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ORIGINAL ARTICLE

A novel deep intronic mutation expands the genotype spectrum of MYH7-related myopathies

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Congenital myopathies are a heterogeneous group of rare inherited muscle disorders. Despite the good sensitivity of whole-exome sequencing in detecting pathogenic variants, many cases remain molecularly unsolved. Here, we present the case of a woman with congenital myopathy that remained unsolved for many years, in which the application of whole-genome sequencing enabled the identification of a novel deep intronic mutation in the *MYH7* gene.

A 22-year-old woman developed muscle weakness since infancy, with frequent falls, toe-walking, and difficulty climbing stairs. Muscle biopsy revealed atrophy of type 1 fibers relative to type 2, consistent with fiber-type disproportion. After a long "molecular odyssey," whole-genome sequencing performed on the patient–parents trio identified a de novo deep intronic variant in *MYH7*.

This case further underscores the importance of pursuing the search for the causative gene to enable more accurate clinical monitoring and tailored health care.

Key words: congenital myopathy, *MYH7*, intron, Whole Genome Sequencing

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Introduction

Congenital myopathies (CMs) are a heterogeneous group of rare inherited muscle diseases that typically present from birth or early infancy with hypotonia, muscle weakness, and skeletal deformities [1]. The main subtypes of CMs include nemaline myopathies, core myopathies, centronuclear myopathies, and congenital fiber-type disproportion (CFTD), with more than thirty causative genes identified to date. CFTD has most frequently been associated with mutations in *TPM3*, *RYR1*, and *ACTA1* [2]. In rarer cases, it can be caused by mutations in *MYH7*, *SEPN1*, *SPEG*, or *TPM2*.

Traditionally, the genetic diagnosis of CMs was performed through a gene-by-gene approach based on clinical and histopathological findings. However, this strategy was limited by incomplete understanding of disease-associated genes and, in some cases, by the large size of implicated genes such as *TTN*, *RYR1*, and *NEB*.

The advent of next-generation sequencing (NGS) – including gene panels, whole-exome sequencing (WES), and whole-genome sequencing (WGS) – has significantly transformed the genetic diagnosis of congenital myopathies. These techniques have led to the discovery of new disease genes, enriched the understanding of the genetic spectrum, and

improved genotype–phenotype correlations[3]. WES has become a key tool in the molecular diagnosis of CMs and has been incorporated into initiatives such as the French MYOCAPTURE project, which aims to identify novel pathogenic variants and genes involved in congenital myopathies [4]. However, WES is limited to coding regions and exon-intron boundaries, leaving deep intronic and regulatory elements unexplored. Although WES achieves a detection rate of approximately 50% for pathogenic variants, many patients remain genetically undiagnosed[4]. Therefore, determining the depth of molecular investigation requires balancing clinical benefit and cost-effectiveness.

As a result, many patients with CMs still lack a confirmed genetic diagnosis, which hampers personalized care, therapeutic opportunities, and reproductive counseling [5]. In this context, we present the case of a woman with CFTD whose diagnosis remained unsolved for many years but was ultimately solved thanks to WGS and transcriptional analysis of muscle biopsy.

Case description

The patient is a 22-year-old woman who received a diagnosis of congenital myopathy at age four. There was no evidence of reduced fetal movements during pregnancy, nor were there signs of neonatal hypotonia. Developmental milestones were achieved on time. Early symptoms included symmetric distal weakness, notably affecting the extensor hallucis longus and tibialis anterior muscles, resulting in an abnormal gait, frequent falls, and toe-walking. Over the years, she developed proximal weakness in the lower limbs and both proximal and distal weakness in the upper limbs. She is of Caucasian descent with no family history of myopathy, and her parents are not consanguineous.

At age four, she underwent neurological evaluation. Serum creatine kinase levels were within normal range. Electromyography revealed a myopathic pattern. Cardiological and cognitive assessments were unremarkable. A muscle biopsy showed type 1 fiber atrophy relative to type 2 fibers, consistent with fiber-type disproportion (Fig. 1A).

