

Dysferlinopathies

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Abstract

Primary dysferlinopathies are due to mutations in the 55-exon gene located on chromosome 2p13 which encodes the protein dysferlin. They are a group of autosomal recessive heterogeneous muscular disorders. Clinical presentations show heterogeneity, ranging from limb girdle muscular dystrophy 2B, Miyoshi myopathy and distal myopathy with anterior tibial onset to isolated hyperCKemia and severe functional disability. Miyoshi myopathy symptoms begin in the posterior muscle compartment of the calf, spreading later to the upper muscles. In limb girdle muscular dystrophy 2B the proximal muscles of the lower limbs are involved. The distal myopathy with anterior tibial onset shows anterior muscle weakness at onset, progressing rapidly to the lower and upper proximal muscles. The onset of these disorders is generally in the teens or early adulthood. The values of CK are always very high. The diagnosis is made by the reduction or absence of dysferlin in muscles of the affected patients performed by immunohistochemistry and immunoblotting with dysferlin monoclonal antibodies. The molecular analysis confirms the diagnosis.

Keywords: Dysferlinopathies; LGMD2B; Miyoshi myopathy; DMAT; Dysferlin complex

Introduction

Primary dysferlinopathies are a rare heterogeneous group of autosomal recessive muscular dystrophies that are caused by mutations in the 55-exon gene encoding the protein dysferlin (*DYSF*, 2p13, MIM#603009) [1,2]. This 237 kDa protein belongs to a class of homologous proteins called “ferlins”. It is a protein located at the sarcolemma and the cytoplasm and, is not associated with the dystrophin-glycoprotein complex [3-5]. The essential role of this protein seems to be the repair of the membrane of skeletal muscle fibers [6-9].

The different presentations of these disorders have been described in various ethnic groups. Miyoshi myopathy (MM, MIM#254130), limb girdle muscular dystrophy 2B (LGMD2B, MIM#253601), distal myopathy with anterior tibial onset (DMAT, MIM#606678), and proximo-distal phenotypes are the most common disorders [10-14]. There are also other presentations ranging from isolated hyperCKemia to severe functional disability [13,15] and in elderly people, it can also be observed spinal muscle degeneration, with or without camptocormia [16]. The case of dysferlinopathy with choreic movements has only been described once and this could have been only by sheer chance since it has never been described again [17].

There is significant phenotypic heterogeneity among the patients sharing the same mutation in the *DYSF* gene so MM, LGMD2B or DMAT can be observed within the same family [13,18,19]. Furthermore, there is intra and interfamilial variability among the patients [2,12,14,15,18-23]. Modifier genes are held responsible for this [20,24].

The onset of LGMD2B and MM is generally in the teens or early adulthood. They usually have a normal life up to age 20 and they are often very good at sports. The muscles primarily affected in MM are the soleus and the gastrocnemius muscles leading to weakness of the thigh and later the upper limb muscles [10,12,13,25]. The early symptoms of these disorders are inability to stand on tip-toe, hop on one leg, run and difficulty to climb stairs [10,13]. These dystrophies progress

slowly [10,13]. DMAT begins with anterior tibial muscle weakness which rapidly progresses to the lower and upper proximal muscles [11]. Approximately 22 years after onset, the patients are generally wheelchair-bound [13].

Creatine kinase (CK) levels are generally very high [10,12-15].

In many cases, it is common to find in the biopsies of the dysferlin deficient patients inflammatory infiltrates suggesting the misdiagnosis of inflammatory myopathy [14,26-31]. It is believed that patients suffering from inflammatory myopathy have a more rapid progression [14,27].

The diagnosis is made by the absence or reduced dysferlin in muscle by immunohistochemistry or immunoblotting [13,32]. The expression level of dysferlin protein can be either measured from blood monocytes [33,34] or Western blot analysis. When in the Western blot dysferlin is absent (0%) or reduced (up to 20%) it can be concluded that the *DYSF* gene is the only one to be held responsible for the variation [35]. The molecular analysis of the dysferlin gene confirms the diagnosis [13]. There are no mutational hotspots and there is no genotype-phenotype correlation [36].

Clinical Features

Common features

The patients have normal milestones. The psychological

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Received April 02, 2013; Accepted April 24, 2013; Published April 27, 2013

Citation: Vernengo L, Carrasco L, Angelini C, Rodriguez MM (2013) Dysferlinopathies. J Genet Syndr Gene Ther 4: 134. doi:[10.4172/2157-7412.1000134](https://doi.org/10.4172/2157-7412.1000134)

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development is normal and many of them have university degrees [13-15,36-39].

There is great variability in the natural history of these disorders: the age of onset as well as muscle involvement at onset and during the course of them. The onset is in the early teens or adulthood. The mean age varies from 12 to 73 years. Weakness, wasting and atrophy are commonly observed (Figure 1) [13,37,38].

