İstanbul Med J 2016; 17: 136-40
DOI: 10.5152/imj.2016.27870

Case Report

Dysferlinopathy: A Case Report and Literature Update

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Abstract

Dysferlinopathy is a rare autosomal recessive myopathy, resulting in the lack or absence of dysferlin production caused by mutations in the encoding gene. Dysferlin is a sarcolemmal membrane protein involved in the repair of membrane damage caused by calcium. There are four identified phenotypic dysferlinopathies, two of which are relatively frequently observed. Miyoshi myopathy and limb-girdle muscular dystrophy type 2B are frequently observed; the two very rare dysferlinopathies are distal anterior compartment myopathy and scapuloperoneal muscular dystrophy (observed in only one case). Serum CK levels are quite high, even in the pre-clinical period. A muscle biopsy typically shows dystrophic patterns, often accompanied with T-lymphocyte-based inflammatory changes. The clinical course of dysferlinopathy is usually much better than that of other recessive trait muscular dystrophies. Dysferlinopathies should be considered in the differential diagnosis of polymyositis to avoid unnecessary and potentially dangerous medications such as oral steroids or immunosuppressive therapies. Here we report the case a 21-year-old Syrian patient diagnosed with dysferlinopathy who has had serious CK elevations from the age of 1 and who had been diagnosed with polymyositis by a muscle biopsy 7 years ago and who therefore used steroids/azathioprine for the following 3 years.

Keywords: Dysferlinopathy, CK, polymyositis

Introduction

Dysferlinopathy is a rare myopathy with autosomal recessive inheritance, which results from the absence or deficiency of dysferlin protein due to a mutation in the gene encoding dysferlin. Dysferlin is the product of a gene that covers a large region of 150 kb and has 55 exons and is a membrane protein with a molecular weight of 237 kDa that plays a role in repairing calcium (Ca^{2+})-mediated sarcolemmal damage. Over 400 mutations identified in the dysferlin gene (*DYSF*, OMIM gene number 603009, chromosome 2p13, Gene Bank NM 003494.2) cause dysferlinopathy. The frequency of dysferlinopathy is reported to be 1/100,000–200,000. Dysferlinopathy is classified based on different phenotypic features into four types, namely distal anterior compartment myopathy; scapuloperoneal muscular dystrophy, which is observed in only one case; and, primarily, Miyoshi myopathy (MM) and limb–girdle muscular dystrophy (LGMD) type 2B (1, 2). MM is an early adult-onset disorder and has a slow progression, with weakness and atrophy, especially in the posterior compartment muscles of the lower limb such as, the gastrocnemius and soleus. Weakness may also occur in the proximal muscles in the later period, but no cardiac involvement is observed. Incidences have mostly been reported in Japan, and there are also reports from Tunisia, Israel, Saudi Arabia, the United States, France, and other Western countries. Very early and late-onset cases with atypical symptoms have also been reported. LGMD type 2B is a juvenile-onset condition; proximal muscle involvement is evident, atrophy in the lower extremity anterior, upper extremity distal, or scapular muscles is remarkable in the later period, and cardiac involvement may rarely be seen. It is clinically similar to LGMD type 2A owing to the lack of calpain-3, which is seen as a result of a mutation in *CAPN3*. Calpain protein levels may also be found to be low in patients with dysferlinopathy (3).

The clinical appearance is relatively benign compared with that of other autosomal recessive muscular dystrophies. A clinical picture may not be seen in every patient diagnosed with a dysferlin mutation, or different phenotypes such as MM and LGMD may be seen in patients of the same family; this suggests that dysferlin may also have a modifying effect via a protein that is the product of another gene. For example, although there is no mutation to cause a lack of calpain-3 in some patients with dysferlin deficiency, the level of calpain is also low (4-6). Nearly all patients have normal intelligence and, in general, no involvement of the cardiac or respi-

This case has been presented at the 17th International Internal Diseases Congress, 14-18 October 2015, Antalya, Türkiye.

