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Muscle Phenotypic Variability in Limb Girdle Muscular Dystrophy 2 G

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Abstract Limb girdle muscular dystrophy type 2 G (LGMD2G) is caused by mutations in the telethonin gene. Only few families were described presenting this disease, and they are mainly Brazilians. Here, we identified one additional case carrying the same common c.157C > T mutation in the telethonin gene but with an atypical histopathological muscle pattern. In a female patient with a long duration of symptoms (46 years), muscle biopsy showed, in addition to telethonin deficiency, the presence of nemaline rods, type 1 fiber predominance, nuclear internalization, lobulated fibers, and mitochondrial paracrystalline inclusions. Her first clinical signs were identified at 8 years old, which include tiptoe walking, left lower limb deformity, and frequent falls. Ambulation loss occurred at 41 years old, and now, at 54 years old, she presented pelvic girdle atrophy, winging scapula, foot deformity with incapacity to perform ankle dorsiflexion, and absent tendon reflexes. The presence of nemaline bodies could be a secondary phenomenon, possibly associated with focal Z-line abnormalities of a long-standing disease. However, these new histopathological findings, characteristic of congenital myopathies, expand muscle phenotypic variability of telethoninopathy.

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Keywords Limb girdle muscular dystrophy · Telethonin · LGMD2G · Nemaline rods · Paracrystalline inclusions

Abbreviations
LGMD Limb girdle muscular dystrophy

Introduction

Limb girdle muscular dystrophy type 2 G was initially described in Brazil (Moreira et al. 1997) through genotyping and linkage analysis to 17q11–12 in families with limb girdle muscular dystrophy. In the same year, a new muscle protein was identified and named telethonin (Valle et al. 1997), which is encoded by the *TCAP* gene. Patients with mutations in the *TCAP* gene showed total deficiency of telethonin in the muscle (Moreira et al. 2000; Vainzof and Bushby 2010).

Telethonin is a sarcomeric protein that is expressed in the skeletal and cardiac muscles, whose deficiency is associated with both limb girdle muscular dystrophy type 2 G (LGMD2G) and dilated cardiomyopathy (Vainzof and Bushby 2010). Telethonin is also called titin cap, as it binds to the titin amino terminus (Gregorio et al. 1998) and is involved with normal sarcomeric regulation and development, activated by MyoD through the promoter proximal E box. It is expressed after myogenin binding during embryogenesis (Zhang et al. 2011).

There is a worldwide small number of known patients with telethoninopathy, either with limb girdle muscular dystrophy type 2 G (Moreira et. al. 1997; Passos-Bueno et al.1999; Moreira et al. 2000; Lima et al. 2005; Yee et al. 2007; Olivé et al. 2008; Negrão et al. 2010; Waddell et al. 2012) or with congenital muscular dystrophy (Ferreiro et al. 2011; Almeida et al. 2012).

Here, we describe a newly identified patient with a relatively slow LGMD2G clinical presentation but with interesting histopathological findings including type 1 fiber predominance and nemaline rods, expanding the muscle phenotype of this disease to several forms of congenital muscular dystrophies.

Patient and Methods

Clinical examination, including muscle strength testing as rated by the Medical Research Council scale, serum creatine kinase (CK) levels, transthoracic echocardiogram, electrocardiogram, electromyography, standard enzyme histochemical, immunofluorescence staining, and transmission electron microscopy, were performed. The study was performed in accordance with the Declaration of Helsinki guidelines with the patient's written informed consent.

Results

A female patient, born of non-consanguineous parents, presented first clinical signs at 8 years old, which include tiptoe walking, left lower limb deformity, and frequent falls. She lost ambulation at 41 years old after a fall that caused bone fractures; at the same time, she noticed difficulty raising her arms. Now, at 54 years old, she presented pelvic girdle atrophy, winging scap-

