ACTA MYOL 2024;XLIII:139-144;
DOI: 10.36185/2532-1900-603

REVIEW

# The *TRIM32* geno-phenotype spectrum: a literature review and 25-year clinical follow-up of two brothers living with sarcotubular myopathy

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**Objectives. Pathogenic *TRIM32* gene variant was first described in 1976 in the Hutterite population of North America, presenting a phenotype of Limb-girdle muscular dystrophy R8 (LGMDR8, formerly termed LGMD2H). In recent years, different pathogenic mutations in this gene have been reported, with a spectrum of phenotypic heterogeneity, causing sarcotubular myopathy (STM), Bardet-Biedl Syndrome (BBS) and scapuloperoneal dystrophy. The genotype-phenotype correlation of this disease has been poorly reported.**
**Methods. Here, we perform a literature review to analyze the genotype-phenotype correlation of the pathogenic variants in the *TRIM32* gene. We also describe the clinical progression of two cases of STM in two patients presenting the D487N mutation in the *TRIM32* gene.**
**Results. We define the variety of LGMDR8 phenotypes associated with the identified *TRIM32* variants so far.**
**Conclusions. *TRIM32* mutations are responsible for a broad spectrum of clinical phenotypes.**

**Key words:** LGMDR8, *TRIM32*, sarcotubular myopathy

Received: August 5 2024
Accepted: November 22, 2024

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**How to cite this article:** Caputo M, Schoser B. The *TRIM32* geno-phenotype spectrum: a Literature Review and 25-year clinical follow-up of two brothers living with sarcotubular myopathy. Acta Myol 2024;43:139-144. https://doi.org/10.36185/2532-1900-603



## Introduction

J.N. Walton and F.J. Nattrass first used the expression limb-girdle muscular dystrophy (LGMD) in 1954 to describe patients with weakness and atrophy of proximal muscles in the limb girdles, sparing of facial muscles, and a moderately rapid course [1].
They classified LGMD as a different clinical entity from the X-linked recessive Duchenne Muscular Dystrophy (DMD) and supposed that LGMD most likely comprises a heterogeneous group of disorders. LGMD inheritance is autosomal [2].
In 1995, a European Neuromuscular Center (ENMC) consortium reached a consensus on a classification of LGMD subtypes based on molecular and genetic criteria, identifying the autosomal dominant loci as LGMD type 1, and the autosomal recessive loci as LGMD type 2 [3]. LGMD nomenclature adopted a progressive alphabetical letter indicating the order of gene mapping identification. The 2nd ENMC workshop, held in March 2017 in Naarden, the Netherlands, reached a consensus to update the definition and to review the classification of LGMD subtypes. Autosomal dominant LGMDs were named D and numbered from 1 to 5, and the recessive forms R numbered from 1 to 23 [3]. As of today, according to the current classification, there are 31 types of LGMD: 5 dominant subtypes (LGMD D1-D5) and 26 recessive subtypes (LGMD R1-R25 and R) [4] (Tab. I, as supplemental).
LGMDR8 (previously termed LGMD2H) is caused by pathogenic variants in the *TRIM32* gene encoding the *TRIM32* protein, an E3 ubiquitin ligase, identified in 2002[5]. The first