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Received:
21.10.2015

Accepted:
17.01.2016

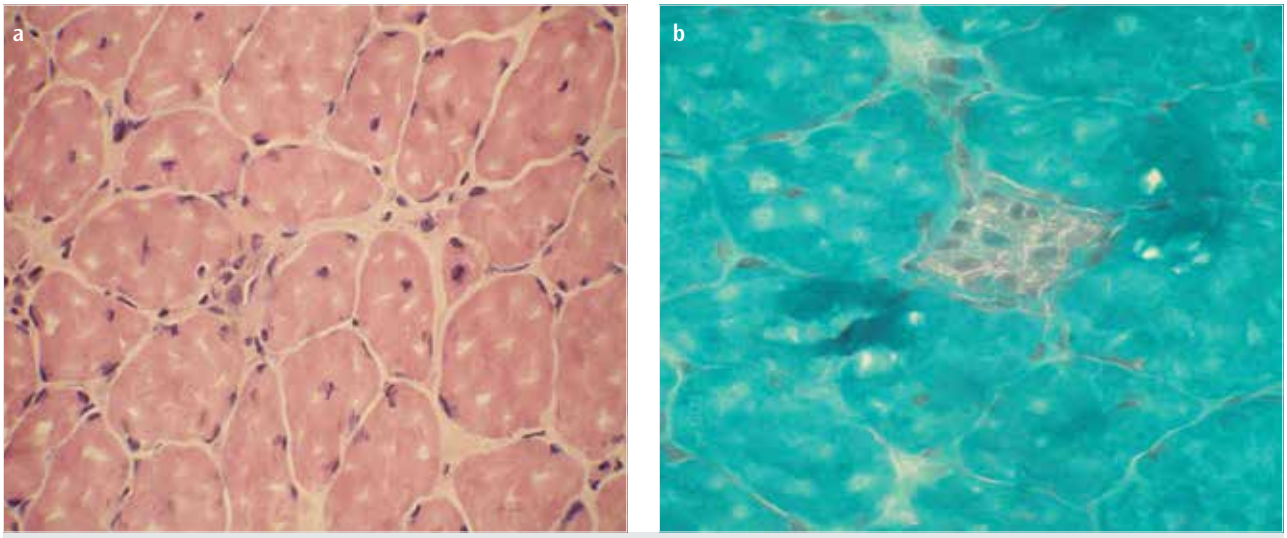

Figure 1. a, b. Differences in diameter and an increase in the amount of internal nuclei were observed in muscle fibers in fresh-frozen sections stained with hematoxylin-eosin (a) and modified Gömöri trichrome (b) (×20). A small number of pyknotic nuclear clusters and one necrotic fiber were detected

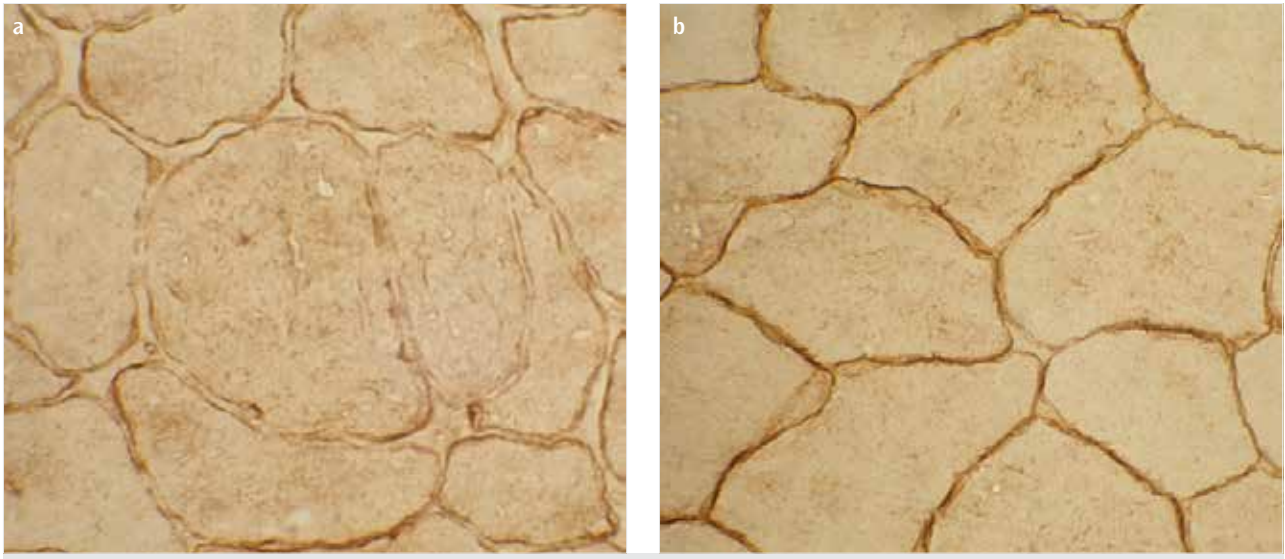

Figure 2. a, b. On staining performed by immunohistochemical methods using anti-dysferlin antibodies, it was observed that samples from the patient (a) were stained less than control samples (b)

