Logical modelling of Dysferlinopathies

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Chapter 1

Biological Background

This section will provide the necessary information to understand dysferlinopathies. It will begin by outlining the structure and function of skeletal muscle, the primary tissue affected by this disease. It will then give a detailed characterisation of dysferlin, the protein whose gene mutation is responsible for dysferlinopathies. Finally, the section will examine the cellular and molecular consequences of dysferlin deficiency, establishing a link between the genetic defect and the pathological manifestations of dysferlinopathies.

1.1 Skeletal Muscles

Skeletal muscles are composed of individual muscle fibres that orchestrate bodily movements. In addition to their essential function in movement, they participate in a wide range of functions, including locomotion, postural maintenance and respiration.

The development of skeletal muscles, myogenesis, is a tightly regulated process that is governed by several transcription factors during embryogenesis. Muscle cells arise from the differentiation of myogenic precursor cells during early embryonic development. These precursor cells, which are guided by various transcription factors, are activated and differentiate into myoblasts. These newly formed myoblasts then proliferate, meaning they rapidly increase in number. Subsequently, they align and fuse together to form multinucleated structures called myotubes. Myotubes further differentiate into mature muscle fibres (myofibrils). Each muscle fibre is an elongated cell with numerous nuclei peripherally located. Mature muscle contains a population of muscle precursor cells, designated as satellite cells. These cells remain quiescent until activated for muscle regeneration purposes.

Muscle fibres are bundled together with associated connective tissues to form a functional

unit. Each muscle is enveloped in a dense connective tissue layer, called the epimysium. This layer provides structural support and facilitates interaction with surrounding tissues. Deeper within the muscle, groups of muscle fibres are organised into fascicles. These fascicles are surrounded by another connective tissue layer, the perimysium. The perimysium further compartmentalises the muscle and houses blood vessels and nerves that supply the muscle fibres. Finally, individual muscle fibres are encased within a thin layer of connective tissue called the endomysium. This hierarchical organisation ensures efficient force generation and coordinated movement.

The cell membrane of a muscle fibre (sarcolemma) contains transmembrane proteins that connect it to the contractile units of the muscle fibre, known as sarcomeres. These connections allow for coordinated force generation upon nerve stimulation. Each muscle fibre contains numerous myofibrils whose intracellular compartment (sarcoplasm) houses the muscle fibre's metabolic machinery, including the sarcoplasmic reticulum (which regulates calcium for contraction), mitochondria (which produce energy), and the Golgi apparatus (which processes and transports proteins). Additionally, the sarcoplasm stores glycogen (which provides fuel for muscle) and myoglobin (which carries oxygen).

The efficient functioning of muscle tissue is dependent on the triads, which are specialised structures containing a transverse tubule (T tubule) and a pair of terminal cisternae derived from the sarcoplasmic reticulum (SR). The SR is responsible for storing calcium, which is essential for contraction. T tubules are invaginations of the sarcolemma that penetrate the myofibril and act as electrical signal conduits. The close proximity of these elements within the triad facilitates excitation-contraction coupling. A nerve impulse travelling along the T tubule triggers calcium release from adjacent SR terminal cisternae, initiating the contractile machinery within the sarcomere. The strategic positioning of triads ensures efficient and coordinated calcium release, enabling synchronised muscle contraction.

Surrounding the muscle fibre is the extracellular matrix (ECM). This specialized network provides critical support and communication functions. The (ECM) can be further divided into two layers: the basal lamina, a thin layer directly associated with the sarcolemma, and the reticular lamina, a more loosely organised outer layer. The ECM is composed of various molecules, including collagens, laminins, and proteoglycans. It offers structural support, elasticity, and facilitates force transmission during muscle contractions. Moreover, the ECM plays a crucial role in cell adhesion and communication, ensuring the coordinated function of skeletal muscle.

Mature muscle fibres possess remarkable plasticity, enabling repair of local damage, particularly disruptions to the sarcolemma caused by muscle contractions. This repair process can be

categorised into two main mechanisms: spontaneous repair (for small lesions) and active repair (for larger lesions). Lesions less than a nanometre in size can be repaired spontaneously. Following injury, the exposed hydrophobic domains of the damaged membrane bilayer rapidly attract surrounding lipids, forming a curved edge that facilitates repair. However, the phospholipid bilayer is also tethered to the myofibril cytoskeleton. Membrane ruptures can lead to retraction at the lesion site due to this attachment, which opposes the force of repair. Therefore, the success of spontaneous repair depends on the lesion diameter. Larger lesions require an active repair process involving patch formation. This mechanism involves the fusion of multiple intracellular vesicles with each other and with the damaged plasma membrane, effectively sealing the hole. Concurrent processes such as cytoskeletal and membrane remodeling also play a role.

These repair mechanisms are reversible and do not result in cell death, thus preserving the integrity of muscle fibres and surrounding tissues. In contrast, more severe injuries, such as traumatic muscle strains or genetic diseases like muscular dystrophies, can trigger necrosis of entire muscle fibre segments. In such scenarios, skeletal muscle regeneration, a multi-stage process mediated by satellite cells (muscle stem cells), is initiated. Muscle fibre necrosis triggers an inflammatory response, marked by the infiltration of macrophages. Necrotic debris is cleared by macrophages, facilitated by the influx of calcium and subsequent activation of calpains (calcium-dependent proteases), which rapidly degrade myofibrils. Additionally, the complement cascade is activated, leading to the recruitment of immune cells, further promoting muscle regeneration through the release of factors that stimulate satellite cell proliferation and differentiation. From day two post-injury onwards, various signals activate satellite cells, including sphingosine-1-phosphate (S1P), nitric oxide (NO), and hepatocyte growth factor (HGF). Satellite cell activation leads to proliferation, generating a pool of undifferentiated daughter cells. The transition from proliferation to differentiation appears to be tightly regulated by the interplay of Wnt and Notch signalling pathways. In the early stages of injury, the Notch pathway is dominant, promoting the expansion and self-renewal of satellite cells to maintain the stem cell pool for future needs. Subsequently, Wnt signaling becomes the primary pathway, driving the differentiation of satellite cells into myogenic precursor cells, which ultimately fuse with either intact muscle fibres or each other to form new myofibrils. The process of skeletal muscle regeneration culminates in the maturation of newly formed myofibrils and the remodeling of regenerated muscle tissue. The remodeling process is influenced by a number of factors, including the nature of the injury, the involvement of blood vessels, and the re-establishment of neuromuscular and myotendinous connections. Of particular importance is the integrity of the basal lamina surrounding muscle

fibres, as its disruption hinders fusion between regenerated myotubes, leading to the formation of smaller muscle fibres. Furthermore, the formation of scar tissue within the damaged area can also hinder the reconstruction of complete muscle fibres, necessitating the formation of new myotendinous junctions.