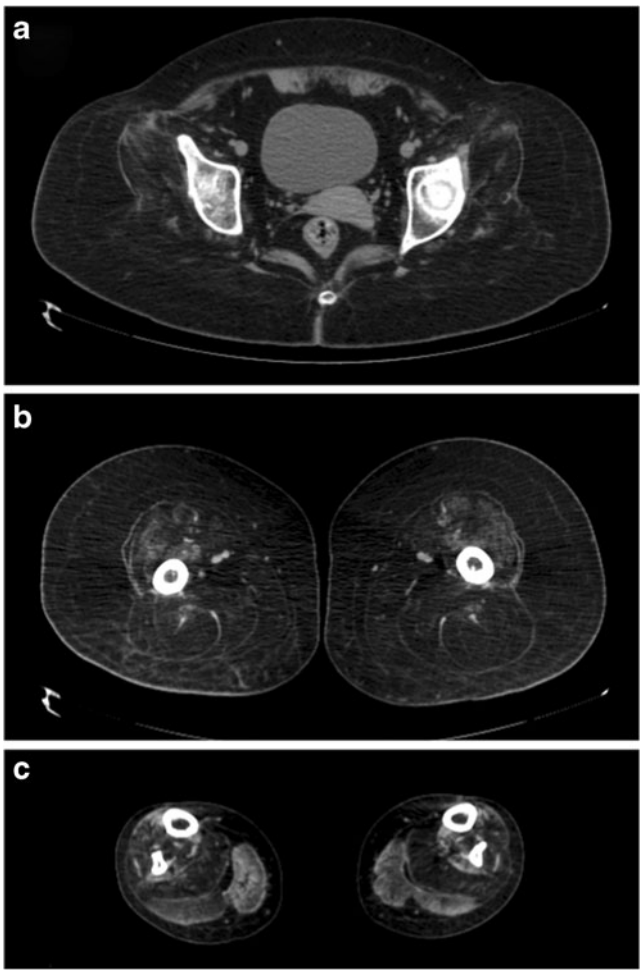

Fig. 1 Computed tomography showing severe fat muscle substitution of the glutei (**a**); anterior, medial and posterior thigh (**b**); and soleus, fibularis, and moderate substitution of tibialis anterior and gastrocnemii (**c**)

ula, foot deformity with incapacity to perform ankle dorsiflexion, and absent tendon reflexes.

There was proximal upper limb weakness and proximal and distal lower limb weakness with incapacity to walk. Her muscle strength was reduced. Transthoracic echocardiogram was normal, and electrocardiogram revealed a right bundle conduction disturbance. Electromyogram was consistent with a myopathic pattern, and CK levels were slightly increased (two times). Image studies demonstrated signs of proximal and distal fat muscle substitution in the upper and lower limbs, which is most prominent in the lower limbs (Fig. 1).

Muscle biopsy demonstrated adipose substitution, type 1 fiber predominance (90.3 %), fiber caliber variance, lobulated fibers, nuclear internalization (45 %), rare nemaline rods, small necrosis and phagocytosis foci (Fig. 2). Transmission electron microscopy revealed rare lipofuscin granules, fiber and myofiber splitting, rods, mitochondrial accumulations with proliferated cristae, dense matrix, and paracrystalline inclusions (Fig. 3). Immunohistochemical studies demonstrated normal reaction for three dystrophin epitopes (rod, carboxy terminus, amino terminus), sarcoglycans (alpha, beta, gamma, delta), and myotilin. Immunohistochemical analysis using anti-telethonin antibody (G-11 sc-25327) showed total deficiency of protein in the sarcomeres, which was confirmed through Western blot analysis (Fig. 4).

Molecular investigation excluded mutations compatible with facioscapulohumeral muscular dystrophy, dystrophinopathy, LGMD2A, LGMD2I, LGMD2C, LGMD2D, LGMD2E, LGMD2F, and spinal muscular atrophy. Sequencing of the two exons of the *TCAP* gene identified the common c.157C>T (Q53X) mutation in homozygous state.

Discussion

The number of patients described with limb girdle muscular dystrophy type 2 G in the world is small. The disease was initially described in Brazil (Moreira et. al. 1997; Passos-Bueno et al. 1999; Moreira et al. 2000; Lima et al. 2005), where it corresponds to approximately 4 % of classified autosomal recessive limb girdle muscular dystrophies (Vainzof and Bushby 2010). LGMD2G was later found in sporadic patients in China (Yee et al. 2007), Moldavia (Olivé et al. 2008), Portugal (Negrão et al. 2010), and Australia (Waddell et al. 2012). The pattern of mutation is, however, variable. The patient from Moldavia presents a TGG-to-TGA substitution in exon 1 that results in prema-

