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# Miyoshi Muscular Dystrophy Due to Novel Splice Site Variants in *DYSF* Gene

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## Abstract

Dysferlinopathies are a group of phenotypically heterogeneous disorders caused by pathogenic variants in the *DYSF* (DYStrophy-associated Fer-1-like) gene encoding dysferlin. The phenotypic spectrum includes Miyoshi muscular dystrophy (MMD), limb-girdle muscular dystrophy type R2, distal myopathy with anterior tibial onset, and isolated hyperCKemia. MMD is characterized by muscle weakness and atrophy predominantly affecting the calf muscles with symptoms onset between 14 and 40 years of age. There is no clear phenotype – genotype correlation for dysferlinopathy. We describe a 15-year-old girl who presented with a phenotype consistent with MMD. However, she was initially treated for presumed polymyositis without improvement. Subsequent genetic testing revealed two novel variants in *DYSF*: c.3225dup (p.Gly1076Trpfs*38) in exon 30 and c.3349-2A > G (Splice acceptor) in intron 30. No dysferlin was detected in a muscle biopsy using immunostains and western blots, a result consistent with dysferlinopathy that supports the pathogenicity of the *DYSF* variants.

## Keywords

myopathy, genetics, next-generation sequencing

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## Introduction

Dysferlinopathies are a group of autosomal recessive, phenotypically heterogeneous neuromuscular disorders caused by pathogenic variants in the *DYSF* (DYStrophy-associated Fer-1-like) gene. Typically, there is a loss of dysferlin protein from skeletal muscle, but there may also be dysfunction or a loss of dysferlin function. The phenotypic spectrum of dysferlinopathy includes Miyoshi muscular dystrophy (MMD) or Miyoshi myopathy, limb-girdle muscular dystrophy type R2 (LGMDR2, previously designated as LGMD2B), distal myopathy with anterior tibial onset (DMAT), and isolated hyperCKemia (elevated serum creatinine kinase).[1,2] MMD is characterized by muscle weakness and atrophy predominantly affecting the calf muscles (gastrocnemius and soleus muscles). Patients typically present with difficulties walking or standing on tiptoes. Symptoms onset can be between 14 and 40 years of age. Calf pain and discomfort can be seen. The disease can slowly progress to involve weakness and atrophy of proximal lower extremity muscles, as well as forearm muscles. LGMDR2 is characterized by slowly progressive proximal muscle weakness and atrophy of the pelvic and shoulder girdle muscles with onset in adolescence or young adulthood. DMAT is a rare form of dysferlinopathy that begins with weakness of the anterior tibial muscle at the age of 14–30 years and rapidly progresses to involve proximal muscles of both the lower and upper extremities. Serum CK level can be highly elevated (typically between 5000 and 20000 IU/L) in

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Search Terms: dysferlinopathy, LGMD, LGMDR2, muscular dystrophy

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dysferlinopathy.[1,2] Cardiac and pulmonary involvement have been noted in some patients with dysferlinopathy.[1,3]

The *DYSF* gene that encodes dysferlin is located at the 2p13.2 locus. Dysferlin is localized predominantly in the sarcolemma of the muscle, and it interacts with numerous other muscle membrane proteins. Dysferlin is critical for muscle repair as well as for maintaining the integrity of the muscle membrane.[2] The diagnosis of dysferlinopathy is established in a patient when there is typical phenotype associated with biallelic pathogenic variants in *DYSF*. Pathological assessment of dysferlinopathy by immunohistochemistry and immunoblotting using anti-dysferlin antibodies commonly reveals an absence or deficiency of dysferlin.[1] There may be clinical evidence of inflammation leading to a misdiagnosis of polymyositis,[4] however, a thorough evaluation of dysferlinopathy muscle biopsies failed to demonstrate consistent histopathologic evidence of myositis.[5] There is no clear phenotype – genotype correlation for dysferlinopathy beyond a few studies that report the variant c.2997G > T in *DYSF* is associated with a milder form of MMD and LGMDR2 and c.3373delG is associated with MMD.[6–8] The absence of a clear correlation between phenotype and genotype even exists within first degree family members.[2]

Here, we describe a teenage girl with MMD phenotype due to novel splice site variants in the *DYSF* gene.