1.2 Dysferlin

1.2.1 Generalities

Dysferlin is a protein encoded by the DYSF gene located at chromosome 2p13. The DYSF gene spans over 230 kb on the short arm of chromosome 2 and contains 55 exons of varying sizes (30-461 bp). DYSF undergoes alternative splicing, a post-transcriptional process where introns are removed and exons are joined to generate transcripts of varying lengths. This process leads to the formation of 14 alternative transcripts encoding different dysferlin isoforms. Four alternative exons (v1, 5a, 17, and 40a) contribute to this transcript diversity, with their presence varying depending on the tissue. The transcripts containing the alternative exons have a low expression level in skeletal muscles (up to 5% of total transcript). Transcriptomic data shows widespread DYSF gene transcript expression, with highest levels in skeletal muscle and placenta. Other tissues like cardiac muscle and spleen show slightly higher transcript levels compared to others.

Mutations within the DYSF locus, leading to dysferlin deficiency, cause a form of muscular dystrophy named dysferlinopathy. Indeed, dysferlin is crucial for proper skeletal muscle function. Dysferlin is a large transmembrane protein (237 kDa) that exhibits isoform-dependent variations in size (2080-2119 amino acids). It possesses a transmembrane segment anchoring it to the plasma membrane, T-tubules, and intracellular vesicles within its expressing cells. Structurally, dysferlin comprises a large cytoplasmic domain containing seven C2 domains (A-G), three Iron domains, three DysF domains, a transmembrane domain, and a small extracellular portion.

The C2 domains interact with positively charged ions like calcium, protein partners, and lipid-rich regions. Notably, the C2A domain binds phospholipids in a calcium-dependent manner, unlike other C2 domains in dysferlin. Moreover, C2A interacts with various protein partners involved in membrane fusion, such as AHNAK, annexin A1, annexin A2, and tubulin. The C2B-FerI-C2C motif appears to regulate dysferlin expression and endocytosis at the plasma membrane. Finally, the C2F-C2G-TM motif has been implicated in muscle cell membrane repair. The specific functions of the Iron and DysF domains remain elusive, warranting further investigation.

Dysferlin is predominantly localized to the sarcolemma (muscle cell membrane) in skeletal

muscle. However, research has revealed its presence in T-tubules and intracellular myofibril vesicles.

Dysferlin protein expression is primarily in skeletal muscle. It was also detected in placenta and kidney in lower concetration. Lower levels of dysferlin protein expression are observed in cardiac muscle (cardiomyocytes), spleen (red pulp), bone marrow (hematopoietic cells), testes (seminiferous tubules and Leydig cells), smooth muscle cells, and colonic endothelial cells. Dysferlin's presence is detectable as early as 5-6 weeks of human embryonic development and its expression persists throughout human life.

1.2.2 Functions

Dysferlin is an essential protein for skeletal muscle function, as it is involved in numerous functions within myofibrils: myoblast/myotube fusion, T-tubule formation and stabilization, vesicle trafficking and muscle cell membrane repair. However, it is also involved in non-muscle functions such as inflammatory processes.

Myoblast/Myotube Fusion

Skeletal muscle development, growth, and regeneration all rely on a crucial process: myoblast fusion, where individual myoblasts merge to form multinucleated myotubes, which then mature into myofibrils. Myoblast fusion is a tightly regulated process involving several key steps. First, myoblasts migrate towards each other, guided by chemotactic factors secreted by muscle cells. Proteins like Fam65b play a role in this process by stabilizing microtubules, essential for cell movement. As myoblasts reach their target, they extend cellular protrusions (lamellipodia and filopodia) to establish contact with neighboring muscle cells. Recognition and adhesion occur through specific molecules like M-cadherin, integrins, and Adam12. For example, the integrin vLA-4 on the myotube membrane interacts with its receptor VCAM-1 on the myoblast membrane, facilitating alignment and adhesion. At the contact sites between fusing cells, proteins like myoferlin (abundant in myoblasts) and dysferlin (more prevalent in myotubes) are highly expressed. Their precise roles remain unclear. Actin cytoskeleton remodeling is crucial for fusion. A dense actin scaffold forms along the long axis of the fusing cells, providing membrane rigidity. Myosin 2A contributes to this process. As fusion progresses, vesicles accumulate and pair at the contact zone, forming fusion pores that allow cytoplasmic continuity between the two cells. Dysferlin, involved in vesicle trafficking, might play a role in transporting vesicles to the fusion site. While several proteins like integrins, myoferlin, dysferlin, and myosin 2A are known to be

involved, further research is needed to fully elucidate the precise steps and individual protein functions in myoblast/myotube fusion.

T-Tubule Formation and Stabilization

The contractile function of myofibrils relies heavily on T-tubules, which are specialized membrane structures. They transmit action potentials from the neuromuscular junction to the sarcoplasmic reticulum's terminal cisternae, triggering the release of calcium ions necessary for muscle fiber contraction. T-tubules formation during embryonic development is a coordinated process involving several key proteins: dysferlin, caveolin-3, dynamin-2, and Bin1. T-tubules are forwmed in two steps:

1. Membrane Invagination: Two key proteins, Bin1 and dynamin-2, are involved in this process. Bin1 binds to the sarcolemma, its SH3 domain interacting with dynamin-2's proline-rich domain. The precise mechanism remains unclear, but the structure of Bin1's BAR domain and this interaction are believed to be responsible for the curvature and invagination of the sarcolemma.

2. Membrane Extension: Dysferlin is likely involved in extending the invaginated membrane. T-tubule membranes are rich in PI(4,5)P2 phospholipids, for which dysferlin has a strong affinity. Caveolin-3, localized to developing T-tubules, may also play a role due to its presence in cholesterol-rich caveolae and T-tubule membranes.

Once formed, T-tubules primarily run longitudinally, parallel to the myofibril axis. At this stage, crucial connections with the sarcoplasmic reticulum are established, mediated by proteins like junctophilin1. T-tubules then undergo rearrangement to acquire a predominantly transverse orientation.

Vesicle Trafficking

Dysferlin seems to play a role in muscle cell vesicle trafficking. This hypothesis is supported by two key observations. Animal models lacking dysferlin exhibit an accumulation of vesicles within their muscle cells. These vesicles can contain various molecules, including cell-signaling receptors. Studies using myoblasts lacking dysferlin show a reduced recycling rate and improper localization of the IGFR1 receptor, a key growth factor receptor. This leads to the formation of receptor aggregates within the cell. While the exact mechanism remains unclear, these observations

suggest dysferlin might be involved in facilitating the movement and proper targeting of vesicles within muscle cells.

Other muscle proteins also contribute to vesicle trafficking. MG53, for example, associates with intracellular vesicles and the inner membrane layer of the muscle cell. These MG53-associated vesicles are readily observed moving across the cell and fusing with the outer membrane, releasing their contents into the extracellular space. Interestingly, caveolin-3 seems to act as a regulator of MG53 activity in vesicle trafficking. When caveolin-3 is present, it reduces the fusion activity of MG53-associated vesicles.

Muscle Membrane Repair

The repair of muscle cell membranes is an active process involving several proteins, in particular calpains, mitsugumin 53 and dysferlin. As disruptions to the plasma membrane are common, this repair process is essential for eukaryotic cells. The influx of calcium, generated by the rupture of the muscle cell membrane, is the main trigger for this active repair mechanism. The increase in intracellular calcium initiates a cascade of reactions, including the remodelling of the cytoskeleton, local activation of calpains, exocytosis and the recruitment of several proteins and certain membrane lipids.

