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Disfunções do envelope nuclear observadas em doentes com Distrofia Miotónica tipo 1

Nuclear envelope dysfunctions observed in patients with Myotonic Dystrophy type 1

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Biomedicina Molecular, realizada sob a orientação científica da Professora Doutora Sandra Maria Tavares da Costa Rebelo, Professora Auxiliar do Departamento de Ciências Médicas da Universidade de Aveiro e coorientação da Professora Doutora Maria Teresa Ferreira Herdeiro, Professora Auxiliar do Departamento de Ciências Médicas da Universidade de Aveiro.

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o júri

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palavras-chave Distrofias musculares, distrofias miotónicas, envelope nuclear, fibroblastos, western blot, imunocitoquímica

resumo

As distrofias musculares são um grupo muito heterogêneo de doenças hereditárias que se caracterizam por fraqueza muscular, perda de massa muscular e, em alguns casos, alterações do sistema nervoso. Até o momento, são conhecidas mais de 30 formas diferentes de distrofias musculares, incluindo distrofia miotónica (DM). A distrofia miotónica possui duas variantes distintas, distrofia miotónica tipo 1 (DM1) e distrofia miotónica tipo 2 (DM2), que resultam de uma expansão anormal do trinucleótido CTG e do tetranucleótido CCTG, respetivamente. O DM1 resulta de uma expansão anormal de CTG na região 3 'não traduzida do gene DMPK. O mRNA DMPK mutante acumula-se no núcleo como focos ribonucleares comprometendo a função nuclear normal. Nos últimos anos, as proteínas do envelope nuclear (NE) têm sido associadas às distrofias musculares e, especificamente, á DM1, sendo um componente essencial na resposta das células a stresses mecânicos externos e na manutenção da integridade das células musculares. Até ao momento, poucos estudos demonstraram que fibroblastos e mioblastos / miotubos de pacientes com DM1 têm estrutura nuclear comprometida, juntamente com localização e níveis intracelulares alterados de algumas proteínas NE. Apesar disso, o papel das proteínas NE no DM1 ainda não está claro e mais estudos são necessários. Portanto, no presente trabalho, analisamos fibroblastos de um controle aparentemente saudável e de pacientes com DM1 com aproximadamente 1000 e 2000 repetições de CTG (representando os fenótipos adulto e congénito, respetivamente) usando as técnicas de western blotting e imunocitoquímica para avaliar os níveis de proteína NE e localização subcelular, respetivamente. Em relação aos níveis intracelulares das proteínas NE, observamos aumento nos níveis intracelulares de LAP1, SUN1, lamina A / C e lamina B1, em pacientes com DM1 quando comparados aos controles. No caso da nesprina-1, foi observada uma diminuição dos níveis intracelulares da proteína. Os níveis intracelulares de nesprina-2 diminuíram apenas em fibroblastos de DM1_2000 quando comparados aos controles. Finalmente, os níveis da proteína emerina foram semelhantes entre os fibroblastos derivados de DM1 e controles. No estudo da imunocitoquímica, constatamos que os núcleos dos fibroblastos derivados do DM1, quando marcados apenas com DAPI, apresentavam aumento de núcleos deformados, acompanhado de aumento significativo da área nuclear (em DM1_2000). Além disso, descobrimos que os núcleos de fibroblastos derivados de DM1 são positivos para proteínas da lâmina A/C. emerina, LAP1 e nesprina-1 também apresentaram aumento no número de núcleos deformados e inclusões nucleares. No caso da lâmina A/C, emerin e LAP1, também houve aumento da imunomarcação do NE e do nucleoplasma. Em resumo, demonstramos que pacientes com DM1 tendem a apresentar alterações nos níveis intracelulares das proteínas NE e que estas são acompanhadas por alterações na arquitetura nuclear. Assim, mais estudos são necessários para elucidar a contribuição dessa estrutura para os mecanismos patológicos do DM1.

keywords

Muscular Dystrophies, Myotonic Dystrophies, Nuclear envelope, fibroblasts, western blot, immunocytochemistry

abstract

Muscular dystrophies are a very heterogeneous group of inherited diseases that are characterized by muscle weakness, loss of muscle mass and, in some cases, alterations in nervous system. To date, more than 30 different forms of muscular dystrophies are known, including myotonic dystrophy (DM). Myotonic dystrophy has two distinct variants, myotonic dystrophy type 1 (DM1) and myotonic dystrophy type 2 (DM2), which result from an abnormal expansion of the CTG trinucleotide and CCTG tetranucleotide, respectively. DM1 results from an abnormal expansion of CTG in the 3 'untranslated region of the *DMPK* gene. The mutant DMPK mRNA accumulates in the nucleus as ribonuclear foci compromising the normal nuclear function. In recent years, nuclear envelope (NE) proteins have been associated with muscular dystrophies and specifically with DM1, as being an essential component in the response of cells to external mechanical stresses and in maintaining the integrity of muscle cells. To date, only few studies have shown that fibroblasts and myoblasts/myotubes from patients with DM1 have a compromised nuclear structure, together with altered location and intracellular levels of some NE proteins. Despite this, the role of NE proteins in DM1 is remain unclear and further studies are needed. Therefore, in present work, we analysed fibroblasts from an apparently healthy control and from patients with DM1 with approximately 1000 and 2000 CTG repeat length (representing the adult and congenital phenotypes, respectively) using the techniques western blotting and immunocytochemistry to evaluate NE protein levels and subcellular localization, respectively. Concerning intracellular levels of NE proteins, we observed an increase in LAP1, SUN1, lamin A/C and lamin B1 intracellular levels, in patients with DM1 when compared with controls. In the case of nesprin-1, a decrease in the intracellular was observed. The intracellular levels of nesprin-2 were decreased only with DM1_2000 fibroblasts when compared with controls. Finally, the emerin proteins levels were similar between the DM1-derived fibroblasts and controls. In the study of immunocytochemistry, we found that the nuclei of DM1-derived fibroblasts, when marked only with DAPI, presented an increase in deformed nuclei, accompanied by a significant increase in nuclear area (DM1_2000). In addition, we found that the nuclei of DM1-derived fibroblasts, positive for lamin A/C proteins. emerin, LAP1 and nesprin-1 showed also an increase in the number of deformed nuclei and nuclear inclusions. In the case of the lamin A/C, emerin and LAP1, there was also an increase in the immunolabelling of the NE and the nucleoplasm. In summary, we demonstrated that DM1 patients tend to have changes in the intracellular levels of NE proteins and that these are accompanied by changes in the nuclear architecture. Thus, further studies are needed to elucidate the contribution of this structure to the pathological mechanisms of DM1.

List of abreviations

BAF – autointegration barrier factor

BCA – Pierce's bicinchoninic acid

BSA – Bovine Serum Albumin

BMD – Becker muscular dystrophy

CMD – Congenital muscular dystrophy

CIC-1 – Chloride channel 1

CNBP/ZNF9 – CCHC-type zinc finger nucleic acid binding protein

CNS- Central nervous system

cTNT – Cardiac troponin T

CUGBP – CUG-RNA binding protein

DAPI – 4',6-diamidino-2-phenolyde

DD – Distal muscular dystrophy

DM – Myotonic dystrophy

DM1 – Myotonic dystrophy type 1

DM2 – Myotonic dystrophy type 2

DMD – Duchenne muscular dystrophy

DMEM – Dulbecco's Modified Eagle Medium

DMPK – Myotonic dystrophy protein kinase

DMWD - Dystrophia Myotonic WD Repeat-Containing Protein

DNA – Desoxiribonucleic acid

EDMD – Emery-Dreifuss muscular dystrophy

ER - Endoplasmatic reticulum

FBS – Fetal Bovine Serum

FSHD – Facioscapulohumeral muscular dystrophy

HRP – Horsedish peroxidase

ICC - Immunocytochemistry

INM – Inner nuclear membrane

IR – Insulin receptor

KASH – Klarsicht/ANC-1/SYNE homology

LAP1 – Lamin-associated with polypeptide 1

LBR – Lamin B receptor

LEM – LAP2-emerin-MAN1 domain

LGMD – Limb-Girdle muscular dystrophy

LGMD1B - Limb-Girdle muscular dystrophy type B

LINC – Linker of nucleoskeleton and cytoskeleton

MBNL – Muscleblind-like proteins

MYPT – Myosin phosphatase

ND – Not determined

NE – Nuclear envelope

NLS – Nuclear localization sequence

NPC – Nuclear pore complex

Nups - Nucleoporins

ONM – Outer nuclear membrane

OPMD – Oculopharyngeal muscular dystrophy

PBS – Phosphate Buffered Saline

PNS – Perinuclear space

PROMM – Proximal myotonic myopathy

qPCR - Quantitative Polymerase chain reaction

Rb - Retinoblastoma

RNA – Ribonucleic acid

SDS – Sodium dodecyl sulfate

SEM – Standard error of the mean

SIX5 - SIX Homeobox 5

SUN – Sad1/unc-84 protein-like

TBS-T – Tris-buffered saline with 0.1% Tween-20

TMEM38a – Trimeric intracellular cation channel Type-A

TNNT2 – Troponin T2

TORP1AIP1 – Torsin-1A-interacting protein 1

UTR – Untranslated region

WB – Western Blot

X-EDMD – X-linked Emery-Dreifuss muscular dystrophy

List of figures

CHAPTER I

CHAPTER III

CHAPTER IV

List of tables

Outlines

CHAPTER I: INTRODUCTION

Nuclear envelope alterations in Myotonic Dystrophies type 1 and type 2

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Abstract

Muscular dystrophies are a heterogeneous group of inherited diseases characterized by muscle weakness and wasting, with or without breakdown of nerve tissue. Myotonic dystrophy (DM), a multisystemic disease, is an example of a pathology belonging to this group and has two distinct forms, namely Myotonic dystrophy type 1 (DM1) and type 2 (DM2). DM1 results from an abnormal expansion of the CTG trinucleotide in the 3' UTR region in *DMPK* gene while DM2 results from an abnormal expansion of the CCTG tetranucleotide in intron 1 of the *CNBP* gene. These expansions result in a breakdown of mRNA metabolism, which is not translated and accumulates in nuclear foci. The mRNA accumulation leads to the sequestration of MBNL protein and hyperphosphorylation of CUGBP1. However, this mechanism fails to explain the totality of symptoms and phenotypes existing in DM and, therefore, new approaches have been explored. The involvement of NE proteins has been demonstrated in several muscular dystrophies over time. The nuclear envelope (NE) proteins emerin, LAP1 and 2, SUN1 and 2, nesprin -1 and -2 as well as lamin A/C, involved in processes of cellular architecture, maintenance of nuclear structure and regulation of gene expression, were associated with muscular dystrophies. Mutations in their encoding genes, or changes in their intracellular levels, result in deformed cells, with less mechanical resistance and changes in nuclear positioning. In this way, these proteins and others from the NE have been evaluated in the particular cases of DM1 and DM2 and interesting changes have already been reported. To date, alterations in emerin, SUN 2, nesprin -1 and -2 and lamin A/C and B1 were investigated particularly in cells of patients with DM1 and changes in emerin, lamin A/C and B1 in cells of patients with DM2 were reported. These findings suggest that NE proteins may play an important role in the alterations of muscle cells of DM patients and more studies must be done to better understand this role.

Keywords

Muscular dystrophies, Myotonic dystrophy type 1, Myotonic dystrophy type 2, Nuclear envelope, nuclear envelope proteins

1. Introduction

Muscular dystrophies are a very heterogeneous group of hereditary diseases from a clinical, biochemical and genetic point of view. They are caused by mutations in different genes and characterized by muscle weakness and wasting with or without nerve tissue commitment [1,2]. To date, more than 30 different forms of muscular dystrophies are known, which are distinguished by age of onset, affected muscle groups, rate of progression and, in some cases, they are associated with heart disease and mental retardation [1]. Concerning muscular dystrophies, nine major forms are considered, namely Duchenne muscular dystrophy (DMD), Becker muscular dystrophy (BMD), Congenital muscular dystrophy (CMD), Myotonic dystrophy (DM), Facioscapulohumeral muscular dystrophy (FSHD), Emery-Dreifuss muscular dystrophy (EDMD), Distal muscular dystrophy (DD), Limb-girdle muscular dystrophy (LGMD) and Oculopharyngeal muscular dystrophy (OPMD) [3,4]. Of note, each of these types involves eventually loss of muscle strength, increasing disability and possibly deformity [5]. Muscular dystrophies diagnosis is made by a combination of clinical signs with muscle histology, biochemical and genetic analyses. Although muscle biopsy is a highly invasive process, the results obtained are important for a differential diagnosis. From a histological point of view, dystrophic muscles present variation in fibres size, presence of round muscle fibres, increase in the number of internal nuclei, muscle necrosis, increase amount of fat and proliferation of connective tissue [1,2,5,6]. These histopathological changes differ according to the severity of the disease and the type of muscular dystrophy.

Some muscular dystrophies also present changes in nuclear envelope (NE) architecture, proposing the involvement of this structure in the molecular mechanisms underlying these pathologies [7]. In individuals not affected by muscle pathologies, myonuclei are spaced across the periphery of the muscle fibre, maximizing the distance between nuclei. In affected individuals, myonuclei are usually located and grouped in the centre of the muscle fibre [8,9]. The movement and positioning of myonuclei involves NE proteins, which are essential for the interaction between the nucleus and the cytoskeleton. Although it is known that nuclear mispositioning is a histopathological marker of muscle disorders and changes in the NE may be involved in the pathophysiological mechanisms of these diseases, its involvement in the muscular dystrophies pathogenesis and its correlation with the observed muscle weakness is still elusive and requires further studies [9,10].

2. Myotonic Dystrophy (DM)

DM is a type of muscular dystrophy. To date, two distinct forms of DM are known, namely myotonic dystrophy type 1 (DM1) and myotonic dystrophy type 2 (DM2). They are

distinguished by the affected gene and consequently by the set of repeated nucleotides as well as by the clinical manifestations presented (Figure 1) [11–18]. Both DM1 and DM2 will be detailed below.

2.1. DM1 classification and clinical manifestations

DM1, also known as classic myotonic dystrophy or Steinert disease, was first described in 1909. It is the most common adult form of muscular dystrophy with a prevalence of 1:8000 individuals worldwide [12,19]. DM1 is characterized by an abnormal unstable expansion of the CTG trinucleotide in the 3' untranslated region (UTR) of the *Myotonic Dystrophy Protein Kinase* (*DMPK*) gene, located on chromosome 19 (Figure 1) [12,14]. Regarding life expectancy, DM1 patients have a reduced life expectancy when compared to DM2 (Figure 1) [20]. The clinical manifestations vary from the prenatal, postnatal period to adulthood. Due to the great heterogeneity of DM1, this disease is often subdivided according to the severity of the symptoms and the age of onset. Therefore, phenotypically, DM1 is divided into severe, classic or mild and, according the age of onset, it is divided into congenital, infantile, juvenile, adult-onset and late-onset [21–23]. Considering the number of CTG repeat length, in a non-pathological situation, the trinucleotide is repeated up to 37 times in the same individual. CTG repeat length between 38-49 is considered pre-mutation. When the number of CTG repetitions is greater than 50, the pathology is expressed [24,25].

Congenital DM1 is the most severe form of this pathology. These patients present a CTG repeat length higher than 1000 and this phenotype appears at birth or before one month of age [21–23]. This form of DM1 has a mortality rate around 30-40% during the neonatal period [26–28]. Clinically, the congenital DM1 symptoms appear even before birth, such as polyhydramnios and reduced foetal movement [29,30]. After birth, the first symptoms to be recognized are hypotonia, immobility, generalized weakness (typically distal) and facial dysmorphia (“carp's mouth”, ptosis, neck and long faces). In addition to these symptoms, new-borns, in most cases, have a weak cry and difficulties in sucking due to weakness of the facial, jaw and palate muscles [29,31–33]. They also present breathing difficulties, which is the main cause of neonatal mortality [33]. The surviving individuals tend to have motor and cognitive delays during their development, ranging from intellectual impairment to selective cognitive impairment, apathy and autism (Figure 1) [18,33].

Patients diagnosed with the infantile form of DM1 have a distinct phenotype from those diagnosed with congenital DM1, with symptoms appearing between the first month and tenth year of life. These individuals have more than 500 CTG repetitions [21–23,33]. These patients have facial and neck muscles weakness (but without the typical appearance of the congenital form), distal muscle weakness, myotonia, hypotonia and, sometimes, a

slight growth delay [29,33]. Despite these muscular alterations, the most evident altered parameters are neurocognitive. These patients have speech and learning difficulties, being the first aspects that lead them to seek medical support during school age [29,34]. They also present psychiatric symptoms, such as anxiety disorder, attention deficit and hyperactivity [34,35], as well as cardiac problems, namely arrhythmias, cardiac conduction defects and cardiomyopathies, which can lead to serious complications and sudden death [21,22]. Other clinical manifestations include respiratory failure, obesity, gastrointestinal symptoms and somnolence (Figure 1) [22,33].

The juvenile form of DM1 appears between 10 and 20 years of age and patients usually present a CTG length higher than 400 repetitions [21–23]. This phenotype is identical to that of infantile DM1, but with some differences in terms of symptoms severity. These patients exhibit gastrointestinal symptoms, daytime sleepiness, overweight/obesity and cardiac conduction defects. Additionally, they present muscle weakness, dysphagia and respiratory insufficiency, but these symptoms are less severe when compared to the infantile DM1 phenotype. However, myotonia is more severe than in juvenile DM1 patients. People diagnosed with this phenotype may still develop cataracts or manifest infertility (Figure 1) [21,22,36].

The adult-onset variant of DM1 is the most common phenotype and appears between 20 and 40 years of age, wherein the CTG repeat length can vary between 150 to 1000 repetitions [21,23,29]. Initial atypical manifestations include abnormal heart rhythms, excessive daytime sleepiness, irritable bowel syndrome and premature baldness in men. From a muscular point of view, the main pathological characteristics are facial weakness, mild bilateral ptosis, myotonia and marked distal weakness [21,34,36], leading to difficulties in the performance of daily tasks. Respiratory problems, such as pulmonary failure and infections, are also common due to weakness of the diaphragm and other respiratory muscles. Cataracts and endocrine abnormalities are also observed (Figure 1) [22,34].

The late-onset phenotype is the mildest form of DM1, wherein patients have the least CTG repeat length (between 50 and 149 repetitions) and symptoms appear after 40 years of age [21–23]. Many of these patients are asymptomatic or have mild symptoms that delay the diagnosis of this pathology, which is often made as a disposal based on family history and not on the symptoms presented [30]. When symptoms develop, the main clinical manifestations are mild myotonia, cataracts, muscle weakness, overweight/obesity, diabetes and excessive daytime sleepiness (Figure 1) [22,30,36].

Given the different clinical manifestations observed in patients with DM1 the understanding the molecular mechanisms underlying these different DM1 forms is of paramount importance. Therefore, a summary of DM1 molecular mechanisms so far proposed for DM1 will be detailed below.

2.2. DM1 Molecular mechanisms

DM1 is caused by a mutation located in the 3' UTR region of the *DMPK* gene, leading to an abnormal expansion of the CTG trinucleotide. Several studies have been performed to unravel the molecular mechanisms involved in this pathology and various hypotheses have emerged, of which the most accepted is the RNA toxic gain-of-function. CTG expansion disrupts mRNA metabolism, since mutant transcripts are not translated and accumulate in the cell nucleus, forming hook-shaped nuclear aggregates [37,38]. These nuclear foci are able to bind and sequester RNA binding proteins/slicing regulators, namely muscleblind-like proteins (MBNL) and CUG RNA binding protein 1 (CUGBP1), which are involved in RNA splicing, stabilization and translation processes [39–42]. The increase in CUG leads to hyperphosphorylation of CUGBP1 and consequent increasing in its activity, which, in turn, results in abnormal alternative splicing of diverse pre-mRNAs, such as cardiac troponin T (cTNT), muscle chloride channel (CIC-1) and insulin receptor (IR) [37,43,44]. Additionally, the increase in CUG leads to MBNL sequestration at nuclear foci, resulting also in alterations in the alternative splicing of pre-mRNAs, namely CIC-1 and troponin T2 (TNNT2) [45]. Abnormal alternative splicing of these pre-mRNAs may justify the appearance of multisystemic manifestations observed in DM1, such as cardiac abnormalities, predisposition to diabetes, insulin resistance and myotonia. However, the loss of function of these two proteins does not explain most of the pathological characteristics of DM1 [46–48].

Two other hypotheses have also been considered: *DMPK* haploinsufficiency and rearrangement of the DM1 locus. Regarding the *DMPK* haploinsufficiency hypothesis, it is known that the abnormal CTG expansion leads to reduced levels of expression and decreased activity of DMPK. Since this protein is an essential protein kinase, which phosphorylates proteins involved in the process of muscle contraction and relaxation, this hypothesis justifies, to some extent, some of DM1 symptoms [49,50]. This pathological relevance was highlighted by the discovery that mice with deletion of the *DMPK* gene present cardiac abnormalities and late onset myopathy [51–53].

Furthermore, the *DMPK* gene is transcribed into sense and anti-sense transcripts. According to the hypothesis of rearrangement of the DM1 locus, anti-sense transcripts may be involved in chromatin structure regulation [54,55]. This remodelling and organization of chromatin at the DM1 locus affects the expression of the neighbouring genes (*Dystrophia Myotonic WD Repeat-Containing Protein (DMWD)* and *SIX Homeobox 5* (*SIX5))* located immediately upstream and downstream of the *DMPK* gene, respectively, [46,47].

The possibility of increased RAN transduction and the defect in myonuclei positioning (resulting from mutations in NE proteins) also being involved in the pathological mechanisms of DM1 has been raised, similar to other muscular dystrophies [8,56–58].

2.3. DM2 clinical manifestations

DM2, also known as proximal myotonic myopathy (PROMM), was discovered in 1994 [59]. DM2 is caused by the unstable expansion of the CCTG tetranucleotide in intron 1 of the *CCHC-type zinc finger nucleic acid binding protein* (*CNBP)* gene, located on chromosome 3 (Figure 1) [59–61]. In non-pathological conditions, the number of CCTG repetitions is less than 30. When the number of CCTG repetitions is between 30 and 54, it is considered pre-mutation and, when it is higher than 55, the clinical manifestations of DM2 are observed [62,63]. Concerning DM2, there is no congenital or early onset forms [64] and life expectancy is not affected being considered normal (Figure 1). Prevalence of DM2 is, to date, unknown. However, *CNBP* mutation has been identified predominantly in the European Caucasian population [65]. In general, DM2 appears to be less common than DM1. Despite this assumption, some studies have suggested that DM2 may be as common as DM1 among people in Germany, Poland and Finland [66].

The clinical evaluation of these patients is difficult, given the huge phenotypic variability among them. A large percentage of DM2 patients only present mild symptoms and remain undiagnosed. The first sign of DM2 may be an asymptomatic increase in creatine kinase or gamma-glutamyltransferase levels even during childhood [17]. DM2 usually appears in adulthood around 30-40 years of age with clinical manifestations such as early cataracts (before 50 years of age), muscle weakness, myotonia and thigh stiffness (Figure 1) [15,16,20]. DM2-associated muscle weakness is typically proximal and axial [16,66]. Regarding myotonia and muscle stiffness, both vary from minimal to moderate severity over time (but may also be absent) [67]. The presence of myotonia in DM2 patients is less apparent than in DM1 patients [68]. Muscle pain is also a clinical feature of DM2. Pain tends to be worse after physical exercise or in colder temperatures. The typical DM2 pain is not continuous and appears and disappears without an obvious cause and varies in intensity and distribution to the limbs (Figure 1) [16,68–70].