At age fifteen, following an episode of bronchopneumonia, she began nocturnal noninvasive ventilation. Over time, she underwent a series of genetic tests, including karyotyping, comparative genomic hybridization (CGH) array, Sanger sequencing of several single genes (*SMN1, SEPN1, ACTA1, TPM2, TPM3)*, and a congenital myopathy gene panel. All yielded negative results. She was first assessed at our Center at age nineteen. Clinical examination showed a waddling gait, bilateral foot drop, and hyperlordosis. There was severe and diffuse muscle atrophy, most pronounced at the scapular girdle with bilateral scapular winging and scoliosis. She could abduct her arms to only 30 degrees (Fig. 2A). To rise from a seated position, she widened her stance and pushed on her thighs; she was unable to rise from the floor or climb stairs. Muscle strength was markedly reduced, especially proximally in the upper limbs and distally in the lower limbs (Tab. I). Craniofacial features included an elongated facies, moderate facial muscle weakness, a high-arched palate, open bite, temporal muscle atrophy, and interphalangeal hyperlaxity.

Muscle MRI of the thighs revealed significant fatty replacement of the quadriceps with relative sparing of the rectus femoris, gracilis, adductors, and semitendinosus muscles. The anterior compartment

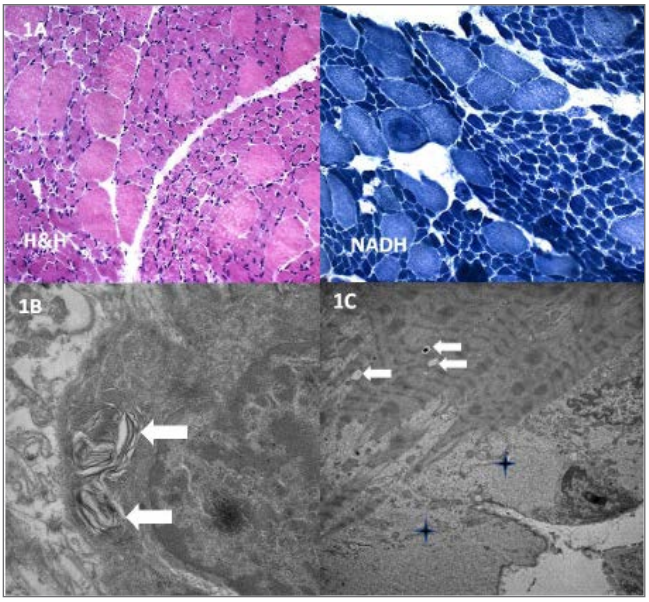


Figure 1. (A). Muscle biopsy. H&E and NADH staining show numerous hypotrophic type 1 fibers (dark blue on NADH) surrounding hypertrophic or normal type 2 fibers (light blue on NADH), suggestive of a fiber-type disproportion myopathy. No central nuclei, cores, fiber degeneration, or increased endomysial connective tissue were observed. (B-C). Ultrastructural analysis. Myelinoid-like elements composed of membranous structures are visible in the subsarcolemmal region (white arrows) (B). A few vacuoles are present in the intermyofibrillar space (white arrows), along with nonspecific granulofilamentous material in the subsarcolemmal region (crosses) (C).

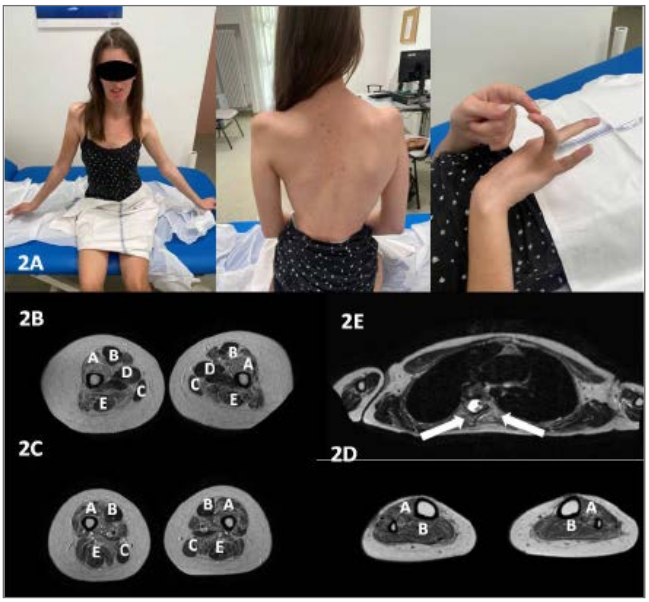


Figure 2. (A). Neurological examination. Diffuse muscle atrophy is evident, along with scapula alata, scoliosis, and distal joint hyperlaxity. (B-E). Muscle MRI (T1-weighted images). Thigh sections (B-C) show hyperintense signal consistent with fatty replacement of the quadriceps (A), with relative sparing of the rectus femoris (B), gracilis (C), adductors (D), and semitendinosus (E) muscles. The leg section (D) demonstrates relatively preserved musculature compared to the thigh, with mild hyperintensity of the tibialis anterior (A) and sparing of the soleus (B). Fatty infiltration of the paraspinal muscles is also visible (arrows) (E).