Rupture of the plasma membrane leads to disorganisation of the actin network and also the microtubule network. Therefore, the remodelling of the cytoskeleton is necessary. To achieve this, a substantial influx of calcium from the extracellular environment, which passes through the lesion, causes local activation of calpains, which cleave two actin partners located on the plasma membrane: vimentin and talin. This cleavage induces membrane depolarisation and enables remodelling of the cytoskeleton by disassembling the damaged actin network, which is an essential early event in membrane repair.

Following the disassembly of the damaged actin network, two GTPases, RhoA and Cdc42, recruit actin and myosin around the lesion to form an actomyosin ring. This ring then attaches to the proteins with which actin and myosin interact, namely talin, vimentin or filamin. The actomyosin ring contracts continuously throughout the repair process until the damaged area is closed. With regard to microtubules, EB1 proteins, which are located at the ends of microtubules, are recruited as a result of the influx of calcium. This facilitates the elongation of microtubules and the transport of vesicles and phospholipids towards the membrane lesion. Secondly, the rupture of the plasma membrane causes the release of membrane tension, which is naturally present in every cell. This leads to retraction of the membrane at the site of injury. This is

because the phospholipid bilayer is attached to the cytoskeleton of the muscle cell, which causes the phospholipids to move towards each other at the site of injury, resulting in the retraction of the membrane. This force is opposite to that of repair.

The massive influx of calcium into the muscle cell induces exocytosis with the aim of reducing membrane retraction. Exocytosis is the process by which intracellular vesicles fuse with the plasma membrane to release their contents into the extracellular environment. To achieve this, intracellular vesicles migrate towards the plasma membrane using motor proteins, including kinesin and dysferlin. The vesicles then fuse with the plasma membrane, for example, lysosomes fuse with the plasma membrane thanks to the presence of synaptotagmin 7 on their surfaces. This fusion of vesicles with the plasma membrane increases the membrane surface area, thereby reducing the membrane shrinkage induced by the lesion. Furthermore, membrane shrinkage is also reduced by the formation of an intracellular annexin 5 network at the lesion.

During the exocytosis process, the lysosomal enzyme ASM is released into the extracellular compartment and acts on the outer leaflet of the plasma membrane by hydrolysing membrane sphingomyelin, generating ceramides on the lipid bilayer. This reaction induces inward bending of the membrane, enabling the formation of endosomes and promoting endocytosis, a mechanism in which certain annexins, the SNARE complex and the ESCRT complex participate.

Concurrently, microtubule elongation facilitates the transport of proteins involved in muscle cell membrane repair. This is the case for vesicles containing dysferlin, which move along microtubules thanks to kinesin and migrate towards the site of injury. At the site of the membrane break, active calpains cleave dysferlin at the protein portion encoded by alternative exon 40a, generating mini-dysferlin. Vesicles containing mini-dysferlin are accumulated beneath the membrane lesion and fuse with the plasma membrane, where MG53 proteins are bound to phosphatidylserine. MG53 detects the change in oxidation of the intracellular environment during membrane damage, oligomerises, binds to phosphatidylserine in the plasma membrane and participates in the trafficking of vesicles to the site of damage. MG53 and dysferlin are both involved in the fusion of vesicles with the plasma membrane. This fusion of the vesicles with the plasma membrane forms of a "membrane patch" which ultimately fills the lesion.

Cellular Inflammation

Dysferlin appears to be involved in cellular inflammation, a fundamental process triggered by internal or external stress and mediated by both innate and adaptive immunity. Studies suggest a compromised immune response in tissues lacking dysferlin. This is evidenced by:

- Reduced Cytokine and Chemokine Secretion: Signaling molecules crucial for immune cell recruitment are diminished.
- Imbalance in Immune Cell Activity: Immune cells arrive late in dysferlin-deficient tissues but remain longer, leading to a prolonged inflammatory response.

Monocytes, a type of immune cell, show increased dysferlin expression during their development. This dysferlin, located on the monocyte membrane, is rapidly internalized upon differentiation. It's believed to form a complex with an integrin, allowing monocytes to adhere to blood vessel cells and migrate to areas of injury. This could explain the delayed arrival of immune cells in dysferlin-deficient muscle tissue.

The prolonged inflammatory response in dysferlin-deficient tissues might be linked to dysfunctions in dysferlin-mediated membrane repair. Monocytes in these tissues exhibit heightened phagocytic activity, potentially contributing to the prolonged response. Moreover, during muscle repair, dysferlin triggers the release of a molecule called ASM through its interaction with other proteins. ASM can regulate inflammation by controlling the release of pro-inflammatory signals. In the absence of dysferlin, this regulatory mechanism might be disrupted, leading to persistent inflammation.

In conclusion, dysferlin seems to play a role in cellular inflammation beyond its established function in muscle. Its involvement in monocyte adhesion and potential role in regulating anti-inflammatory signals warrant further investigation to fully understand its contributions to this complex process.

1.3 Dysferlinopathy

Dysferlinopathies are a group of musclar diseases caused by dysferlin deficiency. They are muscular dystrophies characterized by a broad clinical spectrum but mainly affecting skeletal muscle. Over the past twenty years, the study of dysferlinopathies has seen major advances in pathophysiological understanding, diagnosis, clinical management, and the development of therapeutic strategies.

There are several types of dysferlinopathy. The two main forms are Miyoshi myopathy and limb-girdle muscular dystrophy type R2. There are more atypical forms of dysferlinopathy, including distal tibialis anterior myopathy and Paradas-type congenital myopathy.

Patients with Miyoshi myopathy (MM) present with the first muscular symptoms during adolescence or around the age of 20. Initially, the posterior calf muscles, gastrocnemius and

soleus, are affected, then the pathology progresses to the posterior compartment of the thigh and hip girdle. The extensors of the forearm may also show weakness, but the brachioradialis and intrinsic muscles of the hand are usually spared. The progression of Miyoshi's myopathy is highly variable. Some patients become ambulatory rapidly, within a few years, while others show weakness and muscle atrophy limited to the posterior calf muscles over a longer period of time. However, the majority of patients experience difficulty standing and walking as the disease progresses.

Limb-girdle muscular dystrophies (LGMDs) are a group of muscular dystrophies that primarily affect the pelvic and scapular girdles. This heterogeneous group includes more than 30 muscular dystrophies, each caused by the deficiency of a muscle protein. Limb-girdle muscular dystrophy type R2 (LGMDR2, formerly LGMD2B) is caused by dysferlin deficiency. Like other limb-girdle muscular dystrophies, LGMDR2 manifests as muscle weakness and atrophy of the proximal skeletal muscles that progresses slowly throughout life. Initially, the proximal muscles of the lower limbs are affected, then the pathology progresses to the proximal muscles of the upper limbs. In the lower limbs, the main muscles affected are the biceps femoris, semimembranosus, and adductor magnus. In the upper limbs, the main muscles affected are the biceps and the head of the pectoralis major. The trunk and shoulder girdle are only mildly affected. In many patients, only the head of the pectoralis major is affected. Onset of symptoms in LGMDR2 patients is usually between the ages of 15 and 35. Patients first experience difficulty walking and climbing stairs. Like Miyoshi's myopathy, the progression of LGMDR2 is variable. However, the progression of symptoms is generally slow. For example, a study of 30 LGMDR2 patients showed that one third required a wheelchair, with an average age of about 40 years.