DM2 also presents cardiac problems, such as arrhythmias, atrial fibrillation, conduction defects and cardiomyopathy [71–73]. Regarding CNS involvement, although there are reports mentioning DM2 patients presenting mental retardation, this clinical manifestation is very uncommon. However, mild cognitive abnormalities, reduced blood flow in frontal and temporal brain lobes [66,74,75], some psychological dysfunctions and abnormal personality

traits (Figure 1) are common features of DM2 patients. This pathology can also be characterized by metabolic and endocrine alterations, such as diabetes, insulin resistance, thyroid problems and, occasionally, hypogonadism in adult men. Additional signs/symptoms may include excessive sweating, glucose intolerance, dysphagia, daytime sleepiness, fatigue, tremors and sleep disorders (Figure 1). Exceptionally, DM2 patients may also have respiratory muscle impairment [16,17,68,76,77].

2.4. DM2 Molecular mechanisms

The RNA toxic gain-of-function hypothesis reported for DM1 is also proposed for DM2. This hypothesis proposes that the repeated CCTG expansion, once translated into mutant RNA, exerts a toxic effect on cells. The expanded CCUG transcript tends to accumulate in cell nuclei as nuclear foci [37,42,78]. The RNAs resulting from *CNBP* mutation form an imperfect double-stranded, hook-like structure that is responsible for the dysregulation of RNA binding factors, such as MBNL and CUGBP1 [79]. MBNL and CUGBP1 are regulatory proteins antagonistic to alternative splicing [80]. In DM2, the accumulation of mutant RNAs leads to MBNL1 sequestration at nuclear foci. Furthermore, it also generates the positive regulation of CUGBP1 by hyperphosphorylation, resulting in abnormal alternative splicing [78,80–82]. For example, myotonia and insulin resistance are correlated with the interruption of alternative splicing CIC-1 and IR, respectively. In summary, CCTG expansions lead to a gain in RNA function by changing the location of alternative splicing regulators that are essential in mRNA processing [78,80–82].

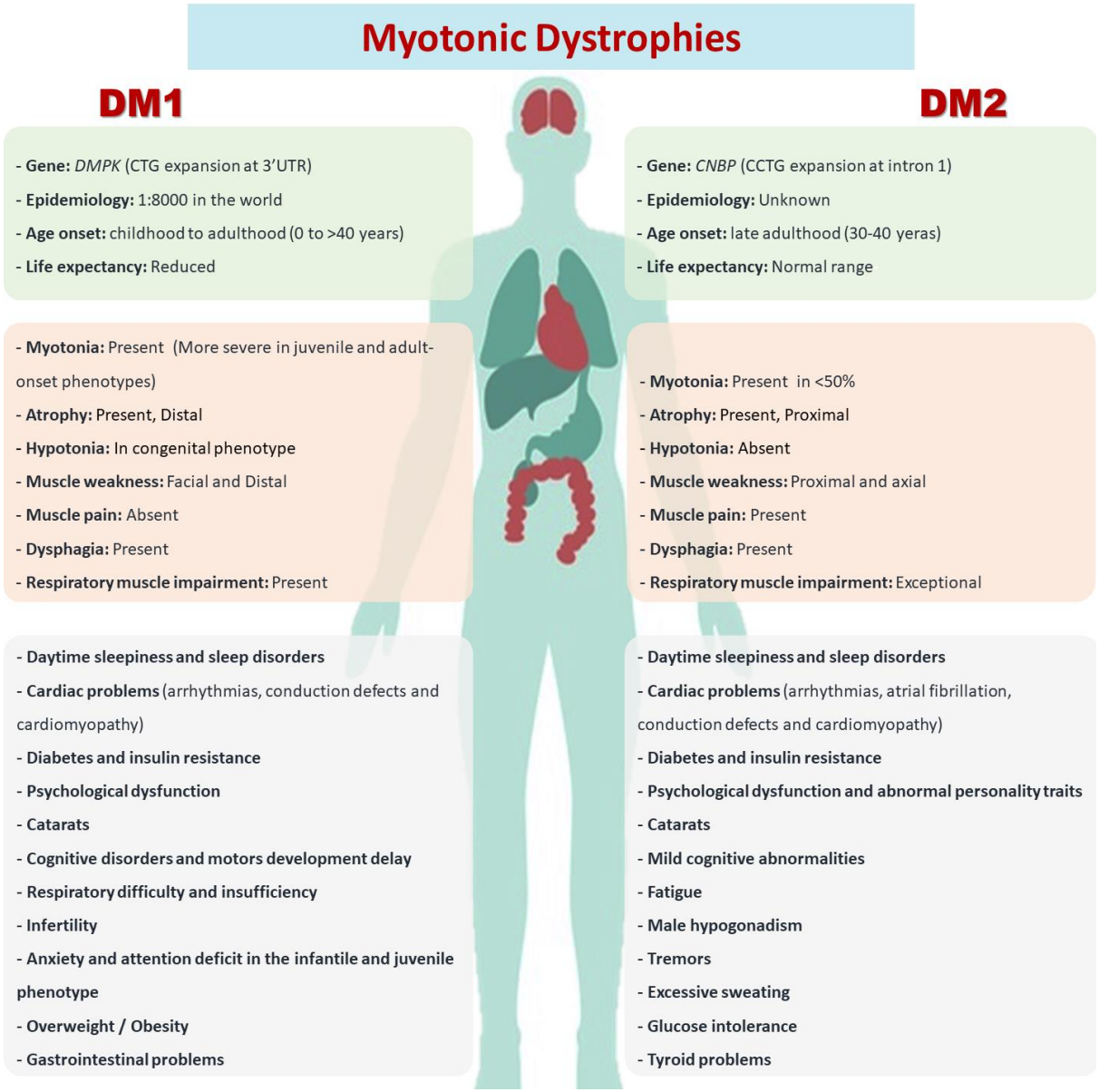

Figure 1: Comparison of clinical manifestations associated with DM1 and DM2. In green boxes, the general characteristics of each disease type are indicated; in pink boxes, the muscular alterations of DM1 and DM2 are represented; and, in grey boxes, the systemic alterations observed in both diseases are summarized.

3. NE proteins previously associated to muscular dystrophies

The NE is responsible for delimiting the genome, being a physical barrier that prevents direct contact between the nucleoplasm and the cytoplasm. This structure is involved in maintaining nuclear integrity, genome organization and gene expression [83,84,85], and can also play important roles in lipid metabolism [86]. The NE is composed of four components, namely the inner nuclear membrane (INM), the outer nuclear membrane (ONM), the

nuclear pore complexes (NPCs) and the nuclear lamina. Between the two membranes, there is the perinuclear space that has continuity with the lumen of the endoplasmic reticulum (RE) [87].

INM is characterized by establishing connections with the nuclear lamins and chromatin [87,88]. In turn, the ONM is located in the outermost part of the NE and establishes several connections with the ER and, therefore, has a similar protein composition [89,90]. INM and ONM are composed of transmembrane proteins that provide support to the nucleus and physical coupling between the nucleoskeleton and the cytoskeleton through the Linker of nucleoskeleton and cytoskeleton (LINC) complex. The LINC complex is composed of Sad1/Unc-84 (SUN) proteins and nesprins [91,92]. This physical connection allows to state that the interruption of the interactions of the SUN-KASH domains leads to an increase in the lumeal space existing between the two membranes, regulating the size of the NE lumen [92,93]. This complex is also involved in cell division, organization of the cytoskeleton and positioning of organelles [91,92,94].

The nuclear lamina is composed by a mesh of proteins called lamins [95]. This structure serves as an anchor point for chromatin factors, playing important roles in signal transduction between the nucleus and the cytoskeleton, gene expression, DNA replication and repair and in chromosomal organization [89,95,96]. The lamins guarantee NE integrity and support the nuclear structure. These proteins, together with the LINC complex, establish a connection between the nucleoskeleton, the cytoskeleton and the genome [97,98]. This structure is made up of type-A lamins and type-B lamins, which we will talk about later [90,92].

Although the NE separates the genomic DNA from the remaining cellular constituents, protecting it from damage and allowing high regulation of the genes, NPCs allow the nucleoplasmic transport of macromolecules. NPCs are made up of nucleoporins (Nups), whose constitution varies in number of proteins and in composition according to the type of cells where they form and depending on the stage of development in which the cell is [99–101]. The structure in NPCs is similar to that of a basket that extends to the nucleoplasm and with cytoplasmic filaments that extend to the cytoplasm [102,103]. NPCs functionally maintain the integrity of the nuclear compartment, preventing uncontrolled and free diffusion of macromolecules inside and outside the nucleus. NPCs are also involved in the regulation of gene expression, cell division, organization of chromatin, organization of internal nuclear architecture and acts as a reservoir for cell cycle proteins when inactive [104,105].

3.1. INM proteins

Emerin is a member of the LAP2-emerin-MAN1 (LEM) domain protein family, which also includes LAP2 and MAN1, and is encoded by the *EMD* gene [106,107]. It has a nucleoplasmic globular N-terminal domain comprising the LEM domain and a C-terminal transmembrane domain [108–110]. The LEM domain mediates the indirect connection of emerin to nuclear lamins and chromatin through barrier-to-autointegration factor (BAF) [111,112]. The interaction of emerin with BAF is important for the regulation of chromatin structure during nuclear assembly, for NE maintenance and for genome stabilization [87,106]. Emerin is also involved in the regulation of gene expression, cell signalling and nuclear architecture [106,113]. Emerin can interact with structural proteins (nuclear actin, nuclear lamins, nesprins, SUN proteins and LAP1), which are predicted to be key regulators of nuclear architecture [111,114–118]. The X-linked EDMD (X-EDMD) was the first human pathology to associate NE defects with muscle disease, being caused by a marked reduction or total absence of emerin in cells (Figure 2) [119–123]. Furthermore, it was shown that emerin "null" cells have a reduced elasticity and a more malleable nuclear membrane. X-EDMD patients have severe nuclear shape abnormalities in skeletal muscle, smooth muscle and fibroblast nuclei [120,121,124].

Regarding Lamin-associated with polypeptide 1 (LAP1), is encoded by the *TOR1AIP1* gene and consists of a nucleoplasmic N-terminal domain, a single transmembrane domain and a luminal C-terminal domain [126]. Two isoforms are known in humans (LAP1B and LAP1C) [124–127]. LAP1 function remains poorly understood, but it is known that it interacts directly with emerin, nuclear lamins and indirectly with chromosomes [117,128]. Therefore, LAP1 may be involved in the preservation of NE integrity as well as the positioning of lamins and chromatin [117,125,128,129]. It is known that LAP1 interacts with torsinA, in the PNS, being associated with the dynamics of NE [130,131]. LAP1 is a phosphoprotein and a PP1 substrate [132]. More recently, the LAP1 interactome has been described, in which several important new putative binding proteins have been identified [133]. Among them is TRF2, which appears to work in conjunction with LAP1 in responding to DNA damage [134].

Skeletal muscle studies using mouse conditional deletion of all LAP1 isoforms, have not shown myopathic features, such as disorganized myofibers and internal myonuclei. However, when the LAP1 deletion occurs in the embryonic stage, dramatic skeletal muscle hypotrophy was observed and leading to 100% mortality. Given this, the expression of LAP1 during initial embryogenesis appears to play an important role in normal postnatal skeletal muscle development and prolonged postnatal survival [135]. In humans, several pathologies caused by mutations in the *TOR1AIP1* gene have been described, including LGMD (with or without cardiac involvement) (Figure 2) [135–137]. These mutations result in the

deficiency or truncation of the LAP1B isoform [136,137]. Patients with truncated LAP1B have higher levels of LAP1C, but this increase does not seem sufficient to counteract the lack of LAP1B [138]. From a histological point of view, the muscle cells of patients with LGMD have dystrophic characteristics (eg. irregular fibers and enlarged internal nuclei) [136].

From the 5 (SUN) isoforms encoded by the human genome (SUN1, SUN2, SUN3, SUN4 and SUN5). All of these isoforms are located at INM [139]. The SUN1 and SUN2 isoforms are found in several cell types while the remaining isoforms are specific to male germ cells [139–142]. These proteins are constituted by an N-terminal domain, located in the nucleoplasm, and a C-terminal domain, consisting of a coiled-coil region, located in the PNS [90,116]. SUN1 and SUN2 are essential components of the LINC complex, playing an important role in the nucleus and cytoskeleton connection, in cell migration and in the positioning of muscle cells nuclei [91,143]. SUN1 is also involved in the process of mRNA exporting, in repairing double-stranded DNA breaks, in chromosomal pairing, in the distribution of NPCs and in nucleolar morphogenesis. In turn, SUN2 is responsible for the formation of perinuclear actin cables involved in nuclear movement and also involved in DNA damage response [91,143,144]. SUN mutations are also found in patients with EDMD (Figure 2), whose cells are characterized by changes in the nuclei positioning and in impaired muscle function, given that a disruption between the nucleus and structural elements of the cytoplasm occurs by LINC disturbance [145].

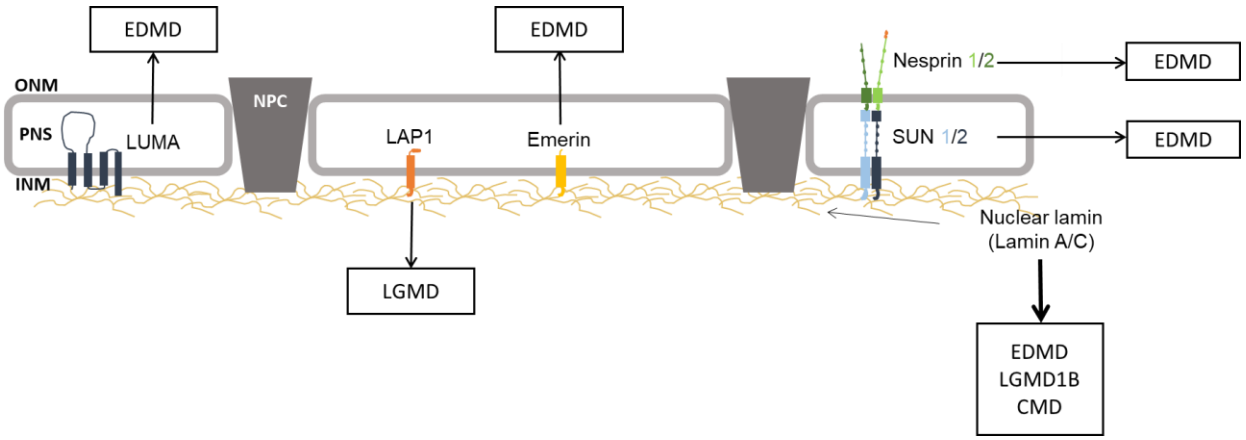

Figure 2: NE proteins previously associated with muscular dystrophies. Only NE proteins associated with muscular dystrophies are shown in this figure. Mutations in the genes encoding these proteins lead to the development of these pathologies. Emerin, LAP1, LUMA, SUN1, SUN2 are proteins of the inner nuclear membrane (INM), nesprins -1 and -2 are in the outer nuclear membrane

(ONM) and lamin A/C is in the nuclear lamina. PNS – Perinuclear space; LGMD - Limb-Girdle Muscular Dystrophy; LGMD1B - Limb-Girdle Muscular Dystrophy Type B; EDMD - Emery-Dreifuss Muscular Dystrophy; CMD - Congenital Muscular Dystrophy.

The LUMA protein is also located in the INM and is encoded by the *TMEM43* gene [146]. This protein consists of 4 transmembrane domains and a hydrophilic domain located in the PNS [147,148]. This protein is known to interact with the nuclear lamins, emerin and SUN2 [146–148]. These interactions, and in particular with emerin, suggest that LUMA plays a crucial role in the spatial and functional organization of INM protein complexes [149]. Mutations in the *TMEM43* gene were first associated with arrhythmogenic cardiomyopathies [150]. In addition, it has also been found that mutations in the gene encoding LUMA may contribute to the development of EDMD-related myopathy, although clinical data are still limited [146,150]. A study carried out in patients with EDMD (without mutations in the *EMD, LMNA, SYNE-1* and *SYNE-2* genes) in which there was an increase in the expression of mutant LUMA, demonstrated an increase in the number of deformed nuclei (Figure 2) [147].

3.2. ONM proteins

Klarsicht/ANC-1/SYNE homology (KASH) proteins, or also called nesprins, are encoded by 5 different genes namely *SYNE-1, SYNE-2, SYNE-3*, *SYNE-4* and *KASH5* giving rise to nesprins -1, -2 ,-3 and -4 and KASH5 proteins, respectively [151–153]. Nesprins are constituted by the cytoplasmatic N-terminal domain and a KASH C-terminal domain containing an highly conserved region which is rooted at the PNS [151,154]. These proteins are expressed, particularly, in cardiac and skeletal muscle and are decisive in the architecture and behaviour of the cell, given that their anchoring functions influence the stability, rigidity, positioning and mechanical migration of the nucleus [155,156]. The nesprin-1 and 2 interact with SUN proteins, emerin, the nuclear lamina proteins and with the actin cytoskeleton as well as with other cytoplasmatic proteins [151,154]. Mutations in *SYNE-1* and *SYNE-2* are associated with EDMD and dilated cardiomyopathy (DCM) (Figure 2) [57,154]. In knockout mice for the KASH domain of nesprin -1 and -2, their death occurred shortly after birth due to respiratory failure. On the other hand, mice bearing a nesprin-1 mutation presented a low survival rate, marked growth delay, defects in neurogenesis and skeletal and cardiac muscle pathologies [57,156,157]. From a cellular point of view, most mutations in the *SYNE 1* and *SYNE 2* genes lead to changes in the nuclear form and presence of giant micronuclei and/or nuclei [57,154].

3.3. Nuclear lamina

The type A lamins (lamins A, C and variants AΔ10 and C2) are encoded by the *LMNA* gene, by alternative splicing given rise lamin A, C and also the variants AΔ10 and C2). The type B lamins are encoded by the *LMNB1* gene given rise to lamin B1) while *LMNB2* gene originates lamin B2 and B3 [90,158–160]. Lamins have a tripartite structure consisting of a helical central stem domain, a globular N-terminal domain ("head") and a longer C-terminal domain ("tail"). The C-terminal domain contains a nuclear localization sequence (NLS), important for the import of these lamins to the nucleus, and an immunoglobulin (Ig) fold [91]. Lamin A, B1, B2 and B3 still have a Caax motif (C: cysteine; a: aliphatic residue; X: any residue) in the C-terminal that is involved in their protein maturation [90,161,162]. The lamins provide structural support to the nucleus and fixation points for the chromatin (through interaction with INM proteins, especially those containing LEM domain) [163–165]. EDMD, CMD and Limb-Girdle muscular dystrophy type B (LGMD1B) result from a mutation in the *LMNA* gene (Figure 2) [90,166,167]. Muscle biopsies performed in patients with CMD, resulting from mutations in the *LMNA* gene, increased variation in fiber size, necrotic fibers and increased internal nuclei [168]. In cells of LGMD patients it was possible to observe nuclear deformations and blebs [168,169]. Null *LMNA* mice developed a phenotype quite similar to that of patients with EDMD, who has severe skeletal myopathy as well as cardiomyopathy [170–172,173].

3.4. DMPK

DMPK is the central protein of DM1 having 7 distinct isoforms (DMPK A to G) in human, which are generated by alternative splicing, and these isoforms are found in skeletal and cardiac muscle cells and in epithelial cells of mammals [174–176]. All DMPK isoforms are constituted by N-terminal rich in leucine, a serine/threonine protein kinase domain, a α-helical coiled-coil region and C-terminal [174–176]. Subcellular localization of these isoforms are affected by the C-terminal of each isoform. DMPK subcellular localization is confined to either endoplasmic reticulum (ER) or nuclear envelope (DMPK A and B), to mitochondria (DMPK C and D) or to cytoplasm (DMPK E, F and G) [174,176,177]. DMPK belongs to the AGC-kinase family and is involved in the modulation of the actin cytoskeleton by regulating the activity of myosin phosphatase (MYPT) or direct phosphorylation of the myosin regulatory light chain [174].

4. NE alterations in Myotonic Dystrophies

NE and NE proteins are essential for gene regulation, nuclear structure and muscle function. As previously demonstrated in some muscular dystrophies, dysfunctions or defects in the NE are involved in the molecular mechanisms responsible for the development of those diseases explaining somehow the loss of muscle mass and also muscle weakening. Previous studies have reported alterations in NE as well as in NE proteins in myotonic dystrophies [178–180].

Although many alterations of the NE and NE proteins in DM have not been demonstrated, the involvement of this structure and these proteins is not excluded from the molecular mechanisms of DM. Therefore, it is important to summarize the nuclear and NE alterations previously reported as altered in patients with DM1 and DM2.

4.1. NE alterations in DM1

The first study that was developed in order to evaluate the role of DMPK in NE stability used HeLa and C2C12 cells as a cell model [181]. A study demonstrated that the removal of DMPK in HeLa and C2C12 cells (loss of DMPK function) led to incorrect location of lamin A/C and B1 [181]. Similar changes were also obtained in cells where there was an increase in DMPK levels (expression gain) [181]. In addition, cells without DMPK form blebs of nuclear material outside the NE and the consequent loss of integrity of the NE [181]. In turn, cells with increased DMPK levels show nuclear fragmentation and multiple micronuclei [181]. Thus, it has been demonstrated that well-regulated DMPK levels are necessary to maintain the location of the lamins A/C and B1 of the NE and preserve the nuclear structure.

In addition to the in vitro study that we have just reported, specific studies of DM1 started using cells derived from DM1 patients.

Previous studies have in fact evaluated both nuclear structure and also several crucial NE proteins previously identified associated to other muscular dystrophies. The following NE protein have already been studied in DM1 Lamin B receptor (LBR), emerin, SUN1, SUN2, nesprin-1, nesprin-2, lamin A/C and lamin B1 [178–180]. In these studies, DM1-derived cell models were used namely DM1-derived fibroblasts and healthy controls [178], DM1-derived myoblasts and myotubes and also controls [179,180].

In relation to LBR, it is known that this protein is involved in the heterochromatin binding to the nuclear periphery at early stages of development contributing to the shape and nuclear architecture in interphase [182,183]. Using DM1-derived myoblasts and controls, there were no changes in the intracellular levels of this protein. Despite this, the nuclei of patients with DM1 when observed by immunocytochemistry show a tendency to have more

nuclear invaginations than the nuclei of healthy controls (Table 1; Figure 3) [180]. However, no quantitative these nuclear invaginations were not quantified by the authors.

Another INM relevant protein was evaluated, namely emerin. This protein was evaluated in DM1-derived fibroblasts and controls as well as DM1-derived myoblasts and controls [178,180]. The authors reported that using DM1-derived fibroblasts an increase in the immunolabelling at both NE and nucleus, increased deformed nuclei and increase of NE invaginations. Interestingly, the latter changes were significantly higher in DM1-derived fibroblast with higher CTG repeat length (CTG 2000) [178]. Unfortunately, the intracellular levels of emerin were not reported.

However, another study evaluated emerin intracellular levels and also the immunolocalization in DM1-derived myoblasts. Essentially, no differences the mentioned parameters were reported for emerin, but an increase in NE invaginations was reported [180]. Further, using DM1-derived myotubes and controls a decrease in the immunolocalization levels were observed. An increase in nuclear invaginations were also reported [180].

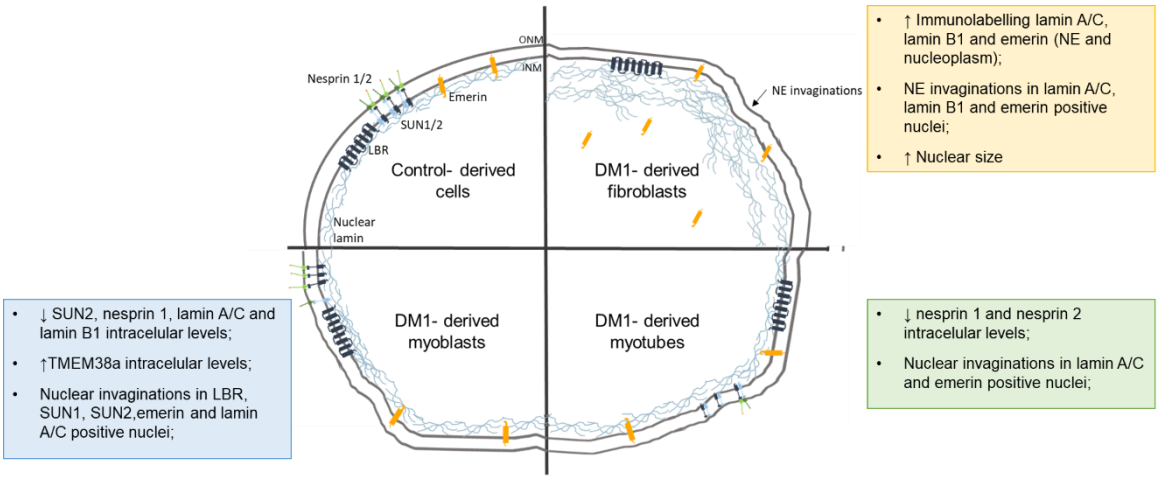

Figure 3: NE and NE proteins alterations related in DM1- derived cells. Schematic representation of NE and NE changes reported in the literature in DM1-derived cells. In yellow we have the changes observed in DM1-derived fibroblasts. In green the changes reported in DM1-derived myotubes. In blue the changes observed in DM1-derived myoblasts. DM1 – Myotonic dystrophy type 1; NE - nuclear envelope.