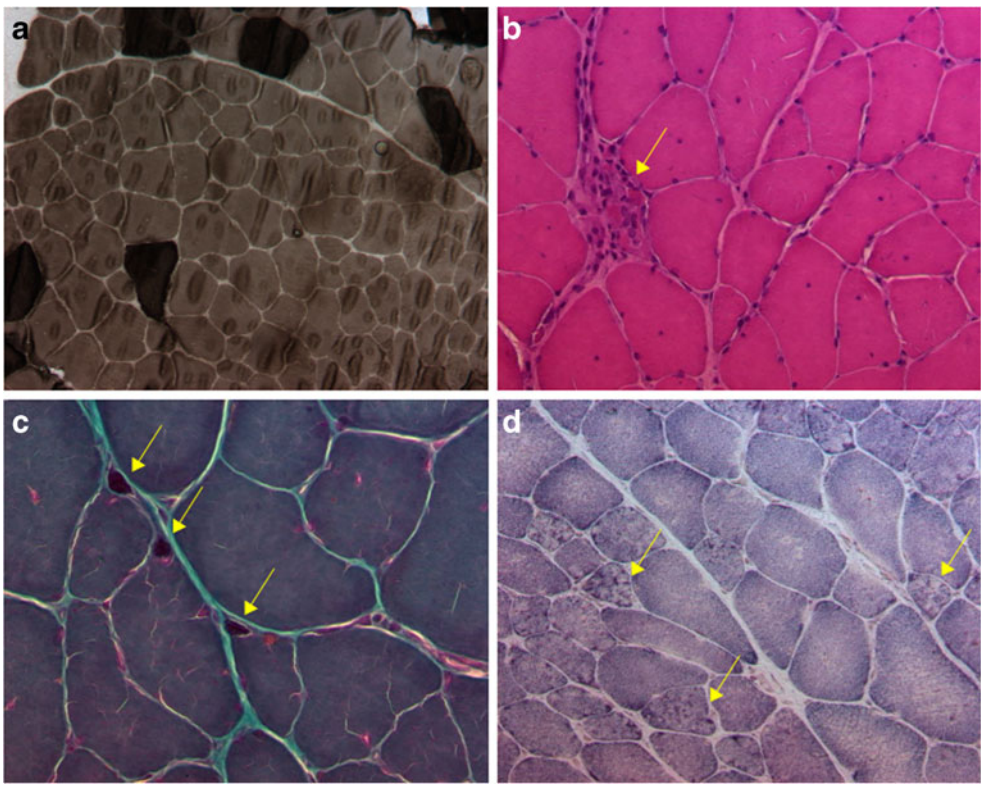

Fig. 2 Muscular tissue with variation in fiber caliber, type 1 fiber predominance (**a**), (*arrows*) necrosis (**b**), (*arrows*) subsarcolemal nemaline rods (**c**), and (*arrows*) lobulated fibers (**d**). Optic microscopy: adenosine triphosphatase pH 9.4, ×100 (**a**); hematoxylin and eosin, ×200 (**b**); Gomori's modified trichrome, ×400 (**c**); and succinate dehydrogenase, 100 (**d**)