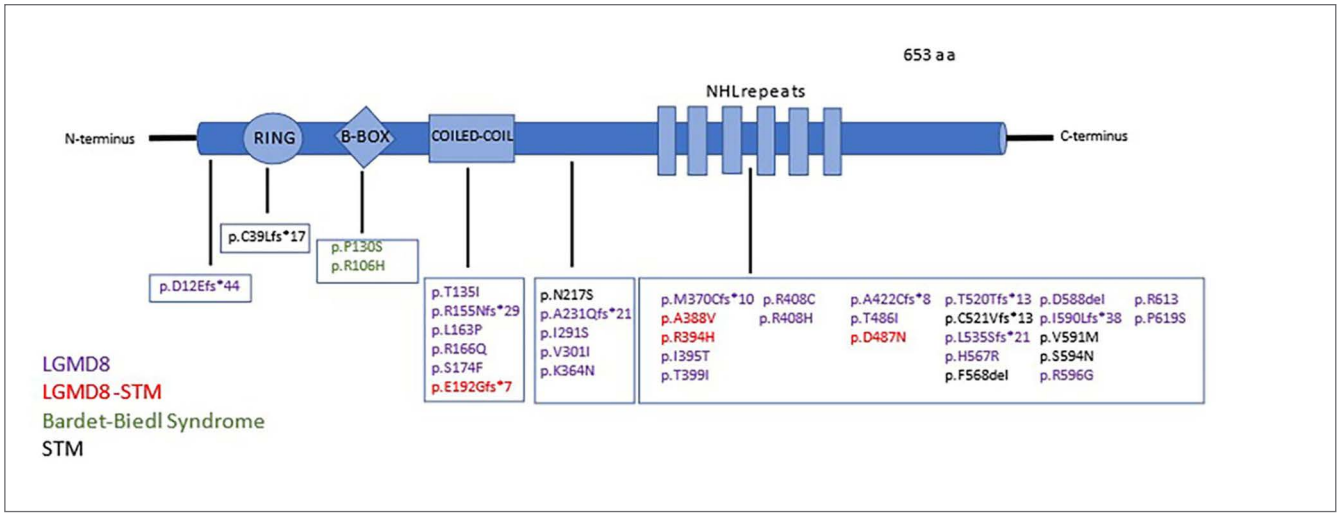


**Figure 1.** A literature review of TRIM32 mutations and phenotypes. Schematic representation of the TRIM-32 protein. Location of all detected variants associated with TRIM32-related myopathy patients (Guan et al., 2023) [14].

mutation (*TRIM32*: c.1459G > A, p.D487N) causing LGMDR8 was identified in 1976 in the Hutterite population of North America, a religious and genetic isolate brotherhood found throughout the North American plains with a high prevalence of LGMD [5].

The *TRIM32* gene encodes a protein of 653 amino acids [6] (Fig.1). The *TRIM32* gene contains the entire open reading frame in the second exon. The N-terminal conserved motif of the *TRIM32* protein consists of a RING structural domain, a B-box and a coiled-coil structural domain, while its C-terminal part consists of six NHL repeats. The RING domain is mainly associated with *TRIM32* E3 ubiquitin ligase activity, the B-box and coiled-coil domains are involved in proper protein folding, and the C-terminal domain mainly mediates *TRIM32* self-association and interactions with its substrates [7] (Fig. 1).

Most mutations causing LGMDR8 are located in the domain of the NHL repeat sequence, while mutations in the domain of the B-box lead to another disease called Bardet-Biedl syndrome (BBS), characterized by obesity, retinitis pigmentosa, polydactyly, renal abnormalities, learning disabilities, and hypogonadism, without the symptoms of myotonic dystrophy, which may imply different pathogenic mechanisms from LGMDR8 [8,9].

## Methods

Clinical, histological and molecular data from the probands were integrated within our literature review of *TRIM32* patients carrying different variants (Tab. II, as supplemental). As far as the literature review is concerned, the following terms were searched through PubMed encompassing the years 2005 to 2024 using the following keywords, filtering for human studies, abstract and full-text availability in English: "(*TRIM32*) AND (myopathy) AND (LGMDR8) AND (sarcotubular myopathy)". We included publications reporting patients of any age and providing clinical, instrumental, and molecular characterization.

## Results

### *Review of the literature*

The term LGMD defines a genetically inherited condition with progressive weakness with onset in the proximal limb girdle muscles, with age at onset of symptoms varying from early childhood (not congenital) to late adulthood. The progression of muscle weakness is usually symmetrical and variable among individuals and genetic types [10]. Other muscles, including the cardiac and respiratory muscles, are often affected. Phenotypically, LGMD subtypes are highly variable in their age of onset, speed of disease progression and overall severity [11].