INM's SUN proteins (SUN1 and SUN2) were also evaluated. The SUN1 and SUN2 were evaluated in DM1-derived myoblasts and control [180]. The authors reported that the use of DM1-derived myoblasts immunolabelling NE invaginations. Despite this, no differences were reported in relation to control [180] (Table 1; Figure 3). The authors also reported that DM1-derived myoblasts have a decrease in intracellular levels of SUN2 with increasing length of CTG repetitions (Table 1; Figure 3). Unfortunately, SUN1 intracellular levels have not been evaluated [180].

Nesprins -1 and -2 have been the NE proteins evaluated in DM1 to date. Since nesprin intracellular levels vary according to the phase of muscle differentiation [184], DM1-derived myoblasts and controls as well as DM1-derived myotubes were used [180]. The authors reported that the immunolabelling (for nesprin -1 and -2) of DM1-derived myoblasts and DM1-derived myotubes showed no obvious differences compared to controls [180]. The authors also reported that DM1-derived myoblasts and DM1- derived myotubes have a decrease in nesprin-1 (giant isoform and N1-α2 isoform) intracellular levels with increasing length of CTG repetitions (Table 1; Figure 3) [180]. Regarding the intracellular levels of nesprin-2, it has been reported that they increase in DM1-derived myotubes and that they do not vary in relation to the control in DM1-derived myoblasts [180].

The lamins A/C and B1 of the nuclear lamin were also evaluated in DM1-derived fibroblasts, in DM1-derived myoblasts and in DM1-derived myoblasts [178,179].

Regarding lamin A/C, the authors reported that the use of DM1-derived fibroblasts increases the immunolabelling in the NE and in the nucleoplasm, increases nuclear invaginations, increases nuclear deformation, apparent nuclear increase and protein mislocalization. Interestingly, the number of deformed nuclei was higher in fibroblasts derived from DM1 with approximately 1000 CTG repeat length (CTG 1000) instead of 2000 CTG repeat length [178] (Table 1; Figure 3). Regarding the levels of expression (mRNA and protein) of lamin A/C, the authors did not report differences between DM1-derived fibroblasts and control [178].

Another study evaluated the intracellular levels of lamin A/C in DM1-derived myoblasts and also immunolocation in DM1-derived myoblasts and DM1-derived myotubes [179]. The authors reported a decrease lamin A intracellular levels and an increase number of nuclear invaginations in DM1-derived myoblasts. Nuclear invaginations have also been reported in DM1-derived myotubes [179] (Table 1; Figure 3). Unfortunately, the intracellular levels of lamin A/C in DM1-derived myotubes were not evaluated.

In relation to lamin B1, the authors reported that, with the use of DM1-derived fibroblasts, there was an increase in immunolabelling both in the NE and in the nucleus, increased deformed nuclei and increase NE invaginations (Table 1; Figure 3). Interestingly, the latest changes were significantly greater in fibroblasts derived from DM1 with longer CTG repeat length (CTG 2000) [178]. Unfortunately, intracellular levels of lamin B1 have not been reported.

However, another study evaluated the intracellular levels of lamin B1 DM1-derived myoblasts [179]. Essentially, DM1-derived myoblasts have lower intracellular levels of lamina B1 than intracellular levels of the control [179] (Table 1; Figure 3). Unfortunately, the immunolocalization of emerin were not reported.

The Trimeric intracellular cation channel type-A (TMEM38a) protein was identified only in the muscle's NE proteome. This protein is expressed abundantly and preferably in muscle cells and can be found in both INM and ONM [185]. It works as a counter ion channel to release calcium, and is therefore essential in the muscle contraction process [185]. TMEM38a was evaluated in DM1-derived myoblasts. The authors reported an increase in the intracellular levels of the ~35kDa band of this protein. The increase in this band was greater the greater the number of CTG repetitions [180]. The authors also reported that there was no immunostaining of NE in DM1-derived myoblasts and controls [180] (Table 1; Figure 3).

4.2. NE alterations in DM2

DM2 is significantly less studied than DM1. The alterations in NE associated with the molecular mechanisms of the disease are still poorly understood, although some studies in this direction are beginning to emerge. To date, only 3 NE proteins namely emerin, lamin A/C and lamin B1 have been studied in DM2 (Table 1) [179].

In relation to emerin, this protein was evaluated in DM2-derived myoblasts and controls as well as DM1-derived myotubes and controls [179]. The authors reported that using DM1-derived myoblasts an increase of nuclear invaginations. No differences the immunolabelling were reported for emerin between DM2-derived myotubes and controls (Table 1) [179]. Unfortunately, the intracellular levels of emerin were not reported.

The same authors evaluated lamin A/C and lamin B1 in DM2-derived myoblasts and DM2-derived myotubes. The authors did not report differences in the lamin A/C and lamin B1 intracellular levels in DM2-derived myoblasts compared to control [179]. The intracellular levels of these lamins were not evaluated in myotubes. In the case of lamin A/C, an increase in nuclear invagination in DM2-derived myoblasts has been reported (Table 1) [179].

Table 1: NE alterations in DM1 and DM2 patients. The proposed function of the NE proteins, as well as the NE alterations and consequent molecular and cellular alterations observed are described. DM1-derived fibroblasts, myoblasts and myotubes were stated as fibroblasts, myoblasts and myotubes.

	Protein	Proposed function	Cellular line	NE expression/protein levels (qPCR/WB)	Molecular and cellular alterations (ICC)	Reference
DM1	LBR	- Involved in tethering heterochromatin to the nuclear periphery at early developmental stages	Myoblasts	- No changes in the intracellular levels;	- Nuclear invaginations (identical to control)	[182,183, 180]
	Emerin	- Connection of the nucleus to the cytoplasm - Regulation of gene expression - Organization of nuclear membrane during cell division	Myoblasts	- No changes in the intracellular levels	- Nuclear invaginations (identical to control)	[87,106,113,178,180]
			Fibroblasts	- ND (fibroblasts)	- Increased Immunolabelling of NE and nucleoplasm; - Nuclear invaginations increased - Deformed nuclei increased	
	SUN1	- Connection of the nucleus to the cytoplasm (LINC complex) - Cell migration - Nuclei positioning in muscle cells	Myoblasts	- ND (myoblasts)	- Nuclear invaginations (identical to control)	[91,143,144,180]
	SUN2	- Connection of the nucleus to the cytoplasm (LINC complex) - Cell migration - Nuclei positioning in muscle cells	Myoblasts	- Decreased intracellular levels	- Nuclear invaginations (identical to control)	[91,143,144,180]
	Nesprin-1	- Connection of the nucleus to the cytoplasm - Nuclear stability	Myoblasts	- Decreased giant isoform intracellular levels - Decreased N1-α2 isoform intracellular levels	- No changes	[155,156, 180]
			Myotubes	- Decreased giant isoform intracellular levels - Decreased N1-α2 isoform intracellular levels	- No changes	
	Nesprin-2	- Connection of the nucleus to the cytoplasm - Nuclear stability	Myoblasts	- No changes in the intracellular levels	- No changes	[155,156, 180]
			Myotubes	- Decreased intracellular levels	- No changes	
	Lamin A/C	- Nucleus structural support - Mechanical stability - Chromatin organization	Myoblasts	- Decreased lamin A intracellular levels	- Increased nuclear invaginations	
			Myotubes	-ND	- Increased nuclear invaginations	

		- Transcriptional regulation - Response to oxidative stress	Fibroblasts	- No changes in the mRNA and protein levels	- Increased Immunolabelling of NE and nucleo-plasm; - Increased nuclear invaginations; - Apparent augmented nuclear; - Increased deformed nucleus; - Protein mislocalization;	[163–165, 178,179]
	Lamin B1	- Nucleus structural support - Mechanical stability - Chromatin organization - Transcriptional regulation - Response to oxidative stress	Myoblasts	- Decreased intracellular protein levels	- ND	[163–165, 178,179]
			Fibroblasts	- ND (fibroblasts)	- Increased Immunolabelling of NE and nucleo-plasm (fibroblasts); - Nuclear invaginations increased - Deformed nuclei increased	
	TMEM38a	- Essential in the muscle contraction process	Myoblasts	- Increased intracellular levels (~35kDa band)	- NE not immunolabelling	[180,185]
DM2	Emerin	- As above	Myoblasts	- ND	- Nuclear invaginations increased	[87,106,113,179]
			Myotubes	- ND	- No changes	
	Lamin A/C	- As above	Myoblasts	- No changes in the intracellular levels;	- Nuclear invaginations increased	[163–165, 179]
			Myotubes	- ND	- No changes	
	Lamin B1	- As above	Myoblasts	- No changes in the intracellular levels;	- ND	[163–165, 179]

Abbreviations: DM1 – Myotonic dystrophy type 1; DM2 – Myotonic dystrophy type 2; qPCR – quantitative Polymerase chain reaction; WB – Western blot; ICC – Immunocytochemistry; LBR – Lamin B receptor; SUN - Sad1/Unc-84; TMEM38a - Trimeric intracellular cation channel type-A; NE – Nuclear envelope; ND – Not determined

5. Future Perspectives

DM affects a large number of people around the world and, therefore, understanding the molecular mechanisms of the disease is extremely important.

It seems clear that changes in the integrity of the NE occur in patients with DM. These changes are associated with alterations in NE proteins and may also be associated with abnormal expansion of the CTG trinucleotide and CCTG tetranucleotide in DM1 and DM2, respectively. Despite this, the mechanisms and pathways associated with changes in the integrity of the NE are still poorly known in these pathologies and should be explored in more detail. Deciphering these mechanisms presents a very interesting challenge that may allow the development of earlier diagnostic techniques and more effective interventional methods to improve the quality of life of these patients.

From this literature review, we realize that very little is known about the involvement of NE proteins in these disease. In this review we found that, for most of the proteins studied in DM1 and DM2, the quantification of expression levels (mRNA and / or protein) or their immunolocation were evaluated. Therefore, it would be interesting in future studies to evaluate the two situations for the same protein. In addition, most of the assessment of intracellular protein levels has been assessed in myoblasts (with the exception of lamin A/C, nesprin-1 and nesprin-2). Since myoblasts are undifferentiated cells, it would also be interesting to evaluate the intracellular levels of NE proteins in differentiated cells to see whether or not there are differences in the intracellular levels of proteins according to the cell type. Another point that would also be an asset, to decipher the involvement of the NE in DM1 and DM2, would be to evaluate other proteins of the NE that have already been associated with other muscular dystrophies, such as, for example, LAP1 and LUMA. Finally, it would be interesting to assess whether the intracellular levels of DMPK and CNBP are actually decreased in the cells of patients with DM1 and DM2, respectively.

6. Concluding remarks

In this review we consolidate the changes in the integrity of the NE and NE proteins observed in cells of patients with DM. However, additional studies must be carried out to confirm these changes and add more information.

Changes in NE and NE proteins have been observed in muscular dystrophies, such as EDMD and LGMD [119–123,135–137,145,147,166,167]. These changes are responsible for nuclear deformations, such as increased nuclear size, increased micronuclei, nuclear fragmentation, which consequently affect the structure and function of muscle cells [120,121,124,136,145,147,154]. Alterations in NE and its proteins were demonstrated in

this review between DM1 and DM2 patients and control groups, although the differences between patients and controls were not very pronounced. These changes are nuclear invaginations, increased nuclear size, nuclear deformations, altered localization and increased immunostaining in the NE and the nucleus, as can be seen in Table 1 in detail for each protein.

The altered levels of NE proteins and the deformations of NE found in patients with DM, may be involved in the multisystemic changes of these diseases and in particular in muscle changes since these proteins are involved in cellular processes such as nuclear positioning, gene expression, migration and response to oxidative stress [87,106,113,117,125,128,129,143,155,156].

To date, two hypotheses have been proposed to justify how defects in NE proteins can give rise to changes in cardiac and skeletal muscle, in muscular dystrophies, which involve models based on genetic expression and mechanical stress [91,186,187]. The gene expression model proposes that changes in NE associated with mutations in emerin and type-A lamins lead to changes in the expression of muscle cell genes. In turn, the mechanical stress model suggests that the nuclei of muscle cells that show a decrease or absence of type-A lamins or emerin may be subject to mechanical damage caused by repetitive muscle contraction cycles. These cells are more fragile and tend to deform more easily [91,188].

NE dysfunction is a registered trademark of several pathologies and in the DM is emerging. Therefore, it is extremely important to unveil the different molecular mechanisms underlying the dysfunction of NE and NE proteins, essential for understanding the most varied phenotypes of myotonic dystrophy.

7. References

[1] I. Dalkilic and L. M. Kunkel, “Muscular dystrophies: Genes to pathogenesis,” *Curr. Opin. Genet. Dev.*, vol. 13, no. 3, pp. 231–238, 2003, doi: 10.1016/S0959-437X(03)00048-0.

[2] A. E. H. Emery, “Fortnightly review: The muscular dystrophies,” *Bmj*, vol. 317, no. 7164, pp. 991–995, 1998, doi: 10.1136/bmj.317.7164.991.

[3] J. M. Bhatt, “The Epidemiology of Neuromuscular Diseases,” *Neurol. Clin.*, vol. 34, no. 4, pp. 999–1021, 2016, doi: 10.1016/j.ncl.2016.06.017.

[4] A. Theadom *et al.*, “Prevalence of muscular dystrophies: A systematic literature review,” *Neuroepidemiology*, vol. 43, no. 3–4, pp. 259–268, 2014, doi: 10.1159/000369343.

[5] E. I. Gisela Gaina, Magdalena Budisteanu, Emilia Manole, “Clinical and Molecular Diagnosis in Muscular Dystrophies,” in *Intech*, vol. i, no. tourism, 2018, p. 13.

[6] N. C. Joyce, B. Oskarsson, and L. Jin, “Muscle Biopsy Evaluation in Neuromuscular Disorders Nanette,” *Phys Med Rehabil Clin N Am*, vol. 23, no. 3, pp. 609–631, 2012, doi: 10.1016/j.pmr.2012.06.006.Muscle.

[7] K. L. Wilson, “The nuclear envelope, muscular dystrophy and gene expression,” *Trends Cell Biol.*, vol. 10, no. 4, pp. 125–129, 2000, doi: 10.1016/S0962-8924(99)01708-0.

[8] E. S. Folker and M. K. Baylies, “Nuclear positioning in muscle development and disease,” *Front. Physiol.*, vol. 4 DEC, no. December, pp. 1–10, 2013, doi: 10.3389/fphys.2013.00363.

[9] L. Gueneau *et al.*, “Mutations of the FHL1 Gene Cause Emery-Dreifuss Muscular Dystrophy,” *Am. J. Hum. Genet.*, vol. 85, no. 3, pp. 338–353, 2009, doi: 10.1016/j.ajhg.2009.07.015.

[10] M. Crisp *et al.*, “Coupling of the nucleus and cytoplasm: Role of the LINC complex,” *J. Cell Biol.*, vol. 172, no. 1, pp. 41–53, 2006, doi: 10.1083/jcb.200509124.

[11] G. Karpati, D. Hilton-jones, K. Bushby, and R. C. Griggs, *Disorders of Voluntary Muscle*, 8th ed. Cambridge, 2001.

[12] J. D. Brook *et al.*, “Molecular basis of myotonic dystrophy: Expansion of a trinucleotide (CTG) repeat at the 3′ end of a transcript encoding a protein kinase family member,” *Cell*, vol. 68, no. 4, pp. 799–808, 1992, doi: 10.1016/0092-8674(92)90154-5.

[13] Y. H. Fu *et al.*, “An unstable triplet repeat in a gene related to myotonic muscular dystrophy,” *Science (80-.).*, vol. 255, no. 5049, pp. 1256–1258, 1992, doi: 10.1126/science.1546326.

[14] M. Mahadevan *et al.*, “Myotonic dystrophy mutation: An unstable CTG repeat in the 3′ untranslated region of the gene,” *Science (80-.).*, vol. 255, no. 5049, pp. 1253–1255, 1992, doi: 10.1126/science.1546325.

[15] G. Meola, V. Sansone, S. Radice, S. Skradski, and L. Ptacek, “A family with an unusual myotonic and myopathic phenotype and no CTG expansion (proximal myotonic myopathy syndrome): A challenge for future molecular studies,” *Neuromuscul. Disord.*, vol. 6, no. 3, pp. 143–150, 1996, doi: 10.1016/0960-8966(95)00040-2.

[16] K. Ricker, M. C. Koch, D. Lehmann-Horn, M. Otto, R. Heine, and R. T. Moxley, “Proximal myotonic myopathy: A new dominant disorder with myotonia, muscle weakness, and cataracts,” *Am. Acad. Neurol.*, no. 44, pp. 1448–1452, 1994, doi: 10.1212/WNL.44.8.1448.

[17] B. Udd, R. Krahe, C. Wallgren-Pettersson, B. Falck, and H. Kalimo, “Proximal myotonic dystrophy - A family with autosomal dominant muscular dystrophy, cataracts, hearing loss and hypogonadism: Heterogeneity of proximal myotonic syndromes?,” *Neuromuscul. Disord.*, vol. 7, no. 4, pp. 217–228, 1997, doi: 10.1016/S0960-8966(97)00041-2.

[18] S. Wenninger, F. Montagnese, and B. Schoser, “Core clinical phenotypes in Myotonic Dystrophies,” *Front. Neurol.*, vol. 9, no. MAY, pp. 1–9, 2018, doi: 10.3389/fneur.2018.00303.

[19] L. P. W. Ranum and J. W. Day, “Myotonic Dystrophy: RNA Pathogenesis Comes into Focus,” *Am. J. Hum. Genet.*, vol. 74, no. 5, pp. 793–804, 2004, doi: 10.1086/383590.

[20] R. Meola, Giovanni, & Cardani, “Myotonic dystrophies: An update on clinical aspects,

genetic, pathology, and molecular pathomechanisms.," *Biochim. Biophys. Acta - Mol. Basis Dis.*, 2015, doi: https://doi.org/10.1016/j.bbadis.2014.05.019.

[21] M. De Antonio *et al.*, "Unravelling the myotonic dystrophy type 1 clinical spectrum: A systematic registry-based study with implications for disease classification," *Rev. Neurol. (Paris).*, vol. 172, no. 10, pp. 572–580, 2016, doi: 10.1016/j.neurol.2016.08.003.

[22] S. Tomé and G. Gourdon, "DM1 phenotype variability and triplet repeat instability: Challenges in the development of new therapies," *Int. J. Mol. Sci.*, vol. 21, no. 2, pp. 1–10, 2020, doi: 10.3390/ijms21020457.

[23] K. Yum, E. T. Wang, and A. Kalsotra, "Myotonic dystrophy: disease repeat range, penetrance, age of onset, and relationship between repeat size and phenotypes," *Curr. Opin. Genet. Dev.*, vol. 44, pp. 30–37, 2017, doi: 10.1016/j.gde.2017.01.007.

[24] H. G. Harley *et al.*, "Size of the unstable CTG repeat sequence in relation to phenotype and parental transmission in myotonic dystrophy," *Am. J. Hum. Genet.*, vol. 52, no. 6, pp. 1164–1174, 1993.

[25] L. Martorell, D. G. Monckton, A. Sanchez, A. Lopez de Munain, and M. Baiget, "Frequency and stability of the myotonic dystrophy type 1 premutation," *Neurology*, vol. 56, no. 3, pp. 328–335, 2001, doi: 10.1212/WNL.56.3.328.

[26] W. Reardon, R. Newcombe, I. Fenton, J. Sibert, and P. S. Harper, "The natural history of congenital myotonic dystrophy: Mortality and long term clinical aspects," *Arch. Dis. Child.*, vol. 68, no. 2, pp. 177–181, 1993, doi: 10.1136/adc.68.2.177.

[27] M. Roig, P. R. Balliu, C. Navarro, R. Brugera, and M. Losada, "Presentation, clinical course, and outcome of the congenital form of myotonic dystrophy," *Pediatr. Neurol.*, vol. 11, no. 3, pp. 208–213, 1994, doi: 10.1016/0887-8994(94)90104-X.

[28] C. Campbell, R. Sherlock, P. Jacob, and M. Blayney, "Congenital Myotonic Dystrophy: Assisted Ventilation Duration and Outcome," *Pediatrics*, vol. 113, no. 4 I, pp. 811–816, 2004, doi: 10.1542/peds.113.4.811.

[29] A. Ekström, "Congenital and Childhood Myotonic Dystrophy type 1 - the impact on central nervous system , visual and motor function," 2009.

[30] C. Turner and D. Hilton-Jones, "The myotonic dystrophies: Diagnosis and management," *J. Neurol. Neurosurg. Psychiatry*, vol. 81, no. 4, pp. 358–367, 2010, doi: 10.1136/jnnp.2008.158261.

[31] B. Echenne, A. Rideau, A. Roubertie, G. Sébire, F. Rivier, and B. Lemieux, "Myotonic dystrophy type I in childhood. Long-term evolution in patients surviving the neonatal period," *Eur. J. Paediatr. Neurol.*, vol. 12, no. 3, pp. 210–223, 2008, doi: 10.1016/j.ejpn.2007.07.014.

[32] A. T. M. Hageman, F. J. M. Gabreëls, K. D. Liem, K. Renkawek, and J. M. Boon, "Congenital myotonic dystrophy; a report on thirteen cases and a review of the literature," *J. Neurol. Sci.*, vol. 115, no. 1, pp. 95–101, 1993, doi: 10.1016/0022-510X(93)90072-7.

[33] G. Ho, "Congenital and childhood myotonic dystrophy: Current aspects of disease and future directions," *World J. Clin. Pediatr.*, vol. 4, no. 4, p. 66, 2015, doi: 10.5409/wjcp.v4.i4.66.

[34] U. Schara and B. G. H. Schoser, "Myotonic Dystrophies Type 1 and 2: A Summary on Current Aspects," *Semin. Pediatr. Neurol.*, vol. 13, no. 2, pp. 71–79, 2006, doi: 10.1016/j.spen.2006.06.002.

[35] J. S. Rubinsztein, D. C. Rubinsztein, S. Goodburn, and A. J. Holland, “Apathy and hypersomnia are common features of myotonic dystrophy,” *J. Neurol. Neurosurg. Psychiatry*, vol. 64, no. 4, pp. 510–515, 1998, doi: 10.1136/jnnp.64.4.510.

[36] S. LoRusso, B. Weiner, and W. D. Arnold, “Myotonic Dystrophies: Targeting Therapies for Multisystem Disease,” *Neurotherapeutics*, vol. 15, no. 4, pp. 872–884, 2018, doi: 10.1007/s13311-018-00679-z.

[37] A. Philips, T. Lubov, T. Timchenko, and E. Al, “Disruption of Splicing Regulated by a CUG-Binding Protein in Myotonic Dystrophy,” *Science (80-.).*, vol. 180, pp. 737–740, 1998.

[38] N. A. Timchenko, Z. J. Cai, A. L. Welm, S. Reddy, T. Ashizawa, and L. T. Timchenko, “RNA CUG Repeats Sequester CUGBP1 and Alter Protein Levels and Activity of CUGBP1,” *J. Biol. Chem.*, vol. 276, no. 11, pp. 7820–7826, 2001, doi: 10.1074/jbc.M005960200.

