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ARTICLE

Limb-girdle muscular dystrophy type 2A in Brazilian children

Distrofia muscular cinturas tipo 2A em crianças brasileiras

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ABSTRACT

Calpainopathy is an autosomal recessive limb girdle muscular dystrophy (LGMD2A) caused by mutations in CAPN3 gene. **Objective:** To present clinical and histological findings in six children with a molecular diagnosis of LGMD2A and additionally the MRI findings in two of them. **Method:** We retrospectively assessed medical records of 6 patients with mutation on CAPN3 gene. **Results:** All patients were female (three to 12 years). The mean of age of disease onset was 9 years. All of them showed progressive weakness with predominance in lower limbs. Other findings were scapular winging, joint contractures and calf hypertrophy. One female had a more severe phenotype than her dizygotic twin sister that was confirmed by muscle MRI. Muscle biopsies showed a dystrophic pattern in all patients. **Conclusion:** In this cohort of children with LGMD2A, the clinical aspects were similar to adults with the same disorder.

Keywords: calpainopathy, LGMD2A, MRI, dystrophy, child.

RESUMO

Calpainopatia é uma distrofia muscular de cinturas autossômica recessiva (LGMD2A) causada por mutações no gene CAPN3. **Objetivo:** Apresentar os aspectos clínicos e histológicos em seis crianças com diagnostico molecular de LGMD2A e adicionalmente os achados na RNM de músculo em duas delas. **Método:** Nos retrospectivamente analisamos os dados de prontuário de seis crianças com mutações no gene CAPN3. **Resultados:** Todos os pacientes eram do sexo feminino (3 a 12 anos). A média de idade de inicio da doença foi de nove anos. Todos mostraram uma fraqueza progressiva com predomínio nos membros inferiores. Outros achados incluíam escapula alada, contratura de tendão de Aquiles e hipertrofia de panturrilhas. Uma menina apresentou um fenótipo mais severo quando comparado a sua irmã gêmea dizigótica o que foi confirmado pelos achados encontrados na RNM de músculo. Em todos os pacientes a biópsia muscular mostrou um padrão distrófico. **Conclusão:** Nesta coorte de crianças com LGMD2A, os aspectos clínicos foram bastante similares a pacientes adultos com a mesma doença.

Palavras-chave: calpainopatia, LGMD2A, RNM, distrofia, criança.

Limb-girdle muscular dystrophies (LGMDs) are a heterogeneous group of disorders characterized by progressive weakness of pelvic and shoulder girdle muscles and a highly variable clinical course[1]. Twenty four autosomal recessive (LGMD2) and eight autosomal dominant (LGMD1) forms have been described to date[2].

Calpainopathy (LGMD2A), one of the most common forms, is caused by mutations in the *CAPN3* gene (locus15q15.1) (OMIM *114240), encoding calpain-3, a calcium dependent protease[3]. So far, approximately 490 unique allelic variants are known to be responsible for LGMD2A (Leiden Open Variation Database: www.dmd.nl)[4]. The phenotypic aspects include involvement of the pelvic, scapular and trunk muscles, but not of cardiac or facial muscles. Mental status is normal. Age at onset vary between 8 and 15 years for at least two-thirds of patients, with a total range of about 2 to 40 years[1,5]. However, studies that enrolled exclusively children with LGMD2A are rare in literature. A high level of phenotypic variability, even within members of the same family has been reported[6,7]. The slowly progressive course of the disease leads to loss of ambulation during adulthood and a near-normal life expectancy. Serum creatine kinase (CK) levels are elevated and muscle biopsy analysis shows a dystrophic pattern with evidence of necrosis, regeneration and lobulated fibers[5,8]. A final diagnosis of LGMD2A requires calpain quantification in the muscle biopsy via immunoblotting or mutation detection in the *CAPN3* gene[9].

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In this study, we present the clinical and histological findings in six cases of a cohort of Brazilian children with a molecular diagnosis of LGMD2A and additionally the MRI findings in two of them.