## Case Report

Our patient is a 15-year-old girl who presented to the neuromuscular clinic with a 2-year history of clumsiness and falls. A few months prior to the clinic visit, she was hospitalized for muscle weakness. She had a 2 year history of tripping and falling. However, a few months before her hospitalization, she was noted to have significant difficulties with standing on her toes, skipping, climbing stairs, and curbs. The falls had worsened. She was born full term after a normal pregnancy. Her growth and developmental milestones were normal. There was no family history of any known neuromuscular disorders. Her medical history was significant for attention deficit disorder without hyperactivity, oppositional defiant disorder, depression, and anxiety. Her serum CK level during the hospitalization ranged between 7396 U/L and 16,102 U/L (reference: 20-128 U/L). Her blood counts, metabolic panel, and inflammatory markers were within normal limits. There was no evidence of rhabdomyolysis. A specific myositis antibody panel was unrevealing. Magnetic resonance imaging (MRI) of the pelvis revealed edema involving the bilateral lumbar, psoas, gluteal and quadriceps muscles. She underwent left vastus lateralis muscle biopsy with preliminary findings suggestive of a myopathic process with patchy, non-specific inflammation. She was treated for presumed polymyositis with pulse intravenous steroids continued by oral steroids. There was no symptomatic improvement following which a neuromuscular consultation was sought. At the time of neuromuscular evaluation, the patient had discontinued oral steroids with no worsening. Neurological examination showed bilateral ankle plantarflexion weakness, inability to toe-walk, difficulty with raising from a low stool, jumping, and running, and abnormal gait with increased hip flexion. The remainder of her examination was within normal limits.

Neuropathologic examination of her muscle biopsy revealed polygonal myofiber size variation, necrosis, regeneration, and internalized nuclei in the absence of significant lymphocytic inflammation, inclusion bodies, vacuoles, ragged red fibers, or neurogenic changes. Loss of dysferlin expression was demonstrated by immunofluorescence with three different antibodies [Romeo (exon 5; Abcam; rabbit monoclonal JAI-1-49-3; ab124684), NCL-Hamlet (exon 53; Leica Biosystems; mouse monoclonal HAM1/7B6), and NCL-Hamlet 2 (exons 11-12; Leica Biosystems; mouse monoclonal HAM3/17B2)] and by Western blot (NCL-Hamlet monoclonal antibody) with normal human skeletal muscle control. Embryonic myosin heavy chain (eMHC; Developmental Studies Hybridoma Bank, The University of Iowa; mouse monoclonal F1.652) was diffusely expressed, MHC class I expression was patchy (HLA-ABC; Agilent (DAKO); clone W6/32), and C5b-9 deposits (Abcam; clone aE11; ab66768) were identified on muscle fiber surfaces by immunofluorescence. Taken together, the neuropathologic findings were consistent with a necrotizing myopathy in the context of a dysferlinopathy (Figure 1).

She underwent next generation sequencing panel genetic testing (Invitae) that included sequencing and deletion/duplication analysis of 330 genes. It revealed two pathogenic variants in *DYSF*: c.3225dup (p.Gly1076Trpfs*38) in exon 30 and c.3349-2A > G (Splice acceptor) in intron 30. Her pulmonary function test, electrocardiogram, echocardiogram were within normal limits. The patient is followed in our multidisciplinary neuromuscular clinic.

## Discussion

The patient described here has a clinical phenotype suggestive of MMD. Muscle histopathology, immunostaining and western blotting confirm dysferlinopathy. Both variants identified in *DYSF* in our patient were novel. The first variant c.3225dup sequence change creates a premature translational stop signal (p.Gly1076Trpfs*38) in the *DYSF* gene. It is expected to result in an absent or disrupted protein product. Loss-of-function variants in *DYSF* are known to be pathogenic.[1,9] In addition, this variant is neither present in population databases nor has it been reported in the literature in individuals affected with DYSF-related conditions. Further, prediction analysis done by the reporting lab on the effect of this sequence changes on RNA splicing suggested that this variant might disrupt the consensus splice site. The second variant c.3349-2A > G affects an acceptor splice site in intron 30 of the *DYSF* gene. It is expected to disrupt RNA splicing. Variants that disrupt the donor or acceptor splice site typically lead to a loss of protein function.[10] In addition, disruption of this splice site has been observed in individuals with clinical features of LGMD.[11] Furthermore, studies have shown that disruption of this splice site has been associated with altered splicing resulting in insertion of 60 bp of intron 30 and the deletion of the 21 nucleotides of exon 31.[12] Thus, both of these novel variants have been predicted to be pathogenic. The neuropathologic findings reflect muscle biopsy changes that can be seen in the context of a dysferlinopathy, such as myofiber size variation, internalized nuclei,