ratory muscles is seen. The serum creatine kinase (CK) level is quite high, even in the preclinical period. Typically, a dystrophic pattern is observed on muscle biopsy and is often accompanied by inflammatory changes (1). Here we report a case of dysferlinopathy in a patient who had been followed up owing to an increased CK level since she was 1 year old and who had received steroid and azathioprine treatment for 3 years with a diagnosis of polymyositis, which was made as a result of a muscle biopsy performed 7 years ago in Syria.

Case Report

A 26-year-old Syrian single female patient presented with a complaint of difficulty in climbing stairs. Necrotic fibers and T-cell-induced inflammation were detected at a rate of 5%–10% with muscle biopsy, which was performed on the patient, who had muscle weakness since she was 1 year old, in Syria 7 years ago and was found to be compatible with polymyositis, and the patient was followed up for 3 years with steroid and azathioprine treatment. Her blood pressure was 130/70 and her temperature was 36.5°C. Respiratory, cardiac, and abdominal examinations were normal. There was apparent muscle weakness at a level of 3/5 in the proximal muscles on neurological examination and no weakness of the distal muscles, myotonia, pathological reflex, or cranial findings were observed. The CK level was found to be 4193 U/L (0–145), lactate dehydrogenase level was 482 U/L (0–248), aspartate aminotransferase level was 69 U/L (0–35), alanine aminotransferase level was 44 U/L (0–35), erythrocyte sedimentation rate was 7 mm/h (0–25), and C-reactive protein level was 3 mg/L (0–5) in the patient, whose hemogram, creatine, calcium, phosphorus, ferritin, thyroid-stimulating hormone, and vitamin B_{12} levels, and antinuclear antibody and extractable nuclear antibody profiles (HI, RIB, JO1, CB, SCL70, Ro-52, SM, SMRNP, SSA, SSB, dsDNA, and NUC) were normal. Myopathic

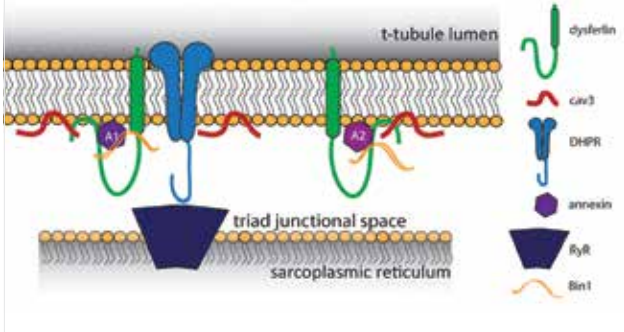

Figure 3. Model of dysferlin in T-tubules, from the article by Kerr et al. (9) entitled Dysferlin regulates transverse tubules at Ca^{2+} homeostasis in skeletal muscle