METHOD

Cases and immunohistochemical analysis

We retrospectively analyzed medical records of children of the Neuromuscular Disorders Section of the Hospital das Clínicas of FMUSP with a clinical and histological diagnosis of LGMD, during the period of three years. Of the initial 39 cases (2 to 18 years), according to the immunohistochemical staining, 15 were diagnosed as sarcoglycanopathies, five as dysferlinopathy and one as caveolinopathy. The other remaining 18 cases were submitted to molecular study for CAPN3 gene by Sanger sequencing of the "hot spot" coding regions. We identified six cases, with six different mutations, corresponding to a 15% of the children with LGMD (second more common form). All cases had an informed consent form signed by the legal guardian.

Molecular study

Cases without a specific muscular dystrophy diagnosis after immunohistochemical staining were screened for the 24 exons of the *CAPN3* gene. DNA was amplified by PCR using oligonucleotideo sequences juxtaposing exon-intron boundaries. PCR products were then sequenced using the Sanger method in an Applied Biosystems ABI 3130 sequencer (Foster City, CA, USA). Sequences were compared to the wild type*CAPN3*sequence and identified variants were matched to variant databases - LOVD, Exome Variant Server (EVS), and Exome Aggregate Consortium (ExAC) - to exclude polymorphisms.

RESULTS

Six cases (all female) from five families were diagnosed as calpainopathy. The age of onset of ranged from three to 12 years (average of nine years). Symptoms and signs at onset included lower limb-predominant proximal muscle weakness in all cases (four manifesting with frequent falls, one with difficulty climbing stairs and one with trouble walking). Winging of the scapulae was observed in five cases, heel tendon contractures in five, hypertrophy of calves in only one and scoliosis in two (Table). CK levels at onset of the disease ranged from 3 to 40 times the normal value. The weakness showed a slow progression in five of six cases and moderate in one case, and no case lost the walking capacity during follow-up. There was no clear association between age of onset and clinical course.

Cases 1 and 2 are twin siblings, dizygotic, and showed a marked difference at progression of the disease (Figure 1). Although in both the onset of symptoms was at 12 years old, the disease showed a faster progression in case 1 than in case 2, demonstrating an intrafamilial variability in calpainopathy. Case 1 initially presented with predominantly proximal weakness of the lower limbs, which assumed a progressive character, leading to significant difficulty in walking. Physical examination revealed winging of the scapulae and calf hypertrophy associated with heel tendon contractures. Her sister, case 2, had a different disease course with slow progression, and showed only minimal weakness, mild heel tendon retractions, winging of the scapulae, but no calf hypertrophy. Interestingly, both patients showed, in serial blood counts, persistent eosinophilia.

Electromyography showed myopathic features in all patients. Electrocardiography, holter, echocardiography and pulmonary function tests were normal in all patients.

Muscle biopsies (muscle biceps braquialis) showed characteristic dystrophic patterns in all cases. In addition to evidence of muscle fiber necrosis and regeneration, in two cases (cases 1 and 4) there were fibers with architectural changes of disorganized intermyofibrillar networks including "moth-eaten" and focal areas that do not react (central cores) (Figure 2).

The identified mutations in *CAPN3* gene analysis are presented in the Table.