[39] J. W. Day and L. P. W. Ranum, “RNA pathogenesis of the myotonic dystrophies,” *Neuromuscul. Disord.*, vol. 15, no. 1, pp. 5–16, 2005, doi: 10.1016/j.nmd.2004.09.012.

[40] M. Fardaei *et al.*, “in vivo with nuclear foci of expanded-repeat transcripts in DM1 and DM2 cells,” vol. 11, no. 7, pp. 805–814, 2002.

[41] J. W. Miller *et al.*, “Recruitment of human muscleblind proteins to (CUG) n expansions associated with myotonic dystrophy,” vol. 19, no. 17, pp. 4439–4448, 2000.

[42] L. T. Timchenko, N. A. Timchenko, C. T. Caskey, and R. Roberts, “Novel proteins with binding specificity for DNA CTG repeats and RNA CUG repeats : implications for myotonic dystrophy,” vol. 5, no. 1, pp. 115–121, 1996.

[43] N. Charlet-b, R. S. Savkur, G. Singh, A. V Philips, E. A. Grice, and T. A. Cooper, “Loss of the Muscle-Specific Chloride Channel in Type 1 Myotonic Dystrophy Due to Misregulated Alternative Splicing,” vol. 10, pp. 45–53, 2002.

[44] R. S. Savkur, A. V. Philips, and T. A. Cooper, “Aberrant regulation of insulin receptor alternative splicing is associated with insulin resistance in myotonic dystrophy,” *Nat. Genet.*, vol. 29, no. 1, pp. 40–47, 2001, doi: 10.1038/ng704.

[45] R. N. Kanadia *et al.*, “A Muscleblind Knockout Model for Myotonic Dystrophy,” vol. 302, no. December, pp. 1978–1981, 2003.

[46] M. Alwazzan, E. Newman, M. G. Hamshere, and J. D. Brook, “Myotonic dystrophy is associated with a reduced level of RNA from the DMWD allele adjacent to the expanded repeat,” vol. 8, no. 8, pp. 1491–1497, 1999.

[47] C. A. Bouchert *et al.*, “A novel homeodomain-encoding gene is associated with a large CpG island interrupted by the myotonic dystrophy unstable (CTG) n repeat,” vol. 4, no. 10, pp. 1919–1925, 1995.

[48] C. A. Thornton, J. Wymer, Z. Simmons, and E. Al, “Expansion of the myotonic dystrophy CTG repeat reduces expression of the flanking DMAHP gene,” *Nat. Genet.*, pp. 407–409, 1997, doi: 10.1038/ng0897-407.

[49] T. A. Lee, Johanna E; Cooper, “Pathogenic mechanisms of myotonic dystrophy,” *Biochem Soc Trans*, vol. 37, no. 0 6, 2009, doi: 10.1042/BST0371281.Pathogenic.

[50] J. J., R. Surez-Snchez, N. Leyva-Garca, B. Cisneros, and O. Hernndez-Hernndez, “Myotonic Dystrophy Protein Kinase: Structure, Function and Its Possible Role in the

Pathogenesis of Myotonic Dystrophy Type 1," *Adv. Protein Kinases*, vol. 1, no. Cdm, 2012, doi: 10.5772/37238.

[51] D. Furling, D. Lemieux, K. Taneja, and J. Puymirat, "Decreased levels of myotonic dystrophy protein kinase (DMPK) and delayed differentiation in human myotonic dystrophy myoblasts," *Neuromuscul. Disord.*, vol. 11, no. 8, pp. 728–735, 2001, doi: 10.1016/S0960-8966(01)00226-7.

[52] S. Salvatori *et al.*, "Decreased expression of DMPK: Correlation with CTG repeat expansion and fibre type composition in myotonic dystrophy type 1," *Neurol. Sci.*, vol. 26, no. 4, pp. 235–242, 2005, doi: 10.1007/s10072-005-0466-x.

[53] P. S. Sarkar, J. Han, and S. Reddy, "In situ hybridization analysis of Dmpk mRNA in adult mouse tissues," *Neuromuscul. Disord.*, vol. 14, no. 8–9, pp. 497–506, 2004, doi: 10.1016/j.nmd.2004.03.012.

[54] G. Sicot, G. Gourdon, and M. Gomes-Pereira, "Myotonic dystrophy, when simple repeats reveal complex pathogenic entities: New findings and future challenges," *Hum. Mol. Genet.*, vol. 20, no. R2, pp. 116–123, 2011, doi: 10.1093/hmg/ddr343.

[55] D. H. Cho and S. J. Tapscott, "Myotonic dystrophy: Emerging mechanisms for DM1 and DM2," *Biochim. Biophys. Acta - Mol. Basis Dis.*, vol. 1772, no. 2, pp. 195–204, 2007, doi: 10.1016/j.bbadis.2006.05.013.

[56] M. A. Wheeler *et al.*, "Distinct functional domains in nesprin-1 α and nesprin-2 β bind directly to emerin and both interactions are disrupted in X-linked Emery – Dreifuss muscular dystrophy," vol. 3, 2007, doi: 10.1016/j.yexcr.2007.03.025.

[57] Q. Zhang *et al.*, "Nesprin-1 and -2 are involved in the pathogenesis of Emery - Dreifuss muscular dystrophy and are critical for nuclear envelope integrity," *Hum. Mol. Genet.*, vol. 16, no. 23, pp. 2816–2833, 2007, doi: 10.1093/hmg/ddm238.

[58] T. Zu *et al.*, "Non-ATG-initiated translation directed by microsatellite expansions," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 108, no. 1, pp. 260–265, 2011, doi: 10.1073/pnas.1013343108.

[59] K. Ricker *et al.*, "Proximal myotonic myopathy: A new dominant disorder with myotonia, muscle weakness, and cataracts," *Neurology*, pp. 1448–1452, 1994, doi: 10.1212 / wnl.44.8.1448.

[60] C. L. Liquori *et al.*, "Myotonic dystrophy type 2 caused by a CCTG expansion in intron l of ZNF9," *Science (80-.).*, vol. 293, no. 5531, pp. 864–867, 2001, doi: 10.1126/science.1062125.

[61] L. P. W. Ranum, P. F. Rasmussen, K. A. Benzow, M. D. Koob, and J. W. Day, "Genetic mapping of a second myotonic dystrophy locus," *Nat. Genet.*, vol. 19, no. 2, pp. 196–198, 1998, doi: 10.1038/570.

[62] K. Ricker *et al.*, "Linkage of proximal myotonic myopathy to chromosome 3q," *Neurology*, vol. 52, no. 1, pp. 170–171, 1999, doi: 10.1212/wnl.52.1.170.

[63] L. L. Bachinski *et al.*, "Premutation allele pool in myotonic dystrophy type 2," *Neurology*, vol. 72, no. 6, pp. 490–497, 2009, doi: 10.1212/01.wnl.0000333665.01888.33.

[64] S. Rudnik-Schöneborn, C. Schneider-Gold, U. Raabe, W. Kress, K. Zerres, and B. G. H. Schoser, "Outcome and effect of pregnancy in myotonic dystrophy type 2," *Neurology*, vol. 66, no. 4, pp. 579–580, 2006, doi: 10.1212/01.wnl.0000198227.91131.1e.

[65] G. Meola and R. Cardani, “Myotonic Dystrophy Type 2: An Update on Clinical Aspects, Genetic and Pathomolecular Mechanism,” *J. Neuromuscul. Dis.*, vol. 2, pp. S59–S71, 2015, doi: 10.3233/JND-150088.

[66] B. Udd *et al.*, “Report of the 115th ENMC workshop: DM2/PROMM and other myotonic dystrophies: 3rd Workshop, 14-16 February 2003, Naarden, The Netherlands,” *Neuromuscul. Disord.*, vol. 13, no. 7–8, pp. 589–596, 2003, doi: 10.1016/S0960-8966(03)00092-0.

[67] J. W. Day *et al.*, “Myotonic dystrophy type 2: Molecular, diagnostic and clinical spectrum,” *Neurology*, vol. 60, no. 4, pp. 657–664, 2003, doi: 10.1212/01.WNL.0000054481.84978.F9.

[68] C. Heatwole *et al.*, “Patient-Reported Impact of Symptoms in Myotonic Dystrophy Type 2 (PRISM-2),” *Neurology*, vol. 85, no. 24, pp. 2136–2146, 2015, doi: 10.1212/WNL.0000000000002225.

[69] J. W. Day, R. Roelofs, B. Leroy, I. Pech, K. Benzow, and L. P. W. Ranum, “Clinical and genetic characteristics of a five-generation family with a novel form of myotonic dystrophy (DM2),” *Neuromuscul. Disord.*, vol. 9, no. 1, pp. 19–27, 1999, doi: 10.1016/S0960-8966(98)00094-7.

[70] M. Satu Auvien, Ms. Tiina Suomenen, M. Pekka Hannonen, L. B. Linda, R. Krahe, and M. Bjarne Udd, “Myotonic Dystrophy Type 2 Found in two of sixty-three persons diagnosed as having fibrmyalgia,” *arthritis rheum.*, vol. 58, no. 11, pp. 3627–3631, 2008, doi: 10.1002/art.24037.

[71] K. I. Suokas, M. Haanpää, H. Kautiainen, B. Udd, and A. J. Hietaharju, “Pain in patients with myotonic dystrophy type 2: A postal survey in Finland,” *Muscle and Nerve*, vol. 45, no. 1, pp. 70–74, 2012, doi: 10.1002/mus.22249.

[72] G. Meola *et al.*, “Proximal myotonic myopathy: A syndrome with a favourable prognosis?,” *J. Neurol. Sci.*, vol. 193, no. 2, pp. 89–96, 2002, doi: 10.1016/S0022-510X(01)00649-9.

[73] V. A. Sansone *et al.*, “The frequency and severity of cardiac involvement in myotonic dystrophy type 2 (DM2): Long-term outcomes,” *Int. J. Cardiol.*, vol. 168, no. 2, pp. 1147–1153, 2013, doi: 10.1016/j.ijcard.2012.11.076.

[74] K. Wahbi *et al.*, “Left ventricular dysfunction and cardiac arrhythmias are frequent in type 2 myotonic dystrophy: A case control study,” *Neuromuscul. Disord.*, vol. 19, no. 7, pp. 468–472, 2009, doi: 10.1016/j.nmd.2009.04.012.

[75] D. T. Franc *et al.*, “Cerebral and Muscle MRI Abnormalities in Myotonic Dystrophy,” vol. 22, no. 6, pp. 483–491, 2013, doi: 10.1016/j.nmd.2012.01.003.

[76] G. Meola *et al.*, “Reduced cerebral blood flow and impaired visual-spatial function in proximal myotonic myopathy,” *Neurology*, vol. 53, no. 5, pp. 1042–1050, 1999, doi: 10.1212/wnl.53.5.1042.

[77] C. Maurage, B. Udd, M. Ruchoux, and E. Al, “Similar brain tau pathology in DM2 / PROMM and DM1 / Steinert disease,” *Neurology*, pp. 1636–1639, 2005, doi: 10.1212/01.wnl.0000184585.93864.4e.

[78] B. G. H. Schoser, W. Kress, M. C. Walter, B. Halliger-Keller, H. Lochmüller, and K. Ricker, “Homozygosity for CCTG mutation in myotonic dystrophy type 2,” *Brain*, vol. 127, no. 8, pp. 1868–1877, 2004, doi: 10.1093/brain/awh210.

[79] A. Mankodi *et al.*, “Muscleblind localizes to nuclear foci of aberrant RNA in myotonic dystrophy types 1 and 2,” *Hum. Mol. Genet.*, vol. 10, no. 19, pp. 2165–2170, 2001,

doi: 10.1093/hmg/10.19.2165.

[80] L. P. W. Ranum and T. A. Cooper, “RNA-mediated neuromuscular disorders,” *Annu. Rev. Neurosci.*, vol. 29, pp. 259–277, 2006, doi: 10.1146/annurev.neuro.29.051605.113014.

[81] A. Botta *et al.*, “Effect of the [CCTG]n repeat expansion on ZNF9 expression in myotonic dystrophy type II (DM2),” *Biochim. Biophys. Acta - Mol. Basis Dis.*, vol. 1762, no. 3, pp. 329–334, 2006, doi: 10.1016/j.bbadis.2005.11.004.

[82] A. Botta *et al.*, “Gene expression analysis in myotonic dystrophy: Indications for a common molecular pathogenic pathway in DM1 and DM2,” *Gene Expr.*, vol. 13, no. 6, pp. 339–351, 2007, doi: 10.3727/000000006781510705.

[83] J. M. Margolis, B. G. Schoser, M. L. Moseley, J. W. Day, and L. P. W. Ranum, “DM2 intronic expansions: Evidence for CCUG accumulation without flanking sequence or effects on ZNF9 mRNA processing or protein expression,” *Hum. Mol. Genet.*, vol. 15, no. 11, pp. 1808–1815, 2006, doi: 10.1093/hmg/ddl103.

[84] E. Hatch and M. Hetzer, “Breaching the nuclear envelope in development and disease,” *J. Cell Biol.*, vol. 205, no. 2, pp. 133–141, 2014, doi: 10.1083/jcb.201402003.

[85] L. W. Michael, “THE NUCLEAR ENVELOPE ITS STRUCTURE AND RELATION TO CYTOPLASMIC MEMBRANES,” *J. Biophys. Biochem. Cytol.*, vol. I, no. 3, pp. 257–270, 1955, [Online]. Available: http://www.ncbi.nlm.nih.gov/pubmed/13242591.

[86] C. Ostlund, A. Hernendez-Ono, and J. Y. Shin, “The Nuclear Envelope in Lipid Metabolism and Pathogenesis of NAFLD,” *Biology (Basel).*, 2020, doi: 10.3390/biology9100338.

[87] S. Rebelo, E. F. Da Cruz e Silva, and O. A. B. Da Cruz e Silva, “Genetic mutations strengthen functional association of LAP1 with DYT1 dystonia and muscular dystrophy,” *Mutat. Res. - Rev. Mutat. Res.*, vol. 766, pp. 42–47, 2015, doi: 10.1016/j.mrrev.2015.07.004.

[88] Y. Gruenbaum, A. Margalit, R. D. Goldman, D. K. Shumaker, and K. L. Wilson, “The nuclear lamina comes of age,” *Nat. Rev. Mol. Cell Biol.*, vol. 6, no. 1, pp. 21–31, 2005, doi: 10.1038/nrm1550.

[89] L. Holmer and H. J. Worman, “Inner nuclear membrane proteins: Functions and targeting,” *Cell. Mol. Life Sci.*, vol. 58, no. 12–13, pp. 1741–1747, 2001, doi: 10.1007/PL00000813.

[90] M. A. D’Angelo and M. W. Hetzer, “The role of the nuclear envelope in cellular organization,” *Cell. Mol. Life Sci.*, vol. 63, no. 3, pp. 316–332, 2006, doi: 10.1007/s00018-005-5361-3.

[91] K. J. Roux and B. Burke, “Nuclear envelope defects in muscular dystrophy,” *Biochim. Biophys. Acta - Mol. Basis Dis.*, vol. 1772, no. 2, pp. 118–127, 2007, doi: 10.1016/j.bbadis.2006.06.001.

[92] Z. Jahed, D. Fadavi, U. T. Vu, E. Asgari, G. W. G. Luxton, and M. R. K. Mofrad, “Molecular Insights into the Mechanisms of SUN1 Oligomerization in the Nuclear Envelope,” *Biophys. J.*, vol. 114, no. 5, pp. 1190–1203, 2018, doi: 10.1016/j.bpj.2018.01.015.

[93] A. Méjat and T. Misteli, “LINC complexes in health and disease,” *Nucleus*, vol. 1, no. 1, pp. 40–52, 2010, doi: 10.4161/nucl.1.1.10530.

[94] A. Janin, D. Bauer, F. Ratti, G. Millat, and A. Méjat, “Nuclear envelopathies: A complex LINC between nuclear envelope and pathology,” *Orphanet J. Rare Dis.*, vol. 12, no. 1, pp. 1–16, 2017, doi: 10.1186/s13023-017-0698-x.

[95] L. Gerace and G. Blobel, “The nuclear envelope lamina is reversibly depolymerized during mitosis,” *Cell*, vol. 19, no. 1, pp. 277–287, 1980, doi: 10.1016/0092-8674(80)90409-2.

[96] A. Fernández-Álvarez and J. P. Cooper, “Chromosomes Orchestrate Their Own Liberation: Nuclear Envelope Disassembly,” *Trends Cell Biol.*, vol. 27, no. 4, pp. 255–265, 2017, doi: 10.1016/j.tcb.2016.11.005.

[97] S. S. Katta, C. J. Smoyer, and S. L. Jaspersen, “Destination: Inner nuclear membrane,” *Trends Cell Biol.*, vol. 24, no. 4, pp. 221–229, 2014, doi: 10.1016/j.tcb.2013.10.006.

[98] G. F. Brady, R. Kwan, J. Bragazzi Cunha, J. S. Elenbaas, and M. B. Omary, “Lamins and Lamin-Associated Proteins in Gastrointestinal Health and Disease,” *Gastroenterology*, vol. 154, no. 6, pp. 1602-1619.e1, 2018, doi: 10.1053/j.gastro.2018.03.026.

[99] A. Senior and L. Gerace, “Integral membrane proteins specific to the inner nuclear membrane and associated with the nuclear lamina,” *J. Cell Biol.*, vol. 107, no. 6 I, pp. 2029–2036, 1988, doi: 10.1083/jcb.107.6.2029.

[100] J. M. Cronshaw, A. N. Krutchinsky, W. Zhang, B. T. Chait, and M. L. J. Matunis, “Proteomic analysis of the mammalian nuclear pore complex,” *J. Cell Biol.*, vol. 158, no. 5, pp. 915–927, 2002, doi: 10.1083/jcb.200206106.

[101] J. A. DeGrasse *et al.*, “Evidence for a shared nuclear pore complex architecture that is conserved from the last common eukaryotic ancestor,” *Mol. Cell. Proteomics*, vol. 8, no. 9, pp. 2119–2130, 2009, doi: 10.1074/mcp.M900038-MCP200.

[102] M. P. Rout, J. D. Aitchison, A. Suprapto, K. Hjertaas, Y. Zhao, and B. T. Chait, “The yeast nuclear pore complex: Composition, architecture, transport mechanism,” *J. Cell Biol.*, vol. 148, no. 4, pp. 635–651, 2000, doi: 10.1083/jcb.148.4.635.

[103] M. Beck *et al.*, “Nuclear pore complex structure and dynamics revealed by cryoelectron tomography,” *Science (80-.).*, vol. 306, no. 5700, pp. 1387–1390, 2004, doi: 10.1126/science.1104808.

[104] D. Stoffler, B. Feja, B. Fahrenkrog, J. Walz, D. Typke, and U. Aebi, “Cryo-electron tomography provides novel insights into nuclear pore architecture: Implications for nucleocytoplasmic transport,” *J. Mol. Biol.*, vol. 328, no. 1, pp. 119–130, 2003, doi: 10.1016/S0022-2836(03)00266-3.

[105] G. Kabachinski and T. U. Schwartz, “The nuclear pore complex - Structure and function at a glance,” *J. Cell Sci.*, vol. 128, no. 3, pp. 423–429, 2015, doi: 10.1242/jcs.083246.

[106] S. R. Wente and M. P. Rout, “The nuclear pore complex and nuclear transport.,” *Cold Spring Harb. Perspect. Biol.*, vol. 2, no. 10, pp. 1–19, 2010, doi: 10.1101/cshperspect.a000562.

[107] A. J. Koch and J. M. Holaska, “Emerin in health and disease,” *Semin. Cell Dev. Biol.*, vol. 29, pp. 95–106, 2014, doi: 10.1016/j.semcdb.2013.12.008.

[108] S. Bione *et al.*, “Identification of a novel X-linked gene responsible for Emery-Dreifuss muscular dystrophy,” *Nat. Genet.*, vol. 8, no. 4, pp. 323–327, 1994, doi: 10.1038/ng1294-323.

[109] N. Wolff, B. Gilquin, K. Courchay, I. Callebaut, H. J. Worman, and S. Zinn-Justin, “Structural analysis of emerin, an inner nuclear membrane protein mutated in X-linked Emery-Dreifuss muscular dystrophy,” *FEBS Lett.*, vol. 501, no. 1–3, pp. 171–176, 2001, doi: 10.1016/S0014-5793(01)02649-7.

[110] J. M. Berk, K. E. Tifft, and K. L. Wilson, “The nuclear envelope LEM-domain protein emerin,” *Nucl. (United States)*, vol. 4, no. 4, pp. 298–314, 2013, doi: 10.4161/nucl.25751.

[111] A. Muchir and H. J. Worman, “The nuclear envelope and human disease,” *Physiology*, vol. 19, no. 5, pp. 309–314, 2004, doi: 10.1152/physiol.00022.2004.

[112] K. Lee, T. Haraguchi, T. Koujin, Y. Hiraoka, and K. Wilson, “Distinct functional domains in emerin bind lamin A and DNA-briging protein BAF,” *Cell*, pp. 4567–4573, 2001, [Online]. Available: https://pubmed.ncbi.nlm.nih.gov/11792821/.

[113] T. Shimi, T. Koujin, M. Segura-Totten, K. L. Wilson, T. Haraguchi, and Y. Hiraoka, “Dynamic interaction between BAF and emerin revealed by FRAP, FLIP, and FRET analyses in living HeLa cells,” *J. Struct. Biol.*, vol. 147, no. 1, pp. 31–41, 2004, doi: 10.1016/j.jsb.2003.11.013.

[114] J. M. Holaska and K. L. Wilson, “Multiple roles for emerin: Implications for Emery-Dreifuss muscular dystrophy,” *Anat. Rec. - Part A Discov. Mol. Cell. Evol. Biol.*, vol. 288, no. 7, pp. 676–680, 2006, doi: 10.1002/ar.a.20334.

[115] E. A. L. Fairley, J. Kendrick-Jones, and J. A. Ellis, “The Emery-Dreifuss muscular dystrophy phonotype arises from aberrant targeting and binding of emerin at the inner nuclear membrane,” *J. Cell Sci.*, vol. 112, no. 15, pp. 2571–2582, 1999.

[116] G. Lattanzi *et al.*, “Association of emerin with nuclear and cytoplasmic actin is regulated in differentiating myoblasts,” *Biochem. Biophys. Res. Commun.*, vol. 303, no. 3, pp. 764–770, 2003, doi: 10.1016/S0006-291X(03)00415-7.

[117] F. Haque *et al.*, “Mammalian SUN protein interaction networks at the inner nuclear membrane and their role in laminopathy disease processes,” *J. Biol. Chem.*, vol. 285, no. 5, pp. 3487–3498, 2010, doi: 10.1074/jbc.M109.071910.

[118] J. Shin *et al.*, “Lamina-associated Polypeptide-1 Interacts with the Muscular Dystrophy Protein Emerin and is Essential for Skeletal Muscle Maintenance,” vol. 26, no. 6, pp. 591–603, 2013, doi: 10.1016/j.devcel.2013.08.012.Lamina-associated.

[119] T. Libotte *et al.*, “Lamin A/C–dependent Localization of Nesprin-2, a Giant Scaffolder at the Nuclear Envelope,” *Mol Biol Cell*, vol. 16, no. july, pp. 3411–3424, 2005, doi: 10.1091/mbc.E04-11-1009.

[120] A. E. Emery and F. E. Dreifuss, “Unusual type of benign x-linked muscular dystrophy.,” *J. Neurol. Neurosurg. Psychiatry*, vol. 29, no. 4, pp. 338–342, 1966, doi: 10.1136/jnnp.29.4.338.

[121] A. Fidziańska and I. Hausmanowa-Petrusewicz, “Architectural abnormalities in muscle nuclei. Ultrastructural differences between X-linked and autosomal dominant forms of EDMD,” *J. Neurol. Sci.*, vol. 210, no. 1–2, pp. 47–51, 2003, doi: 10.1016/S0022-510X(03)00012-1.