The “diamond on quadriceps” is a typical sign of these disorders, which is not observed in other muscular dystrophies [40]. A simple semiological maneuver is needed to observe it. The patients are required to stand with the knees flexed so the quadriceps muscles are in mild contraction. Then, abnormal asymmetrical bulges with the shape of a diamond may be observed on the anterolateral part of the quadriceps muscles and are made more prominent by the wasting of the surrounding muscles [40,41].

It is frequent to misdiagnose dysferlinopathy with inflammatory myopathy [14,26-31,34]. Inflammation and severity of the disorder correlate with a more rapid progression [14,27]. It has also been demonstrated that inflammation cannot only cause muscle atrophy but it can also originate secondary muscle disorders, general organic diseases such as metabolic, endocrine, vascular and paraneoplastic myopathies [42].

Although there is not an overt cardiac involvement, there have been descriptions of affected patients that have cardiac impairment [43-45].

Patients have excelled or practiced sports that involve physical exertion. The majority of them show good muscle strength before the onset of the disease which generally takes place in the second decade of their lives [13,14,38,39]. As sport is associated with eccentric muscle exercise, aggravation of the disease might be due to the delivery of cytokines in muscle which eventually can cause myoedema and inflammation that can be observed in the MRI. [38,39]. The muscle membrane breaks down and the mutant dysferlin is incapable of repairing it [36,46].

The progression rate is variable and patients need to use a cane or crutches fifteen years after onset and they are wheelchair-bound approximately 22 years after onset [13,14].

LGMD2B

At onset, patients with LGMD2B have weakness and wasting in the hip and thighs. The shoulder girdle muscles are later involved during the progression of the disease. Sometimes the first symptoms are fatigue while walking, difficulty in running and climbing stairs. The pelvic muscles (glutei, tensor fascia latae) and the posterior compartment of the thigh (adductors, hamstrings) are the first ones to be affected. Proximal muscles of the legs can be also involved leading to a peculiar gait and they cannot stand on tiptoe [12,13,25,37]. The disorder can spread from the lower to the upper limbs. The muscles of the shoulder girdle (supraspinatus, infraspinatus) and the upper limbs (biceps brachia) are less frequently and mildly affected. Progressive muscular wasting is also observed [13,37]. Cardiac involvement with secondary dilated cardiomyopathy has been described [43,44].

Miyoshi myopathy

The flexor muscles (gastrocnemius and soleus muscles) are predominantly involved. At onset patients complain that they cannot stand on tiptoe due to weakness of the gastrocnemius muscles, but they can stand on their heels [10,13]. There is symmetrical muscle weakness

and atrophy as well as moderate or marked wasting of the lower part of the legs. There can be pseudohypertrophy of the affected muscles [10,13,14]. Other distal symptoms are difficulty in climbing stairs, ankle subluxations and foot drop. Patients sometimes complain of pain on the legs [10,13,14].

Many years after onset the patients are unable to stand from squatting position [13,14].

In MM, when muscle wasting is observed, the “calf-heads on trophy sign” has been described [47].

It has been reported that patients suffer from subclinical cardiac impairment and some even have fibrosis of the cardiac muscle [45].

DMAT

In the distal myopathy with onset in the tibialis anterior (DMAT) or distal anterior compartment myopathy (DACM), the muscles to be involved are the anterior tibial muscles [11]. Early contractures of the ankle have also been observed [48]. As the disease progresses, muscle weakness extends to the posterior compartment involving the gastrocnemius. Although the age of onset, CK levels, and histological changes are similar to MM, the muscle weakness distribution is significantly different [11,48]. Sometimes the patients have to wear calipers to oppose the foot and be able to move around [13]. This phenotype has only been observed in Spaniards and Japanese patients [4,11].

Proximo-distal phenotypes

The proximo-distal phenotypes account for 35% of the cases. The variation of the disorder is not due only to the mutations in the *DYSF* gene but also to environmental factors [13,15] (Figure 1). Either proximal and a distal muscle weakness can be observed at onset, but the course is different in family members.

Congenital phenotypes

This type of inheritance is very rare. When it occurs, patients show delayed cephalic control support, generalized hypotonia, weakness of the limbs and difficulty in walking, running and climbing stairs. CK can be increased [49].

Prevalence

The prevalence is estimated between 1/100,000 and 1/200,000 [50] varying in the different populations. It is about 1/1300 on Libyan Jews [51] while in the Japanese population it has not been possible to determine [52].



Figure 1: Atrophy and wasting of the supraspinatus muscle and hypertrophy of deltoid muscle.

Inheritance

Dysferlinopathies are inherited in an autosomal recessive pattern, being all the parents obligatory heterozygous. Carriers are generally asymptomatic, but some patients have been described to have symptoms of either LGMD2B or MM [36,53,54].

Serum CK

In the active phase, serum CK level is markedly elevated, up to 50-100 fold or more above the upper limit of normal value (170 IU/l). The level of CK decreases as the disease progresses [13,14,37].