The clinical course and the expressivity may be variable, ranging from severe forms with rapid onset and progression to very mild forms, allowing affected people to have fairly normal life spans and activity levels [12].

The classification of LGMD included dystrophies with proximal or distal-proximal involvement, with evidence at muscle biopsy of fiber degeneration and splitting, high creatine kinase (CK) values, muscle MRI imaging showing degenerative change and fibro-fatty infiltration. Degenerative changes on muscle MRI consist in replacement of skeletal muscle with adipose tissue as detected on standard T1 weighted axial images. Dystrophic changes in muscle histology include internalization of myonuclei, fibre necrosis, regeneration, and increased endomysial and perimysial fibrosis and adipose tissue [12]. Furthermore, patients must achieve independent walking; this can distinguish LGMD from congenital muscular dystrophies [13].

LGMDD, i.e., the autosomal dominant forms, usually have an adult-onset and a milder course because affected parents are usually in quite good health until their third decade. They are relatively rare, representing less than 10% of all LGMD [12].

The autosomal recessive forms (LGMDR) are much more common, having a cumulative prevalence of 1:15,000 [12]. It is estimated that the prevalence of LGMD is between 1 in 14,500 and 1 in 123,000 for all subtypes, with a carrier frequency of 1:211, but the prevalence of LGMD varies by region of the world [14].

LGMDR8 is an autosomal recessive limb-girdle muscular dystrophy.

Clinically, it is described as having wide variability without a specific hallmark of the disease. The onset is usually within the 2nd or 3rd decade of life, and the progression is slow; most patients remain ambulatory into the 6th decade of life [5].

It is a slowly progressive disease characterized by proximal muscle weakness, atrophic wasting of the lower extremities and mild to moderate CK elevation. Facial weakness, pterygoid scapulae, hypertrophic calves, and contracted Achilles tendons are not uncommon[10].

The clinical manifestations widely range from nearly asymptomatic to a more severe wheelchair-bound phenotype[11].

In recent years, different pathogenic mutations in this gene have been reported, with a spectrum of phenotypic heterogeneity. These mutations also cause sarcotubular myopathy (STM).

The two disorders are difficult to distinguish by clinical criteria because of the extreme phenotypic variability within each patient group and the common findings of proximal muscle weakness and wasting and increased serum CK levels. Still, STM generally has an earlier onset, in most cases reported during childhood, characterized by more severe weakness than LGMDR8.

## A 25-year follow-up report of two brothers

Here, we report and update the clinical progression of two German brothers presenting with a sarcotubular myopathy (STM), already described by Müller et al. in 1999, who made this diagnosis based on the histological findings in 1999. Later, in 2005, Schoser et al. genotyped these two brothers, finding the D487N mutation in the *TRIM32* gene, until that moment only described in LGMDR8 patients, thus addressing for the first time the genetic basis for this disorder to *TRIM32* mutation.

### *Case 1*

The motor and developmental stages of development were normally achieved. He could sit unsupported at seven months and started to walk at 17 months. He noticed his first symptoms at the age of six when he started complaining of weakness and pain when walking uphill and needed to stop every 100 m due to muscle pain. After exercise, he reported severe and long-lasting muscle aching. He complained of weakness in the arms, which did not interfere with