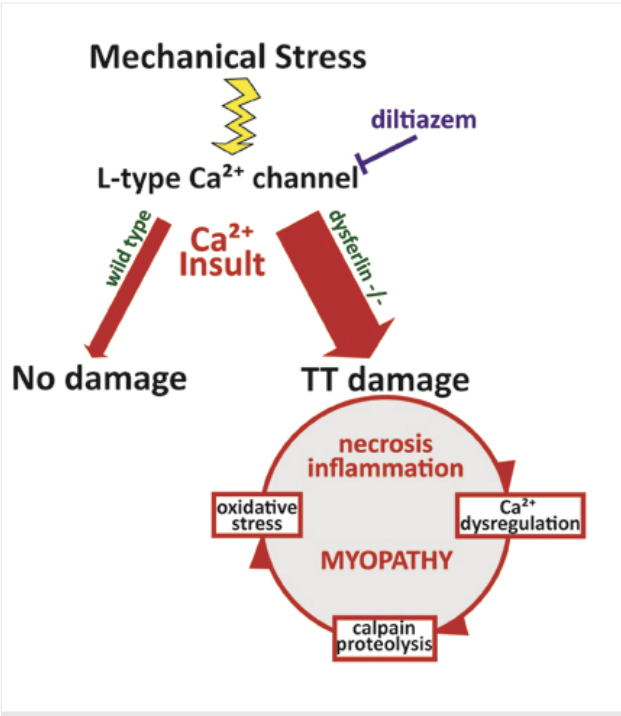

Figure 4. Pathophysiology of dysferlin deficiency, from the article by Kerr et al. (9) entitled Dysferlin regulates transverse tubules at Ca^{2+} homeostasis in skeletal muscle

involvement; intense myotonic discharges; and polyphasic, brief, low-amplitude, and early interfering motor unit potentials in the voluntary muscles were observed on electromyography; nerve conduction studies were normal and denervation was not detected. Immunohistochemical staining of the muscle biopsy showed that patient samples with anti-dysferlin antibodies were stained weaker than control group samples, and there was sporadic necrosis in the muscle fibers and mild inflammatory cells rich in T-cells (Figure 1, 2). Echocardiography was found to be normal.

Discussion

In the event that the integrity of a muscle cell membrane is impaired, a "patch" is formed by the exocytosis of intracellular vesicles in the torn region of the membrane. During patch formation, dysferlin plays a role along with, and in coordination with, a group of proteins such as annexin, caveolin-3, tubulin, Bin 1, dihydropyridine receptor, and ryanodine receptor in remodeling the cell skeleton (Figure 1). The etiology of some idiopathic myopathies has been elucidated by the recent identification of some of these proteins.

Dysferlin plays a role in the repair of muscle cell membranes and regeneration of muscle cells by participating in the fusion of vesicles and membranes. Although vesicle formation occurs in injured muscle fibers in the absence of dysferlin, ultrastructural studies showed that membranes could not be repaired because patches could not be formed (4, 7, 8). In the absence of dysferlin, myofibrils are inadequate for Ca^{+2} homeostasis during stress, cytosolic Ca^{2+} levels increase abnormally, and proteolysis and oxidative stress are stimulated, with the activation of various pathways; consequently, the development of necrosis, inflammation, and myopathy occurs.

Kerr et al. (9) considered T-tubules as responsible for the damage in dysferlinopathies by drawing attention to the location of dysferlin, especially in T-tubules, and suggested that the Ca^{2+}-mediated process is extremely important in disease progression (Figure 4). In their recent cell culture studies, they showed an increase in structural separation in T-tubules in a group with dysferlin deficiency in the adult muscle fibers, in which they created mild damage due to osmotic shock, and they also found a decrease in the amplitude of Ca^{2+} transitions due to a dramatic increase in cytosolic Ca^{2+} levels (9). These effects displayed an improvement after blockage of L-type calcium channels (LTCC) by diltiazem. After these in vitro findings, a regression in loss of function with diltiazem was shown in vivo. On day 3 after injury, necrosis and inflammation decreased with diltiazem and a decrease was seen in fibers with a central nucleus. It is believed that the healing activity was caused by the effect of diltiazem on LTCC and the protection that it afforded in the formation of T-tubules. Kerr et al. (9) also showed that dysferlin protects T-tubules from damage after mechanical stress, which suggests that dysferlin may have a protective effect by binding the annexins A1 and A2. In the last study, it was also shown that the production of free oxygen radicals stimulated by NADPH oxidase increased with mechanical stress and that the mechanosensitive Ca^{2+} channels became active in T-tubules in this case. Signs of an increase in free oxygen radicals were shown in fibers with dysferlin deficiency. These pathways may be therapeutic targets in the future (Figure 3). In the results of the study, inhibition of LTCC was shown to lead to a significant improvement in patients with dysferlinopathy (9).

Studies conducted using human fetal tissue indicate that dysferlin is expressed even during the early developmental periods, when the legs undergo regional differentiation. It is also possible that dysferlin plays a role in the fetus during the construction of the proximal and distal muscles. Dysferlin also plays a role in the maturation of regenerated fibers (4).