Morphological Finding

Histopathology

The muscle biopsy shows non-specific myopathic changes that include: variability in fiber size and fiber splitting, multiple internal nuclei, small vacuoles and necrotic and regenerative fibers (H&E, Gomori Trichrome and NADH) [29] (Figures 2A, 2C and 2D). Lobulated fibers [14,29,55] and ring fibers have also been observed [55,56] with oxidative enzyme reactions.

Proliferation of endomysial and perimysial connective tissue as well as loss of muscle fibers with fatty replacement and fibrosis at the advance stages can be found. Lymphocytes and macrophages can be seen surrounding and invading necrotic fibers. Inflammatory myopathy is sometimes misdiagnosed because inflammatory infiltrates are observed at the earlier stages of the disorder [13,14,26,28-30]. In most cases with active dystrophy an increased inflammatory response either moderate or marked in MHC-1 and/or macrophage reaction has been observed [29] (Figures 2A and 2B).

Mosaic pattern was seen on ATPase reaction. Both type 1 (slow-twitch) and type 2 (fast-twitch) fibers are normally distributed. In patients with an advanced-stage of dystrophy, there is a marked predominance of type 1 fibers [29], but it has also been observed that type 2C fibers were increased in number [10,57].

The Congo red shows amyloid deposits in the perimysial connective tissue, sarcolemmally and in the blood vessel walls [58,59].

Hypertrophied cardiomyocytes with swollen nuclei and severe diffuse perivascular and interstitial fibrosis can be observed [43].

When compared with other muscular dystrophies studied with the same methodology, it can be stated that in dysferlinopathies the percentage of the degenerating fibers is similar to that of sarcoglynoopathies and lower than in Duchenne muscular dystrophy and myositis, but regarding regenerating fibers the percentage is higher than in sarcoglynoopathies and lower than in Duchenne muscular dystrophy or myositis [29].

Inflammation is not only observed in the biopsies of dysferlinopathies, but also in polymyositis and other muscular dystrophies such as Duchenne muscular dystrophy and fascioscapulohumeral dystrophy. While in the biopsies of dysferlinopathies the macrophages are twice as many as in polymyositis, in the latter it is the CD8+ cells that double in number [28]. In Duchenne dystrophy macrophages and lymphocytes T can be observed in necrotic fibers and in fascioscapulohumeral dystrophy lymphocytes B and lymphocytes CD4+ can be observed in perivascular sites of necrotic fibers [60].

Immunohistochemistry

Dysferlin is generally absent in the sarcolemma of both skeletal and

cardiac muscles. Patchy sarcolemmal and diffuse cytoplasmic staining can be observed in skeletal muscle (Figure 3). In cardiomyocytes the dysferlin seems to be trapped inside them [13,14,47].

Electron Microscopy

At the early stages, subsarcolemmal vesicles and vacuoles are found. There are multilayered areas in the basal lamina, papillary projections and aggregates of subsarcolemmal vesicles, some of them filled with electron dense material (Figure 4). The nonspecific sarcoplasmic alterations found can be focal disruption of myofilaments filled with mitochondria, rough endoplasmic reticulum, free ribosomes subsarcolemmally and streaming of Z line [14,56,61].

Western Blot

The immunoblot analysis of muscle biopsy is undoubtedly the most useful tool to diagnose dysferlinopathy. This technique seems more reliable than the immunohistochemistry technique [13]. Even

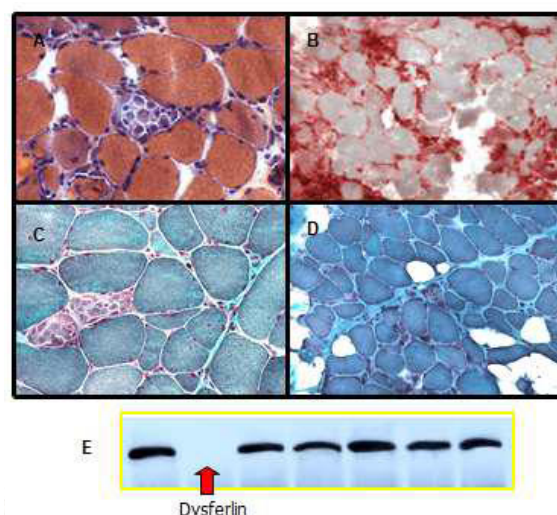


Figure 2: A) HE, B) Acid phosphatase, C,D) Trichrome. Muscle shows diffuse lymphocytic reaction and macrophagic infiltration (A), degenerating and regenerating muscle fibers (A, C). Increased MHC class 1 molecules (B). Muscle shows fibro-fatty replacement (D). (Magnification 40X) Absence of dysferlin in multiplex Western Blot (E).



Figure 3: Absence of dysferlin in most of the fibers. Patchy sarcolemmal and diffuse cytoplasmic dysferlin (Magnification 10X).

