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Novel *TRIM32* mutation in sarcotubular myopathy

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Tripartite motif-containing protein 32 (*TRIM32*) is a member of the TRIM ubiquitin E3 ligases which ubiquitinates different substrates in muscle including sarcomeric proteins. Mutations in *TRIM32* are associated with Limb-Girdle Muscular Dystrophy 2H. In a 66 old woman with disto-proximal myopathy, we identified a novel homozygous mutation of *TRIM32* gene c.1781G > A (p. Ser594Asn) localised in the c-terminus NHL domain. Mutations of this domain have been also associated to Sarcotubular Myopathy (STM), a form of distal myopathy with peculiar features in muscle biopsy, now considered in the spectrum of LGMD2H. Muscle biopsy revealed severe abnormalities of the myofibrillar network with core like areas, lobulated fibres, whorled fibres and multiple vacuoles. Desmin and Myotilin stainings also pointed to accumulation as in Myofibrillar Myopathy. This report further confirms that STM and LGMD2H represent the same disorder and suggests to consider *TRIM32* mutations in the genetic diagnosis of Sarcotubular Myopathy and Myofibrillar Myopathy.

Key words: *TRIM32*, LGMD2H, sarcotubular myopathy, spheroids bodies, myotilin, desmin

Introduction

The *TRIM32* gene is composed of two exons encoding for a protein of 653 amino acids which is a member of the TRIM ubiquitin E3 ligases. *TRIM32* is characterized by a N-terminal conserved motif composed of a RING domain followed by a B-box and a Coiled-Coil domain, while its C-terminal portion presents 6 NHL repeats (1). The RING domain confers E3 ligase activity to *TRIM32*, the B-box and Coiled-Coil domains help the correct folding of the protein and the C-terminal domain mediates the interaction of *TRIM32* with its substrates. The main role of *TRIM32* consists in ubiquitination of different specific substrates (2, 3). Among these are included many muscular proteins such as actin, alpha-actinin, desmin, tropomyosin and dysbindin, thus indicating a role of *TRIM32* in promoting ubiquitin-dependent degradation of target proteins. Interestingly, *TRIM32* was reported to localize around the Z-line in skeletal muscle of guinea pig, showing a potential role of *TRIM32* in the maintenance and physiology of the sarcomere (4). Nevertheless *TRIM32* is involved in ubiquitination of cell cycle regulators (c-Myc, MYCN, p53) and the cell growth and transformation factor, Abi2 (5, 6), involving *TRIM32* in other signaling mechanisms such as the regulation of muscle satellite cells renewal and differentiation.

TRIM32 mutations were initially described in the Manitoba Hutterite population (41 patients) of North America presenting with a LGMD2H phenotype and the first mutation identified was the c.1459G > A (p. Asp487Asn) (7). LGMD2H is an autosomic recessive limb girdle muscular dystrophy associated with mildly to moderately increased creatine kinase (CK), presenting with a wide clinical presentation spectrum, ranging from virtually asymptomatic patients to rarely wheelchair-bound in the late course of their disease. The same mutation reported by Frosk et al. (7) was also identified in four pa-