There are also several atypical forms of dysferlinopathy, the best described of which is distal tibialis anterior myopathy. This myopathy is characterized by muscle weakness affecting the anterior tibial muscles. Patients with this form of dysferlinopathy show progressive impairment of the flexor muscles of the wrist and fingers, as well as the biceps brachii. Between the ages of 30 and 50, patients with distal tibialis anterior myopathy lose the ability to walk. On the other hand, Paradas-type congenital myopathy is another atypical form of dysferlinopathy that manifests early, between birth and two months of age, with weakness of the lower limbs and neck flexors. This myopathy results in delayed motor development.

Despite the variability of the clinical spectrum of dysferlinopathies, certain clinical features are common, in particular muscle weakness. In fact, the first symptom of patients with dysferlinopathies is often the onset of muscle weakness. This muscle weakness affects a variety of

skeletal muscles depending on the type of dysferlinopathy, resulting in difficulty climbing stairs, standing, getting up from the floor, or even standing on tiptoe. Some patients also report muscle aches. On the other hand, muscle atrophy is often observed in patients with dysferlinopathies.

Depending on the type of dysferlinopathy, affected skeletal muscles show a reduction in volume due to necrosis of certain muscle fibers. This muscular atrophy progresses continuously due to the degeneration of many muscle fibers, resulting in the muscle weakness observed in patients with dysferlinopathies. Histologically, several features are common to the different forms of dysferlinopathies: inflammation, fibrosis, muscle atrophy, irregularity of muscle fiber size, and mislocalization of muscle fiber nuclei are common histological findings in patients with dysferlinopathies. Indeed, muscle biopsies from patients reveal the abnormal presence of mononuclear inflammatory cells in the connective tissue surrounding necrotic muscle fibers. In addition, the connective tissue around the muscle fibers is abnormally enlarged: this is known as fibrosis.

Finally, in patients with dysferlinopathy, the nuclei that are normally located at the periphery of the muscle fibers are located in the center of the fibers, indicating a process of degeneration/regeneration of the muscle fibers. A clinical sign common to most muscular dystrophies is the elevation of creatine kinase, an energy metabolism protein present in muscle. Damaged muscle cells release large amounts of creatine kinase. This is the case in dysferlinopathies, where elevated levels of creatine kinase are found in the bloodstream.

Apart from these muscular clinical signs, no extra-muscular phenotype has been described in patients with dysferlinopathies. Patients with dysferlinopathies show normal motor development and an absence of early signs. In fact, the age of onset is highly variable, ranging from 3 to 60 years, although the average age is about 22 years. Symptoms develop progressively. In the decade following the onset of symptoms, patients often require assistive devices such as canes, walkers, or intermittent use of a wheelchair. In fact, progressive muscle degeneration and atrophy leads to increasing muscle weakness in patients.

Despite differences in clinical phenotypes, all these muscular pathologies share pathogenic variants within the DYSF gene. Dysferlinopathies are genetic diseases where the DYSF gene has one or more pathogenic variants that cause the total or partial absence of dysferlin. Genetic pathologies are characterized as rare diseases because each genetic pathology affects a small proportion of the population. Miyoshi myopathy and LGMDR2 each affect between one and nine people per million. Distal tibialis anterior myopathy and Paradas congenital myopathy each affect less than one person in a million.

To date, more than 400 pathogenic variants of the DYSF gene have been described. They

are distributed along the entire length of the gene, with no single site showing a higher frequency of pathogenic variants. Furthermore, there is no difference in localization between pathogenic variants causing Miyoshi myopathy and pathogenic variants causing LGMDR2. Some studies have also shown that the same pathogenic variant can cause Miyoshi myopathy in some patients and LGMDR2 in others. Thus, there seems to be no genotype/phenotype correlation in dysferlinopathies. As dysferlinopathies are genetic diseases, they are inherited and transmitted within the same family.

The mode of transmission of dysferlinopathies is autosomal recessive and it is necessary for both alleles of the DYSF gene to carry a pathogenic variant to cause the pathology. This mode of transmission means that the disease affects as many girls as boys, and that there is not necessarily an affected individual in every generation. However, there are a few rare muscular dystrophy patients in whom no family member is a carrier of a pathogenic variant in the DYSF gene. These sporadic cases results from an error during gametogenesis. In the case of dysferlinopathies, very few patients are sporadic because it is necessary to carry two pathogenic variants, one on each allele.

Several pathogenic mechanisms underlie the clinical phenotype of dysferlinopathies. Since dysferlin is involved in several skeletal muscle functions, its deficiency has several consequences. First, T-tubule dysfunction has been observed in patients with dysferlinopathies. In addition, abnormalities in cytoskeletal remodeling and vesicle trafficking and fusion have been observed during membrane repair in dysferlin-deficient tissues. Taken together, these observations suggest that the membrane repair function of muscle cells is severely compromised in patients with dysferlinopathies, which may underlie the necrosis of certain muscle fibers and thus muscular dystrophy and weakness. Additionaly, the muscles of these patients are highly inflamed. Indeed, certain inflammatory cells, particularly monocytes, appear to have increased proliferation and phagocytic activity when dysferlin is deficient. Similarly, the inflammasome, a protein complex involved in innate immunity, is greatly increased in patients with dysferlinopathies, explaining the presence of inflammatory infiltrates in the muscles of patients. Finally, lipid accumulation is seen in the muscles of patients with dysferlinopathies. It was shown that dysferlin-deficient muscles have an early accumulation of lipids within muscle fiber bundles, which are subsequently replaced by adipocytes. This fat accumulation may be caused by the expression of certain connexins by dysferlin-deficient muscle cells. Indeed, treatment with a connexin channel blocker showed inhibition of this fat accumulation in dysferlin-deficient muscles.

Currently, there is no curative treatment for dysferlinopathies. Palliative care is the ba-

sis of clinical management of patients with dysferlinopathies. The goal is to ensure patient comfort by preventing and relieving physical pain, symptoms, and psychological distress. In dysferlinopathies, physical therapy with stretching and muscle strengthening exercises is crucial.

Chapter 2

Problematic

Dysferlinopathies are a group of rare genetic muscle diseases caused by mutations in the DYSF gene. This project aims to unravel the disease's pathophysiology through logical modeling. We want to understand how these mutations disrupt various muscle functions and ultimately lead to the symptoms observed in dysferlinopathy patients. Additionally, the model may identify potential therapeutic targets.

Logical modeling, particularly Boolean and multi-valued network models, offers a powerful tool to simulate biological processes. These models integrate existing knowledge from scientific literature with data generated by high-throughput omics technologies (genomics, transcriptomics, proteomics, etc.). A key advantage of logical modeling is that it doesn't require precise values for molecular concentrations, gene expression levels, or kinetic constants. It solely relies on documented pairwise regulations.

