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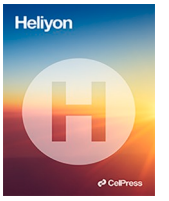

Case report

Clinical description of a homozygous Lys 1169* variant in the DYSF gene associated with autosomal recessive Miyoshi muscular dystrophy type 1: A familial case report

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ABSTRACT

Miyoshi Muscular Dystrophy Type 1 is a rare autosomal recessive myopathy caused by mutations in the *dysferlin (DYSF)* gene. This disease presents with progressive distal lower limb weakness, such as gastrocnemius and soleus muscles resulting in difficulty standing on tiptoes, walking, and climbing stairs. We describe a family consisting of 6 siblings, 2 affected males, 1 affected female, 1 affected-death female, and 2 unaffected females. The affected members of this family have lived without an appropriate diagnosis for more than 20 years. Our patients have a homozygous nonsense pathogenic variant of the *DYSF* gene with 0 frequency in the Genome Aggregation Database. Our study shows that genetic testing provides a crucial aid to doctors when the physical examination and the clinical history are insufficient. It also emphasizes that a precise and accurate diagnosis prompts the correct management of a complex case.

1. Introduction

Muscular dystrophies are inherited myogenic disorders characterized by progressive muscle wasting with variable distribution and weakness. These conditions are classified into different groups according to the affected muscles, the progression of the weakness, the age of appearance, and the associated genes [1].

Miyoshi Muscular Dystrophy Type 1 (MMD1), (Online Mendelian Inheritance in Man Catalog (OMIM) # 254130)) is a rare autosomal recessive myopathy caused by *dysferlin* (*DYSF*) gene mutations. MMD1 phenotype presents with progressive distal lower limb weakness, commonly affecting muscles such as gastrocnemius and soleus, resulting in difficulty standing on tiptoes, walking, and climbing stairs [2].

Dysferlin is a member of the ferlin-1-like protein family, weighs 237 kDa, and is mainly expressed in muscle, cardiomyocytes, blood monocytes, prefrontal cortex, placenta, and bone marrow. Dysferlin localizes in the cytoplasmic vesicles and sarcolemma. The main function is to repair the cell membrane mainly by processes involving calcium regulation and maintaining the integrity of the muscle membranes. In muscles, Dysferlin coordinates cytoskeleton remodel and sarcolemma repair [3–5].

Animal studies have shown that transgenically restoring muscle with dysferlin prevents muscular dystrophy from developing. Dysferlin secretes cytokines and chemokines from monocytes. This protein dysfunction contributes to the progression of the disease due to the increased secretion of these compounds that end up activating the inflammasome pathway. A hypothesis is that *DYSF* mutations alter the homeostasis of the muscular membrane, activating inflammatory pathways involving calcium-dependent proteases

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playing a role in cell damage and fibrosis, resulting in muscular dysfunction [6,7].

The first report of MMD1 was in 1967 by Miyoshi et al. describing a distal lower limb involvement case. However, Miyoshi et al., in 1986 reported some cases of this disease with distal forearm involvement.

Geographically, most cases are in Asia, especially in Japan. Followed by Spain and Italy in Europe and the United States in North America. Reports of Limb-Girdle Muscle Dystrophy R2 (LGMDR2) show a similar presentation to MMD1, except for a proximal lower muscle involvement.

Different phenotypes are associated with *DYSF* mutations, including the Miyoshi Muscular Dystrophy type 1, limb-girdle muscular dystrophy autosomal recessive 2 (OMIM #253601), and the distal myopathy with anterior tibial onset (OMIM #606768), presented in Table 1. Additionally, there are other phenotypes of Miyoshi Muscular Dystrophy caused by different genes, Miyoshi Muscular Dystrophy type 2 (MMD2, OMIM # 613318) and Miyoshi Muscular Dystrophy type 3 (MMD3, OMIM # 613319) [9,10].