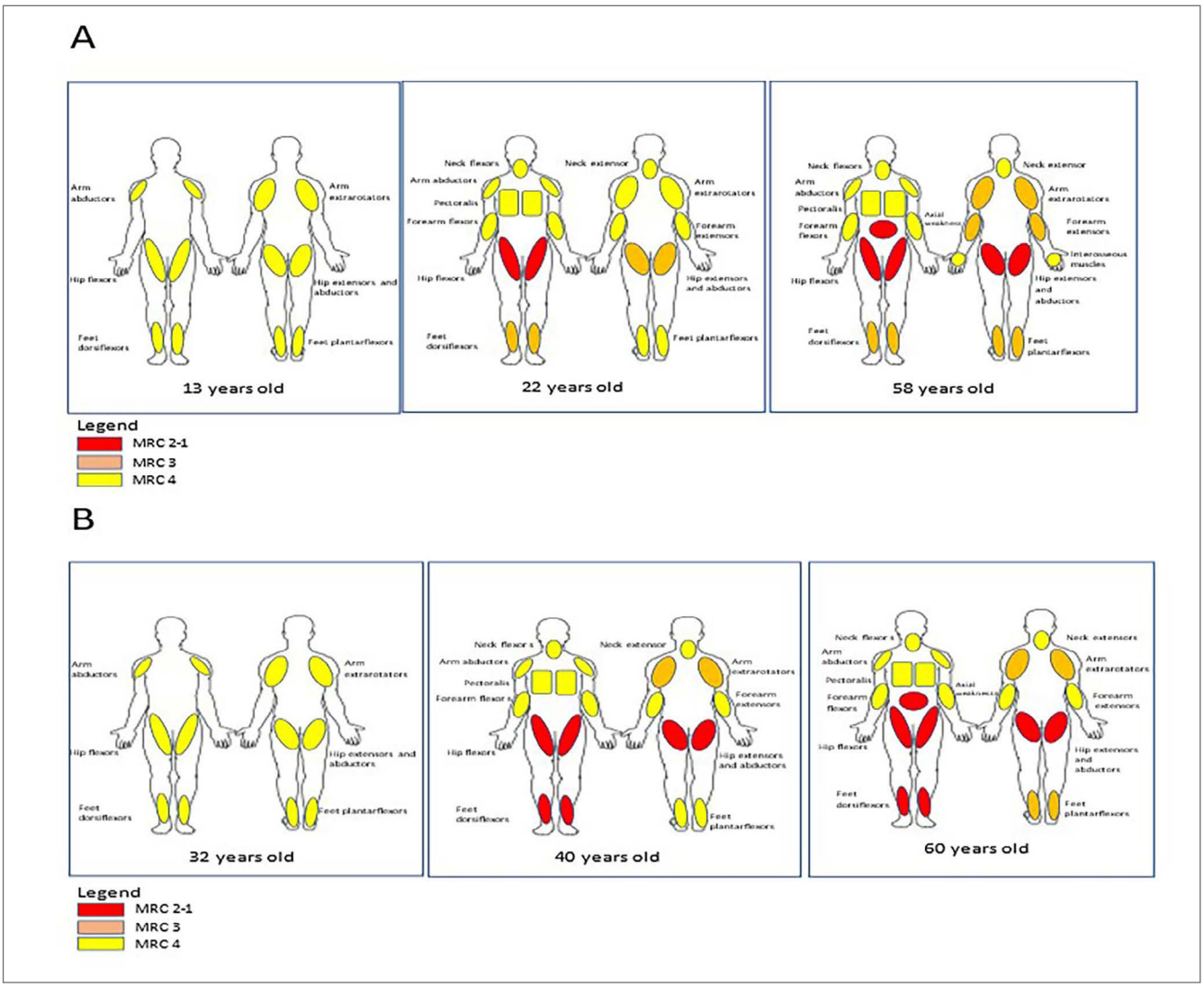


**Figure 2.** Iconic representation of the progression of the muscle weakness in the two brothers. (A) case 1, (B) case 2. Muscle strength has been assessed through the Medical Research Council Scale (MRC).

activities of daily living. A neurological examination at age 8 showed a moderate weakness of the proximal muscles of the arms and legs. Gowers' sign was positive. Winging of the scapula and moderate hypertrophy of the calf were also present. Tendon reflexes were absent. At 11, he needed help to rise from the floor. At age 26, he was unable to walk more than 50 m. He described post-exercise myalgia without muscle cramps. Clinical follow-up over the years showed that at age 31, he could not walk more than 10–20 m. The slight pelvic girdle paresis slightly progressed, and at age 38, he was wheelchair-bound (Fig. 2A). Respiratory insufficiency was present from the age of 44, thus requiring the use of non-invasive ventilation (NIV).