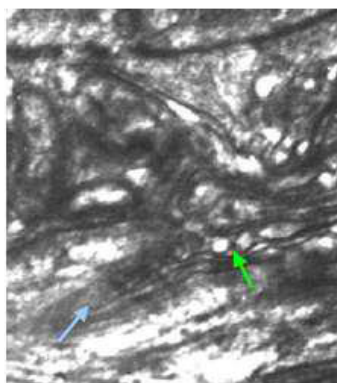


Figure 4: Papillary projections (blue arrow) and multiple vesicles (green arrow) (Magnification 20000X).

though the immunoblotting using anti-dysferlin antibody is sufficient for the diagnosis of dysferlinopathy (Figure 5B), the Multiplex Western blot allows to make the differential diagnosis with dystrophinopathies, sarcoglycanopathies, calpainopathy (LGMD2A) [13] (Figures 2E and 5A). In LGMD2L, the recessive muscular dystrophy caused by anoctamin-5, patients show a normal dysferlin [62]

When dysferlin is absent (0%) or reduced (up to 20%), the deficiency is due to the *DYSF* gene [35]. A 50% reduction has been observed in heterozygous patients [53]. Calpain can be either normal or reduced, but dystrophin and sarcoglycans are normal [3].

Electrocardiogram, Echocardiogram and Cardiovascular Magnetic Resonance

Some authors believe the heart is not affected [12,15]. Others have demonstrated that when the heart is involved, the ECG shows sinus rhythm, anterior ventricular block, ventricular hypertrophy and alterations in the repolarization [45,63].

The standard echocardiogram shows patients have a preserved or decreased left ventricular systolic function, hypokinesis of the inferior wall and some can have dilated cardiomyopathy [43,44]. The two dimensional longitudinal strain imaging by the automated function imaging (AFI) technique shows subclinical involvement of the heart. While there is a normal left ventricular ejection fraction, the peak systolic longitudinal strain can be decreased in the anteroapical wall, the anterolateral wall or the inferior wall [45].

In the cardiovascular magnetic resonance with late gadolinium it has been observed fibrosis of the different cardiac walls [45].

Electromyogram

Dysferlinopathies generally have a myopathic pattern. Small amplitude polyphasic potentials with full recruitment, positive sharp waves, fibrillations and low voltage small unit potentials can be seen [13,64].

Pulmonary Function

On the one hand, there are reports where the tests have shown that vital capacity is generally not affected, nor have they shown any nocturnal hypoventilation [36].

On the other hand there are reports that state quite the opposite. A case of a patient with dysferlinopathy and chronic obstructive

pulmonary disease (COPD) was described. It was suggested that the respiratory muscle impairment can be due to the fact that the systemic inflammatory response of COPD could influence the phenotypic expression of dysferlin [65]. As the disease has a protracted course patients are at risk of developing severe respiratory failure since the respiratory muscles can be affected [66].

CT and MRI

CT and MRI can be used to observe muscle atrophy, but the images of MRI have proven to be better to ascertain dystrophic muscle changes and myoedema of the different muscle compartments [11,49,64,67,68]. On the STIR sequences of the MRI studies, the muscle involvement can be observed as a hyper-density prior to clinical symptoms [38,69]. The muscles earlier affected are the gastrocnemius medialis and adductor magnus. Later, the muscles involved are the vastus lateralis and the soleus muscles. Diffuse muscle wasting is observed in the final stages of the disease [38].

The pattern described above, allows distinguishing between dysferlinopathies and other myopathies and muscular dystrophies [38].

Molecular Genetics

The 55-exon *DYSF* gene (NM_001130978) is a 6,243 base-pair, located on chromosome 2p13, spanning a genomic region of 150 Kb. It is ubiquitously expressed in stomach, lung, kidney, uterus, placenta, cerebellum, brain stem, spinal cord, sciatic nerve, liver and spleen and is more prominent in skeletal and heart muscles [1-3,33,70].

Dysferlin (Fer-1L1), which is a type II transmembrane protein, belongs to the ferlin family and does not belong to the dystrophin-glycoprotein complex. There is homology to Fer-1 of *Caenorhabditis elegans*. Fer-1 is needed for the fusion of the membrane during spermatogenesis and a mutation of this gene results in infertility [71,72]. The other members of the family in mammals are otoferlin (Fer-1L2; 2p23.1), myoferlin (Fer-1L3; 10q.24), Fer-1L4 (20q11.22), Fer-1L5 (2q11.2) and Fer-1L6 (8q24.13) [8,72-75] (Figure 6).

Dysferlin is found in the T-tubules and in the plasma membrane of five to six week embryos [3,76] and seems to play a role in T-tubulogenesis and monocyte fagocytosis. It repairs the sarcolemma pathways which can range from intracellular vesicle trafficking and fusion to sealing breaches [6,75-79].