The autosomal recessive MMD1 is caused by mutations in the *DYSF* gene. The phenotype starts from aged 15–25, with distal muscle weakness more prominent in the lower limbs. Common findings are decreased or absent ankle reflexes, muscle wasting in lower limbs, difficulty climbing stairs, rising from a squatting position, and toe walking. One of the most affected muscle groups is the gastrocnemius and soleus. Heel standing is preserved along with the sparing of the anterior tibialis muscle and small hand and finger muscles. Forearm muscles, weakness, atrophy, and decreased grip strength are expected. The muscle biopsy shows dystrophic and inflammatory changes and decreased or absent dysferlin staining. MRI of the affected muscles shows increased signal intensity congruent with fatty infiltration and inflammatory changes. Our patients have all the clinical features mentioned above except for the preservation of heel standing, explained by the long course of the disease in males A and B [2].

The MMD2 phenotype has a similar clinical presentation to MMD1, except for asymmetry present more commonly in MMD2. Two studies mapped the *MMD2* gene to chromosome 10 [5].

The MMD3 phenotype presents in patients aged 20–51 with distal muscle weakness and inability to stand on tiptoes, run, climb stairs, and eventually, problems rising from a chair later in the disease. This phenotype starts in the distal muscles and continues to the proximal muscles, such as the quadriceps, in the later stages of the disease. Other findings are calf hypertrophy early in the disease, atrophy later, and extensor digitorum brevis muscles hypertrophy. Muscle weakness and atrophy tend to be asymmetric. Muscle biopsy frequently reports disruption of the sarcolemmal membrane. CK in serum is high. This disorder is autosomal recessive and caused by mutations in the *anoctamin 5* gene (*ANO5*) located on chromosome 11 [10].

We describe a family with progressive muscular weakness for more than 20 years consisting of 6 siblings, 2 affected males, 1 affected female, 1 affected-death female, and 2 unaffected females. There are more than sixty reports, with more than one hundred patients until nowadays; however, to our knowledge, we are the fifth report in Latin America.

2. Methodology

We used a sequencing gene panel of neuromuscular disorders from peripheral blood samples on members of a family affected with a distal myopathy to determine the cause of their disease. The test was positive for a pathogenic variant in the *DYSF* gene. We report this familial case.

2.1. Case/case SERIES presentation

The affected male A (II-6) enjoyed good health throughout his childhood. However, from the age of 15, he began to experience lower muscular weakness, which initially presented itself during physical exercise. Over the course of three years, his condition worsened to the point where he could no longer climb stairs, and after four years, he was no longer able to walk without assistance. By the six-year mark, he had become dependent on a wheelchair for mobility. He has a history of hypertension and an unspecified severe traumatic brain injury that required surgery with minimal sequelae. The patient drinks alcohol and smokes socially every weekend (CAGE questionnaire 3/4). His brother, male individual B (II-12), came to the genetic outpatient clinic for progressive muscular

Table 1
Differential diagnoses of Miyoshi myopathy.

Characteristic	Miyoshi Muscular Dystrophy 1 [2,9,10,14,15]	Limb-Girdle Muscular Dystrophy R2 [10–12]	Distal Myopathy with Anterior Tibial Onset [2,10–12]
Manifestation Age	15–25	20–30	14–28
Inheritance	Autosomal Recessive	Autosomal Recessive	Autosomal Recessive
Main location	Lower limbs, distal, posterior compartment	Lower limbs, proximal and distal	Lower limbs, distal, anterior compartment
Muscular Dystrophy	Yes	Yes	Yes
Progression	Variable	Yes	Yes
Genes	*DYSF, MMD2, ANO5*	*DYSF*	*DYSF*
Creatine Kinase Levels	High	High	High
Muscle Biopsy	Extensive fibrosis and adipose tissue replacement with loss of most muscle fibers. *DYSF* is expressed normally in the muscle.	Variable degrees in fibers' size, muscle fibers' necrosis, and growth of connective tissue. Changes are non-specific.	Variable degrees in fibers' size, muscle fibers' necrosis, and growth of connective tissue. Changes are non-specific.

weakness. He had a history of childhood poliomyelitis affecting his right side, but he was otherwise healthy. At the age of 15, the patient also began to experience muscular weakness in his lower limbs, a symptom that progressed in a manner similar to that of his brother's. Presently, the patient is unable to stand or walk independently and has become reliant on a wheelchair for mobility. Over the course of approximately 15 years, his condition has gradually deteriorated, leading to his current state of wheelchair dependence. He drinks socially and does not smoke (CAGE questionnaire 2/4).