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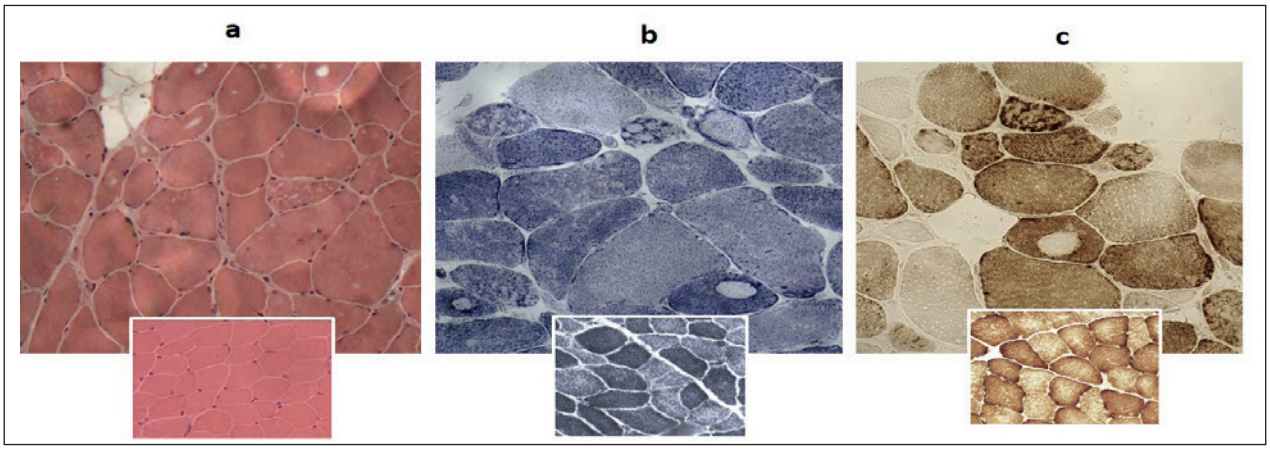

Figure 1. Muscle biopsy. a-c) Representative images of H&E (a), NADH (b) and COX (c) stainings from patient muscle biopsy are shown. Lobulated fibres, whorled fibres and multiple vacuoles containing amorphous material are evident. In small boxes pictures from a normal control biopsy with the same staining are presented for comparison.

tients, affected by Sarcotubular Myopathy (STM), a form of autosomal recessive myopathy (8). Schoser et al. (8) hypothesized that STM and LGMD2H represent different severity presentations of the same disease, since STM and LGMD2H present with clinical and histological overlapping findings. Later, additional mutations in *TRIM32* has been identified in LGMD2H patients of non-Hutterite origins (9-14).

Patients harbouring mutations in *TRIM32* share common features at muscle biopsy, such as increased fiber size variation, marked increase of internal nuclei and typical small, irregularly slit-shaped vacuoles that appeared empty. Electron microscopy showed the vacuoles to originate from focal dilations of the sarcoplasmic reticulum. The membranes limiting the vacuoles also showed sarcoplasmic reticulum-associated ATPase reactivity, confirming that the vacuoles arose from the cytoplasmatic organelles.

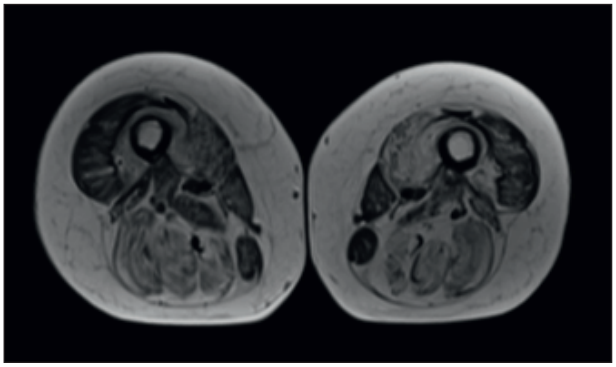

Figure 2. Muscle MRI. Muscle MRI of lower limbs showed a severe involvement of adductors longus, magno and brevis (Score 4), glutei and tight posterior muscles (Score 3).

In some muscle fibres small vacuoles were tightly packed and the membranes were partially disrupted resulting in larger vacuoles. Based on these findings muscle biopsies from patients with mutations in *TRIM32* gene have been defined as Sarcotubular Myopathy pattern (8, 15-18). Occasionally mild increase of endomysial fibrous connective tissue, necrotic fibers and fiber splitting were also reported. In two further reports, authors defined unspecific findings at muscle biopsy with no signs of sarcotubular aggregates in patients with TRIM2 gene mutations (12, 14).

The present work describes the clinical, histological and radiological features of a LGMD2H patient due to a novel homozygous mutation in the *TRIM32* gene with a typical Sarcotubular Myopathy pattern at muscle biopsy.

Case report

The proposita, a 66-year- old woman, was the second child of healthy unrelated parents. She was born at term after uneventful pregnancy and normal delivery. Psychomotor development was reported normal and she did not refer motor defects during her childhood nor early adulthood.