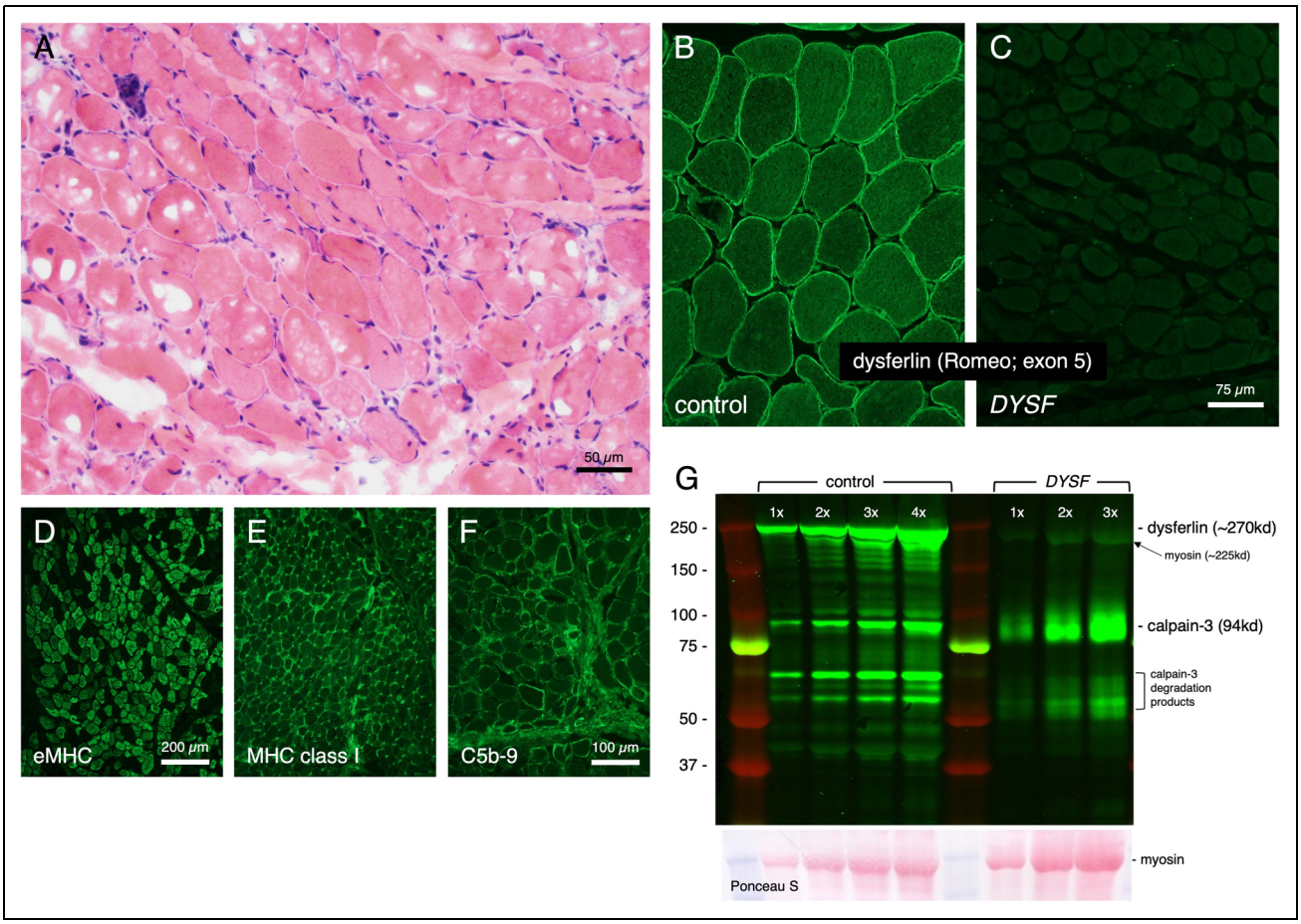


**Figure 1.** A. Frozen section stained with hematoxylin and eosin (H&E) demonstrates prominent size variation of polygonal myofibers and a few basophilic, regenerating fibers. B-C. Dysferlin expression is lost (C) with adequate control (B) by immunofluorescence. D-F. The majority of myofibers express embryonic myosin heavy chain, a marker of regeneration (D), MHC class I sarcolemmal expression is present (E), and C5b-9 deposition on muscle fiber surfaces is identified (F). G. Western blot shows loss of dysferlin (Hamlet monoclonal antibody) with adequate controls, while calpain-3 (full-length 94kd and degradation products) appears Normal (anti-calpain-3 mouse monoclonal antibody 12A2 from Leica Biosystems). The myosin band observed in the immunoblot and the Ponceau S-stained membrane is used to compare the amounts of protein loaded in the lanes of the gel.

myonecrosis, and regeneration. As such, neuropathologic review supports the pathogenicity of the novel *DYSF* variants in this teenage girl with a clinical phenotype of MMD.

We suggest that both variants, c.3225dup and c.3349-2A > G, can be added to the repertoire of variants in *DYSF* responsible for MMD phenotype of dysferlinopathy. This report highlights the importance of muscle biopsy pathologic evaluation in establishing a precise diagnosis when novel variants are detected by the genetic testing. Further studies are needed to better elucidate the phenotype genotype correlation of these variants.

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## Declaration of Conflicting Interests

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