On physical examination, the brothers have a distal symmetrical weakness, qualified by the Medical Research Council (MRC) muscle scale as 3 in the distal muscle groups of the upper and lower limbs. Their feet are classified as 2 on the same scale, and the intrinsic hand muscles are unaffected. Weakness and pseudomyotonia were observed with the handgrip release test in the hand muscle groups. There are decreased reflexes and strength. Sensitivity is preserved. Their proximal muscle groups in the upper and lower limbs are also affected (MRC 3–4) but to a lesser degree than their distal muscles. The "calf heads on a trophy" sign cannot be evaluated due to weakness in their shoulders' muscles. No facial weakness or dysphagia is present. Cardiac and respiratory function are preserved.

Male A has symmetric muscle dystrophy and atrophy, and male B has asymmetric muscle dystrophy and atrophy of his body, predominant on his right side, due to the mentioned poliomyelitis.

Their family history is relevant for osteoarthritis in his mother and alcoholic cirrhosis in his father. Both parents died, their mother from malnutrition as a COVID-19 complication, and their father from hepatic failure as an alcoholic cirrhosis complication. The brothers had an affected sister (II-8) with similar symptoms that died in 2020 at age 35 due to COVID pneumonia and leukemia, as reported by some family members. The brothers have another affected sister (II-10) with similar symptoms but could not be contacted (Fig. 1).

We used a sequencing gene panel of neuromuscular disorders from peripheral blood samples. This panel used the Genome Reference Consortium Human Build 38 (GRCh38) and the NM_003494.4:c.3504dup nucleotide sequence. Both patients (Male A (II-6) and Male B (II-12)) have a homozygous nonsense pathogenic variant c.3504dup (p.Lys1169Glnfs*6) in exon 32 of the *DYSF* gene. There is no information about the frequency in the Genome Aggregation Database (gnomAD). Both patients have normal white blood count (WBC) and normal potassium levels to evaluate their pseudomyotonia. Serum creatinine kinase (CK) is elevated >2000 U/l. Both sisters II-8 and II-10, were not tested for reasons already mentioned. We will study the rest of the unaffected family members using DNA sequencing by Sanger method.

Every day they move in a wheelchair and manage to drive stick-shift cars to keep working and providing for their families. We are gathering physical therapists to develop a muscular rehabilitation program for these patients. Since they live in a rural community, access to permanent physical therapy is challenging. However, patients are currently performing exercises that were provided by a physiatrist. Pain is managed by over-the-counter painkillers such as acetaminophen on demand. Additionally, genetic counseling for these patients and their families was done.

Besides physical therapy, which plays a crucial role in maintaining muscle strength and mobility, and assistive devices and orthotics aiding in daily activities, there is still no definitive treatment for this disorder. Several options are currently under investigation in mouse models, such as myoblast transplantation, gene editing, and gene therapy with Adeno-Associated Virus dual-vector. Two molecules have shown promising results in mice: Ezetimibe, which demonstrated a significant reduction in fat accumulation in muscles, and recombinant human galectin-1 (rHsGal-1), which improved muscle membrane repair. However, further studies are needed to determine appropriate options for humans [8].

3. Discussion

We report the case of a family with two brothers suffering from muscular weakness that started at age 15 in the distal lower extremities and has progressed for over 20 years. They consulted various physicians without a diagnosis before visiting our outpatient clinic. The timeline of the cases of Male A (II-6) and Male B (II-12) are described in Fig. 2.