At 40 years she incidentally documented a moderate hyperckemia (4X) without any muscle symptoms. When she was 46 years old she showed proximal weakness, particularly at the pelvic girdle, leading to weakness while climbing stairs. In the following 20 years she presented with a slowly progressive lower limb girdle muscle weakness, being the upper limb performances less affected. She has never complained about respiratory symptoms nor cardiological involvement occurred.

At 49 years, a muscle biopsy from quadricep was performed, which revealed severe abnormalities of the

myofibrillar network with core-like areas, lobulated fibres, whorled fibres and multiple vacuoles containing amorphous material (Fig. 1a-c). The histological findings pointed out a sarcotubular myofibrillar disorder.

Latest neurological examination at the age 66 showed minimal hypotrophy at scapular girdle muscles without muscle strength impairment, pelvic girdle muscle weakness (quadriceps MRC 4, adductors MRC 3), waddling gait aided with a stick. Deep tendon reflexes were reduced in all limbs. Pseudo-hypertrophy of calves was evident. Rigid spine was also noted. Respiratory muscles function was spared with normal spirometry. Lower limb muscle MRI was performed at 64 years according to the protocol previously described (19). The MRI disclosed complete atrophy and fat substitution of adductors longus, magnus and brevis (Goutallier score 4), severe involvement of glutei and hamstrings muscles (Goutallier score 3) and a selective sparing of gracilis, sartorius and quadriceps muscles (Goutallier Score 2) (Fig. 2).

Recently this case was included in a group of undiagnosed muscular dystrophy patients to be analyzed by Limb Girdle Panel, an extended NGS testing panel which investigates the coding regions of 44 genes linked to LGMDs. We identified a novel homozygous mutation of *TRIM32*, NM_012210.3: c.1781G > A, (p. Ser594Asn) c.1781G > A/p.Ser594Asn, localized in the C-terminus NHL domain. Unfortunately patient's parents were not available for segregation study. Thus, to exclude a possible deletion of the second allele as previously reported (17) we performed qualitative and quantitative analysis of *TRIM32* cDNA and we didn't identify alternative transcripts. (data not shown).

The molecular model of *TRIM32* refined with YASARA (Yet Another Scientific Artificial Reality Application; www.yasara.org) showed that this mutation alters specifically the correct conformation of the NHL domain (Fig. 3a). Mutations of this domain have been also associated to Sarcotubular Myopathy (STM), a form of distal myopathy with peculiar features in muscle biopsy, now considered in the spectrum of LGMD2H.

Since different muscular-relevant proteins have been identified as *TRIM32* substrates, Desmin and Myotilin stainings were performed and the results pointed to accumulation of these proteins within the muscle fibers (Fig. 3b-c). Furthermore, Western blot analysis with anti-*TRIM32* antibody showed a modest reduction of *TRIM32* expression compared to the control (Fig. 3d).