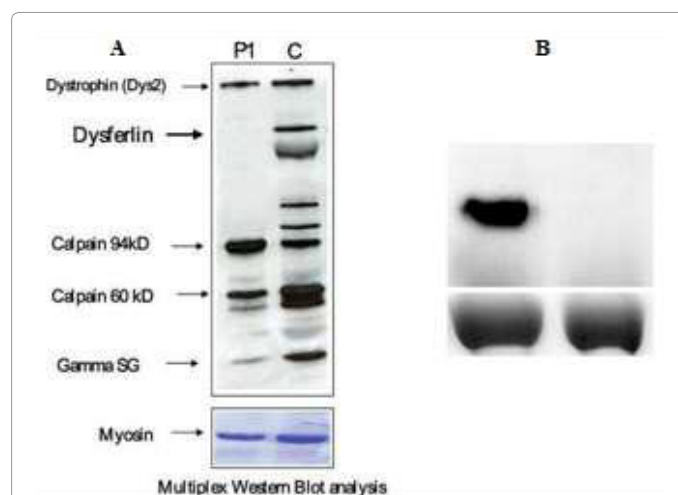


Figure 5: A) Multiplex Western Blot showing absence of dysferlin and a reduction of calpain 3. B) Dysferlin is absent while is present in control biopsies.

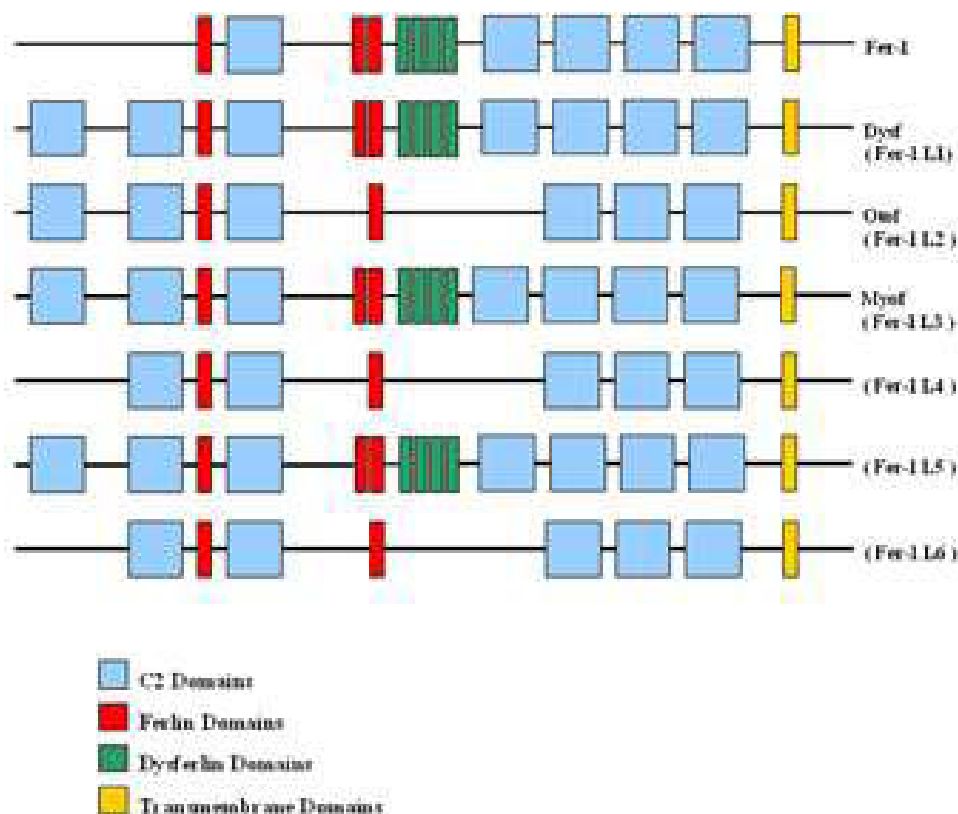


Figure 6: Schematic representation of the different members of the Ferlin family showing the homology between *Caenorhabditis elegans* (Fer-1) and the various ferlin genes in humans. The common structural motifs: the multiple C2 domains, the ferlin conserved domains and the transmembrane segments are depicted in all Fer-1-related proteins.

The gene has seven C2 domains and a single C-terminal transmembrane domain which anchors them to the membrane. It also has the FerA and FerB domains and the DysfN and DysfC domains [8,79,80]. The C2 domains have homology to the synaptotagmin family and protein kinase C. There are two types of C2 domains. Type 1 domains are C2A, C2B and C2E and type 2 are C2C, C2D, C2F and C2G. Their interaction with calcium, phospholipids and other proteins regulate membrane trafficking [8,75,79] (Figure 7). The function of some C2 domains is not yet known [79]. Dysferlin also appears to have a potential role in cell adhesion, metabolism, mitochondrial association and immune cell function [81].

The transcript diversity on the *DYSF* gene can be observed in the 55-exon transcript and those transcripts from the usual or conventional splicing events such as in exon 1, exon 5a and exon 40a, and the fourteen splicing isoforms which have been reported (exons 5a, 17 and 40a) [82].