Bibliography

Abreu-Blanco, M. T., J. J. Watts, J. M. Verboon, and S. M. Parkhurst (Aug. 2012). "Cytoskeleton responses in wound repair". In: *Cell Mol Life Sci* 69.15, pp. 2469–2483. DOI: 10.1007/s00018-012-0928-2.

Alloush, J., S. R. Roof, E. X. Beck, M. T. Ziolo, and N. Weisleder (Oct. 2013). "Expression levels of sarcolemmal membrane repair proteins following prolonged exercise training in mice". In: *Indian J Biochem Biophys* 50.5, pp. 428–435.

Ammendolia, D. A., W. M. Bement, and J. H. Brumell (Apr. 13, 2021). "Plasma membrane integrity: implications for health and disease". In: *BMC Biol* 19.1, p. 71. DOI: 10.1186/s12915-021-00972-y.

Anwar, S., F. Mir, and T. Yokota (Apr. 3, 2023). "Enhancing the Effectiveness of Oligonucleotide Therapeutics Using Cell-Penetrating Peptide Conjugation, Chemical Modification, and Carrier-Based Delivery Strategies". In: *Pharmaceutics* 15.4, p. 1130. DOI: 10.3390/pharmaceutics15041130.

Anwar, S. and T. Yokota (Feb. 21, 2024). "The Dysferlinopathies Conundrum: Clinical Spectra, Disease Mechanism and Genetic Approaches for Treatments". In: *Biomolecules* 14.3. Publisher: MDPI AG, p. 256. DOI: 10.3390/biom14030256.

Azakir, B. A., S. Di Fulvio, C. Therrien, and M. Sinnreich (Apr. 12, 2010). "Dysferlin Interacts with Tubulin and Microtubules in Mouse Skeletal Muscle". In: *PLoS ONE* 5.4. Ed. by N. A. Hotchin. Publisher: Public Library of Science (PLoS), e10122. DOI: 10.1371/journal.pone.0010122.

Báez-Matus, X., C. Figueroa-Cares, A. M. Gónzalez-Jamett, H. Almarza-Salazar, C. Arriagada, M. C. Maldifassi, et al. (Dec. 19, 2019). "Defects in G-Actin Incorporation into Filaments in Myoblasts Derived from Dysferlinopathy Patients Are Restored by Dysferlin C2 Domains". In: *Int J Mol Sci* 21.1, E37. DOI: 10.3390/ijms21010037.

Ballouhey, O., S. Courrier, V. Kergourlay, S. Gorokhova, M. Cerino, M. Krahn, et al. (Nov. 23, 2021). "The Dysferlin Transcript Containing the Alternative Exon 40a is Essential for Myocyte Functions". In: *Front. Cell Dev. Biol.* 9. Publisher: Frontiers Media SA. DOI: 10.3389/fcell.2021.754555.

Barzilai-Tutsch, H., M. Dewulf, C. Lamaze, G. Butler Browne, M. Pines, and O. Halevy (Aug. 15, 2018). "A promotive effect for halofuginone on membrane repair and synaptotagmin-7 levels in muscle cells of dysferlin-null mice". In: *Human Molecular Genetics* 27.16, pp. 2817–2829. DOI: 10.1093/hmg/ddy185.

Becker, N., S. A. Moore, and K. A. Jones (Dec. 2022). "The inflammatory pathology of dysferlinopathy is distinct from calpainopathy, Becker muscular dystrophy, and inflammatory myopathies". In: *acta neuropathol commun* 10.1. Publisher: Springer Science and Business Media LLC. DOI: 10.1186/s40478-022-01320-z.

Begam, M., A. F. Collier, A. L. Mueller, R. Roche, S. S. Galen, and J. A. Roche (June 2018). "Diltiazem improves contractile properties of skeletal muscle in dysferlin-deficient BLAJ mice, but does not reduce contraction-induced muscle damage". In: *Physiol Rep* 6.11, e13727. DOI: 10.14814/phy2.13727.

Benn, K. W., O. G. Bhalala, T. J. Day, and C. R. French (Aug. 2024). "Diagnosis of dysferlinopathy masked by a superimposed hypothyroid myopathy". In: *BMJ Case Rep* 17.8. Publisher: BMJ, e260986. DOI: 10.1136/bcr-2024-260986.

Biondi, O., M. Villemeur, A. Marchand, F. Chretien, N. Bourg, R. K. Gherardi, et al. (June 2013). "Dual Effects of Exercise in Dysferlinopathy". In: *The American Journal of Pathology* 182.6, pp. 2298–2309. DOI: 10.1016/j.ajpath.2013.02.045.

Bouchard, C. and J. P. Tremblay (Sept. 16, 2023). "Portrait of Dysferlinopathy: Diagnosis and Development of Therapy". In: *JCM* 12.18. Publisher: MDPI AG, p. 6011. DOI: 10.3390/jcm12186011.

Bulgart, H. R., M. A. Lopez Perez, A. Tucker, G. N. Giarrano, K. Banford, O. Miller, et al. (Oct. 31, 2024). "Plasma membrane repair defect in Alzheimer's disease neurons is driven by the reduced dysferlin expression". In: *The FASEB Journal* 38.20. Publisher: Wiley. DOI: 10.1096/fj.202401731rr.

Cacciottolo, M., G. Numitone, S. Aurino, I. R. Caserta, M. Fanin, L. Politano, et al. (Sept. 2011). "Muscular dystrophy with marked Dysferlin deficiency is consistently caused by primary dysferlin gene mutations". In: *Eur J Hum Genet* 19.9, pp. 974–980. DOI: 10.1038/ejhg.2011.70.

Cai, C., P. Lin, H. Zhu, J.-K. Ko, M. Hwang, T. Tan, et al. (May 29, 2015). "Zinc Binding to MG53 Protein Facilitates Repair of Injury to Cell Membranes". In: *J Biol Chem* 290.22, pp. 13830–13839. DOI: 10.1074/jbc.M114.620690.

Cai, C., H. Masumiya, N. Weisleder, N. Matsuda, M. Nishi, M. Hwang, et al. (Jan. 2009). "MG53 nucleates assembly of cell membrane repair machinery". In: *Nat Cell Biol* 11.1, pp. 56–64. DOI: 10.1038/ncb1812.

Cai, C., H. Masumiya, N. Weisleder, Z. Pan, M. Nishi, S. Komazaki, et al. (2009). "MG53 regulates membrane budding and exocytosis in muscle cells". In: *Journal of Biological Chemistry* 284.5. ISBN: 0021-9258 Publisher: ASBMB, pp. 3314–3322.

Cai, C., N. Weisleder, J.-K. Ko, S. Komazaki, Y. Sunada, M. Nishi, et al. (2009). "Membrane repair defects in muscular dystrophy are linked to altered interaction between MG53, caveolin-3, and dysferlin". In: *Journal of Biological Chemistry* 284.23. ISBN: 0021-9258 Publisher: ASBMB, pp. 15894–15902.