The clinical criteria for MMD1 include an autosomal recessive inheritance, a young adulthood onset, a preferential involvement of the distal lower limb compartment muscles, and a high serum creatinine kinase. The prognosis of this disease includes a progression of

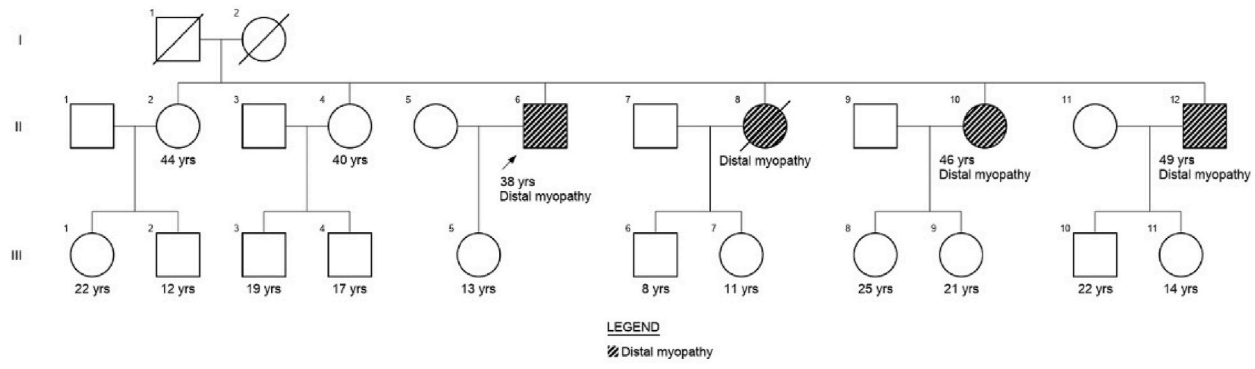

Fig. 1. Family Pedigree. Four out of six siblings have a distal myopathy presentation. Male A (II-6) AND Male B (II-12) have a homozygous nonsense pathogenic variant c.3504dup (p.Lys1169Glnfs*6) in exon 32 of the DYSF gene.

10–30 years between the initial symptoms and becoming wheelchair-dependent [2,11]. Our patients fulfil the clinical criteria. However, their physical examination was confusing at first encounter due to their past medical history.

In 2021, Moore et al. demonstrated that MMD1 and LGMDR2 are the same diseases. However, MMD1 is more commonly diagnosed in Asia, while LGMDR2 is more commonly diagnosed in Europe and America [11]. Most cases have a consanguinity background, but we could not get information of the preceding the parents, and they were not blood-related [12]. We must mention that the town where the family lives is small, and other autosomal recessive disorders like phenylketonuria have a high frequency.

Our case is important because the patients have an atypical neurological physical exam compared to a regular Miyoshi Muscular Dystrophy Type 1 due to other neurological comorbidities. This report supports the article of Moore et al. providing a phenotype of this disease with the presentation of Limb-Girdle Muscle Dystrophy R2 in America. Additionally, we offer differential diagnoses to consider in small populations with a high risk of consanguinity within ancestors.

Table 2 broadly compares previous studies and their variability depending on the country/region. The age of onset is a variable with an inconstant range, representing a change in the typical age of onset reported classically by Miyoshi's criteria. Furthermore, it is notorious that most studies report more cases of Limb Girdle Muscular Dystrophy (LGMD); however, the studies performed in Japan consistently report more cases of Miyoshi Myopathy (MM). These facts support the multiple phenotypes of a unique disease and emphasize the discussion of Moore et al., 2021, as previously mentioned.

Accurate clinical descriptions of variants are essential to the international medical community, especially for diseases with limited information about their complete spectrum. Dysferlinopathies are one such disease, with varying nomenclature depending on location, emphasizing the importance of precise and comprehensive clinical descriptions of associated variants to improve diagnosis and management.

Supplementary Table 1 presents 187 DYSF nonsense variants included in the ClinVar database, incorporating clinical descriptions of 14 variants reported in the literature identified in the Supplementary Fig. 1. The lack of consensus due to inadequate clinical descriptions can lead to Miyoshi Myopathy being overdiagnosed in Asian countries, while Limb Girdle Muscular Dystrophy may be more prevalent elsewhere. Additionally, multiple authors may describe the same variant as different diseases due to the diagnosis being based on the initial clinical presentation, even though it is the same disease.