The allelic heterogeneity is revealed by point mutations, deletions, insertions and frame-shift mutations that have been described among most of the 400 deleterious mutations or non pathogenic polymorphisms reported to the Leiden Muscular Dystrophy database (<http://www.dmd.nl>) [14,32,83-85]. 83% of the reported pathogenic missense mutations in dysferlin seem to affect the evolutionary conserved amino acids [8,80].

There must be a prominent reduction of dysferlin in order to affect muscle repair. A reduction of the protein to 20% can be considered pathognomonic of LGMD2B [35].

The mutations on the *DYSF* gene cause a misfolding of the protein, which in turn leads to dysfunction and degradation [8,43,80,81].

The complement system is involved in inflammatory disorders and dysferlin is reduced in muscles [9]. Dysferlin is also expressed in CD14⁺ macrophages [34,35].

It has also been demonstrated that there is accumulation of dysferlin in some non muscular diseases such as Alzheimer's disease [75,86]. In Multiple Sclerosis dysferlin reactivity is induced in endothelial cells, being its expression associated with vascular leakage of serum proteins [75,87].

It has also been suggested that dysferlin might be essential for glomerular epithelial stability [88].

Binding Partners of Dysferlin

The *DYSF* gene interacts with other genes through several different pathways.

Annexins A1 and A2

Dysferlin usually associates with both annexins which bind to phospholipids, Ca²⁺ and actin. They are involved in membrane trafficking, channel activity and cell-matrix interactions. The impaired dysferlin affects the capability of the healing process. Both annexins have different roles in the repair process and this can be observed when the sarcolemma is injured. Annexin 2 is not altered whereas the relationship with A1 is damaged [75,89-93]. The alterations of A1 in the A1-null mice show a great inflammatory response in muscles [9].

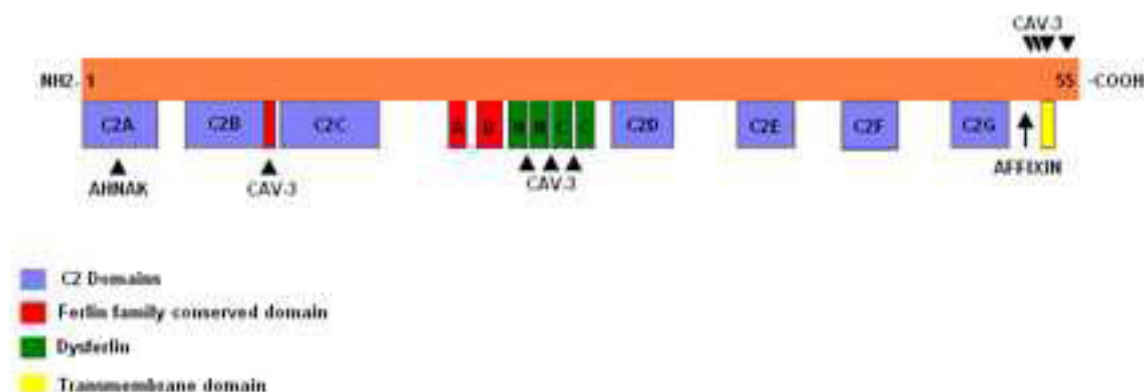


Figure 7: The dysferlin gene and the different protein domains and their interaction with dysferlin.

AHNAK 1 and 2

These large proteins, located at the enlargesome surface, and the costameric network, co-localize with dysferlin at the sarcolemmal membrane where they bind to dysferlin at the C2A domain. When there is a reduction or absence of dysferlin, there is also a reduction of AHNAK. In conjunction with dysferlin, both proteins take part in membrane repair and seem to affect transverse muscle fiber stiffness [75,91,94,95].

Calpain-3

Calpain-3 is a skeletal muscle-specific, non-lysosomal and Ca²⁺-dependent cysteine protease, localized in the sarcomere, and its function seems to be the regulation of the AHNAK proteins by dysferlin. In primary dysferlinopathies, there is a reduction of dysferlin as well as a moderate calpain-3 diminishment in some cases. In secondary dysferlinopathies, the reciprocal has been observed [75,96]. The cleavage of AHNAK fragments by CAPN3 leads to a loss of affinity for dysferlin [97].

Cavin or Polymerase I and transcript release factor (PTRF)

Cavin-1 or PTRF localizes to caveolae at the plasma membrane. Muscular dystrophy as well as generalized lipodystrophy in man and mice is due to its absence [85,98,99].

Caveolin-3

It is found at the junction of the sarcolemma and the T-tubules and acts as a chaperone to dysferlin. The caveolins are required for dysferlin trafficking. Mutations in caveolin-3 gene lead to accumulation of dysferlin in both LGMD1C muscle [100] and C2C12 transfectants [101]. Their mutations lead to accumulation of dysferlin in the Golgi apparatus resulting in an abnormal localization of Mitsugumin 53 and dysferlin thus affecting membrane repair. Dysferlin is reduced when there is a deficit of caveolin-3, but the opposite does not take place [101]. The reason may be that caveolin-3 is found at the plasma membrane. A similar change happens with cavin. It can be inferred that while dysferlin needs caveolin-3 and cavin to have a correct position at the plasma membrane, the opposite does not happen [75,85,101,102].