In the muscle fibers, the absence of dysferlin on immunohistochemistry or immunoblotting is essential for accurate diagnosis. The diagnosis of dysferlinopathy can be made and the differential diagnosis of the other types of LGMD becomes pos-

sible via immunohistochemistry (4, 7). It is difficult to conduct molecular examination of the large-sized *DYSF* for a definitive diagnosis of dysferlinopathies, and detailed molecular analysis is not cost-effective. Molecular analysis can be suggested only in cases where genetic counseling will be received and for shaping future treatment strategies. In addition, screening of the dysferlinopathies is not suitable. However, if these analyses can be performed in Turkey, it will be useful for identifying the mutations in this country and to show the increased incidence in some regions (4).

The skeleton has an active cell repair system to cope with the harmful effects of contractions. Via the acute membrane repair system, damaged fibers are protected from necrosis and severe tissue damage is prevented by the immune system stimulated by "danger" signals. With a reduction in danger signals, inflammatory cells are not seen in many types of musculoskeletal dystrophy, except for macrophages. T-lymphocyte-induced inflammation draws attention to dysferlinopathies among muscular dystrophies (4, 7).

Polymyositis is extremely important in differential diagnosis. In both diseases, there is a very prominent increase in CK levels, and the pathologist may remain suspicious, like the clinician, in patients with intense inflammation. Normally, the expression of major histocompatibility complex (MHC) class 1 molecules is seen neither in normal nor in myopathic muscle cells. The expression of MHC class 1 molecules in inflammatory myopathies is classified as "upregulation," but it is not known whether this situation results from the nature of the disease or is a nonspecific response that develops after cell damage. Increased expression occurs in active inflammatory myopathies and chronic inactive inflammatory myopathies. In contrast, there is no overexpression of MHC class 1 molecules in dysferlinopathies. In this regard, the MHC class 1 complex may be a valuable marker for differential diagnosis.

The role of the complement system in the pathology of muscles in dysferlinopathies is important; recovery from muscular pathology was shown in mice with dysferlin deficiency after genetic deterioration of the central component C3 of the complement system. The C5b-9 membrane attack complex (MAC) marker is the most highly expressed marker in patients with dysferlinopathy. The expression of MAC and downregulation of the inhibitor CD55 (decay-accelerating factor) suggest the importance of the involvement of the complement system in the pathogenesis of dysferlinopathy in SJL/J mice (4, 10, 11). The primary antigenic target in polymyositis is the endomysial capillary endothelial cell. C5b-9 MAC accumulates in capillaries after the activation of complement; thus, destruction of the muscle fibers occurs with capillary necrosis, perivascular inflammation, and ischemia. The appearance of MAC deposits in non-necrotic fibers in patients with dysferlinopathy may be helpful in distinguishing from idiopathic inflammatory myopathies.

Twenty-five genetically diagnosed patients with dysferlinopathy were followed up by Walter et al. in a natural course for 1 year. After a 2% reduction was detected in muscle strength on the Clinical Investigation of Duchenne Dystrophy scale 1 year later, the patients were divided into steroid (deflazacort at 1 mg/kg/day for 1 month, then 2 mg/kg every other day for 6 months) and placebo groups in a double-blinded method. It was reported at the end of the study that the steroid did not display the benefit that it provided in Duchenne muscular dystrophy and, on the contrary, adverse effects were observed in the steroid group on the severity of disease, loss in muscle strength, and quality of life scales (1). Thus, the importance of differentiation from polymyositis was emphasized once again and it was reported that the initiation of unnecessary steroid treatment adversely affected the disease.

Conclusion

Considering the relative frequency and diversity in clinical appearance of dysferlinopathy among the musculoskeletal dystrophies, it is extremely important to consider dysferlinopathies in the differential diagnosis of polymyositis to avoid unnecessary and potentially hazardous interventions such as long-term oral steroid or immunosuppressive treatment. The results of clinical trials carried out by administering diltiazem therapy for the purpose of LTCC blockage can shed therapeutic light on this disease, of which the treatment is not known.

Informed Consent: Written informed consent was not received due to the retrospective nature of this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - O.K.; Design - O.K., İ.Ü., B.R., Z.A.; Supervision - O.K., H.E.; Funding - O.K., İ.Ü.; Materials - C.E.B.K., Z.A., A.D.K., O.K.; Data Collection and/or Processing - O.K., C.E.B.K.; Analysis and/or Interpretation - O.K.; Literature Review - O.K.; Writing - O.K.; Critical Review - O.K., C.E.B.K.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

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