This highlights the critical need for accurate and detailed clinical descriptions of variants associated with diseases like Dysferlinopathies, even when they are already in a database without a clinical description. By establishing a consensus on clinical descriptions, the medical community can improve disease diagnosis and management, preventing misdiagnosis and improving patient outcomes.

Some limitations of our study are that we did not perform electromyography or muscle biopsy as the genetic findings are enough to diagnose the condition. The death cause of the deceased affected sister could not be expanded as the family does not have contact with her husband and daughters. The other affected sister could not be contacted either. However, we are performing genetic studies on other members of the family.

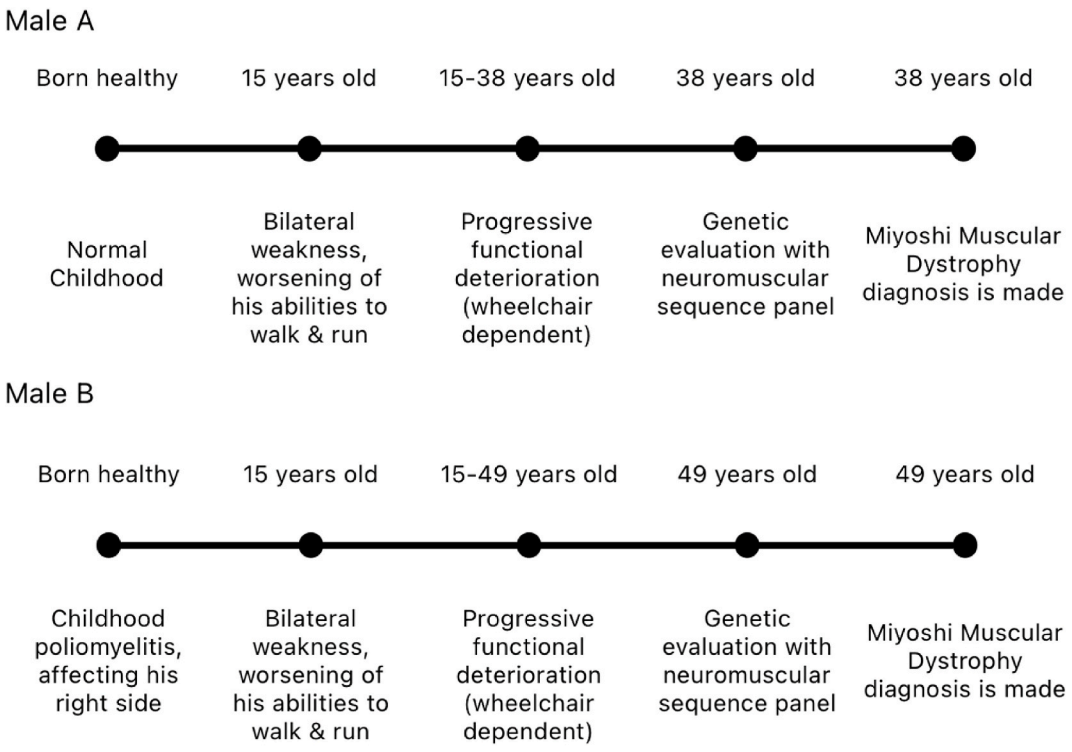

Fig. 2. Timelines of the cases of Male A and B.

Table 2
Comparison of previous studies and our study.

Study	Our Study	Miyoshi et al., 1986 [13]	Weiler et al., 1996 [14]	Cagliani et al., 2005 [15]	Nanlini et al., 2008 [16]	Klinge et al., 2010 [17]	Jin et al., 2016 [18]	Chakravorty et al., 2020 [19]	Izumi et al., 2020 [20]	Jacobs et al., 2021 [21]	Nashi et al., 2023 [22]
Number of Patients	2	17	9	27	28	36	89	27	209	187	124
Age of Onset	15	16–20	14–25	14–55	11–37	7–73	10–49	13–33	10–63	0–60	13–50
Geographic location	Ecuador	Japan	Canada	Italy	India	United Kingdom	China	India	Japan	United States of America	India
Consanguinity	Low	High	High	–	Low	–	–	Low	–	–	High
Diagnosed as MMD or LGMD (most patients)[a]	MMD 2	MMD 17	LGMD 7, MMD 2	MMD 15, LGMD 9	MMD 12, LGMD 12	LGMD 22, MMD 11	LGMD 45, MMD 31	LGMD 21, MMD 3	MMD 104, LGMD 82	LGMD 116, MMD 54	LGMD 64, MMD 29