[122] A. Fidziańska, D. Toniolo, and I. Hausmanowa-Petrusewicz, “Ultrastructural abnormality of sarcolemmal nuclei in Emery-Dreifuss muscular dystrophy (EDMD),” *J. Neurol. Sci.*, vol. 159, no. 1, pp. 88–93, 1998, doi: 10.1016/S0022-510X(98)00130-0.

[123] J. R. W. Yates *et al.*, “Genotype-phenotype analysis in X-linked Emery-Dreifuss

muscular dystrophy and identification of a missense mutation associated with a milder phenotype," *Neuromuscul. Disord.*, vol. 9, no. 3, pp. 159–165, 1999, doi: 10.1016/S0960-8966(98)00121-7.

[124] A. Nagano *et al.*, "Emerin deficiency at the nuclear membrane in patients with Emery-Dreifuss muscular dystrophy," *Nat. Genet.*, vol. 12, no. 3, pp. 254–259, 1996, doi: 10.1038/ng0396-254.

[125] A. C. Rowat, J. Lammerding, and J. H. Ipsen, "Mechanical properties of the cell nucleus and the effect of emerin deficiency," *Biophys. J.*, vol. 91, no. 12, pp. 4649–4664, 2006, doi: 10.1529/biophysj.106.086454.

[126] L. Martin, C. Crimaudo, and L. Gerace, "cDNA cloning and characterization of lamina-associated polypeptide 1C (LAP1C), an integral protein of the inner nuclear membrane," *Journal of Biological Chemistry*, vol. 270, no. 15. pp. 8822–8828, 1995, doi: 10.1074/jbc.270.15.8822.

[127] M. Santos, P. Costa, F. Martins, E. F. da Cruz e Silva, O. A. B. da Cruz e Silva, and S. Rebelo, "LAP1 is a crucial protein for the maintenance of the nuclear envelope structure and cell cycle progression," *Mol. Cell. Biochem.*, vol. 399, no. 1–2, pp. 143–153, 2015, doi: 10.1007/s11010-014-2241-x.

[128] M. Santos *et al.*, "Identification of a novel human LAP1 isoform that is regulated by protein phosphorylation," *PLoS One*, vol. 9, no. 12, pp. 1–32, 2014, doi: 10.1371/journal.pone.0113732.

[129] R. Foisner and L. Gerace, "Integral membrane proteins of the nuclear envelope interact with lamins and chromosomes, and binding is modulated by mitotic phosphorylation," *Cell*, vol. 73, no. 7, pp. 1267–1279, 1993, doi: 10.1016/0092-8674(93)90355-T.

[130] R. S. H. Brown, C. Zhao, A. R. Chase, J. Wang, and C. Schlieker, "The mechanism of Torsin ATPase activation," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 111, no. 45, pp. E4822–E4831, 2014, doi: 10.1073/pnas.1415271111.

[131] B. Sosa, E. Demircioglu, and J. et al Chen, "How lamina-associated polypeptide 1 (LAP1) activates Torsin, Elife," pp. 1–13, 2014, doi: 10.7554/eLife.03239.

[132] M. Santos *et al.*, "The Nuclear Envelope Protein, LAP1B, Is a Novel Protein Phosphatase 1 Substrate," *PLoS One*, vol. 8, no. 10, pp. 1–13, 2013, doi: 10.1371/journal.pone.0076788.

[133] J. B. Serrano, O. A. B. da Cruz e Silva, and S. Rebelo, "Lamina associated polypeptide 1 (LAP1) interactome and its functional features," *Membranes (Basel).*, vol. 6, no. 1, pp. 1–19, 2016, doi: 10.3390/membranes6010008.

[134] C. D. Pereira, F. Martins, M. Santos, T. Müeller, O. A. B. da Cruz E Silva, and S. Rebelo, *Nuclear Accumulation of LAP1:TRF2 Complex during DNA Damage Response Uncovers a Novel Role for LAP1*, vol. 9, no. 8. 2020.

[135] J. Y. Shin *et al.*, "Lamina-associated polypeptide 1 is dispensable for embryonic myogenesis but required for postnatal skeletal muscle growth," *Hum. Mol. Genet.*, vol. 26, no. 1, pp. 65–78, 2017, doi: 10.1093/hmg/ddw368.

[136] X. Feng *et al.*, "Muscular involvement and tendon contracture in limb-girdle muscular dystrophy 2Y: A mild adult phenotype and literature review," *BMC Musculoskelet. Disord.*, vol. 21, no. 1, 2020, doi: 10.1186/s12891-020-03616-4.

[137] R. Ghaoui *et al.*, "TOR1AIP1 as a cause of cardiac failure and recessive limb-girdle muscular dystrophy," *Neuromuscul. Disord.*, vol. 26, no. 8, pp. 500–503, 2016, doi:

10.1016/j.nmd.2016.05.013.

[138] G. Kayman-Kurekci *et al.*, “Mutation in TOR1AIP1 encoding LAP1B in a form of muscular dystrophy: A novel gene related to nuclear envelopathies,” *Neuromuscul. Disord.*, vol. 24, no. 7, pp. 624–633, 2014, doi: 10.1016/j.nmd.2014.04.007.

[139] A. Fridkin, A. Penkner, V. Jantsch, and Y. Gruenbaum, “SUN-domain and KASH-domain proteins during development, meiosis and disease,” *Cell. Mol. Life Sci.*, vol. 66, no. 9, pp. 1518–1533, 2009, doi: 10.1007/s00018-008-8713-y.

[140] D. M. Hodzic, D. B. Yeater, L. Bengtsson, H. Otto, and P. D. Stahl, “Sun2 is a novel mammalian inner nuclear membrane protein,” *J. Biol. Chem.*, vol. 279, no. 24, pp. 25805–25812, 2004, doi: 10.1074/jbc.M313157200.

[141] V. C. Padmakumar *et al.*, “The inner nuclear membrane protein Sun1 mediates the anchorage of Nesprin-2 to the nuclear envelope,” *J. Cell Sci.*, vol. 118, no. 15, pp. 3419–3430, 2005, doi: 10.1242/jcs.02471.

[142] X. Shao, H. A. Tarnasky, J. P. Lee, R. Oko, and F. A. Van Der Hoorn, “Spag4, a novel sperm protein, binds outer dense-fiber protein Odf1 and localizes to microtubules of manchette and axoneme,” *Dev. Biol.*, vol. 211, no. 1, pp. 109–123, 1999, doi: 10.1006/dbio.1999.9297.

[143] C. Frohnert, S. Schweizer, and S. Hoyer-Fender, “SPAG4L/SPAG4L-2 are testis-specific SUN domain proteins restricted to the apical nuclear envelope of round spermatids facing the acrosome,” *Mol. Hum. Reprod.*, vol. 17, no. 4, pp. 207–218, 2011, doi: 10.1093/molehr/gaq099.

[144] K. Lei *et al.*, “Inner nuclear envelope proteins SUN1 and SUN2 play a prominent role in the DNA damage response,” *Curr. Biol.*, vol. 22, no. 17, pp. 1609–1615, 2012, doi: 10.1016/j.cub.2012.06.043.

[145] P. Meinke *et al.*, “Muscular Dystrophy-Associated SUN1 and SUN2 Variants Disrupt Nuclear-Cytoskeletal Connections and Myonuclear Organization,” *PLoS Genet.*, vol. 10, no. 9, 2014, doi: 10.1371/journal.pgen.1004605.

[146] M. Dreger, L. Bengtsson, T. Schöneberg, H. Otto, and F. Hucho, “Nuclear envelope proteomics: Novel integral membrane proteins of the inner nuclear membrane,” *Proc. Natl. Acad. Sci. U. S. A.*, vol. 98, no. 21, pp. 11943–11948, 2001, doi: 10.1073/pnas.211201898.

[147] W. C. Liang *et al.*, “TMEM43 mutations in emery-dreifuss muscular dystrophy-related myopathy,” *Ann. Neurol.*, vol. 69, no. 6, pp. 1005–1013, 2011, doi: 10.1002/ana.22338.

[148] A. Brull, B. M. Rodriguez, G. Bonne, A. Muchir, and A. T. Bertrand, “The pathogenesis and therapies of striated muscle laminopathies,” *Front. Physiol.*, vol. 9, no. OCT, pp. 1–18, 2018, doi: 10.3389/fphys.2018.01533.

[149] L. Bengtsson and H. Otto, “LUMA interacts with emerin and influences its distribution at the inner nuclear membrane,” *J. Cell Sci.*, vol. 121, no. 4, pp. 536–548, 2008, doi: 10.1242/jcs.019281.

[150] N. D. Merner *et al.*, “Arrhythmogenic Right Ventricular Cardiomyopathy Type 5 Is a Fully Penetrant, Lethal Arrhythmic Disorder Caused by a Missense Mutation in the TMEM43 Gene,” *Am. J. Hum. Genet.*, vol. 82, no. 4, pp. 809–821, 2008, doi: 10.1016/j.ajhg.2008.01.010.

[151] T. Mukai *et al.*, “Emery-Dreifuss muscular dystrophy-related myopathy with TMEM43 mutations,” *Muscle and Nerve*, vol. 59, no. 2, pp. E5–E7, 2019, doi:

10.1002/mus.26355.

[152] W. Lu *et al.*, “Nesprin interchain associations control nuclear size,” *Cell. Mol. Life Sci.*, vol. 69, no. 20, pp. 3493–3509, 2012, doi: 10.1007/s00018-012-1034-1.

[153] Q. Zhang *et al.*, “Nesprins: A novel family of spectrin-repeat-containing proteins that localize to the nuclear membrane in multiple tissues,” *J. Cell Sci.*, vol. 114, no. 24, pp. 4485–4498, 2001.

[154] H. F. Horn *et al.*, “A mammalian KASH domain protein coupling meiotic chromosomes to the cytoskeleton,” *J. Cell Biol.*, vol. 202, no. 7, pp. 1023–1039, 2013, doi: 10.1083/jcb.201304004.

[155] A. Janin and V. Gache, “Nesprins and lamins in health and diseases of cardiac and skeletal muscles,” *Front. Physiol.*, vol. 9, no. SEP, pp. 1–12, 2018, doi: 10.3389/fphys.2018.01277.

[156] D. Rajgor and C. M. Shanahan, “Nesprins: from the nuclear envelope and beyond,” *Expert Rev. Mol. Med.*, vol. 15, no. July, pp. 1–17, 2013, doi: 10.1017/erm.2013.6.

[157] M. J. Puckelwartz *et al.*, “Disruption of nesprin-1 produces an Emery Dreifuss muscular dystrophy-like phenotype in mice,” vol. 18, no. 4, pp. 607–620, 2009, doi: 10.1093/hmg/ddn386.

[158] J. Zhang *et al.*, “Nesprin 1 is critical for nuclear positioning and anchorage,” *Hum. Mol. Genet.*, vol. 19, no. 2, pp. 329–341, 2009, doi: 10.1093/hmg/ddp499.

[159] K. H. Schreiber and B. K. Kennedy, “When lamins go bad: Nuclear structure and disease,” *Cell*, vol. 152, no. 6, pp. 1365–1375, 2013, doi: 10.1016/j.cell.2013.02.015.

[160] F. Lin and H. J. Worman, “Structural organization of the human gene (LMNB1) encoding nuclear lamin B1,” *Genomics*, vol. 27, no. 2. pp. 230–236, 1995, doi: 10.1006/geno.1995.1036.

[161] K. Furukawa and Y. Hotta, “cDNA cloning of a germ cell specific lamin B3 from mouse spermatocytes and analysis of its function by ectopic expression in somatic cells,” *EMBO J.*, vol. 12, no. 1, pp. 97–106, 1993, doi: 10.1002/j.1460-2075.1993.tb05635.x.

[162] A. Peter and R. Stick, “Evolution of the lamin protein family: What introns can tell,” *Nucleus*, vol. 3, no. 1, pp. 44–59, 2012, doi: 10.4161/nucl.18927.

[163] T. Dittmer and T. Misteli, “The lamin protein family,” *Genome Biol.*, vol. 12, no. 5, 2011, doi: 10.1186/gb-2011-12-5-222.

[164] R. D. Moir and T. P. Spann, “The structure and function of nuclear lamins: Implications for disease,” *Cell. Mol. Life Sci.*, vol. 58, no. 12–13, pp. 1748–1757, 2001, doi: 10.1007/PL00000814.

[165] G. E. Morris, “The role of the nuclear envelope in Emery-Dreifuss muscular dystrophy,” *Trends Mol. Med.*, vol. 7, no. 12, pp. 572–577, 2001, doi: 10.1016/S1471-4914(01)02128-1.

[166] L. Mounkes, S. Kozlov, B. Burke, and C. L. Stewart, “The laminopathies: Nuclear structure meets disease,” *Curr. Opin. Genet. Dev.*, vol. 13, no. 3, pp. 223–230, 2003, doi: 10.1016/S0959-437X(03)00058-3.

[167] A. Méjat *et al.*, “Lamin A/C - mediated neuromuscular junction defects in Emery-Dreifuss muscular dystrophy,” *J. Cell Biol.*, vol. 184, no. 1, pp. 31–44, 2009, doi: 10.1083/jcb.200811035.

[168] Y. Fan *et al.*, “ Clinical spectrum and genetic variations of LMNA -related muscular

dystrophies in a large cohort of Chinese patients ," *J. Med. Genet.*, p. jmedgenet-2019-106671, 2020, doi: 10.1136/jmedgenet-2019-106671.

[169] A. Muchir *et al.*, "Nuclear envelope alterations in fibroblasts from LGMD1B patients carrying nonsense Y259X heterozygous or homozygous mutation in lamin A/C gene," *Exp. Cell Res.*, vol. 291, no. 2, pp. 352–362, 2003, doi: 10.1016/j.yexcr.2003.07.002.

[170] B. M. Paschal and J. B. Kelley, "Nuclear Lamina," *Encycl. Biol. Chem. Second Ed.*, pp. 310–313, 2013, doi: 10.1016/B978-0-12-378630-2.00477-1.

[171] W. H. Raharjo, P. Enarson, T. Sullivan, C. L. Stewart, and B. Burke, "Nuclear envelope defects associated with LMNA mutations cause dilated cardiomyopathy and Emery-Dreifuss muscular dystrophy," *J. Cell Sci.*, vol. 114, no. 24, pp. 4447–4457, 2001.

[172] G. Bonne *et al.*, "Clinical and molecular genetic spectrum of autosomal dominant Emery-Dreifuss muscular dystrophy due to mutations of the lamin A/C gene," *Ann. Neurol.*, vol. 48, no. 2, pp. 170–180, 2000, doi: 10.1002/1531-8249(200008)48:2<170::AID-ANA6>3.0.CO;2-J.

[173] T. Sullivan *et al.*, "Loss of A-type lamin expression compromises nuclear envelope integrity leading to muscular dystrophy," *J. Cell Biol.*, vol. 147, no. 5, pp. 913–919, 1999, doi: 10.1083/jcb.147.5.913.

[174] R. E. M. A. van Herpen *et al.*, "Divergent Mitochondrial and Endoplasmic Reticulum Association of DMPK Splice Isoforms Depends on Unique Sequence Arrangements in Tail Anchors," *Mol. Cell. Biol.*, vol. 25, no. 4, pp. 1402–1414, 2005, doi: 10.1128/mcb.25.4.1402-1414.2005.

[175] P. J. T. A. Groenen, "Constitutive and regulated modes of splicing produce six major myotonic dystrophy protein kinase (DMPK) isoforms with distinct properties," *Hum. Mol. Genet.*, vol. 9, no. 4, pp. 605–616, Mar. 2000, doi: 10.1093/hmg/9.4.605.

[176] D. G. Wansink, R. E. M. A. van Herpen, M. M. Coerwinkel-Driessen, P. J. T. A. Groenen, B. A. Hemmings, and B. Wieringa, "Alternative Splicing Controls Myotonic Dystrophy Protein Kinase Structure, Enzymatic Activity, and Subcellular Localization," *Mol. Cell. Biol.*, vol. 23, no. 16, pp. 5489–5501, 2003, doi: 10.1128/mcb.23.16.5489-5501.2003.

[177] T. Mateus, F. Martins, A. Nunes, T. Herdeiro, and S. Rebelo, "Metabolic alterations in Myotonic Dystrophy type 1 and their correlation with Lipin function," Int. J.Environ. Res. Public Health, vol.18, no.4, 2021, doi: 10.3390/ijerph18041794

[178] R. Rodríguez, O. Hernández-Hernández, J. J. Magaña, R. González-Ramírez, E. S. García-López, and B. Cisneros, "Altered nuclear structure in myotonic dystrophy type 1-derived fibroblasts," *Mol. Biol. Rep.*, vol. 42, no. 2, pp. 479–488, 2015, doi: 10.1007/s11033-014-3791-4.

[179] P. Meinke, S. Hintze, S. Limmer, and B. Schoser, "Myotonic dystrophy-A progeroid disease?," *Front. Neurol.*, vol. 9, no. JUL, pp. 1–12, 2018, doi: 10.3389/fneur.2018.00601.

[180] S. Hintze, L. Knaier, S. Limmer, B. Schoser, and P. Meinke, "Nuclear envelope transmembrane proteins in myotonic dystrophy type," *Front. Physiol.*, vol. 9, no. OCT, pp. 1–7, 2018, doi: 10.3389/fphys.2018.01532.

[181] E. B. Harmon, M. L. Harmon, T. D. Larsen, J. Yang, J. W. Glasford, and M. B. Perryman, "Myotonic dystrophy protein kinase is critical for nuclear envelope integrity," *J. Biol. Chem.*, vol. 286, no. 46, pp. 40296–40306, 2011, doi:

10.1074/jbc.M111.241455.

[182] E. Nikolakaki, I. Mylonis, and T. Giannakouros, “Lamin B Receptor: Interplay between Structure, Function and Localization,” *Cells*, vol. 6, no. 3, p. 28, 2017, doi: 10.3390/cells6030028.

[183] I. Solovei *et al.*, “LBR and lamin A/C sequentially tether peripheral heterochromatin and inversely regulate differentiation,” *Cell*, vol. 152, no. 3, pp. 584–598, 2013, doi: 10.1016/j.cell.2013.01.009.

[184] N. T. Duong *et al.*, “Nesprins: Tissue-specific expression of epsilon and other short isoforms,” *PLoS One*, vol. 9, no. 4, 2014, doi: 10.1371/journal.pone.0094380.

[185] J. I. de las Heras *et al.*, “Tissue specificity in the nuclear envelope supports its functional complexity,” *Nucl. (United States)*, vol. 4, no. 6, pp. 460–477, 2013, doi: 10.4161/nucl.26872.

[186] H. J. Worman and J. C. Courvalin, “Nuclear envelope, nuclear lamina, and inherited disease,” *Int. Rev. Cytol.*, vol. 246, no. 05, pp. 231–279, 2005, doi: 10.1016/S0074-7696(05)46006-4.

[187] D. J. Lloyd, R. C. Trembath, and S. Shackleton, “A novel interaction between lamin A and SREBP1: Implications for partial lipodystrophy and other laminopathies,” *Hum. Mol. Genet.*, vol. 11, no. 7, pp. 769–777, 2002, doi: 10.1093/hmg/11.7.769.

[188] J. Lammerding, J. Hsiao, P. C. Schulze, S. Kozlov, C. L. Stewart, and R. T. Lee, “Abnormal nuclear shape and impaired mechanotransduction in emerin-deficient cells,” *J. Cell Biol.*, vol. 170, no. 5, pp. 781–791, 2005, doi: 10.1083/jcb.200502148.

CHAPTER II: AIMS OF DISSERTATION

1. Aims of dissertation

Muscular dystrophies are a heterogeneous group of inherited diseases that are characterized by muscle weakness and wasting with or without nerve tissue commitment and in some cases may also manifest mental retardation and heart problems [1,2]. DM1 is the muscular dystrophy that most adult individuals affect to date, with a prevalence of 1 to 8000 worldwide. This pathology is multisystemic and autosomal dominant and is essentially characterized by myotonia (delayed muscle relaxation after muscle contraction), progressive muscle weakness, progressive muscle wasting, defects in cardiac conduction, respiratory impairment, gastrointestinal problems, and cataracts [3–5]. This pathology is caused by a mutation leading to abnormal expansion of the CTG trinucleotide in the 3 'untranslated region (UTR) of the *DMPK* gene [3,4]. DM1 is a disease that presents very heterogeneous symptoms and with degrees of severity that are also quite different. Based on the phenotype, this disease can be divided into 3 (congenital, adult and mild). Clinically, DM1 is subdivided into 5 different groups (congenital, infantile, juvenile, adult-onset and late-onset) [6–8]. The nuclear envelope (NE) proteins that have been most associated with changes in muscle function and nuclear position in muscle diseases are emerin, LAP1, SUN and lamin A/C [9,10]. Mutations in the genes of the mentioned proteins, result in changes in the normal functioning of muscle differentiation and regulation of nuclear movement [9,10]. The few studies carried out already indicate the existence of changes in the structure and function of NE in patients with DM1, suggesting that this cellular structure may have an important role in the molecular mechanisms of DM1 and that need to be investigated [11–14].

Bearing this in mind, the main objective of this dissertation is to characterize changes in the intracellular levels of NE proteins as well as changes in the organization of NE structure in patients with DM1. For this purpose, human fibroblasts from patients with DM1 will be used as cell models. This study will include healthy control fibroblasts - with CTG repeat between 5 and 27- and DM1-derived fibroblasts representing the adult and congenital phenotypes (with approximately 1000 and 2000 CTG repeat length, respectively).

The specific aims of this dissertation are:

1. Summarize the nuclear envelope alterations associated to DM1 previously reported in literature.

2. Determine the intracellular protein levels of several nuclear envelope proteins using skin DM1-derived and control fibroblasts (DM1 vs control);

3. Evaluate the nuclear envelope profile using DM1-derived and control fibroblasts (DM1 vs control);

4. Determine the subcellular localization of NE proteins using DM1-derived and control fibroblasts (DM1 vs control);

2. References

[1] I. Dalkilic and L. M. Kunkel, "Muscular dystrophies: Genes to pathogenesis," *Curr. Opin. Genet. Dev.*, vol. 13, no. 3, pp. 231–238, 2003, doi: 10.1016/S0959-437X(03)00048-0.

[2] A. E. H. Emery, "Fortnightly review: The muscular dystrophies," *Bmj*, vol. 317, no. 7164, pp. 991–995, 1998, doi: 10.1136/bmj.317.7164.991.

[3] J. D. Brook *et al.*, "Molecular basis of myotonic dystrophy: Expansion of a trinucleotide (CTG) repeat at the 3′ end of a transcript encoding a protein kinase family member," *Cell*, vol. 68, no. 4, pp. 799–808, 1992, doi: 10.1016/0092-8674(92)90154-5.

[4] M. Mahadevan *et al.*, "Myotonic dystrophy mutation: An unstable CTG repeat in the 3′ untranslated region of the gene," *Science (80-.).*, vol. 255, no. 5049, pp. 1253–1255, 1992, doi: 10.1126/science.1546325.

[5] L. P. W. Ranum and J. W. Day, "Myotonic Dystrophy: RNA Pathogenesis Comes into Focus," *Am. J. Hum. Genet.*, vol. 74, no. 5, pp. 793–804, 2004, doi: 10.1086/383590.

[6] M. De Antonio *et al.*, "Unravelling the myotonic dystrophy type 1 clinical spectrum: A systematic registry-based study with implications for disease classification," *Rev. Neurol. (Paris).*, vol. 172, no. 10, pp. 572–580, 2016, doi: 10.1016/j.neurol.2016.08.003.

[7] K. Yum, E. T. Wang, and A. Kalsotra, "Myotonic dystrophy: disease repeat range, penetrance, age of onset, and relationship between repeat size and phenotypes," *Curr. Opin. Genet. Dev.*, vol. 44, pp. 30–37, 2017, doi: 10.1016/j.gde.2017.01.007.