Cea, L. A., J. A. Bevilacqua, C. Arriagada, A. M. Cárdenas, A. Bigot, V. Mouly, et al. (May 24, 2016). "The absence of dysferlin induces the expression of functional connexin-based hemichannels in human myotubes". In: *BMC Cell Biol* 17 Suppl 1, p. 15. DOI: 10.1186/s12860-016-0096-6.

Cea, L. A., G. Fernández, G. Arias-Bravo, M. Castillo-Ruiz, R. Escamilla, M. C. Brañes, et al. (Aug. 21, 2020). "Blockade of Hemichannels Normalizes the Differentiation Fate of Myoblasts and Features of Skeletal Muscles from Dysferlin-Deficient Mice". In: *IJMS* 21.17, p. 6025. DOI: 10.3390/ijms21176025.

Chen, H.-Y. and D. E. Michele (Dec. 26, 2024). "Syntaxin 4-enhanced plasma membrane repair isindependent of dysferlin in skeletal muscle". In: *American Journal of Physiology-Cell Physiology*. Publisher: American Physiological Society. DOI: 10.1152/ajpcell.00507.2024.

Chiu, Y.-H., M. A. Hornsey, L. Klinge, L. H. Jørgensen, S. H. Laval, R. Charlton, et al. (June 1, 2009). "Attenuated muscle regeneration is a key factor in dysferlin-deficient muscular dystrophy". In: *Human Molecular Genetics* 18.11. Publisher: Oxford University Press (OUP), pp. 1976–1989. DOI: 10.1093/hmg/ddp121.

Codding, S. J., N. Marty, N. Abdullah, and C. P. Johnson (July 8, 2016). "Dysferlin Binds SNAREs (Soluble N-Ethylmaleimide-sensitive Factor (NSF) Attachment Protein Receptors) and Stimulates Membrane Fusion in a Calcium-sensitive Manner". In: *J Biol Chem* 291.28, pp. 14575–14584. DOI: 10.1074/jbc.M116.727016.

Cohen, T. V., J. E. Cohen, and T. A. Partridge (July 2012). "Myogenesis in dysferlin-deficient myoblasts is inhibited by an intrinsic inflammatory response". In: *Neuromuscular Disorders* 22.7. Publisher: Elsevier BV, pp. 648–658. DOI: 10.1016/j.nmd.2012.03.002.

Davenport, N. R., K. J. Sonnemann, K. W. Eliceiri, and W. M. Bement (July 15, 2016). "Membrane dynamics during cellular wound repair". In: *MBoC* 27.14. Ed. by D. G. Drubin. Publisher: American Society for Cell Biology (ASCB), pp. 2272–2285. DOI: 10.1091/mbc.e16-04-0223.

Defour, A., J. H. Van der Meulen, R. Bhat, A. Bigot, R. Bashir, K. Nagaraju, et al. (2014). "Dysferlin regulates cell membrane repair by facilitating injury-triggered acid sphingomyelinase secretion". In: *Cell death & disease* 5.6. ISBN: 2041-4889 Publisher: Nature Publishing Group, e1306–e1306.

Diaz-Manera, J., R. Fernandez-Torron, J. LLauger, M. K. James, A. Mayhew, F. E. Smith, et al. (Oct. 2018). "Muscle MRI in patients with dysferlinopathy: pattern recognition and implications for clinical trials". In: *J Neurol Neurosurg Psychiatry* 89.10, pp. 1071–1081. DOI: 10.1136/jnnp-2017-317488.

Donen, G. S., Z. White, E. Sauge, M. Ritso, M. Theret, J. Boyd, et al. (Oct. 2022). "Thermoneutral Housing and a Western Diet Combination Exacerbates Dysferlin-Deficient Muscular Dystrophy". In: *Muscle and Nerve* 66.4, pp. 513–522. DOI: 10.1002/mus.27680.

Escobar, H., S. Di Francescantonio, J. Smirnova, R. Graf, S. Müthel, A. Marg, et al. (Jan. 2, 2025). "Gene-editing in patient and humanized-mice primary muscle stem cells rescues dysferlin expression in dysferlin-deficient muscular dystrophy". In: *Nat Commun* 16.1. Publisher: Springer Science and Business Media LLC. DOI: 10.1038/s41467-024-55086-0.

Fanin, M. and C. Angelini (2002). "Muscle pathology in dysferlin deficiency". In: *Neuropathology and applied neurobiology* 28.6. ISBN: 0305-1846 Publisher: Wiley Online Library, pp. 461–470.

Fanin, M. and C. Angelini (Nov. 2016). "Progress and challenges in diagnosis of dysferlinopathy: Dysferlinopathy Diagnosis". In: *Muscle Nerve* 54.5, pp. 821–835. DOI: 10.1002/mus.25367.

Fernández, G., G. Arias-Bravo, J. A. Bevilacqua, M. Castillo-Ruiz, P. Caviedes, J. C. Sáez, et al. (Aug. 1, 2020). "Myofibers deficient in connexins 43 and 45 expression protect mice from skeletal muscle and systemic dysfunction promoted by a dysferlin mutation". In: *Biochim Biophys Acta Mol Basis Dis* 1866.8, p. 165800. DOI: 10.1016/j.bbadis.2020.165800.

Fernández-Simón, E., C. Lleixà, X. Suarez-Calvet, J. Diaz-Manera, I. Illa, E. Gallardo, et al. (Dec. 2020). "Proteasome inhibitors reduce thrombospondin-1 release in human dysferlin-deficient myotubes". In: *BMC Musculoskelet Disord* 21.1. Publisher: Springer Science and Business Media LLC. DOI: 10.1186/s12891-020-03756-7.

Fontelonga, T., A. J. Hall, J. L. Brown, Y. L. Jung, M. S. Alexander, J. A. Dominov, et al. (Dec. 2023). "Tetraspanin CD82 Associates with Trafficking Vesicle in Muscle Cells and Binds to Dysferlin and Myoferlin". In: *Advanced Biology* 7.12. Publisher: Wiley. DOI: 10.1002/adbi.202300157.

Foxton, R. M., S. H. Laval, and K. M. D. Bushby (Feb. 2004). "Characterisation of the dysferlin skeletal muscle promoter". In: *Eur J Hum Genet* 12.2, pp. 127–131. DOI: 10.1038/sj.ejhg.5201092.

Gallardo, E., A. Ankala, Y. Núñez-Álvarez, M. Hegde, J. Diaz-Manera, N. D. Luna, et al. (Aug. 2014). "Genetic and Epigenetic Determinants of Low Dysferlin Expression in Monocytes". In: *Human Mutation* 35.8. Publisher: Hindawi Limited, pp. 990–997. DOI: 10.1002/humu.22591.

Gallardo, E., N. De Luna, J. Diaz-Manera, R. Rojas-García, L. Gonzalez-Quereda, B. Flix, et al. (Dec. 16, 2011). "Comparison of Dysferlin Expression in Human Skeletal Muscle with That in Monocytes for the Diagnosis of Dysferlin Myopathy". In: *PLoS ONE* 6.12. Ed. by M. Schuelke. Publisher: Public Library of Science (PLoS), e29061. DOI: 10.1371/journal.pone.0029061.