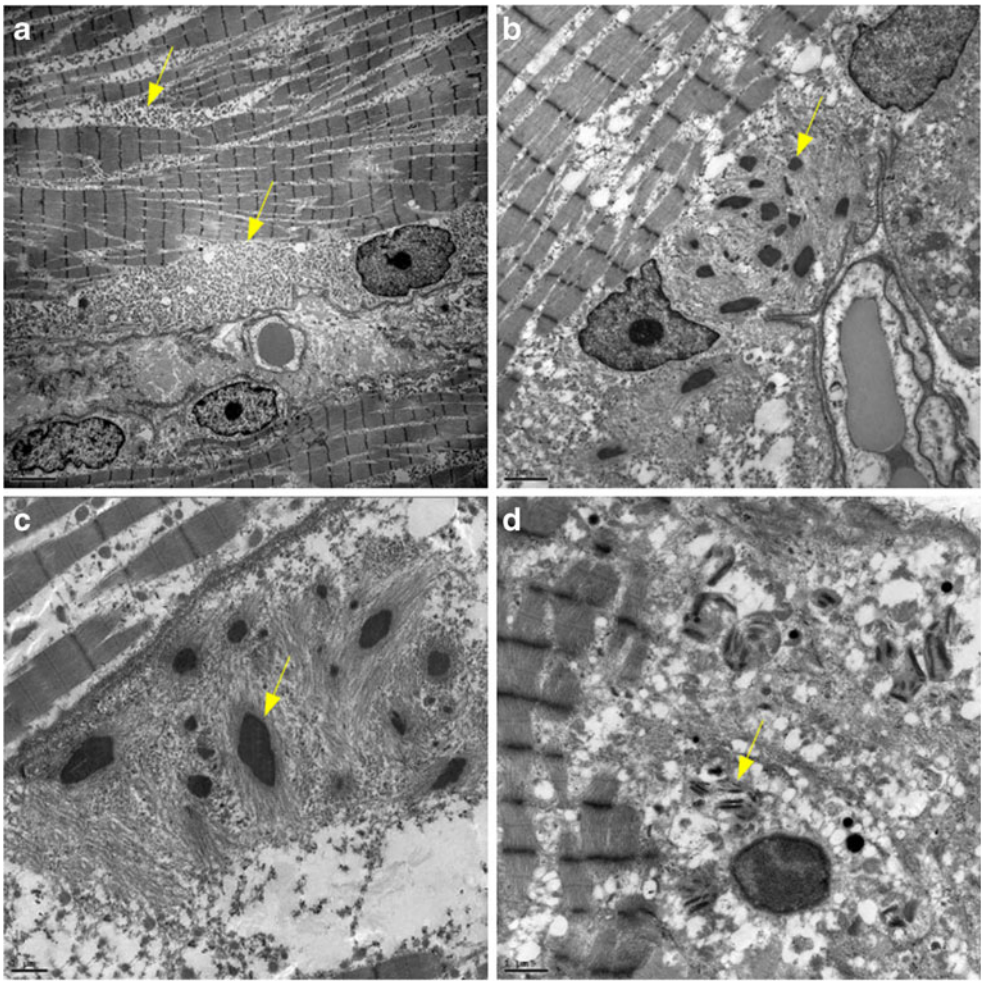

Fig. 3 Muscular tissue with (**a**) lobulated fibers with subsarcolemal and intermyofibrillary mitochondrial accumulations (*arrows*), (**b, c**) subsarcolemal nemaline rods (*arrows*), and (**d**) accumulations of abnormal mitochondria with paracrystalline inclusions (*arrows*). Transmission electron microscopy. ×2,500 (**a**); ×6,000 (**b**); ×10,000 (**c**); ×12,000(**d**)

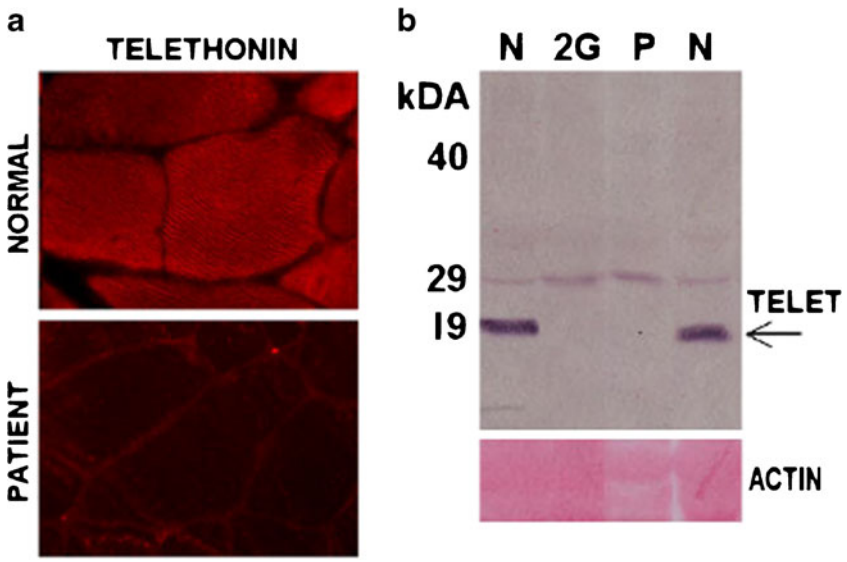

Fig. 4 **a** Immunofluorescence histochemical reaction showing telethonin deficiency in the patient, compared with normal control; magnification ×200. **b** Western blot analysis showing the 19-kDa band in normal control (*N*), and the absence of this band in a patient with LGMD2G (*2 G*) and in the patient of this study (*P*). Actin band in the ponceau pre-stained band is a control of the amount of loaded muscle proteins. (Antibody anti-telethonin Telethonin (G-11): sc-25327, Santa Cruz Biotechnology, Inc., at 1:50 dilution)