Mitsugumin 53 (MG53)

MG53 co-localizes with dysferlin and annexin A1 to the T-tubule network and seems to be at the longitudinal T-tubules. It is necessary for extracellular vesicle trafficking and for membrane repair [8,103,104]. Its interaction with dysferlin and caveolin-3 rules membrane repair

in skeletal muscles. It accumulates at an injured site of patients with muscular dystrophy in response to oxidative stress different from dysferlin and the membrane repair complex apparently targets the longitudinal T-tubules where there is membrane damage [8,104,105]. The MG53-deficient mice are not capable of resealing disruptions of the membrane [85,105].

Affixin (beta parvin)

It is a binding protein which co-localizes with dysferlin and ILK (integrin linked kinase) at the sarcolemma. While affixin is reduced from the sarcolemma its total amount remains normal. Affixin has a binding site at the C-terminal region of dysferlin and probably takes part with the latter in membrane repair [75,81,106].

Other binding proteins

Several other proteins are involved with the dysferlin complex involved either in membrane repair or intracellular vesicle trafficking.

Gelsolin is a protein needed for the cleavage of calpain-3 thus affecting the AHNAK- complex [85,107].

Moesin (MSN) (membrane-organizing extension spike protein) is localized to filopodians and other membranous proteins taking part in cellular movements. It is also apparently involved in vesicle trafficking [85,108].

Vinculin co-localizes with dysferlin at the sarcolemma and interacts with it in the focal adhesion process [81].

By performing the FLIM - FRET technique there seems to be no interaction between caveolin 3, myomesin-2 or calsequestrin and dysferlin [109].

Correlation between Genotype and Phenotype

There is no correlation between the genotype and the phenotype, nor has it been possible to correlate the clinical variability the patients present and the variation in the amount of dysferlin [37]. When there is inflammation, a more rapid progression and a more severe clinical course can be observed [14,15,27].

It has been reported that in MM, the mutation G3370T has proven to have a milder form whereas mutation G3510A has more severe symptomatology [110,111].

Population Genetics

Several founder mutations of the dysferlin gene have been reported

in different geographic regions and populations. Undoubtedly, the mutations in native Canadians [20], Libyan Jews [51] and Jews of the Caucasus [112] were caused by endogamy. The mutations of the Mediterranean Latin populations: Italy [24], Spain [19], Portugal [84] and Portugal-Uruguay [14] have been demonstrated experimentally and are associated with a specific haplotype.

Animal Models

Animal models are useful to identify the different features that accelerate or delay the dystrophic damage in the different skeletal muscles and the new therapies that will be useful for these disorders.

Many murine models have proven the dystrophic features that appear in humans. Human beings and mice have similar muscular dystrophic characteristics. As there is more than 90% amino acid sequence homology between them [113] mice are used as animal models for dysferlinopathies [114-116].

The two naturally occurring murine models are the SJL and A/J mice. These models are useful because the clinical features of the affected mice are similar to those humans have. The SJL mice have an in-frame RNA deletion that makes them lose 57 amino acids in the highly conserved region of the C2E domain and the A/J mice have a ETn retrotransposon insertion in intron 4 [117].

Studies have shown that there are differences between the two models regarding the muscles affected and how the disease progresses [117]. The variability of the dystrophic changes back up the hypothesis that the progression of the muscular disease is not only due to the mutation on the DYSF gene, but also to modifier genes [114,115].

The first model to have proven features compatible with a deficient muscular dystrophy was the SJL mice. Dysferlin splicing mutations in SJL mice (SJL/-) define a natural model for LGMD2B and MM. The SJL/J mice model has a disease progression that is similar to other Dysf-/- and C57BL/10.SJL mice models, but faster than in A/J mice. As in both A/J and Dysf-/- models, the proximal muscles are more severely affected than the distal muscles [113,114,118,119]. Most of the degenerative fibers of the SJL/J model are type2 (fast-twitch fibers) [113,114].

The inbred A/J model shows no dysferlin expression. The disease progression in the abdominal muscles was similar to those the Dysf-/- model had. An increased frequency of rhabdomyosarcomas was also observed [118].

Comparative studies of the SJL/J and A/J models have shown that there is an earlier onset of the disease and the course of the disease is faster in the SJL/J model [114]. In both models there is an increase of macrophages and lymphocytes T, being CD4+ cells more abundant than CD8+ cells [120]. There is controversy regarding the involvement of the diaphragm while a report states that only the SJL/J mice is affected another states it is affected in both models [121].