Table I. Manual Muscle Testing

Muscle	MRC (right/left)
Upper Limb	
Deltoid	1/1
Biceps brachii	4+/4
Triceps brachii	4/4
Flexor carpi radialis	4/4
Extensor carpi	4/4
Flexor digitorum profundus and superficialis	4/4
Interosseous muscles	4/4
Lower Limb	
Hip flexors	3+/3+
Quadriceps	4+/4
Hip adductors	4/4
Hamstrings	4/4
Tibialis anterior and extensor digitorum longus	2/2
Soleus and gastrocnemius (medialis and lateralis)	4/4

*Only abnormal muscles are reported in the table. Score is based on Medical Research Council scale (MRC).

of the leg showed more pronounced involvement compared to the posterior. Paraspinal muscles also exhibited fatty degeneration (Fig. 2B-E).

Ultrastructural analysis of a muscle biopsy confirmed fiber size variability with both small and atrophic fibers. Many fibers contained myelinoid-like elements composed of membranous structures, predominantly located in the subsarcolemmal region, possibly derived from the sarcotubular system. Some intermyofibrillar vacuoles and rare accumulations of nonspecific granulofilamentous material were also observed (Fig. 1B-C).

Whole-exome sequencing, analyzing 117 genes associated with congenital myopathies and 209 genes linked to neuromuscular disorders, was inconclusive. Additional *in silico* reanalysis with eight more genes rarely linked to fiber-type disproportion (*COL12A, CRPPA, MPDU1, RXYLT1, GOSR1, MSTO1, CACNA1H, B4GAT1*) did not reveal any causative mutations. A targeted analysis of a known pathogenic intron 11 variant in *COL6A1* and MLPA analysis for *MYH7* were also negative.

Eventually, trio-based whole-genome sequencing, including both parents, identified a novel de novo heterozygous deep intronic variant (NM_000257.4:c.5791-421C > G) within intron 39 of the *MYH7* gene (Fig. 3A). Transcriptional analysis performed on a muscle biopsy (using Illumina polyA selection kit) confirmed an abnormal splicing event with the in-frame insertion of a novel 117 bp exon between exons 39 and 40. This novel exon was not present in healthy control muscle and was located at genomic coordinates Chr14(GRCh38):g.23413293-23413409 (Fig. 3B-C-D).

This insertion is predicted to result in a *MYH7* protein with an extended tail segment: p.Lys1930_Gly1931insArgCysGlyPheThrMetLeuAlaArgLeuValLeuAsnSer*. In silico prediction tools supported the pathogenic splicing effect (Supplementary Fig. 1). According to the American College of Medical Genetics criteria[6], the variant was classified as pathogenic based on strong evidence of de novo occurrence (PS2), confirmation via functional studies (PS3), and absence in population controls (PM2).