García-Campos, P., X. Báez-Matus, C. Jara-Gutiérrez, M. Paz-Araos, C. Astorga, L. A. Cea, et al. (June 16, 2020). "N-Acetylcysteine Reduces Skeletal Muscles Oxidative Stress and Improves Grip Strength in Dysferlin-Deficient Bla/J Mice". In: *IJMS* 21.12, p. 4293. DOI: 10.3390/ijms21124293.

Golbek, T. W., S. C. Otto, S. J. Roeters, T. Weidner, C. P. Johnson, and J. E. Baio (Jan. 14, 2021). "Direct Evidence That Mutations within Dysferlin's C2A Domain Inhibit Lipid Clustering". In: *J. Phys. Chem. B* 125.1. Publisher: American Chemical Society (ACS), pp. 148–157. DOI: 10.1021/acs.jpcb.0c07143.

Gushchina, L. V., S. Bhattacharya, K. E. McElhanon, J. H. Choi, H. Manring, E. X. Beck, et al. (Oct. 2017). "Treatment with Recombinant Human MG53 Protein Increases Membrane Integrity in a Mouse Model of Limb Girdle Muscular Dystrophy 2B". In: *Molecular Therapy* 25.10, pp. 2360–2371. DOI: 10.1016/j.ymthe.2017.06.025.

Han, R. (2011). "Muscle membrane repair and inflammatory attack in dysferlinopathy". In: *Skeletal Muscle* 1.1, p. 10. DOI: 10.1186/2044-5040-1-10.

Haynes, V. R., S. N. Keenan, J. Bayliss, E. M. Lloyd, P. J. Meikle, M. D. Grounds, et al. (Aug. 2019). "Dysferlin deficiency alters lipid metabolism and remodels the skeletal muscle lipidome in mice". In: *Journal of Lipid Research* 60.8. Publisher: Elsevier BV, pp. 1350–1364. DOI: 10.1194/jlr.m090845.

Hernández-Deviez, D. J., M. T. Howes, S. H. Laval, K. Bushby, J. F. Hancock, and R. G. Parton (Mar. 7, 2008). "Caveolin regulates endocytosis of the muscle repair protein, dysferlin". In: *J Biol Chem* 283.10, pp. 6476–6488. DOI: 10.1074/jbc.M708776200.

Hnilicova, P., M. Grendar, M. Turcanova Koprusakova, A. Trancikova Kralova, J. Harsanyiova, M. Krssak, et al. (Aug. 20, 2024). "Brain of miyoshi myopathy/dysferlinopathy patients presents with structural and metabolic anomalies". In: *Sci Rep* 14.1. Publisher: Springer Science and Business Media LLC. DOI: 10.1038/s41598-024-69966-4.

Hogarth, M. W., A. Defour, C. Lazarski, E. Gallardo, J. Diaz Manera, T. A. Partridge, et al. (June 3, 2019). "Fibroadipogenic progenitors are responsible for muscle loss in limb girdle muscular dystrophy 2B". In: *Nat Commun* 10.1, p. 2430. DOI: 10.1038/s41467-019-10438-z.

Huang, H.-L., G. Grandinetti, S. M. Heissler, and K. Chinthalapudi (Nov. 7, 2024). "Cryo-EM structures of the membrane repair protein dysferlin". In: *Nat Commun* 15.1. Publisher: Springer Science and Business Media LLC. DOI: 10.1038/s41467-024-53773-6.

Jacobs, M. B., M. K. James, L. P. Lowes, L. N. Alfano, M. Eagle, R. Muni Lofra, et al. (May 2021). "Assessing Dysferlinopathy Patients Over Three Years With a New Motor Scale". In: *Annals of Neurology* 89.5, pp. 967–978. DOI: 10.1002/ana.26044.

Kwok, E., S. C. Otto, P. Khuu, A. P. Carpenter, S. J. Codding, P. N. Reardon, et al. (Sept. 2023). "The Dysferlin C2A Domain Binds PI(4,5)P2 and Penetrates Membranes". In: *Journal of Molecular Biology* 435.17. Publisher: Elsevier BV, p. 168193. DOI: 10.1016/j.jmb.2023.168193.

Li, Z. and G. S. Shaw (Feb. 27, 2023). "Role of calcium-sensor proteins in cell membrane repair". In: *Bioscience Reports* 43.2. Publisher: Portland Press Ltd. DOI: 10.1042/bsr20220765.

Lloyd, E. M., R. C. Crew, V. R. Haynes, R. B. White, P. J. Mark, C. Jackaman, et al. (Aug. 9, 2024). "Pilot investigations into the mechanistic basis for adverse effects of glucocorticoids in dysferlinopathy". In: *Skeletal Muscle* 14.1. Publisher: Springer Science and Business Media LLC. DOI: 10.1186/s13395-024-00350-6.

Lloyd, E. M., M. S. Hepburn, J. Li, A. Mowla, J. H. Jeong, Y. Hwang, et al. (Dec. 2024). "Multimodal three-dimensional characterization of murine skeletal muscle micro-scale elasticity, structure, and composition: Impact of dysferlinopathy, Duchenne muscular dystrophy,

and age on three hind-limb muscles". In: *Journal of the Mechanical Behavior of Biomedical Materials* 160. Publisher: Elsevier BV, p. 106751. DOI: 10.1016/j.jmbbm.2024.106751.

Lostal, W., M. Bartoli, C. Roudaut, N. Bourg, M. Krahn, M. Pryadkina, et al. (2012). "Lack of correlation between outcomes of membrane repair assay and correction of dystrophic changes in experimental therapeutic strategy in dysferlinopathy". In: *PloS one* 7.5. ISBN: 1932-6203 Publisher: Public Library of Science San Francisco, USA, e38036.

Matsuda, C., Y. K. Hayashi, M. Ogawa, M. Aoki, K. Murayama, I. Nishino, et al. (Aug. 15, 2001). "The sarcolemmal proteins dysferlin and caveolin-3 interact in skeletal muscle". In: *Hum Mol Genet* 10.17, pp. 1761–1766. DOI: 10.1093/hmg/10.17.1761.

McNeil, P. L. (2002). "Repairing a torn cell surface: make way, lysosomes to the rescue". In: *Journal of cell science* 115.5. ISBN: 1477-9137 Publisher: Company of Biologists, pp. 873–879.

Muriel, J., V. Lukyanenko, T. Kwiatkowski, S. Bhattacharya, D. Garman, N. Weisleder, et al. (Apr. 2022). "The C2 domains of dysferlin: roles in membrane localization, Ca^{2+} signalling and sarcolemmal repair". In: *The Journal of Physiology* 600.8. Publisher: Wiley, pp. 1953–1968. DOI: 10.1113/jp282648.

Ng, R., G. B. Banks, J. K. Hall, L. A. Muir, J. N. Ramos, J. Wicki, et al. (2012). "Animal models of muscular dystrophy". In: *Progress in molecular biology and translational science* 105. ISBN: 1877-1173 Publisher: Elsevier, pp. 83–111.