[8] S. Tomé and G. Gourdon, "DM1 phenotype variability and triplet repeat instability: Challenges in the development of new therapies," *Int. J. Mol. Sci.*, vol. 21, no. 2, pp. 1–10, 2020, doi: 10.3390/ijms21020457.

[9] K. L. Wilson, "The nuclear envelope, muscular dystrophy and gene expression," *Trends Cell Biol.*, vol. 10, no. 4, pp. 125–129, 2000, doi: 10.1016/S0962-8924(99)01708-0.

[10] S. R. Wente and M. P. Rout, "The nuclear pore complex and nuclear transport.," *Cold Spring Harb. Perspect. Biol.*, vol. 2, no. 10, pp. 1–19, 2010, doi: 10.1101/cshperspect.a000562.

[11] E. B. Harmon, M. L. Harmon, T. D. Larsen, J. Yang, J. W. Glasford, and M. B. Perryman, "Myotonic dystrophy protein kinase is critical for nuclear envelope integrity," *J. Biol. Chem.*, vol. 286, no. 46, pp. 40296–40306, 2011, doi: 10.1074/jbc.M111.241455.

[12] R. Rodríguez, O. Hernández-Hernández, J. J. Magaña, R. González-Ramírez, E. S. García-López, and B. Cisneros, “Altered nuclear structure in myotonic dystrophy type 1-derived fibroblasts,” *Mol. Biol. Rep.*, vol. 42, no. 2, pp. 479–488, 2015, doi: 10.1007/s11033-014-3791-4.

[13] P. Meinke, S. Hintze, S. Limmer, and B. Schoser, “Myotonic dystrophy-A progeroid disease?” *Front. Neurol.*, vol. 9, no. JUL, pp. 1–12, 2018, doi: 10.3389/fneur.2018.00601.

[14] S. Hintze, L. Knaier, S. Limmer, B. Schoser, and P. Meinke, “Nuclear envelope transmembrane proteins in myotonic dystrophy type,” *Front. Physiol.*, vol. 9, no. Oct, pp. 1–7, 2018, doi: 10.3389/fphys.2018.01532.

CHAPTER III: Nuclear envelope alteration of DM1 models

Nuclear envelope alterations in myotonic dystrophy type 1 patients-derived fibroblasts

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Abstract

Myotonic dystrophy type 1 (DM1) is the most common adult-onset muscular dystrophy with an estimated prevalence of 1:8000. DM1 is caused by an abnormal expansion of the CTG trinucleotide in the 3'UTR region of the *DMPK* gene. This pathology is characterized, among other features, by myotonia, muscle weakness and loss of muscle mass. The molecular mechanisms involved in this disease are not yet well known, although there are already some hypotheses that explain the multisystemic signs of DM1. The most recent studies have evaluated the role of NE dysfunction in muscular dystrophies, particularly in DM1. Therefore, the main objectives of the present study are to summarize the previous reported studies that demonstrated an impaired nuclear structure; to evaluate the nuclear envelope profile; to determine the NE protein levels and to evaluate the subcellular distribution of NE proteins, using DM1-derived and control fibroblasts.

In present study, we demonstrated that DM1-derived fibroblasts exhibited altered intracellular protein levels of lamin A/C, lamin B1, LAP1, SUN1, nesprin-1 and nesprin-2 in relation to control-derived fibroblasts. In addition, nuclei staining positive for lamin A/C, emerin, LAP1 and nesprin-1 showed an altered location of these NE proteins, accompanied by the presence of nuclear deformations (blebs, lobes and invaginations) and an increased number of nuclear inclusions. Regarding the nuclear profile, fibroblasts derived from patients with DM1 tended to have a larger nuclear area as well as a higher number of deformed nuclei and micronuclei than control fibroblasts.

All together the results reinforce the idea that NE dysfunction is a common pathological characteristic found in DM1.

Keywords

Myotonic dystrophy type 1, nuclear envelope, DMPK, nuclear profile, lamin A/C, lamin B1, emerin, LAP1, SUN1, nesprin-1, nesprin-2

1. Introduction

Myotonic dystrophy type 1 (DM1) is a slowly progressive multisystemic disease, from the group of muscular dystrophies, which is characterized by myotonia, muscle weakness as well as visual (cataracts), cardiac (conduction problems leading to cardiomyopathy) and metabolic (insulin insensitivity and diabetes) alterations [1–3]. This is the most common adult-onset muscular dystrophy with an estimated prevalence of 1:8000 [4,5]. DM1 is caused by a mutation leading to an abnormal unstable expansion of the CTG trinucleotide in the 3'UTR of the *Myotonic Dystrophy Protein Kinase (DMPK)* gene [4,6]. DMPK is the central protein of DM1 and 7 distinct isoforms are known (DMPK A to G) in humans, which are generated by alternative splicing. DMPK subcellular localization is confined to either endoplasmic reticulum or nuclear envelope (NE) (DMPK A and B), to mitochondria (DMPK C and D) or to cytoplasm (DMPK E, F and G) [7–9].

Several studies have been carried out to unravel the molecular mechanisms involved in this pathology. There are three hypothesis accepted as molecular mechanisms of DM1 (RNA toxic gain-of-function, haploinsufficiency of DMPK and rearrangement of the DM1 locus) [9–12]. Despite this, none of these hypotheses can explain the multisystemic signs and symptoms. Thus, the possibility of a defect in the positioning of myonuclei, resulting from alterations in NE proteins has also been raised, to also be involved in the pathological mechanisms of DM1, similarly to other muscular dystrophies [13–16].

Previous studies have reported that some muscular dystrophies result from alterations in NE stability. A common feature of these diseases is the presence of nuclei usually located and grouped in the muscle cells' centre, compromising myonuclear movement [13,17]. NE proteins are essential for gene regulation, nuclear structure and muscle function [18,19]. In the case of DM1, some studies have also been developed in order to assess the role of NE proteins in the mechanisms of this disease [20–22]. To date, NE proteins lamin A/C, lamin B1, emerin, SUN1, SUN2, LBR, TMEM38a, nesprin-1 and nesprin-2 have already been evaluated in DM1 patients-derived cells [20–22]. Despite this, the results obtained regarding the NE dysfunction in DM1 patients are still not very robust and clear.

The main objectives of this study were to evaluate the intracellular protein levels and immunolocalization of the disease-associated protein DMPK and others NE proteins, namely lamin A/C, lamin B1, emerin, LAP1, SUN1, nesprin-1 and nesprin-2, in DM1-derived and control fibroblasts. The results obtained here will provide new insights on the potential contribution of NE dysfunction to DM1 pathogenesis.

2. Materials and Methods

2.1. Human samples

Fibroblasts derived from skin biopsies of adult male DM1 donors with different numbers of CTG repeats as well as a healthy control subject were obtained from the Coriell Institute for Medical Research, New Jersey, EUA. The clinically affected patients' cell lines selected for this study included two cell lines with approximately 1000 CTG repeats, hereafter referred to as DM1_1000 (1) (GM04033) and DM1_1000 (2) (GM04647), and two cell lines with approximately 2000 CTG repeats, hereafter designated DM1_2000 (1) (GM03759) and DM1_2000 (2) (GM03989). In turn, the control cell line used in this study comprised between 5 and 27 CTG repeats (GM02673).

2.2. Cell culture

Fibroblast cultures were maintained in T75 flasks with Dulbecco's Modified Eagle Medium (DMEM; Gibco™) supplemented with 15% fetal bovine serum (FBS; Gibco™), at 37ºC in a humidified atmosphere with 5% CO_2. The medium was changed every other day and all washes performed using Dulbecco's phosphate buffered saline (PBS) (Thermo Scientific). Whenever fibroblast cultures reached a confluence of 80-90%, they were subcultured using 0.05% trypsin-EDTA, plated in complete medium and maintained at 37ºC in a CO_2 incubator [23].

2.3. Antibodies

The list of primary and secondary antibodies used for Western blotting and immunocytochemistry are summarized in Table 1.

Table 1. Primary antibodies, the target and specific dilution according to the technique used.

Protein target	Reference	Type	Dilution
Lamin A/C	Cell Signalling Technology (4777T)	Mouse, monoclonal	WB – 1:4000 ICC – 1:250
Lamin B1	Santa Cruz Biotechnology ((B-10): sc-374015)	Mouse, monoclonal	WB - 1:500
LAP1	Provided by Dr. Dauer [24]	Rabbit, polyclonal	WB - 1:20000
	Atlas Antibodies (HPA050546)	Rabbit, polyclonal	ICC – 1:150
Emerin	Santa Cruz Biotechnology ((H-12) sc-25284)	Mouse, monoclonal	WB – 1:1000 ICC – 1:500
SUN1	Provided by Ya-Hui Chi [25]	Rabbit, polyclonal	WB - 1:2000
Nesprin-1	Developmental Studies Hybridoma Bank (MANNES1E 8C3)	Mouse, monoclonal	WB - 0.4 µg/ml ICC - 1.5 µg/ml
Nesprin-2	Developmental Studies Hybridoma Bank (MANNES2A 11A3)	Mouse, monoclonal	WB - 0.3 µg/ml
DMPK	Developmental Studies Hybridoma Bank (MANDM1 6G8)	Mouse, monoclonal	WB - 0.2 ug/ml
Antibody secondary ICC	Invitrogen (A-11001)	Alexa Fluor 488-conjugated goat anti-mouse IgG	1:300
Antibody secondary ICC	Invitrogen (A-11012)	Alexa Fluor 594-conjugated goat anti-rabbit IgG	1:300
Antibody secondary WB	Cell Signalling Technology (7076)	HRP-linked horse anti-mouse IgG	1:10000
Antibody secondary WB	Cell Signalling Technology (7074)	HRP-linked goat anti-rabbit IgG	1:10000

Abbreviations: ICC – Immunocytochemistry; WB – Western Blot; LAP1 - Lamin-associated with polypeptide 1; SUN - Sad1/Unc-84; DMPK - Myotonic Dystrophy Protein Kinase; HRP – Horsedish peroxidase

2.4. Western blotting

Fibroblast cultures were grown in T75 flasks until they reached a confluence of 80-90%. Cell lysates were collected in a 1% sodium dodecyl sulphate (SDS) solution and boiled at 90 °C for 10 minutes. The total protein content was quantified using Pierce's bicinchoninic acid (BCA) protein assay kit (Thermofisher Scientific). Protein samples were separated on a 5-20% SDS-PAGE gradient gel and electrotransfered onto nitrocellulose membranes. The total protein content in each sample was determined by reversible staining of nitrocellulose membranes with Ponceau S (Sigma-Aldrich), followed by scanning in a calibrated image densitometer GS-800 (Bio-Rad, San Jose, CA, USA) [26].

Immunoblotting analysis of target proteins started by membrane blocking with 5% bovine serum albumin (BSA; Nzytech)/1x Tris-buffered saline with 0.1% Tween-20 (TBS-T) for 3 hours. The primary antibodies (Table 1) were prepared in 3% BSA/1x TBS-T and incubated with the membrane for 2 hours at room temperature, followed by overnight incubation at 4ºC. In the following day, the membrane was incubated with the appropriate secondary antibody (Table 1) for 2 hours at room temperature. For the detection of target proteins, the enhanced chemioluminescence (ECL™) select western blotting detection reagent (GE Healthcare) was used and immunoblots were scanned in a ChemiDoc Imaging System (Bio-Rad) [27].

Quantification of intracellular protein levels was achieved with the ImageLab software (Bio-Rad, Hercules, California, USA) using Ponceau S staining as a protein loading control for data normalization [27]. Relative protein levels were calculated by comparing the DM1 patients' samples with control ones.

2.4.1 Statistical analysis

Statistical significance analysis was conducted using the GraphPad Prism 9 software (GraphPad Software, San Diego, California, EUA) and data were analysed using one-way ANOVA followed by Tukey's multiple comparison test. Quantitative data were presented as mean ± standard error of the mean (SEM) of, at least, three independent experiments. Values of $p < 0.05$ were considered statistically significant.

2.5. Immunocytochemistry

Fibroblast were plated in 6-well plates containing glass coverslips (Corning) at a cell density of 75000 cells/well for 24 hours. Then, cells were fixed using 4% paraformaldehyde for 20 minutes and permeabilized with 0.2% Triton X-100/1x PBS for 10 minutes. After blocking with 3% BSA/1x PBS for 1 hour to avoid unspecific binding, cells were incubated with specific primary antibodies (Table 1) in 3% BSA/1x PBS for 2 hours at room temperature, followed by incubation with the appropriate secondary antibody (Table 1) in 3% BSA/1x PBS for 1 hour in the dark. Coverslips were mounted on a microscope slide using Vectashield® mounting medium with 4',6-diamidino-2-phenolyde (DAPI) (Vector Laboratories) [26,27]. Image acquisition was performed using an epifluorescence microscopy Zeiss AxioImager Z1 (Zeiss) motorized microscope equipped with a Plan-ApoCHROMAT 63x/1.4 oil objective lens. Microphotograph images were taken with a digital AxioCam HR3 (Soft Imaging System).

2.5.1 Morphological analysis

Two hundred nuclei from each cell line were analysed. From the morphological point of view, the nuclear form and the number of nuclear inclusions were evaluated. The number of nuclear inclusions was assessed globally and by categories (1-2 inclusions and ≥ 3 inclusions). Of note, the control-derived fibroblasts nuclei, when nuclear inclusions are present, usually the one or two inclusions. The nucleus was considered normal when they present a typical ring immunostaining for NE proteins or that had an ellipsoid shape when stained with DAPI. The nuclei were considered deformed when they presented nuclear alterations/deformations, such as invaginations, blebs, lobes and micronuclei. Additionally, we subdivide the deformations into two different categories the mild (very soft deformations observed) and the moderate invaginations (severe deformations observed). Representative images of either mild (Figure 3 - DM1_2000) and moderate invaginations are presented in (Figure 5 - DM1_2000).

2.5.2 Morphometric analysis

For a morphometric analysis, four hundred nuclei from each cell line were evaluated. Quantitative analyses of the circularity [$(4\pi \times \text{area})/\text{perimeter}^2$], nuclear area and crossed diameter ratio (length/width) were performed automatically using the Fiji/ImageJ software.

2.5.3 Statistical analysis

Statistical significance analysis was conducted using the GraphPad Prism 9 software and data were analysed using one-away ANOVA followed by Tukey's multiple comparison test. Quantitative data were presented as mean ± SEM. Values of $p < 0.05$ were considered statistically significant.

3. Results

3.1. Evaluation of intracellular DMPK protein levels in DM1 patients-derived and control fibroblasts

DM1 seems to be caused by different molecular mechanisms. The toxic gain of function of expanded CUG repeats of mutant DMPK mRNA and the haploinsufficiency are two well accepted proposed mechanisms, reviewed in [9]. As a consequence, the mRNA and protein levels of DMPK are found to be decreased in adult DM1 tissues [28]. To confirm

these changes in intracellular DMPK protein levels in DM1 patients-derived fibroblasts, the Western blotting technique was used.

The intracellular protein levels of DMPK were significantly decreased in fibroblasts from patients with DM1_1000 (p = 0.0332) and DM1_2000 (p = 0.0332) when compared to control fibroblasts (Figure 1).

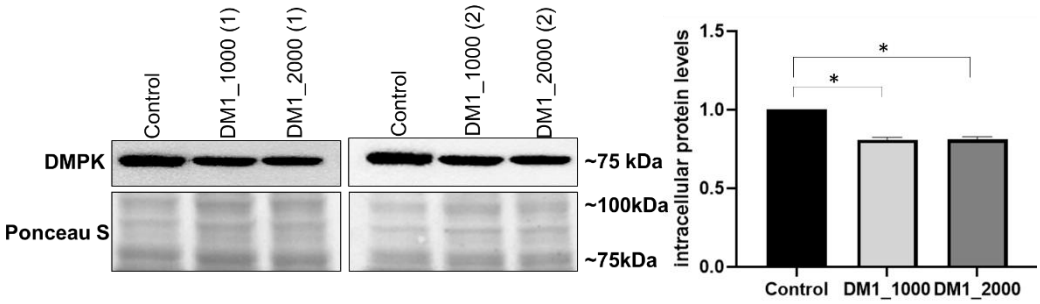

Figure 1: Intracellular DMPK protein levels in DM1 patients-derived and control fibroblasts. The intracellular protein levels in DM1 patients-derived fibroblasts were estimated in relation to protein levels detected in the control condition and are presented as mean ± SEM of 4 independent experiments. To compare intracellular protein levels between groups, one-way ANOVA test followed by the Tukey's test. *p <0.05; DM1 – Myotonic dystrophy type 1; DMPK - Myotonic Dystrophy Protein Kinase

Concerning the significant alterations in the DMPK protein levels observed in DM1-derived and control fibroblasts we decide to explore their functional implications in terms of nuclear architectural structure and function through evaluation of NE proteins.

3.2. Evaluation of the nuclear profile in DM1 patients-derived and control fibroblasts

The presence of nuclear architectural alterations in DM1 patients-derived fibroblasts was assessed through DAPI staining, followed by monitorization of several nuclear parameters, namely occurrence of nuclear deformations, number of micronuclei, nuclear circularity, crossed diameter ratio and nuclear area. Nuclear circularity is a quantitative measure that assesses the circular shape of nuclei, with a maximum value of 1 corresponding to a perfect circle. Concerning nuclear deformations, the existence of blebs, lobed nuclei, micronuclei and nuclear invaginations was taken into consideration. Several visible small nuclei were quantified as micronuclei.

Regarding the presence of nuclear deformations (Figure 2A and 2B), there was a significant increase in the percentage of deformed nuclei in DM1 patients-derived fibroblasts (DM1_1000, p = 0.0066; DM1_2000, p = 0.0012) relative to control-derived fibroblasts.

These results indicate that DM1 patients-derived fibroblasts carrying a higher number of CTG repeats show a higher incidence of nuclear deformations (Figure 2B). The number of micronuclei also tends to be higher in DM1 patients-derived fibroblasts in comparison to control ones (Figure 2C) and seems to be correlated with the increasing number of CTG repeats. The analysis of nuclear circularity revealed no significant differences between DM1 patients-derived and control fibroblasts (Figure 2D). Regarding the crossed diameter ratio, we found that this parameter is significantly increased in the fibroblast nuclei derived from DM1_1000 ($p = 0.0328$) and DM1_2000 ($p = 0.0127$) in relation to the control (Figure 2E). Finally, the mean nuclear area of DM1 patients-derived fibroblasts appears to be larger than the control (Figure 2F). Knowing that the average nuclear area of fibroblasts is around 200 μm^2 [29] (Figure 2F), we started to quantify the number of cells with nuclear area <200 μm^2 and ≥ 200 μm^2. Interestingly, it was found that there is a significant increase in the nuclear area in DM1_2000 patients compared to control ($p = 0.0041$) (Figure 2F).

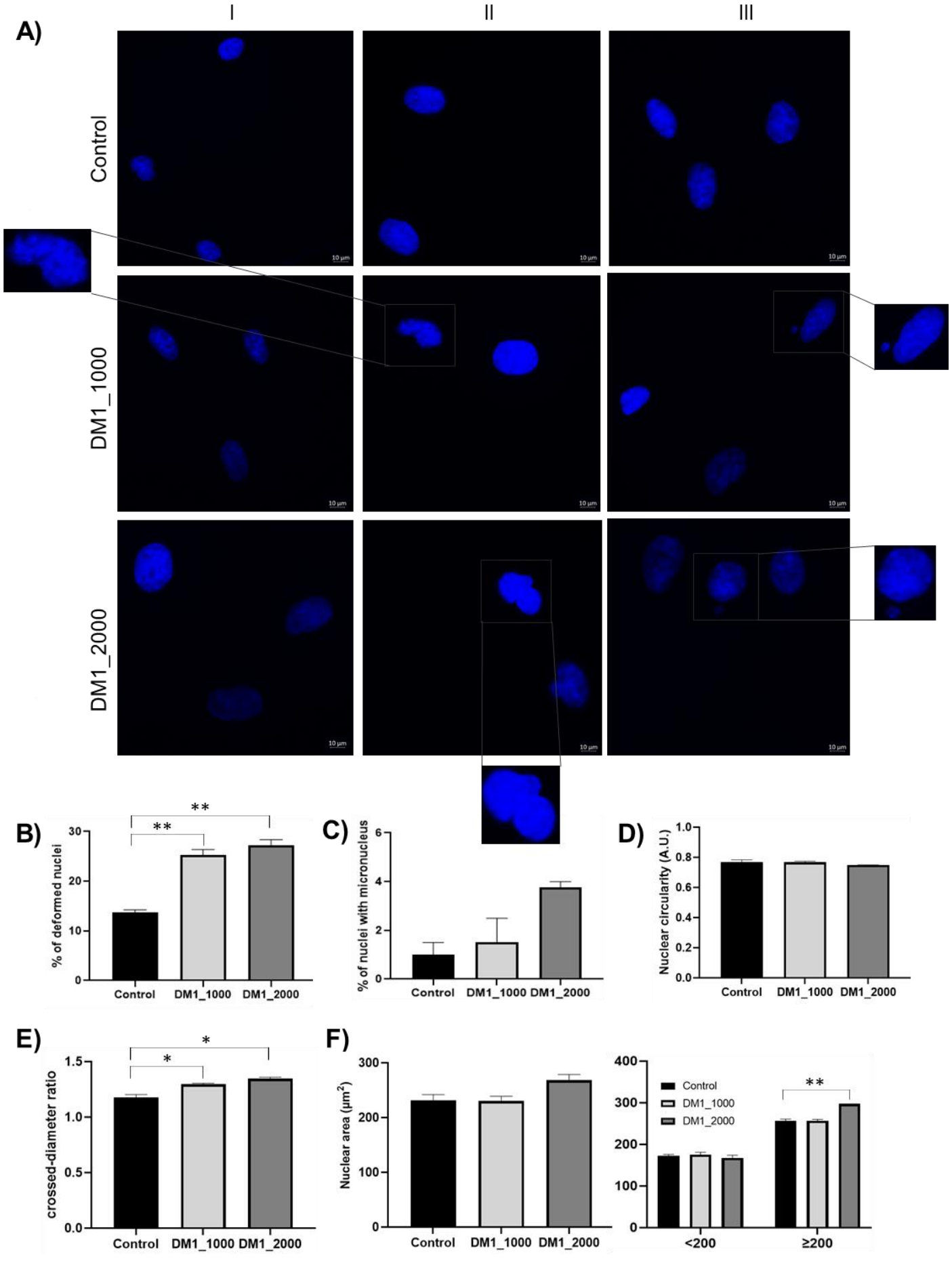
A)
I
II
III
Control
DM1_1000
DM1_2000
10 µm
B)
% of deformed nuclei
Control
DM1_1000
DM1_2000
**
**
C)
% of nuclei with micronucleus
Control
DM1_1000
DM1_2000
D)
Nuclear circularity (A.U.)
Control
DM1_1000
DM1_2000
E)
crossed-diameter ratio
Control
DM1_1000
DM1_2000
*
*
F)
Nuclear area (µm²)
Control
DM1_1000
DM1_2000
Control
DM1_1000
DM1_2000
**
<200
≥200

Figure 2: Fibroblasts nuclear profile (DM1 patients-derived and control fibroblasts). (A) The nuclear profile of DM1 patients-derived and control fibroblasts was analysed using fluorescence microscopy. The fibroblast nuclei were marked with DAPI (blue). Quantitative evaluation of **(B)** deformed nuclei, **(C)** micronuclei, **(D)** nuclear circularity, **(E)** crossed diameter ratio and **(F)** nuclear area. The quantitative data are presented as mean ± SEM and was obtained by analysing 100 cells per condition from 4 independent experiments. Statistical analysis was performed by comparison between the control, DM1_1000 and DM1_2000 fibroblasts using one-way ANOVA test followed by the Tukey's test. *$p<0.05$, **$p<0.01$. **(I)** – Nuclear area representation, **(II)** – Deformed nuclei representation, **(III)** – Micronuclei representation. (A.U.= Arbitrary Units). Scale bar, 10µm; DM1 – Myotonic dystrophy type 1

Once important nuclear changes were observed in DM1 patients-derived fibroblasts in relation to the control, it was decided to evaluate some proteins of the NE as well, since these changes may be correlated with alterations in the nuclear architecture.