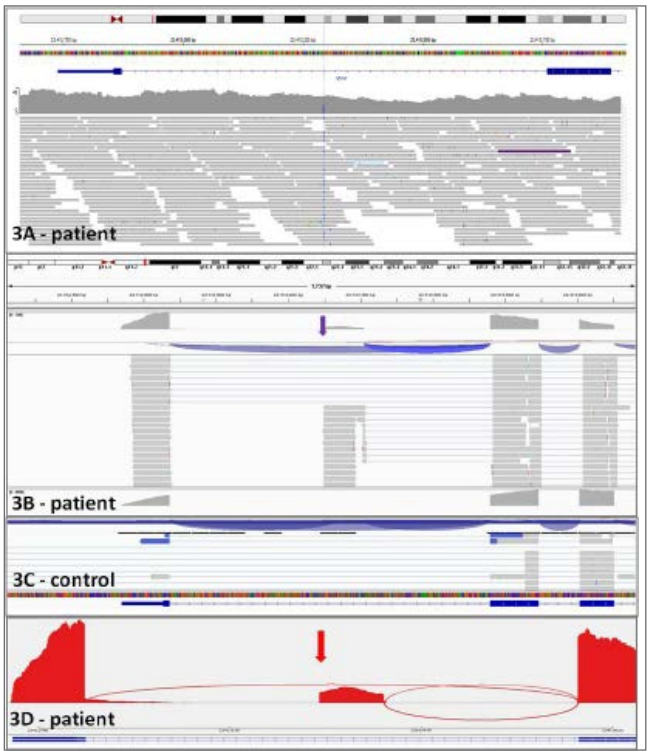


Figure 3. (A). Visualization of the patient's WGS bam file using the Integrative Genomics Viewer (IGV), showing the de novo c.5791-421C > G variant located in intron 39 of the MYH7 gene. (B-C). MYH7 transcript profiles of the proband and a healthy control, focusing on the region between exons 39 and 40. Visualization of the proband's RNA (B) sequencing bam file with IGV reveals intron retention, in contrast to the canonical splicing pattern observed in the healthy control (C). (D). Sashimi plot illustrating aberrant splicing caused by the intronic mutation. Arrow indicate the position of the c.5791-421C > G variant in the MYH7 gene.

Discussion

MYH7-related myopathies (RMs) are rare and clinically diverse early-onset conditions that can involve cardiac and respiratory systems, with a wide spectrum of pathological features. These include myosin storage myopathy, Laing distal myopathy, scapuloperoneal or limb-girdle myopathies [7], multiminicore disease, and congenital fiber-type disproportion. *MYH7* mutations can arise de novo or follow a dominant inheritance pattern, although rare autosomal recessive cases have also been described [8].

The *MYH7* gene encodes the slow/β-cardiac myosin heavy chain (β-MHC), a conserved protein expressed in cardiac muscle and slow-twitch (type 1) skeletal muscle fibers. Typically, mutations in the head and neck regions of myosin are associated with cardiomyopathies, while dominant mutations in the elongated C-rod domain are linked to skeletal muscle diseases[8]. The distribution of muscle involvement in *MYH7*-RMs appears related to the location of the mutation within the myosin tail. Mutations in the middle or proximal C-rod region are known to cause Laing distal myopathy [9] and are associated with dominant central core disease with eccentric cores. Mutations in the distal C-rod domain have been found in myosin storage myopathy and congenital fiber-type disproportion.

In our case, the intronic mutation results in the inclusion of a cryptic exon in the distal C-rod region, leading to elongation of the β-MHC tail and further validating the genotype–phenotype correlations previously proposed.

The pathological findings in *MYH7*-RMs are highly variable, ranging from protein aggregates in the form of tubulofilamentous hyaline inclusions (as seen in myosin storage myopathy), sometimes with rimmed vacuoles, to cores, multiminicores, and fiber-type disproportion. The vacuoles observed in our patient are consistent with *MYH7*-RMs, whereas the myelinoid structures have uncertain significance and have not been previously described in this context.

A recent study by Bahout and colleagues identified 26 different pathogenic or likely pathogenic *MYH7* variants in a cohort of 57 patients, highlighting the gene's high allelic heterogeneity and the need for clinicians to carefully interpret its variants [10].

The use of whole-genome sequencing is reducing the proportion of unresolved hereditary myopathies by enabling access to previously unexplored non-coding regions [10]. Nonetheless, interpreting WGS results remains a complex task, requiring integration of clinical findings with molecular data and, in many cases, validation through functional studies. Muscle biopsy plays a key role, particularly when muscle-derived RNA is needed to confirm splicing abnormalities. Trio-based WGS is crucial for identifying de novo and deep intronic variants with confidence.

In conclusion, applying a strategy of broadening sequencing coverage while narrowing down candidate variants is an effective approach in unresolved congenital myopathies. Persisting in the search for a genetic cause, even in clinically stable cases, remains worthwhile as it enables better monitoring for complications, provides accurate reproductive counseling, and may offer access to targeted therapies or clinical trials.

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Conflict of interest statement

The authors declare no conflict of interest.

Authors contributions

A.B. contributed to conceptualization, data curation, writing & editing of the manuscript. L.M. and L.M.N. contributed to data curation and editing of the manuscript. M.I. contributed to conceptualization, data curation. F.G. contributed to conceptualization, data curation, writing & editing of the manuscript.

Ethical consideration

The authors obtained consensus to publish data and photo.

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