Nishimura, A., C. Nelke, M. Huber, A. Mensch, A. Roth, C. Oberwittler, et al. (Aug. 2024). "Differentiating idiopathic inflammatory myopathies by automated morphometric analysis of MHC-1, MHC-2 and ICAM-1 in muscle tissue". In: *Neuropathology Appl Neurobio* 50.4. Publisher: Wiley. DOI: 10.1111/nan.12998.

Ono, H., N. Suzuki, S.-i. Kanno, G. Kawahara, R. Izumi, T. Takahashi, et al. (Apr. 2020). "AMPK Complex Activation Promotes Sarcolemmal Repair in Dysferlinopathy". In: *Molecular Therapy* 28.4, pp. 1133–1153. DOI: 10.1016/j.ymthe.2020.02.006.

Paulke, N. J., C. Fleischhacker, J. B. Wegener, G. C. Riedemann, C. Cretu, M. Mushtaq, et al. (Aug. 16, 2024). "Dysferlin Enables Tubular Membrane Proliferation in Cardiac Hypertrophy". In: *Circulation Research* 135.5. Publisher: Ovid Technologies (Wolters Kluwer Health), pp. 554–574. DOI: 10.1161/circresaha.124.324588.

Poudel, B. H., S. Fletcher, S. D. Wilton, and M. Aung-Htut (May 21, 2024). "Limb Girdle Muscular Dystrophy Type 2B (LGMD2B): Diagnosis and Therapeutic Possibilities". In: *IJMS* 25.11. Publisher: MDPI AG, p. 5572. DOI: 10.3390/ijms25115572.

Quinn, C. J., E. J. Cartwright, A. W. Trafford, and K. M. Dibb (May 2024). "On the role of dysferlin in striated muscle: membrane repair, t-tubules and Ca^{2+} handling". In: *The Journal of Physiology* 602.9, pp. 1893–1910. DOI: 10.1113/JP285103.

Redpath, G. M. I., N. Woolger, A. K. Piper, F. A. Lemckert, A. Lek, P. A. Greer, et al. (Oct. 1, 2014). "Calpain cleavage within dysferlin exon 40a releases a synaptotagmin-like module for membrane repair". In: *Mol Biol Cell* 25.19, pp. 3037–3048. DOI: 10.1091/mbc.E14-04-0947.

Redpath, G. M., R. A. Sophocleous, L. Turnbull, C. B. Whitchurch, and S. T. Cooper (2016). "Ferlins Show Tissue-Specific Expression and Segregate as Plasma Membrane/Late Endosomal or Trans-Golgi/Recycling Ferlins". In: *Traffic* 17.3. ISBN: 1398-9219 Publisher: Wiley Online Library, pp. 245–266.

Roche, J. A., R. M. Lovering, and R. J. Bloch (Oct. 29, 2008). "Impaired recovery of dysferlin-null skeletal muscle after contraction-induced injury in vivo". In: *Neuroreport* 19.16, pp. 1579–1584. DOI: 10.1097/WNR.0b013e328311ca35.

Roche, J. A., R. M. Lovering, R. Roche, L. W. Ru, P. W. Reed, and R. J. Bloch (2010). "Extensive mononuclear infiltration and myogenesis characterize recovery of dysferlin-null skeletal muscle from contraction-induced injuries". In: *American Journal of Physiology-Cell Physiology* 298.2. ISBN: 0363-6143 Publisher: American Physiological Society Bethesda, MD, pp. C298–C312.

Scott, I. L., M. J. Dominguez, A. Snow, F. M. Harsini, J. Williams, K. L. Fuson, et al. (Apr. 26, 2023). *Pathogenic Mutations in the C2A Domain of Dysferlin form Amyloid that Activates the Inflammasome*. DOI: 10.1101/2023.04.24.538129.

Sreetama, S. C., G. Chandra, J. H. Van Der Meulen, M. M. Ahmad, P. Suzuki, S. Bhuvanendran, et al. (Sept. 2018). "Membrane Stabilization by Modified Steroid Offers a Potential Therapy for Muscular Dystrophy Due to Dysferlin Deficit". In: *Molecular Therapy* 26.9, pp. 2231–2242. DOI: 10.1016/j.ymthe.2018.07.021.

Thapa, R. and P. A. Keyel (Apr. 15, 2023). "Patch repair protects cells from the small pore-forming toxin aerolysin". In: *Journal of Cell Science* 136.8. Publisher: The Company of Biologists. DOI: 10.1242/jcs.261018.

Uapinyoying, P., M. Hogarth, S. Battacharya, D. A. Mázala, K. Panchapakesan, C. G. Bönnemann, et al. (Aug. 2023). "Single-cell transcriptomic analysis of the identity and function of fibro/adipogenic progenitors in healthy and dystrophic muscle". In: *iScience* 26.8. Publisher: Elsevier BV, p. 107479. DOI: 10.1016/j.isci.2023.107479.

Vincent, A. E., H. S. Rosa, C. L. Alston, J. P. Grady, K. A. Rygiel, M. C. Rocha, et al. (Nov. 2016). "Dysferlin mutations and mitochondrial dysfunction". In: *Neuromuscular Disorders* 26.11. Publisher: Elsevier BV, pp. 782–788. DOI: 10.1016/j.nmd.2016.08.008.

Waddell, L. B., F. A. Lemckert, X. F. Zheng, J. Tran, F. J. Evesson, J. M. Hawkes, et al. (Apr. 2011). "Dysferlin, Annexin A1, and Mitsugumin 53 Are Upregulated in Muscular Dystrophy and Localize to Longitudinal Tubules of the T-System With Stretch". In: *J Neuropathol Exp Neurol* 70.4, pp. 302–313. DOI: 10.1097/NEN.0b013e31821350b0.

Wallace, M. A., N. W. Aguirre, G. R. Marcotte, A. G. Marshall, L. M. Baehr, D. C. Hughes, et al. (Apr. 2021). "The ketogenic diet preserves skeletal muscle with aging in mice". In: *Aging Cell* 20.4, e13322. DOI: 10.1111/acel.13322.

Wang, Y., R. Tadayon, L. Santamaria, P. Mercier, C. J. Forristal, and G. S. Shaw (Jan. 15, 2021). "Calcium binds and rigidifies the dysferlin C2A domain in a tightly coupled manner". In: *Biochemical Journal* 478.1. Publisher: Portland Press Ltd., pp. 197–215. DOI: 10.1042/bcj20200773.

Wenzel, K., J. Zabojszcza, M. Carl, S. Taubert, A. Lass, C. L. Harris, et al. (2005). "Increased susceptibility to complement attack due to down-regulation of decay-accelerating factor/CD55 in dysferlin-deficient muscular dystrophy". In: *The Journal of Immunology* 175.9. ISBN: 0022-1767 Publisher: Am Assoc Immnol, pp. 6219–6225.

Zhang, H., Y. Li, Q. Cheng, X. Chen, Q. Yu, and Z. Li (Feb. 4, 2021). "Abnormal Expression of Dysferlin in Blood Monocytes Supports Primary Dysferlinopathy in Patients Confirmed by Genetic Analyses". In: *Front. Neurol.* 11. Publisher: Frontiers Media SA. DOI: 10.3389/fneur.2020.540098.