,17,18].

One novel and important result from our study is that while LGMD2A patients can be subdivided into heterogeneous clinical patterns of progression (one with early onset, severe muscle involvement in adulthood and rapid disease course, and another with juvenile or adult onset and slow course), LGMD2B patients had always a juvenile or adult onset and a more homogeneous disease course, which was rapid in most cases. In agreement with this observation, the levels of serum creatine kinase are reported to be higher in LGMD2B than in LGMD2A, indicating a more aggressive and active process of muscle wasting.

The dissimilar clinical pattern observed in the two disorders is probably related to a differential involvement of muscle groups. Muscle atrophy and wasting occur mainly in the proximal muscle groups in LGMD2A[20], where the walking ability seems to be lost at a later age than in dysferlinopathy. Among LGMD2B patients, a more rapid course has been observed in cases with the distal Miyoshi phenotype than in LGMD phenotype, though in MM subgroup, the onset occurs later than in the proximal phenotype.

The explanation for the differential pattern of muscle involvement in the two forms of LGMD is likely to rely in the different pathogenetic mechanisms that underlie these disorders.

According to observations in recent studies, calpain-3 deficiency would affect the sarcomere organization and its maintenance in adult muscle fibers[3,7]. Skeletal muscles must constantly adapt to respond to various physiological conditions (metabolic, mechanical, hormonal) by a complex process called sarcomere remodeling, which involves protein synthesis and degradation by the intervention of the proteolytic system.

In an initial phase of sarcomere remodeling, the ubiquitous calpains and the ubiquitin/proteasome system would disassemble and degrade myofibrils, whereas in the recovery phase, the calpain-3 defective muscles would fail to regain their full weight[7].

Dysferlinopathy results from abnormal plasma membrane repair mechanism[12]. Small membrane lesions occur mostly after eccentric contractions and various degrees of exercise and mechanical stress; therefore, a defective membrane repair mechanism easily results in the necrosis of muscle fibers.

The repairing capacity of plasmalemma is dependent on the extracellular presence of Ca^{++}. The mechanism by which intracellular vesicles successfully repair plasmalemma damage is the formation of a 'patch' of internally derived membrane over a disruption site. Membrane resealing is triggered by Ca^{++} entry through the membrane gap, which causes a rapid fusion of vesicles in the subsarcolemmal region[12,21].

The bi-articular muscles (gastrocnemius, semintendinosus, semimembranosus) involvement that occurs in LGMD2B could be explained by the fact that these muscle groups undergo shortening and lengthening cycles during exercise that causes active muscle regeneration–degeneration, but the failure of muscle repair due to dysferlin deficiency causes the failure to patch the membrane.

While the abnormal sarcomere remodeling due to the enzyme defect of calpain-3 might account for the clinical features of a chronic myopathy in LGMD2A, the severe damage to muscle fibers caused by the defect in an integral plasmalemmal protein, such as dysferlin, would explain a more active and rapidly evolving muscular dystrophy in LGMD2B.

Implications for future research or clinical practice

Since different pathogenetic mechanisms are at play in the different types of LGMD, it is conceivable that various types of therapeutic interventions are required to slow down muscle wasting. Up to now, several preclinical studies have been conducted: gene replacement has been tried in calpainopathy using viral vectors[22,23]. Possible therapeutic approaches are focused to counteract muscle wasting enhancing muscle mass, using inhibitors of myostatin in LGMD2A[24] or inhibiting the complement membrane attack complex in LGMD2B, where decay-accelerating factor is down-regulated[25]. Furthermore, strenuous physical exercise and prolonged muscle activity should be discouraged since they are likely to cause an exacerbation of physical damage to the plasmalemma and acceleration of dystrophic process.

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