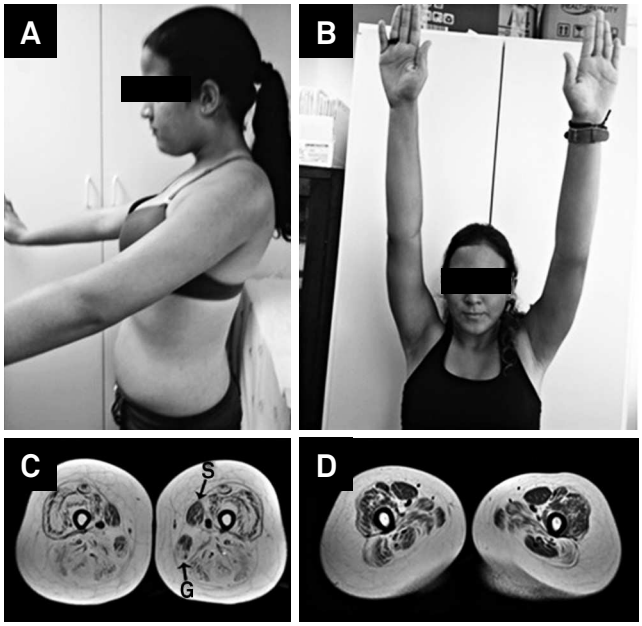

Figure 1. Twin sisters with calpainopathy, showing intrafamilial variability. (A) Case 1 showing proximal weakness in upper limbs; (B) minimal functional motor impairment of Patient 2. (C) Muscle MRI imaging in patient 1 show a striking involvement of the posterior thigh muscles with relative preservation of muscles sartorius and gracilis (arrows) when compared to images from her twin sister with milder symptoms (D). An informed consent form was signed by the legal guardian to publish images.

Muscle magnetic resonance imaging

Muscle MRI imaging was performed in the two sisters (cases 1 and 2) and showed symmetric involvement of the posterior thigh muscles with relative sparing of vastuslateralis, sartorius, and gracilis (Figure 1). Case 1, more severely affected, showed a greater involvement of these muscle groups when compared to her twin sister with milder symptoms.

DISCUSSION

We have described a series of Brazilian children affected with LGMD2A. Even though calpainopathy still seems to be the most frequent form of LGMD in different populations[10,11], in Brazil it only corresponds to approximately 30% of known cases[12]. In the present study, carried out in a sample population composed exclusively of children, we showed that it seems to be the second more frequent, after sarcoglycanopathies. In other studies, including some conducted in Brazil, it was also shown that sarcoglicanopathy is the most common form, particularly when taking into account more severe forms of the disease and forms with earlier onset[13,14].

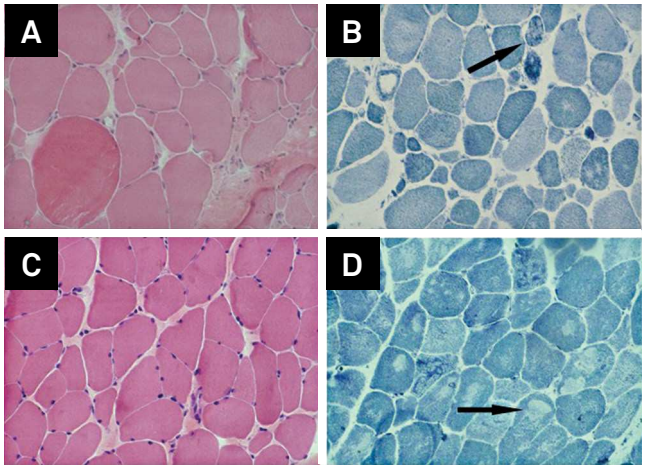

Figure 2. Muscle biopsies in children with calpainopathies. (A) and (C) Dystrophic patterns demonstrated by increased variability in fiber size, increased internalization of nuclei, split fibers and necrosis with regeneration; (B) and (D) Changes in internal intermyofibrillar architecture of fibers, characterized by "moth-eaten" and cores central (arrows).

In a study carried out by a European consortium in 2005 by Saenz et al.[15], which included 484 LGMD patients from different geographic regions, screening for CAPN3 mutations was positive in approximately 50% of cases. In the same study, it was observed that, when specific clinical criteria were used, 80% of cases could be correctly anticipated to have *CAPN3* mutations.

In our cohort, the age of onset ranged from 3 to 12 years with a median of 9 years. Clinically, our cases demonstrated a classic phenotype characterized by muscle weakness with symmetric involvement in a limb girdle distribution. In five of six cases the most prominent findings were winging of scapulae and heel tendon contractures. Serum CK levels at onset ranged from 3 to 40 times the reference value. It is important to note that high CK levels are found in all forms of autosomal recessive LGMD.

There was a slow progression of weakness in most of them. Despite one case having presented a faster progression, no case lost the walking capacity until the last evaluation. Cardiac and respiratory exams were normal in all cases, as described in the literature. Cardiac involvement has only been reported occasionally in LGMD 2A[16].