CK levels were in the range of 200-400 U/l. (normal < 180 U/L) Electromyographic examination performed at the age of 31, showed no spontaneous activity, with a slight myopathic pattern during voluntary activation with low-amplitude and short-duration muscle action potentials. Muscle ultrasound revealed markedly increased echo intensity in both proximal and distal muscles of the limbs. A muscle biopsy of the quadriceps femoris was performed and a light microscopic evaluation showed rounded muscle fibres with an increased number of internal nuclei, an increased variation in fibre size and small foci of Z-disk streaming. A slight type I fiber predominance was detected. NADH reductase reaction showed focal areas with loss of enzyme activity. No increase in acid phosphatase reaction was found. Immunostains with antibodies against cytoskeleton proteins, including dystroglycan, indicated normal findings.

Electron microscopy showed numerous vacuoles surrounded by membranes. No structures were seen within the vacuoles. Electron microscopy studies showed that the smallest abnormal spaces arose from focal dilations of the sarcoplasmic reticulum. Coalescence of the smaller vacuoles caused larger ones, of which the limiting membranes often degenerated. The membranes limiting the vacuoles showed sarcoplasmic reticulum-associated ATPase reactivity confirming that the vacuoles arose from the sarcoplasmic reticulum. Degenerating or necrotic fibers were not observed, and inflammatory changes were absent [15,16].

### *Case 2*

The patient didn't complain of any disturbance until the age of 32 years. From that age, he started complaining of aching of the legs without muscle cramps after physical exercise, other than weakness, after 30 minutes of running. Neurological examination showed a slight pelvic girdle paresis, with moderate hypertrophy of the calves and absence of tendon reflexes. The patient could rise from a prone position without using his arms. Gowers' was negative. He also reported difficulty climbing stairs and running for long distances. When he was 34, he started complaining of proximal upper limb weakness, but without explicit functional limitation. At age 40, he was able to walk unaided for 50 m. He started having numerous falls. Therefore, he started using a wheelchair outside, and after a few months, he also needed a stick for small distances at home. Symptoms had an initial rapid course, leading the patient to a loss of autonomy in 1.5 years. After a few months, the patient was utterly wheelchair-bound (Fig. 2B). Currently, at age 58, the patient can stand up for a very short time and can do some steps with double support. At age 52, he started complaining of respiratory symptoms. Therefore, the use of NIV was necessary.

CK levels were elevated between 900 and 1500 U/l. Electromyographic examination at the age of 32, showed a slight myopathic pattern in the quadriceps femoris, tibialis anterior, and biceps brachii muscles without spontaneous activity. Muscle ultrasound revealed markedly increased echo intensity in both proximal and distal muscles of the limbs.

A muscle biopsy of the quadriceps femoris was performed when he was 32 years old, and a light microscopic evaluation showed rounded muscle fibres with an increased number of internal nuclei and increased variation in fibre size. A slight type I fiber predominance was detected. NADH reductase reaction showed focal areas with loss of enzyme activity. No increase in acid phosphatase reaction was found. Electron microscopy showed numerous vacuoles surrounded by membranes. Only occasionally were some glycogen particles included. No structures were seen within the vacuoles [16]. Degenerating fibers showed increased activity of acid phosphatase. Immunohistochemistry revealed normal findings for dystrophin, b-dystroglycan, a-sarcoglycan, and g-sarcoglycan.

## Discussion

Here, we provide the long-term follow-up of two brothers with different disease progressions. Both patients presented first with exercise-induced myalgia and proximal weakness. In both patients, histological examination revealed a vacuolar myopathy with small vacuoles in some muscle fibers.

Clinically, lower limbs are mainly involved. Conversely, upper limbs are mildly involved, with mild functional impairment.