3.3. Evaluation of intracellular levels and localization of NE proteins in DM1 patients-derived and control fibroblasts

To investigate the intracellular protein levels and localization of NE proteins in DM1-derived and control fibroblasts, the western blotting and immunocytochemistry techniques were used, respectively. Essentially, the following NE proteins were evaluated, two nuclear lamina proteins, namely lamin A/C (Figure 3) and lamin B1 (Figure 4); three inner nuclear membrane proteins, such as emerin (Figure 5), LAP1 (Figure 6) and SUN1 (Figure 7); and two outer nuclear membrane proteins, including nesprin-1 (Figure 8) and nesprin-2 (Figure 9).

The lamin A/C is part of the nuclear lamina and the latter is functionally associated to inner nuclear membrane. Therefore, these important NE components will be evaluated.

Regarding the intracellular protein levels of the lamin A/C, an increase in the fibroblasts of patients DM1_1000 and DM1_2000 ($p = 0.0058$) is observed in relation to the control, this increase is proportional to the CTG length repetition (Figure 3A). Our results also demonstrate mislocalization of the lamin A/C and increased NE and nucleoplasm immunolabelling in DM1 patients-derived fibroblasts (Figure 3B). The increase nuclear inclusions in DM1 patients-derived fibroblasts were evident, with a significant increase between DM1_2000 and control ($p = 0.0254$) (Figure 3C). The number of DM1-derived fibroblast nuclei with 1-2 inclusions tended to increase compared to control fibroblasts and with 3 or more inclusions (≥3) the increase was significant between DM1_2000 and control ($p = 0.0012$) (Figure 3C).

Concerning to deformed nuclei, DM1 patients-derived fibroblasts showed more deformations than control-derived fibroblasts, this increase being significant between DM1_2000 and control ($p = 0.0328$) (Figure 3D). Regarding nuclear invaginations, fibroblasts of patients DM1_1000 and DM1_2000 have a higher number of nucleus invaginations than controls, with this difference being significant in DM1_2000 in relation to the control ($p = 0.033$) (Figure 3E). DM1 patients-derived fibroblasts also showed a tendency to increase the number of mild and moderate nuclear invaginations with the increase in the number of CTG repeats. The increase in moderate invaginations was significant between DM1_2000 and control ($p = 0.0014$) (Figure 3E).

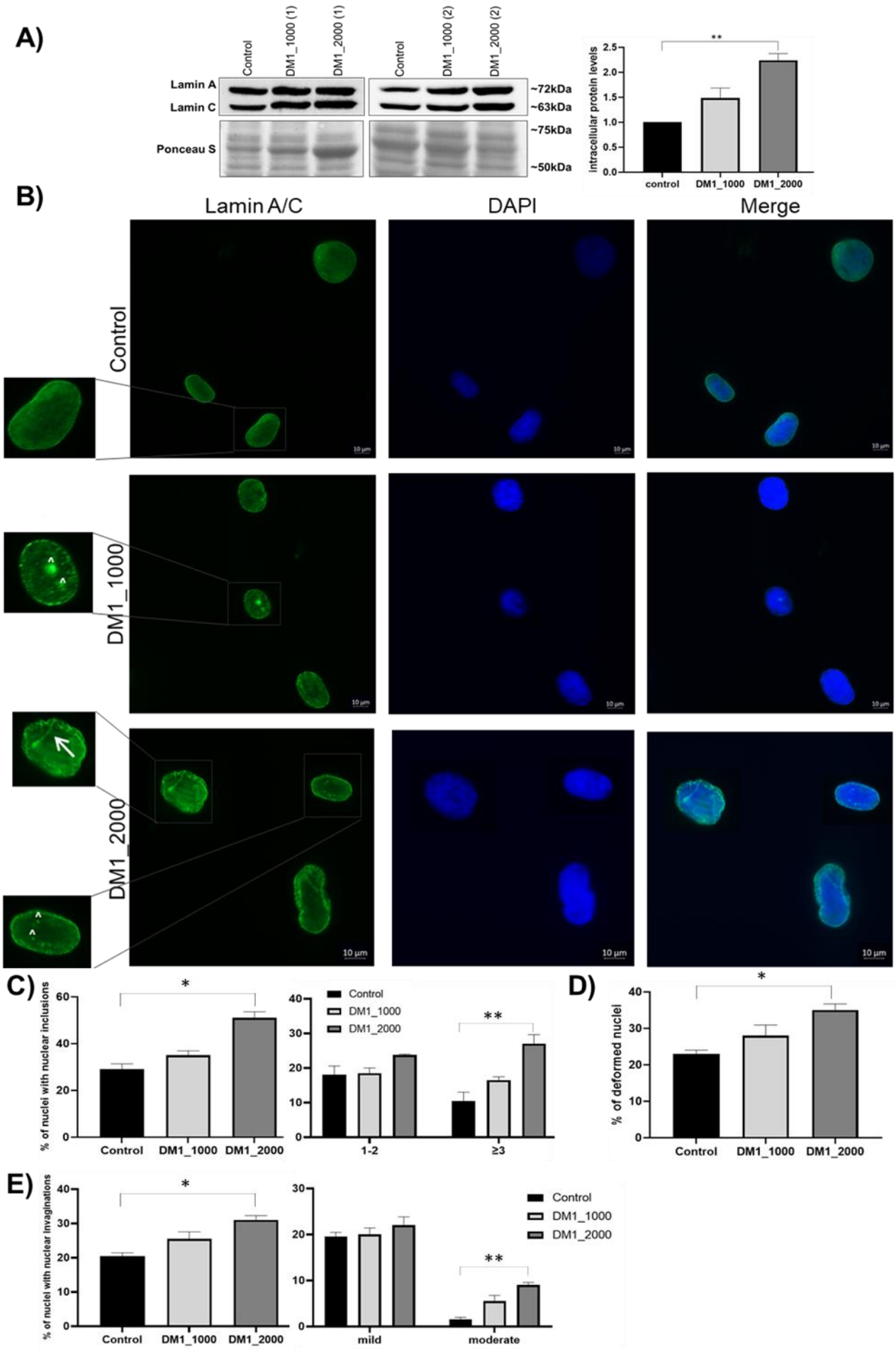
A)
Control
DM1_1000 (1)
DM1_2000 (1)
Control
DM1_1000 (2)
DM1_2000 (2)
Lamin A
Lamin C
Ponceau S
~72kDa
~63kDa
~75kDa
~50kDa
intracellular protein levels
control
DM1_1000
DM1_2000
B)
Lamin A/C
DAPI
Merge
Control
DM1_1000
DM1_2000
10 µm
C)
% of nuclei with nuclear inclusions
Control
DM1_1000
DM1_2000
1-2
≥3
D)
% of deformed nuclei
E)
% of nuclei with nuclear invaginations
mild
moderate

Figure 3: Intracellular lamin A/C protein levels and nuclear localization in DM1 patients-derived and control fibroblasts (A) Intracellular lamin A/C protein levels in DM1-derived and control fibroblasts. The intracellular protein levels in DM1 patients-derived fibroblasts were estimated in relation to protein levels detected in the control condition and are presented as mean ± SEM of 4 independent experiments. To compare intracellular protein levels between groups, one-way ANOVA test followed by the Tukey's test. **p<0.01 **(B)** Subcellular distribution of lamin A/C human fibroblasts from the control and from two DM1 patients was analysed using fluorescence microscopy. Lamin A/C was detected using an anti-mouse Alexa-488 conjugated secondary antibody (green). Nucleic acids were stained using DAPI (blue). **(C)** Evaluation of nuclear inclusions, **(D)** deformed nuclei and **(E)** nuclear invaginations**.** The quantitative data are presented as mean ± SEM and was obtained by analysing 50 cells per condition from 4 independent experiments. Statistical analysis was performed by comparison between the control, DM1_1000 and DM1_2000 fibroblasts by using one-way ANOVA test followed by the Tukey's test. *p<0.05; **p<0.01; Scale bar, 10µm; ↑ - represent nuclear invaginations; ^ - represent nuclear inclusions; DM1 – Myotonic dystrophy type 1

Another nuclear lamina protein evaluated, was lamin B1. The intracellular protein levels in patients DM1_1000 (p = 0.0393) and DM1_2000 increase when compared to control, although the increase is independent of the number of CTG repetitions (Figure 4).

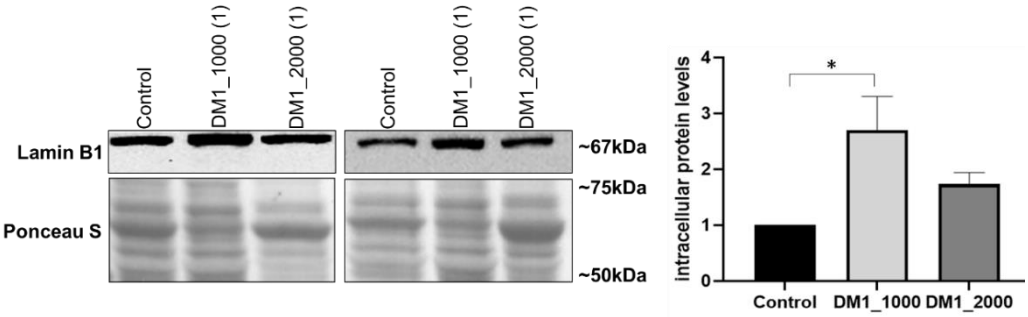

Figure 4: Intracellular lamin B1 protein levels in DM1 patients-derived and control fibroblasts. The intracellular protein levels in DM1 patients-derived fibroblasts were estimated in relation to protein levels detected in the control condition and are presented as mean ± SEM of 3 independent experiments. To compare intracellular protein levels between groups, one-way ANOVA test followed by the Tukey's test. *p <0.05; DM1 – Myotonic dystrophy type 1

Upon nuclear lamina evaluation several important alterations were on in both type A and type B lamins indicating that the NE structure and function could be compromised. Therefore, the analysis of the functional partners of nuclear lamina was performed.

The intracellular levels of emerin remain apparently unchanged in DM1 patients-derived fibroblasts when compared with control ones (Figure 5A). In addition, our results demonstrated an altered emerin localization and increased immunolabelling at both NE and

nucleoplasm in DM1 patients-derived fibroblasts (Figure 5B). Regarding the presence of nuclear inclusions in the global, DM1 patients-derived fibroblasts tend to increase the number of nuclear inclusions in relation to control fibroblasts. When assessing the number of nuclear inclusions divided by the two categories, the trend remained in both, with the number of fibroblasts with 3 or more inclusions significantly higher between fibroblasts of DM1_1000 ($p = 0.0154$) and the control and between DM1_2000 ($p = 0.0033$) and control (Figure 5C).

Additionally, observed that the DM1 patients-derived fibroblasts presented a higher percentage of deformed nucleus than the control fibroblasts (DM1_1000 vs control: $p = 0.0245$; DM1_2000 vs control: $p=0.0032$) (Figure 5D). Our results, also showed that the DM1_1000 ($p = 0.0040$) and DM1_2000 ($p = 0.0026$) presented a significant higher number of nuclei with invaginations, than controls (Figure 5E). The fibroblasts derived from DM1 showed a significant increase in the number of mild (DM1_1000 vs control: $p = 0.0033$; DM1_2000 vs control: $p = 0.0062$) and moderate (DM1_2000 vs control: $p = 0.0089$) in relation to control-derived fibroblasts (Figure 5E).

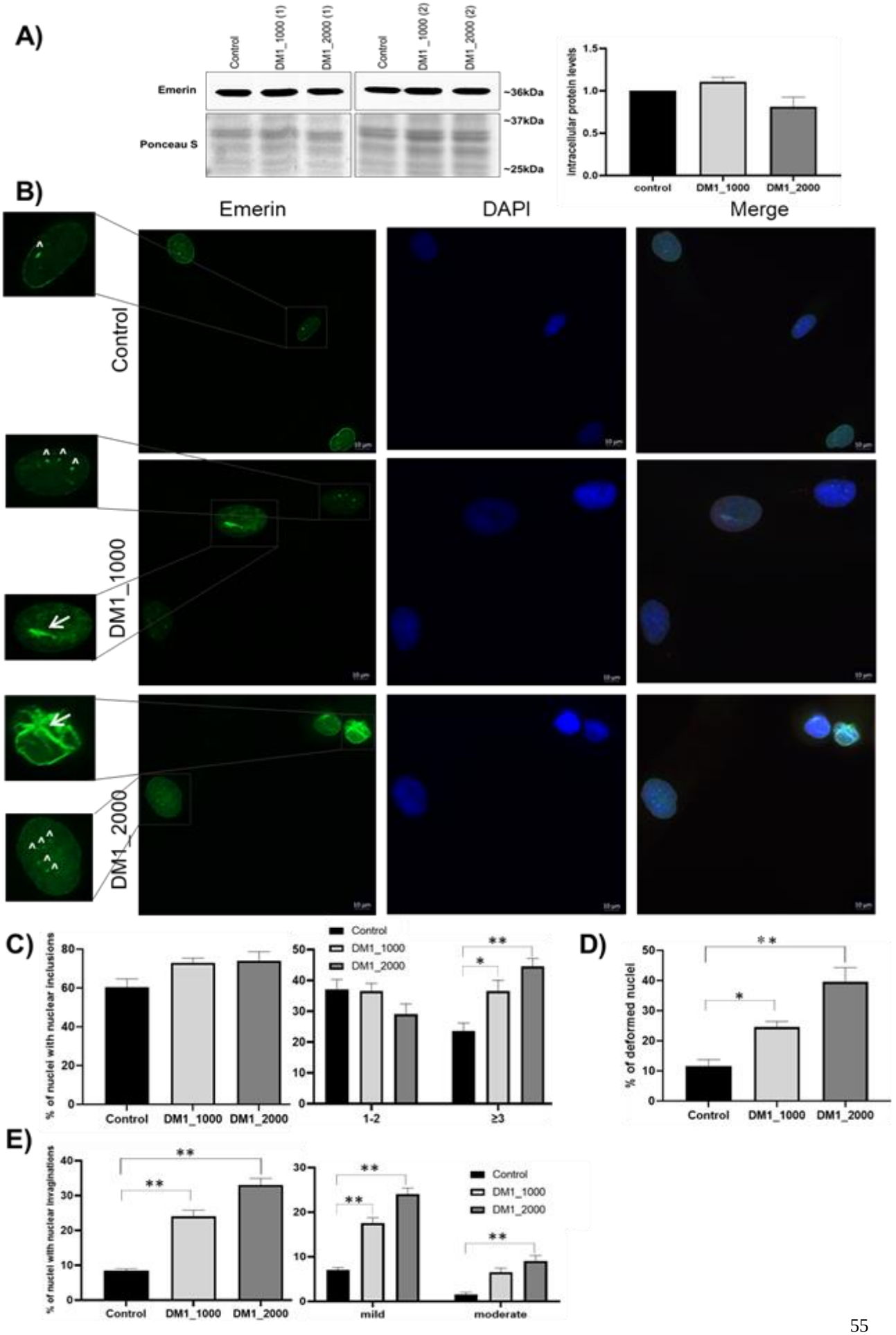
A)
Control
DM1_1000 (1)
DM1_2000 (1)
Control
DM1_1000 (2)
DM1_2000 (2)
Emerin
Ponceau S
~36kDa
~37kDa
~25kDa
intracellular protein levels
control
DM1_1000
DM1_2000
B)
Emerin
DAPI
Merge
Control
DM1_1000
DM1_2000
C)
% of nuclei with nuclear inclusions
Control
DM1_1000
DM1_2000
1-2
≥3
D)
% of deformed nuclei
E)
% of nuclei with nuclear invaginations
mild
moderate

Figure 5: Intracellular emerin protein levels and nuclear localization in DM1 patients-derived and control fibroblasts (A) Intracellular emerin protein levels in DM1 patients-derived and control fibroblasts. The intracellular protein levels in DM1 patients-derived fibroblasts were estimated in relation to protein levels detected in the control condition and are presented as mean ± SEM of 4 independent experiments. To compare intracellular protein levels between groups, one-way ANOVA test followed by the Tukey's test. **(B)** Subcellular distribution of emerin in DM1 patients-derived fibroblasts and control was analysed using fluorescence microscopy. Emerin was detected using an anti-mouse Alexa-488 conjugated secondary antibody (green). Nucleic acids were stained using DAPI (blue). **(C)** Evaluation of nuclear inclusions, **(D)** deformed nuclei and **(E)** nuclear invaginations**.** The quantitative data are presented as mean ± SEM and was obtained by analysing 50 cells per condition from 4 independent experiments. Statistical analysis was performed by comparison between the control, DM1_1000 and DM1_2000 fibroblasts by using one-way ANOVA test followed by the Tukey's test. *p<0.05, **p<0.01; Scale bar, 10µm; ↑ - represent nuclear invaginations; ^ - represent nuclear inclusions; DM1 – Myotonic dystrophy type 1

LAP1 is another important inner nuclear membrane protein, belonging to a dynamic and complex network of interactions spanning the perinuclear space and connecting the nuclear lamina, the NE, the cytoskeleton and nucleoskeleton. At least two human LAP1 isoforms are known namely LAP1B and LAP1C [30,31]. LAP1 interacts with several proteins [32] such as nuclear lamins and emerin, also relevant for this study.

The intracellular protein levels of total LAP1, LAP1B and LAP1C were shown to be increased in fibroblasts derived from DM1, proportional to the number of CTG repetitions and significant between DM1_2000 patients and the control fibroblasts (total LAP1: $p = 0.0302$; LAP1B: $p = 0.0137$; LAP1C: $p = 0.0210$) (Figure 6A). Further our results also showed LAP1 mislocalization and increased NE and nucleoplasm immunolabeling in DM1 patients-derived fibroblasts (Figure 6B). Additionally, number of nuclear inclusions in DM1 patients-derived fibroblasts are identical. However, when we analysed the two inclusions categories, observed that cells with number of inclusions between 1 and 2, tend to decrease in DM1_1000 and DM1_2000 ($p = 0.0310$) patients when compared to control fibroblasts. Also observed a significant increase in the number of fibroblast nuclei DM1_2000 ($p = 0.0472$) with 3 or more nuclear inclusions in relation to the control. The percentage of deformed nuclei in DM1 patients tends to be higher than the control and this increase is proportional to the number of CTG repetitions (DM1_2000 vs control: $p = 0.0301$) (Figure 6D). Finally, patients DM1_1000 ($p = 0.0449$) and DM1_2000 ($p = 0.0051$) present percentage of nuclei with invaginations superior to the control-derived fibroblasts (Figure 6E). Mild and moderate invaginations are found in higher numbers in DM1 patients-derived fibroblasts than in control-derived fibroblasts, being apparently proportional to the increasing in CTG repeats length. Significant

differences are observed between DM1_2000 and the control (p = 0.0412) for mild invaginations.

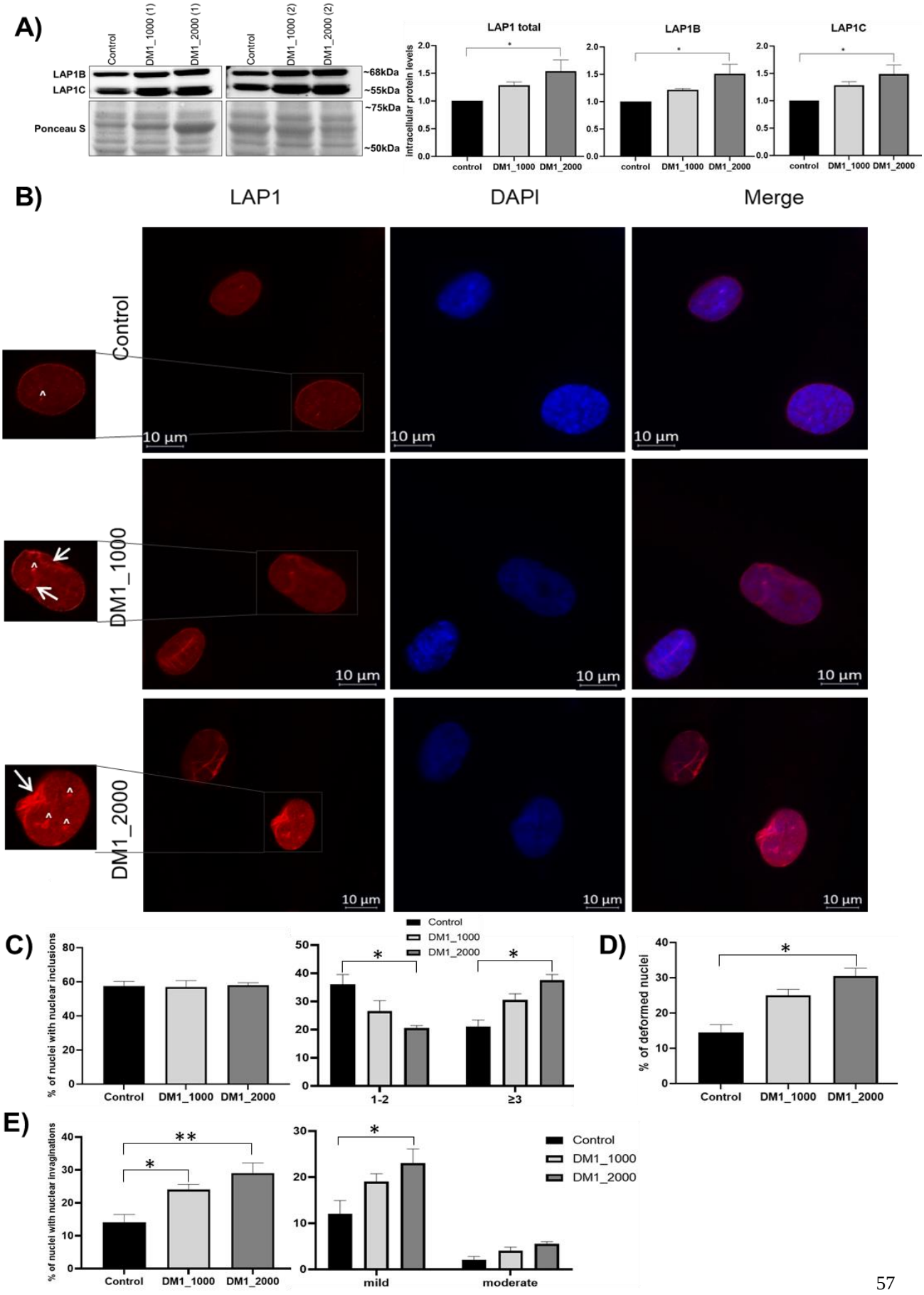

Figure 6: Intracellular LAP1 protein levels and localization in DM1 patients-derived and control fibroblasts (A) Total LAP1, LAP1B and LAP1C intracellular protein levels in DM1 patients-derived and control fibroblasts. The intracellular protein levels in DM1 patients-derived fibroblasts were estimated in relation to protein levels detected in the control condition and are presented as mean ± SEM of 4 independent experiments. To compare intracellular protein levels between groups, one-way ANOVA test followed by the Tukey's test. *p <0.05 **(B)** Subcellular distribution of LAP1 in DM1 patients-derived and control fibroblasts was analysed using fluorescence microscopy. LAP1 was detected using an anti-mouse Alexa-594 conjugated secondary antibody (red). Nucleic acids were stained using DAPI (blue). **(C)** Evaluation of nuclear inclusions, **(D)** deformed nuclei and **(E)** nuclear invaginations**.** The quantitative data are presented as mean ± SEM and was obtained by analysing 50 cells per condition from 4 independent experiments. Statistical analysis was performed by comparison between the control, DM1_1000 and DM1_2000 fibroblasts by using one-way ANOVA test followed by the Tukey's test. *p<0.05, **p<0.001; Scale bar, 10µm; ↑ - represent nuclear invaginations; ^ - represent nuclear inclusions; DM1 – Myotonic dystrophy type 1; LAP1 – lamin-associated with polypeptide 1

SUN1, was another protein evaluated by INM. There was a significant increase in intracellular protein levels of SUN1 in the fibroblasts of patients DM1_1000 (p = 0.0082) compared to control-derived fibroblasts (Figure 7).