[a] If not mentioned, the rest of the patients are classified as other Dysferlinopathy with a minor percentage.

4. Conclusion

In conclusion, we report the clinical description of a pathogenic variant in the *DYSF* gene. The patients waited more than 20 years for a diagnosis due to limited access to genetic testing and to the nonspecific signs and symptoms common in rare neurological diseases. Genetic testing provides a crucial aid to doctors when the physical examination and the clinical history are insufficient and emphasizes that a precise and accurate diagnosis prompts the correct management.

Data availability

The data associated with this study have been deposited into a public available repository. The data of the variant c.3504dup (p. Lys1169Glnfs*6) is available in the genetic database ClinVar with the code SUB12695495 since January 02, 2023.

Ethics statement

Written informed consent was obtained from the individuals for the publication of any potentially identifiable images or data included in this article. Approval by an ethics committee was not necessary.

CRediT authorship contribution statement

Alex S. Aguirre: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Vanessa I. Romero:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e35333.

References

[1] E. Mercuri, C.G. Bönnemann, F. Muntoni, Muscular dystrophies, Lancet 394 (2019) 2025–2038, https://doi.org/10.1016/S0140-6736(19)32910-1.
[2] A. Ivanova, S. Smirnikhina, A. Lavrov, Dysferlinopathies: clinical and genetic variability, Clin. Genet. (2022) 14216, https://doi.org/10.1111/cge.14216 cge.
[3] E. Harris, C.L. Bladen, A. Mayhew, et al., The Clinical Outcome Study for dysferlinopathy: an international multicenter study, Neurol Genet 2 (2016) e89, https://doi.org/10.1212/NXG.0000000000000089.
[4] Ja Urtizberea, G. Bassez, F. Leturcq, K. Nguyen, M. Krahn, N. Levy, Dysferlinopathies, Neurol. India 56 (2008) 289, https://doi.org/10.4103/0028-3886.43447.
[5] M. Cacciottolo, G. Numitone, S. Aurino, et al., Muscular dystrophy with marked Dysferlin deficiency is consistently caused by primary dysferlin gene mutations, Eur. J. Hum. Genet. 19 (2011) 974–980, https://doi.org/10.1038/ejhg.2011.70.
[6] D.P. Millay, M. Maillet, J.A. Roche, M.A. Sargent, E.M. McNally, R.J. Bloch, J.D. Molkentin, Genetic Manipulation of dysferlin expression in skeletal muscle, Am. J. Pathol. 175 (2009) 1817–1823, https://doi.org/10.2353/ajpath.2009.090107.
[7] R. Rawat, T.V. Cohen, B. Ampong, D. Francia, A. Henriques-Pons, E.P. Hoffman, K. Nagaraju, Inflammasome up-regulation and activation in dysferlin-deficient skeletal muscle, Am. J. Pathol. 176 (2010) 2891–2900, https://doi.org/10.2353/ajpath.2010.090058.
[8] C. Bouchard, J.P. Tremblay, Portrait of dysferlinopathy: diagnosis and development of therapy, JCM 12 (2023) 6011, https://doi.org/10.3390/jcm12186011.
[9] W.H.J.P. Linssen, M. de Visser, N.C. Notermans, et al., Genetic heterogeneity in Miyoshi-type distal muscular dystrophy, Neuromuscul. Disord. 8 (1998) 317–320, https://doi.org/10.1016/S0960-8966(98)00020-0.
[10] S. Penttilä, J. Palmio, T. Suominen, et al., Eight new mutations and the expanding phenotype variability in muscular dystrophy caused by ANO5, Neurology 78 (2012) 897–903, https://doi.org/10.1212/WNL.0b013e31824c4682.
[11] U. Moore, H. Gordish, J. Diaz-Manera, et al., Miyoshi myopathy and limb girdle muscular dystrophy R2 are the same disease, Neuromuscul. Disord. 31 (2021) 265–280, https://doi.org/10.1016/j.nmd.2021.01.009.
[12] K. Miyoshi, H. Kawai, M. Iwasa, K. Kusaka, H. Nishino, Autosomal recessive distal muscular dystrophy as a new type of progressive muscular dystrophy. Seventeen cases in eight families including an autopsied case, Brain 109 (Pt 1) (1986) 31–54, https://doi.org/10.1093/brain/109.1.31.
[13] A. Sula, A.R. Cole, C. Yeats, C. Orengo, N.H. Keep, Crystal structures of the human Dysferlin inner DysF domain, BMC Struct. Biol. 14 (2014) 3, https://doi.org/10.1186/1472-6807-14-3.
[14] Y. Yamanouchi, E. Ozawa, I. Nonaka, Autosomal recessive distal muscular dystrophy: normal expression of dystrophin, utrophin and dystrophin-associated proteins in muscle fibers, J. Neurol. Sci. 126 (1994) 70–76, https://doi.org/10.1016/0022-510x(94)90096-5.