In the model B6.A-Dysf^{prmd}/GeneJ (Bla/J mice) the disease progression is similar to A/J but slightly slower than in SJL/J, in Dysf-/- and C57BL/10.SJL models. As SJL/J and Dysf-/- models, it shows that the proximal muscles are more severely affected than the distal muscles while the abdominal muscles are affected like Dysf-/- model [120]. Another model developed the transfer of full-length dysferlin which was achieved by AAV concatemerization [122].

The C57BL/6J-Chr6A/J/NaJ model has chr 6 from the A/J line with an ETn retrotransposon (5-6 kb) inserted in intron 4 of the dysferlin

gene, while the remaining chromosomes are from C57BL/6J. The symptoms are very similar to the A/J mice have [123].

In the Dysf-/-^(129.Dysfml1Kcam)/J model the disease progression is similar to SJL/J, Dysf-/-, and B10.SJL-Dysf^{fm}/AwaJ, and faster than in the A/J mice. This knockout model has demonstrated that under stress there can be cardiac involvement. Dysferlin-null mice develop mild cardiomyopathy under mechanical stress since it was demonstrated that dysferlin is needed to repair cardiomyocytes' membranes [124]. The B10.SJL-Dysf^{fm}/AwaJ model the progression of the disease is similar to the SJL/J mice [125].

The A/J mice were used for gene therapy using adeno-associated virus. The size of the minidysferlin was within the right packaging size of rAAA vectors [126].

Experiments regarding transgenic mice in order to test whether gene replacement therapy is a good candidate for treatment have demonstrated that the amount of dysferlin is related to the phenotype the mice have. An over-expression of dysferlin in mice leads to kyphosis, atypical gait, and reduced muscle mass and strength [127].

There are other non murine models that are being used. The *Drosophila melanogaster* has only one single ferlin gene called misfire. It has been demonstrated that mutations in this gene produce infertility in both males and females [128]. *C. elegans* is a model where new muscle-specific functions of the dysferlin protein can be explored as Fer-1 is expressed in muscle [129]. The zebrafish model by morpholino knockdown results in muscles being affected [130].

Treatment

Today there is no curative treatment for dysferlinopathies.

There is controversy whether patients should practice sports. We believe that they should not be encouraged to practice sports as in other muscular disorders. Exercise may worsen the breakdown of muscles [38].

To date, there is not a specific pharmacological treatment for dysferlinopathies. Steroids have been used to treat patients and have proven to be inefficient [14,35,131,132]. The administration of intravenous immunoglobulin (IV-Ig) has been tested apparently with success [133].

Rituximab (a monoclonal antibody directed against CD20⁺ antigen on B cells) has been reported to be useful, apparently increasing muscle strength in MM [134].

For both IV-Ig and rituximab, we can state the lack of sustained effect (personal observations).

Dantrolene has shown to decrease CK, but the clinical conditions do not improve [135]. It is possible that some stop codon mutations might be treated with Ataluren (PTC124), the oligonucleotide antisense and nonsense suppression drug [135].

Currently, several trials leading to gene therapy have been tested. A treatment with a dysferlin full-length gene transfer might not be easy, but the results have been encouraging. In mice treated with it, the muscle histological aspects, the capability to repair the muscle membrane and the locomotive activity have been improved [122]. Mini-dysferlin seems to be the most promising treatment since it has been proven that it plays an efficient role in membrane repair *in vitro* [126,127]. DYSF-exon skipping also seems to be very promising, especially for those mutations in exon 32 [136-139]. There is

hope that the new approach using proteosomal inhibitor for mis-sense mutated dysferlin will allow to restore the function of dysferlin [140]. Dysferlinopathies represent a particular challenge to define natural history in order to plan relevant clinical trials. Several studies have dealt with either inbred populations [51] or the natural history in genetically defined patients by Gardner Medwin and Walton's modified score, MMT or quantitative muscle testing [38,49]. Nevertheless, the use of different functional tests and of various protocols makes it difficult to compare results meaningfully and to approach a "trial" with a well defined natural history and significant clinical outcome measures. The Jain Foundation, which is trying to "orchestrate" a treatment for dysferlinopathies, has undertaken the task to promote a multicenter prospective study coordinated by Dr Bushby in 16 different European and US centers. This study will investigate both clinical signs and MRI changes in 150 patients with the following specific objectives: 1) to define the natural history and progression in several patients group by a MRI protocol and a physiotherapy protocol in both ambulant and non ambulant patients; 2) to study a selection of possible outcome measures in this multicenter evaluation; 3) to extend and implement already existing registries on LGMD 2B and Miyoshi myopathy (i.e., Jain Foundation, Telethon, TREAT-NMD). A common follow-up clinical/ MRI protocol for the evaluation of 150 dysferlinopathy patients in various Centres of excellence for Muscular Dystrophies will be carried out from January 2013 to Feb 2016

Conclusion

Dysferlinopathies are rare genetic disorders that show heterogeneity and a perplexing clinical course. The promising treatments by mini-dysferlin and exon skipping must still undergo more research and trials. The more we understand how the dysferlin complex works, the greater our chances will be to develop a treatment for these distressing disorders.

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