Table 1 Clinical, laboratorial, and morphological findings in telethoninopathy (LGMD2G)

	Moreira 1997, 2000; Lima 2005	Negrão 2010	Olive 2008	Ferreiro 2011	Present Case
Number of patients	12; 4	1	1	1	1
Start of symptoms (age in years)	9–15	20	15	Congenital	8
Age at diagnosis (years)	27–43	50	27	4	54
Illness duration at diagnosis (years)	15–27	30	12	4	46
Total CPK	3–30× (diminishes with time)	474 (2.6×)	1826 (10.9×)	1130 (4.2×)	269 (1.6×)
Loss of ambulation	Fourth decade	No	No	No	41 years
Necrosis	Yes	Rare	No	Yes	Rare
Regeneration	Yes	Rare	No	Yes	Rare
Vacuoles	Yes	Frequent	No	No	No
Lobulated fibers	No	Frequent	Atrophic type 1	No	Frequent
Nemaline rods	No	No	No	No	Yes

ture stop codon (Trp25X). In China, two mutations were described; one is a heterozygous deletion (c.45_46delTG). The Australian patient has the same duplication identified in China. The patient from Portugal presented the c.157C > T mutation. In the Brazilian population, with exception of one family, all the remaining identified 10 patients present the same c.157C > T (Q53X) mutation, which was also identified in the patient described here. It is important to note that Portuguese ancestry is frequent in Brazil, and the identification of the same common c.157C > T mutation in both populations may reflect a common ancestry (Negrão et al. 2010). Congenital muscular dystrophy telethoninopathy presentation was described in France (Ferreiro et al. 2011) and Brazil (Almeida et al. 2012).

The relatively slow LGMD2G clinical presentation, as well as the interesting histopathological findings in this patient, makes it necessary to perform a differential diagnosis with congenital myopathies. This patient presented type 1 fiber predominance, which is a common finding in congenial myopathies and was previously described in two patients with LGMD2G (Vainzof and Bushby 2010). Additionally, for the first time in LGMD2G, isolated fibers with nemaline rods were observed. Telethonin is present in the rods in nemaline myopathy muscle fibers, confirming its localization to the Z line of the sarcomere (Vainzof et al. 2002). Nemaline rods are associated with proliferative abnormalities of the Z lines, and they may be found in regenerating and denervating fibers in diverse conditions (Banker and Engel 2004; Engel and Banker 2004; Wallgren-Pettersson 2011). In this patient, nemaline bodies were considered a secondary phenomenon, possibly associated with focal Z-line abnormalities and long-standing disease. This patient presents the longest duration of symptoms described, and rods were not observed in the other patients (Table 1) (Moreira et al. 1997; Moreira et al. 2000; Lima et al. 2005; Olivé et al. 2008; Negrão et al. 2010; Ferreiro et al. 2011).

Lobulated fibers may be found in calpainopathy (LGMD2A), facioscapulohumeral muscular dystrophy, dystrophinopathy carrier, α-sarcoglycanopathy, myotonic dystrophy (Banker and Engel 2004), late onset nemaline myopathy (Irodenko et al. 2009; Wengert et al. 2011), and telethoninopathy (LGMD2G) (Olivé et al. 2008; Negrão et al. 2010). They may be related to improper mitochondrial anchoring mechanisms in intermyofibrillary spaces, common in prolonged chronic processes (Chae et al. 2001). Prominent secondary mitochondrial abnormalities, including paracrystalline inclusions, have been previously described in muscular dystrophies (Gamez et al. 2001). No vacuoles were observed in this patient's muscle biopsy (Table 1).

This report significantly expands the phenotypic variability of telethoninopathy. Later studies will be necessary to elucidate the physiopathological mechanisms involved in the development of limb girdle muscular dystrophy with deficient telethonin expression, related to the clinical, morphological, and imaginal phenotypic variability.

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