In the dizygotic twin cases, we detected one of the recurrent mutation in the Brazilian population, p.Arg110* in exon 2 in one allele and another mutation c258dupT on Exon 1. They showed slightly different clinical picture and evolution. Although in both the onset of symptoms was at 12 years, case 1 showed a faster progression, confirming the intra-family variability in calpainopathies, which had already been demonstrated in other studies[6,7]. Interestingly, both cases showed, in serial blood counts, persistent eosinophilia, which has been reported in patients with mutations in the *CAPN3* gene and eosinophilic myositis[17].

Regarding the muscle biopsy findings, the presence of typical features of muscular dystrophy was noted in all cases. In two patients we found "moth eaten" fibers and areas with disruptions in the intermyofibrillar organization, central

Table. Clinical and molecular findings in six patients with LGMD2A.

Cases	1	2	3	4	5	6
Sex/age (y)	F/16	F/16	F/16	F/7	F/17	F/10
Age of onset(y)	12	12	9	3	4	3
First symptoms	Frequent falls	Walking on tip toe	Difficulties in climbing stairs	Frequent falls	Frequent falls	Frequent falls
CK (X normal)	40x	40x	10x	4x	3x	3x
Lost walking capacity	No	No	No	No	No	No
Contractures	Heel	Heel	Heel	Heel	No	Heel
Others findings	Winging of the scapulae, calfhypertrophy	Winging of the scapulae	Winging of the scapulae	Winging of the scapulae, scoliosis	No	Winging of the scapulae, scoliosis
CAPN3 Mutation	c.328C>T - E2	c258dupT - E1 and c.328C>T - E2	c.2050+1G>A and c.2288A>G	c.258dupT	c.78G>A - E1	c.706G>A - E5

CK: creatine kinase; LGMD2A: limb-girdle muscular dystrophy type 2A.

cores. This finding is a nonspecific histological abnormality which is observed in different neuromuscular diseases[18].

MRI studies have been used to facilitate the diagnosis of LGMD. The distinction between the most common subtypes in adults (LGMD2A and LGMD2I) that account for up to 80% of recessive LGMD disorders (in some ethnic groups) is now possible using imaging techniques[19]. LGMD2A is characterized by the involvement of gluteus maximus, the posteromedial thigh area and the selective involvement of medial calf muscles. This is in marked contrast to LGMD2I where calf muscles are non-selectively involved. In addition, winging of scapulae and calf atrophy are more evident in calpainopathies than in LGMD2I. The anterior thigh muscles are more affected than the posterior ones in α-sarcoglycanopathy, where the calf muscles are relatively spared. This is in contrast to dystrophinopathies, where early and striking changes in the gastrocnemius muscles are prominent[19,20].

Muscle MRI performed in twin dizygotic cases of our study showed a striking involvement of the posterior thigh muscles. One case (case 2), with minimal functional motor impairment, showed a predominant involvement of the adductors and semimembranosus muscles, while her sister, with more restricted ambulation, had a more diffuse involvement of the posterolateral muscles of the thigh and of the vastus intermedius, with relative sparing of the vastus lateralis, sartorius and gracilis. Mercuri et al. reported clinical and MRI findings in seven LGMD2A patients who had prominent and early contractures and demonstrated that all patients showed a predominant involvement of the posterior thigh muscles. Furthermore, patients who presented minimal functional motor impairment showed a predominant involvement of the adductors and semimembranosus muscles, and patients with restricted ambulation had a more diffuse involvement of the posterolateral muscles with relative sparing of the vastuslateralis, sartorius and gracilis[21]. This is the same pattern found in our cases.

Our study suggests that, similarly to adults, LGMD2A in one of the more frequent forms of LGMD in children. Some aspects of clinical, such as, the presence of winging of scapulae, heel tendon contractures and muscle weakness with symmetric involvement in a lower limb girdle distribution suggest LGMD2A. The muscle MRI study emerges as a new method that may be helpful in establishing diagnostic strategies in LGMD children.

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