[15] T. Weiler, C.R. Greenberg, E. Nylen, W. Halliday, K. Morgan, D. Eggertson, K. Wrogemann, Limb-girdle muscular dystrophy and Miyoshi myopathy in an aboriginal Canadian kindred map to LGMD2B and segregate with the same haplotype, Am. J. Hum. Genet. 59 (1996) 872–878.
[16] A. Nalini, N. Gayathri, Dysferlinopathy: a clinical and histopathological study of 28 patients from India, Neurol. India 56 (2008) 379–385.
[17] L. Klinge, J. Harris, C. Sewry, R. Charlton, L. Anderson, S. Laval, Y.-H. Chiu, M. Hornsey, V. Straub, R. Barresi, H. Lochmüller, K. Bushby, Dysferlin associates with the developing T-tubule system in rodent and human skeletal muscle, Muscle Nerve 41 (2010) 166–173, https://doi.org/10.1002/mus.21166.
[18] S.Q. Jin, M. Yu, W. Zhang, H. Lyu, Y. Yuan, Z.X. Wang, Dysferlin gene mutation spectrum in a large cohort of Chinese patients with dysferlinopathy, Chin Med J (Engl). 129 (19) (2016 Oct 5) 2287–2293, https://doi.org/10.4103/0366-6999.190671. PMID: 27647186; PMCID: PMC5040013.
[19] S. Chakravorty, B.R.R. Nallamilli, S.V. Khadilkar, M.B. Singla, A. Bhutada, R. Dastur, P.S. Gaitonde, L.E. Rufibach, L. Gloster, M. Hegde, Clinical and genomic evaluation of 207 genetic myopathies in the Indian subcontinent, Front. Neurol. 11 (2020 Nov 5) 559327, https://doi.org/10.3389/fneur.2020.559327. PMID: 33250842; PMCID: PMC7674836.
[20] R. Izumi, T. Takahashi, N. Suzuki, et al., The genetic profile of dysferlinopathy in a cohort of 209 cases: genotype-phenotype relationship and a hotspot on the inner DysF domain, Hum. Mutat. 41 (9) (2020) 1540–1554, https://doi.org/10.1002/humu.24036.
[21] M.B. Jacobs, M.K. James, L.P. Lowes, et al., Assessing dysferlinopathy patients over three years with a new motor scale, Ann. Neurol. 89 (5) (2021) 967–978, https://doi.org/10.1002/ana.26044.
[22] S. Nashi, K. Polavarapu, M. Bardhan, et al., Genotype-phenotype correlation and natural history study of dysferlinopathy: a single-centre experience from India, Neurogenetics 24 (1) (2023) 43–53, https://doi.org/10.1007/s10048-022-00707-3.