Neurological examination showed minimal hypotrophy at scapular girdle muscles with slight muscle strength impairment, but progressive pelvic girdle muscle weakness (glutei MRC 4, iliopsoas MRC 3), and distal lower limbs (tibialis anterior and hamstrings MRC 3/5), waddling gait aided with a stick, requiring later the use of the wheelchair. Deep tendon reflexes were reduced in all limbs. Pseudo-hypertrophy of calves was evident. Respiratory function was later in the course of the disease also involved, thus requiring the use of NIV.

The two brothers' clinical course differed, particularly regarding the age at onset and the severity of symptoms. The first patient showed rapidly progressive disease with loss of ambulation at age 30. At the same time, his younger brother had a slower course, becoming wheelchair-bound after several years from disease onset.

These two cases, hence harbouring the same genetic mutation of LMGDR8 (D487N) in the gene *TRIM32*, present a different clinical course, presenting an earlier age of onset and a faster rate of progression, thus being according to the literature data.

The D487N mutation involves one of six NHL domains of *TRIM32*, critical for the recognition of protein targets to be ubiquitinated by this E3 ubiquitinated ligase [15]. This mutation could abolish this interaction, thus leading to the missed ubiquitination of the proteins, which could not be degraded by the proteasome machinery and would accumulate to greater concentrations in the cell [5]. How these observations could cause the different phenotypes is unknown.

A literature review shows that different mutations in *TRIM32* are associated with a broad phenotypic heterogeneity. Tab. II summarizes

*TRIM32* gene mutations and their associated phenotypes [17-30].
The majority of patients described complained about proximal lower limb weakness, usually self-reported as difficulty in walking/running and climbing the stairs, and furthermore impairment in the scapular girdle, but without fair functional limitations. Other reported muscular symptoms were myalgias and exercise intolerance. Physical examination also revealed lower limb (calves or quadriceps) hypertrophy, as we reported in our two cases. Scapular winging was also noted, and one patient presented with a scapuloperoneal phenotype. The course of the disease results in a slight progression, mostly involving the ability to walk, which requires unilateral or bilateral aid at the beginning. Then, over the years, patients become wheelchair-bound. Cardiac involvement is rarely reported, while respiratory failure can occur. CK values range from normal to x20 the normal values.
Electromyography usually shows a myopathic pattern, often associated with neurogenic signs. MRI shows fibroadipose degeneration with fatty replacement. Histologically, in some cases, a dystrophic pattern is displayed; in others, small vacuolar myopathy is reported in cases of STM.
One case reported an association with MS [17], while another case reported Bardet Biedl Syndrome (BBS) [8]. It is interesting to note that the phenotypes of LGMDR8 and Bardet-Biedl syndrome are highly dissimilar: there is no muscle involvement in the very pleiotropic Bardet–Biedl syndrome, and the major symptoms of BBS (retinal degeneration, renal anomalies, polydactyly, and obesity) do not occur in LGMDR8.
*TRIM32* mutations have also been shown to be responsible for a different clinical phenotype: the sarcotubular myopathy syndrome, a form of autosomal recessive myopathy [15]. STM and LGMDR8 present with clinical and histological overlapping findings, but STM generally has an earlier onset characterized by more severe weakness than LGMDR8.

## Conclusion

Here, we define the variety of LGMDR8 phenotypes associated with the identified *TRIM32* variants by performing a literature review and describing the clinical progression of two cases of STM in two patients reporting the D487N mutation in the *TRIM32* gene. This review could help clinicians become aware of the different clinical pictures of this clinical spectrum of disorders.

### *Conflict of interest statement*

The authors declare no conflict of interest. The funders had no role in the study's design, data collection, analysis, interpretation, manuscript writing, or decision to publish the results.

### *Funding*

Not applicable

### *Author contribution*

MC and BS reviewed the literature and collected the data together. Both developed and wrote the manuscript.

### *Ethical consideration*

This study was performed under the LMU Munich Ethic Board approval no. 224-0242.

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