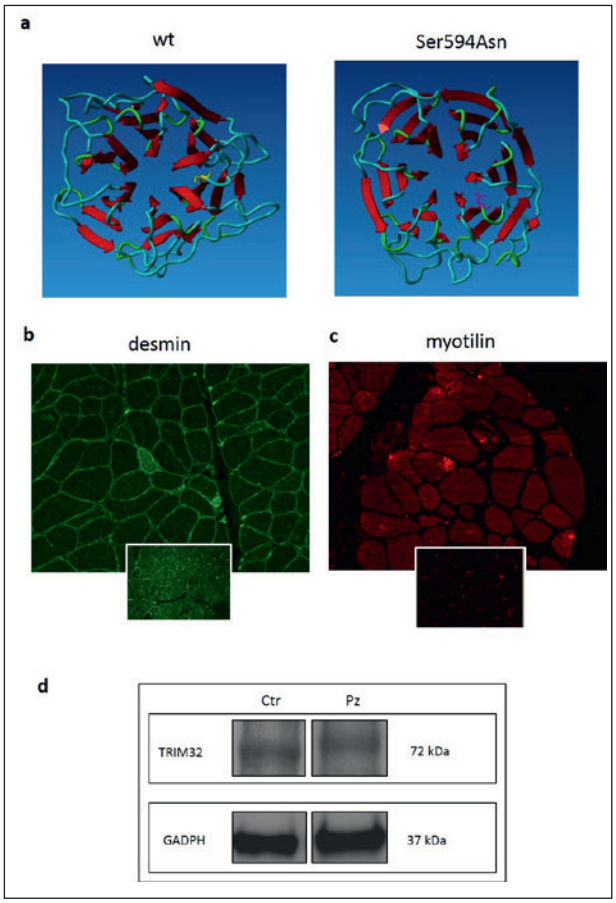

Figure 3. *TRIM32* and its substrates. a) *TRIM32* modeling of WT and mutated protein was performed YASARA. Mutation p.Ser594Asn alters specifically the correct conformation of the NHL domain. b-c) Desmin and Myotilin stainings pointed out accumulation of these proteins within the muscle fibers. In small boxes pictures from a normal control biopsy immunofluorescence with same antibody d) *TRIM32* protein levels were analyzed by Western blot. In the patient, the amount of *TRIM32* protein in muscle was only slightly reduced compared to control

Discussion

We describe a novel mutation in *TRIM32* gene in an adult patient who presented with a mild limb girdle muscle weakness without respiratory nor cardiac involvement. Muscle biopsy was suggestive of sarcotubular myopathy or myofibrillar myopathy. MRI findings are similar to those described in literature (12, 14), showing a preferential affection of the posterior thigh compartment with the sparing of sartorius, gracilis and the adductor longus.

Recently Johnson et al. (14) described 9 patients carrying pathogenetic mutations in *TRIM32*. Muscle biopsies showed non-specific myopathic or dystrophic changes in most patients, whereas scattered vacuoles were noted only in 3 cases. These were described as rimmed vacuoles containing basophilic membranes. Our patient, dissimilarly from this report, displays mainly myofibrillar network abnormalities with core-like areas, lobulated fibers, whorled fibers. Additionally, multiple

large vacuoles containing amorphous material/deposits similar to cytoplasmic hyaline bodies or spheroid bodies are present.

Interesting, spheroids bodies have been described in association with myotilin mutations (20) and a myotilin defect is also responsible of a form of Myofibrillar Myopathy. Hyaline bodies myopathies is a blurred definition of pathology alterations that, over the years, has been linked to mutations in several genes such as MYH7 in the form of myosin storage myopathy and FHL1 in the form of reducing bodies. In the whole, these myopathies with spheroid bodies, hyaline bodies but also cap and cytoplasmic bodies, have been referred as Surplus Protein Myopathies indicating an excess of proteins present in a granular or filamentous form (21). In this scenario we speculate that the mutation here described abolishes the interaction between *TRIM32* and its target proteins, which leads to a decreased ubiquitination and degradation by the proteasome machinery, thus inducing their accumulation to greater concentrations in the cytoplasm. To our knowledge, this is the first report which identified some of the proteins accumulated in the vacuoles in patient with *TRIM32* mutation. Indeed, immunostainings for Desmin and Myotilin, which are substrates of the *TRIM32* E3 ligase, pointed to their accumulation in the cytoplasm. We interpreted these findings as result of altered ubiquitination of these proteins which are known substrates of *TRIM32*.

Furthermore our patient, harbouring a mutation localized in the NHL domain, strengthens the previous findings according to which all the point mutations associated with LGMD2H are clustered in the C-terminus NHL domain, thus indicating a possible specific activity/property intrinsic to the NHL domain in the muscular tissue. The NHL domain is postulated to be critical for the recognition of protein targets to be ubiquitinated by this E3 ligase.

In conclusion, this report further confirms that STM and LGMD2H represent the same disorder and suggests to consider *TRIM32* mutations in the genetic diagnosis of Sarcotubular Myopathies and Myofibrillar Protein Myopathies. We also provided evidence that Desmin and Myotilin represent the contents of the vacuoles in a muscle biopsy from a *TRIM32* mutated patient.

Conflict of interest

The Authors declare to have no conflict of interest.

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