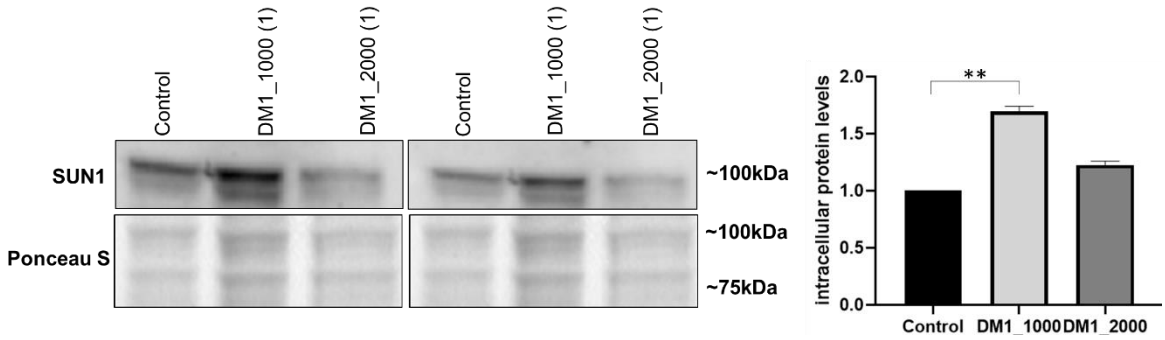

Figure 7: Intracellular SUN1 levels in DM1 patients-derived and control fibroblasts. The intracellular protein levels in DM1 patients-derived fibroblasts were estimated in relation to protein levels detected in the control condition and are presented as mean ± SEM of 3 independent experiments. To compare intracellular protein levels between groups, one-way ANOVA test followed by the Tukey's test. *p <0.05; DM1 – Myotonic dystrophy type 1; SUN - Sad1/Unc-84;

Upon assessment of both nuclear lamins and inner nuclear membrane proteins, we carry with the evaluation of two important outer nuclear membrane proteins namely nesprin-1 and -2.

Regarding the nesprin-1, it was observed a statistical significant decrease in nesprin-1 intracellular protein levels in DM1_1000 ($p = 0.0179$) and DM1_2000 ($p = 0.0129$) in relation control fibroblasts (Figure 8A). Regarding the results obtained by immunochemistry, this study demonstrates an altered location of nesprin-1 in DM1 patients-derived fibroblasts (Figure 8B). Our results demonstrate an increase in the number of nuclear inclusions in DM1 patients-derived fibroblasts, this increase being significant between DM1_2000 and control ($p = 0.0226$) (Figure 8C). When we analysed the inclusions by groups, we found that cells with inclusions 1-2, tend to decrease in DM1 patients-derived fibroblasts when compared to control-derived fibroblasts and that there is a significant increase in the percentage of fibroblasts in DM1_1000 patients ($p = 0.0281$) and DM1_2000 ($p = 0.0002$) with ≥3 nuclear inclusions in relation to the control fibroblasts (Figure 8D).Taking into account the nuclear deformity, the DM1-derived fibroblast nuclei are significantly more deformed than the control nuclei (DM1_1000 vs control: $p = 0.0280$; DM1_2000 vs control: $p = 0.0287$) (Figure 8D). Regarding nuclear invaginations, patients DM1_1000 ($p = 0.0280$) and DM1_2000 ($p = 0.0106$) present a percentage of nuclei with invaginations significantly superior to the control fibroblasts (Figure 8E). When we distinguish between mild and moderate invaginations, there is an increase in patients and was significant between the control and DM1_2000 for mild ($p = 0.0498$) and moderate invaginations ($p = 0.044$).

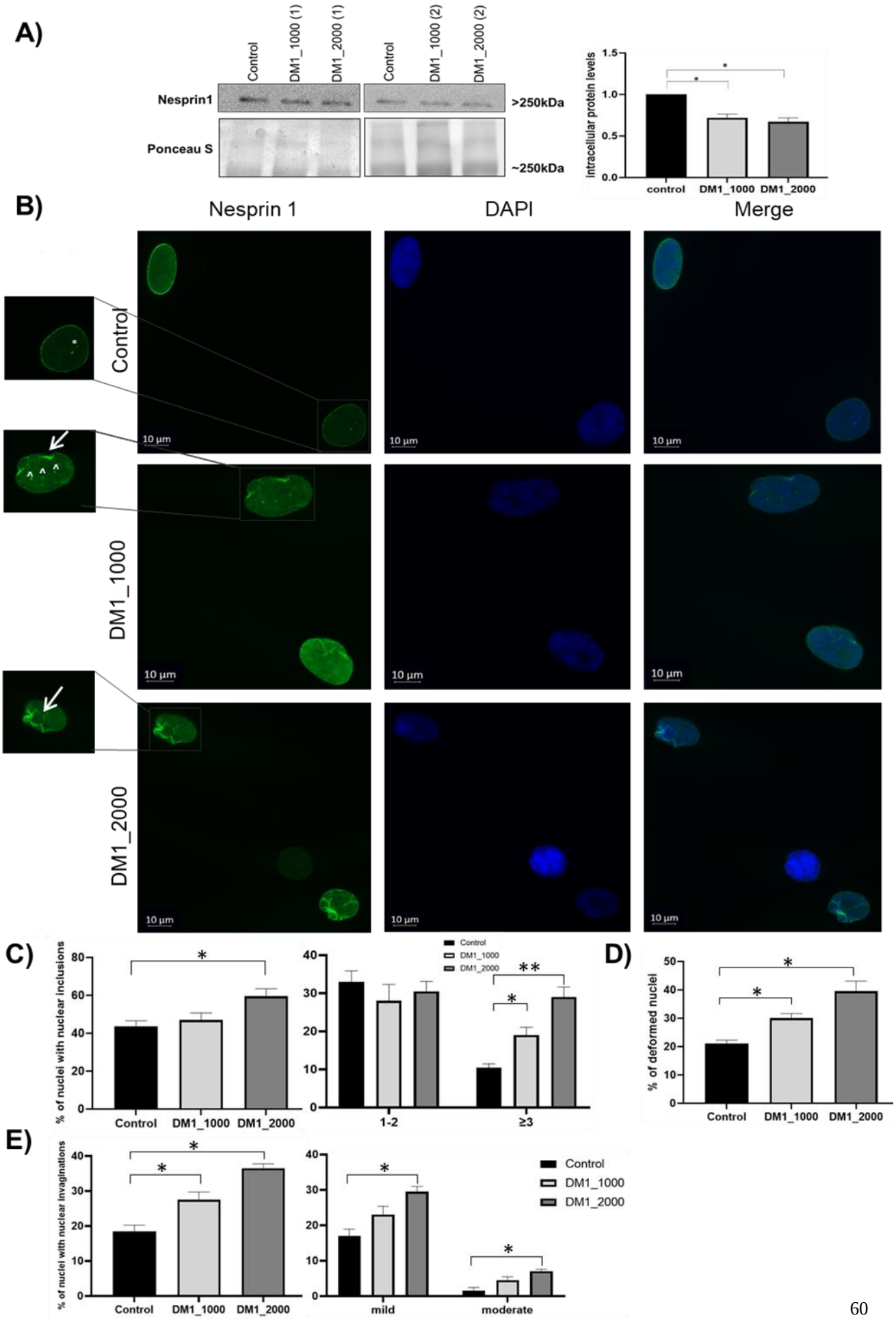
A)
Control
DM1_1000 (1)
DM1_2000 (1)
Control
DM1_1000 (2)
DM1_2000 (2)
Nesprin1
>250kDa
Ponceau S
~250kDa
intracellular protein levels
control
DM1_1000
DM1_2000
B)
Nesprin 1
DAPI
Merge
Control
DM1_1000
DM1_2000
10 µm
C)
% of nuclei with nuclear inclusions
Control
DM1_1000
DM1_2000
1-2
≥3
D)
% of deformed nuclei
E)
% of nuclei with nuclear invaginations
mild
moderate

Figure 8: Intracellular nesprin-1 protein levels and localization in DM1 patients-derived and control fibroblasts (A) Intracellular nesprin-1 protein levels in DM1 patients-derived and control fibroblasts. The intracellular protein levels in DM1 patients-derived fibroblasts were estimated in relation to protein levels detected in the control condition and are presented as mean ± SEM of 4 independent experiments. To compare intracellular protein levels between groups, one-way ANOVA test followed by the Tukey's test. **p <0.01 **(B)** Subcellular distribution of nesprin-1 in DM1 patients-derived and control fibroblasts was analysed using fluorescence microscopy. Nesprin-1 was detected using an anti-mouse Alexa-488 conjugated secondary antibody (green). Nucleic acids were stained using DAPI (blue). **(C)** Evaluation of nuclear inclusions, **(D)** deformed nuclei and **(E)** nuclear invaginations**.** The quantitative data are presented as mean ± SEM and was obtained by analysing 50 cells per condition from 4 independent experiments. Statistical analysis was performed by comparison between the control, DM1_1000 and DM1_2000 fibroblasts by using one-way ANOVA test followed by the Dunnett's test. *p<0.05, **p<0.01; Scale bar, 10µm; ↑ - represent nuclear invaginations; ^ - represent nuclear inclusions; DM1 – Myotonic dystrophy type 1

In relation to nesprin-2, the DM1_2000 patients showed a significant decrease compared to control fibroblasts (p = 0.0059) (Figure 9).

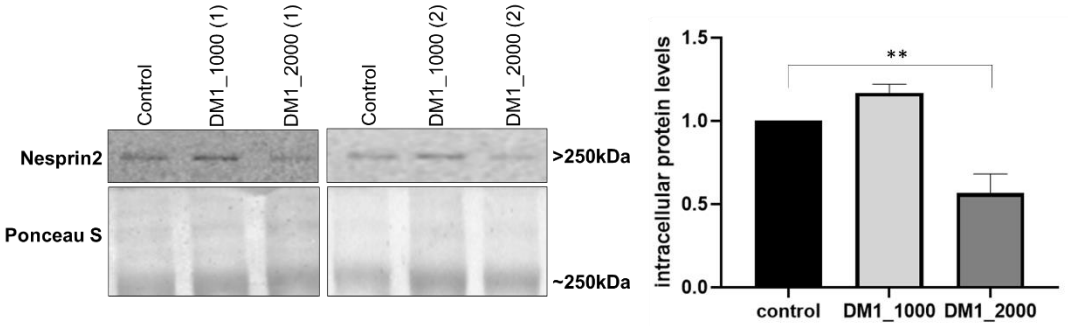

Figure 9: Intracellular nesprin-2 protein levels in DM1 patients-derived and control fibroblasts. The intracellular protein levels in DM1 patients-derived fibroblasts were estimated in relation to protein levels detected in the control condition and are presented as mean ± SEM of 4 independent experiments. To compare intracellular protein levels between groups, one-way ANOVA test followed by the Tukey's test. ** p<0.01; DM1 – Myotonic dystrophy type 1

4. Discussion

In this study demonstrated that the DM1 patients-derived fibroblasts nuclei present an aberrant nuclear morphology and alterations in the NE proteins. DMPK, lamin A/C, lamin

B1, emerin, LAP1, SUN1, nesprin-1 and nesprin-2 were evaluated in DM1 patients-derived fibroblasts and in the one apparently healthy control (Figure 1-9).

4.1. DMPK intracellular levels in DM1 patients-derived fibroblasts

This study first presents the intracellular protein levels of DMPK in DM1 patients-derived and control fibroblasts, to confirm that there is a decrease in the intracellular levels of this protein in DM1 (Figure 1). This was considered a strong point of our study, since we are not previously reported in DM1-patients derived fibroblasts.

As expected, the DMPK protein is decreased in these patients (Figure 1). This protein is encoded by the *DMPK* gene which is mutated in the 3'UTR region in DM1. According to the RNA toxic gain-of-function and haploinsufficiency hypothesis, mutant transcripts with abnormal CUG expansion are not transported to the cytoplasm, being accumulated in the cell nuclei, and are therefore not translated into protein [9,10,33–35]. Thus, DMPK protein intracellular levels are reduced in patients with DM1, independently of CTG repeat length tested, as shown in our results (Figure 1).

4.2. Nuclear profile in DM1 patients-derived fibroblasts

In relation to the nuclear profile, our study demonstrated that DM1 patients-derived fibroblasts tend to have a greater number of deformed nuclei, an increase in the number of micronuclei, an increase in the cross diameter ratio and an increase in the nuclear area (Figure 2). Regarding the nuclear circularity, also evaluated in this study, the results between the control and the patients were quite identical. The fact that the percentage of deformed nuclei is not high, may justify that there are no significant differences in the nuclear circularity of our global sample between DM1 patients-derived fibroblasts and control-derived fibroblasts. Further, our results demonstrated that fibroblasts nuclei of patients with DM1 tend to have an increased number of deformed nuclei, which shows us that nuclear abnormality is a relevant feature in this disease (Figure 2A and 2B). Nuclear deformations are related to changes in chromatin and nuclear lamina [36–38]. The expanded RNA in the cells of DM1 patients may explain changes in nuclear integrity. That is, the expanded mutant RNA exerts an action on the chromatin dynamics, remodelling the chromatin [39] and changing the positioning of the nucleosomes [40–42]. One of the effects of these chromatin conformational changes is the decrease in BAF available in cells (negative regulation of BAF) [43]. Our results showed also that patients with DM1 tend to have a greater number of cells with micronuclei (Figure 2C). Eukaryotic cells need to break the NE to enter mitosis. When the process is finished, the NE is reassembled around each

of the new cells. An altered reassembly of the NE accompanied by the abnormal incorporation of chromosomes results in the encapsulation of separate and smaller genetic material, which is called a micronucleus [44]. Therefore, the increase in the number of cells with micronuclei may be due to errors in the NE reassembly process after cell division. Defects in NE reassembly can happen associated with the NE reassembly defect is the BAF deletion [44]. This transcription factor interacts with the proteins of the NE emerin, MAN1, LAP2 and lamin A/C [45–47]. With this in mind, the expanded mutant RNA and changes in these proteins may explain the increase in the number of deformed and micronucleus nuclei in DM1 patients.

Another's parameters evaluated were the crossed diameter ratio and nuclear area (Figure 2E and 2F). The cross-diameter ratio was greater in the patients compared to the control, indicating that the nuclei of the patients are larger (Figure 2E). When we look at the mean nuclear area of the fibroblasts, we find that the results are quite identical between the control and the patients, although it demonstrates a tendency for DM1 patients to increase the nuclear area. However, we know that human fibroblasts without abnormalities have a nuclear area of approximately 200 μm^2 [29] and, for this reason, we decided to divide our results regarding the nuclear area into two groups (<200 μm^2 and ≥200 μm^2). Interestingly, when we analyse the nuclear area for an average area of <200μm^2 and for ≥200μm^2, we found that the nuclei of our DM1_2000 patient's tend to have a larger nuclear area (Figure 2F). Therefore, our results of the cross diameter and nuclear area are in agreement regarding the increase in nuclear size in cells of patients with DM1. Microtubules are constituents of the cellular cytoskeleton that play an important role in the positioning of the nucleus (dependent on the LINC complex) and are involved in the disassembly process of the NE during the initial mitosis [48–50]. In addition, it has been suggested that the LINC complex dampens forces in the NE while preserving nuclear morphology and restricting nuclear expansion [51]. With this in mind, changes in the LINC complex may be responsible for the poor nuclear localization and the increase in the area of fibroblasts in patients with DM1 directly or through the defective action of microtubes in cell division [52].

4.3. Nuclear envelope proteins intracellular levels and localization in DM1 patients-derived fibroblasts

Our study demonstrated increased intracellular protein levels of lamin A/C, mislocalization in the NE, and nuclear deformations in DM1 patients-derived fibroblasts (Figure 3).

In the literature, there are two studies where the intracellular protein levels of lamin A/C have been evaluated. One study, which used fibroblasts as a cell model, found no

differences between patients' cells and controls [20]. The second study, which used myoblasts (undifferentiated cells) found a decrease in intracellular lamin A protein levels in patients with DM1 [21]. Low intracellular protein levels of lamin A/C lead to nuclei with aberrant morphology, altered gene expression and increased cell senescence [38,53–60]. Mutations in the *LMNA* gene, which lead to the absence or reduction of lamin A/C in cells, have been reported in muscular dystrophies such as EDMD and LGMD1B [54,55,61] that share clinical characteristics with DM1, such as muscle weakness, atrophy and cardiomyopathy [62]. During the skeletal muscle differentiation process, there is usually an increase in the expression levels of emerin and lamin A/C, accompanied by structural changes in chromatin (through BAF) and by the induction of muscle gene expression [46,63–68]. Therefore, the loss of function of one of these proteins results in changes in the cytoskeleton organization, chromosomal position and chromatin mobility, with consequent impaired nuclear architecture [69].

On the other hand, a study carried out on HeLa epithelial cells and C2C12 myoblasts demonstrated that variations in DMPK expression (increase or decrease in relation to control) leads to changes in the location of the lamin A/C in the NE and a break in the lamina network with consequent nuclear fragmentation, increased number of micronuclei and nuclear instability [70]. This demonstrates that DMPK is an essential protein in maintaining the stability of the NE and that strict regulation of DMPK is necessary so that there is no change in the stability of this structure [70].

Therefore, the NE structural alterations that we observed (deformed nuclei, increased nuclear invaginations, increased number of inclusions, lamin A/C mislocalization and increased NE and nucleoplasm immunolabelling) (Figure 3B-3E) may not be directly related to the intracellular protein levels of the lamin A/C, but with the decrease intracellular protein levels of the DMPK. This hypothesis becomes even more robust when we find that the study already reported (which used DM1-derived fibroblasts) did not show changes in the intracellular protein levels of the lamin A/C but reported nuclear deformations (increased NE and nucleoplasm immunolabelling, lamin A/C mislocalization, nuclear deformation and increase nuclear size) [20]. However, further studies are needed to develop the role of increasing the lamin A/C in DM1.

In relation to lamin B1 our results demonstrated an increase in the intracellular protein levels of lamin B1 in DM1 patients-derived fibroblasts (Figure 4). This increase was independent of the CTG repeat length. Intracellular levels of this protein have never been evaluated in DM1 patients-derived fibroblasts, but NE alteration (increased NE and nucleoplasm immunolabelling, lamin B1 mislocalization and nuclear deformations) have already been reported in DM1-derived fibroblasts [20]. Distal muscle cells derived from patients with congenital DM1 showed a reduction in the proliferative capacity caused by

p16-dependent premature senescence [71,72]. Previous studies were used myoblasts, the intracellular protein levels of lamin B1 tend to decrease in DM1 patients, independent of the CTG repeat length [21], suggesting that lamin B1 may be a more relevant senescence marker in DM1 (for any phenotype).

The senescence cellular is accompanied by a reduction in the levels of the lamin B1 protein (detected in fibroblasts derived from patients with progeria) [73,74]. Despite this, it has been reported that increased levels of lamin B1 protein in primary fibroblasts also lead to cellular senescence [75,76]. Therefore, lamin B1 is a marker of cellular senescence, although it is not clear why lamin B1 levels vary. Whether the increase in intracellular levels of lamina B1 in DM1 is associated or not with cellular senescence is unknown. Studies will have to be developed in order to discover it.

Regarding intracellular emerin protein levels, our results did not show significant differences between DM1 patients-derived and control fibroblasts (Figure 5A). In addition, we verified that the nuclei of the DM1-positive patients for emerin, presented emerin mislocalization, accompanied by an increase in the number of nuclear inclusions and nuclear deformations (Figure 5B-5E). These results are in line with those reported in a previous study that also used fibroblasts as a cell model [20]. The nuclei of muscle cells, from patients with X-EDMD, devoid of emerin or with low intracellular protein levels, have a destabilization of the nuclear lamina that leads to greater nuclear fragility [77,78]. This fragility results in an increase in deformed nuclei or nuclear ruptures accompanied by abnormal chromatin organization and chromatin extrusion out of the nucleus or an increase in cell death [78,79]. These changes can robustly explain the phenotypic changes observed in muscle tissue. That is, as the muscle nuclei present a weakened nuclear lamina, when the muscles are exposed to external physical forces during the contraction process, these muscle cells are subject to mechanical destruction.

In DM1, until now, LAP1 has not been evaluated, which is another strength of this study. Our study demonstrated that the intracellular levels of total LAP1, LAP1B and LAP1C are increased in DM1 and this increase is greater the greater the number of CTG repetitions (Figure 6A). In addition, we also see a mislocalization of the protein, an increase NE and nucleoplasm immunolabelling, an increase nuclear inclusions and deformed nuclei (Figure 6B-6E). LAP1, is an INM protein from NE that has been associated in the processes of regulation of the development and maintenance of skeletal muscle and in the integrity of NE [80–82]. Mutations in this protein or in the gene that encodes it lead to the development of muscle diseases such as LGBMD, and there are changes in the NE structure (eg. nuclear fragmentation) [82–85]. Emerin “null” mice do not develop evident signs of muscle changes because of significantly higher levels of LAP1 [86,87]. In our results the intracellular protein levels of emerin are identified in DM1 patients-derived and control fibroblasts. Therefore,

the intracellular protein levels of LAP1 in our sample are not increased to compensate for the lack of emerin. Thus, the cause that leads to the increase of this protein remains unknown.

LAP1 and torsin A interact with each other, with LAP1 stimulating the activity of torsin A as ATPase AAA + [88]. Torsin A and LAP1 were identified as mediators of the assembly of the LINC complex as well as being responsible for the location of nesprins in the NE [89–92]. In turn, SUN1 is responsible for locating torsin A in the NE [90,92]. Given this information, torsin A and, indirectly, LAP1, belong to a dynamic network of interactions that connect the nuclear lamina, the NE and the cytoskeleton [89,90,93]. The altered location of LAP1 and increased deformed nuclei observed in our study may be associated with an abnormal positioning of the nuclei and / or with nuclear deformations resulting from the mechanical stress exerted on cells that present weakened NE due to changes in the nuclear lamins and LINC complex proteins.

SUN1, is involved in the connection of the nucleoplasm with the cytoskeleton, in nuclear anchorage and in nuclear migration [94–96]. To date, SUN1 intracellular protein levels have not been evaluated in DM1. Our results demonstrated an increase in the intracellular protein levels of SUN1 in DM1 patients-derived fibroblasts when compared to the control fibroblasts (Figure 7). Mutations in SUN1 (with decreased intracellular protein levels) have been associated with the EDMD, is manifested histologically by the alteration of the nuclear position and, consequently, degradation of muscle function [97]. The reported mutations for SUN1 in EDMD alone were not able to cause disease, however, EDMD individuals who have these mutations associated with *LMNA* and *EMD* mutations, have worsened disease severity [97]. This suggests that SUN may function as a modifier in muscle diseases [97]. On the other hand, lamin A mutations are associated not only with EDMD but also with Hutchinson-Gilford progeria syndrome (HGPS) (laminopathy) [98]. In these two pathologies there is an interruption of the interaction of SUN1 with lamin A, but the location of SUN1 in the inner nuclear membrane is not changed [98]. Interestingly, it was found that HGPS fibroblasts with lamin A mutation shows a significant increase in SUN1 in the NE [98]. Given this information and changes in the location of the lamin A/C reported in DM1, SUN1 may be increased due to the impossibility of interacting with the lamin A/C (mimicking what occurs in HGPS fibroblasts with mutated lamin A), thus sharing mechanisms molecular with other laminopathies. This hypothesis is reinforced when SUN1-positive DM1 patients myoblasts do not show changes in the protein's location [22]. However, it should be noted that the myoblasts and cells used in this study are separate lines and that the results may differ. However, studies are needed to assess this hypothesis.

As for nesprins -1 and -2, they are part of the LINC complex, which is crucial in the process of movement and positioning of muscle cell nuclei through associations with

nucleoskeleton components [99–102]. Our results showed a tendency for intracellular protein levels to decrease in DM1 patients-derived fibroblasts (Figure 8A and 9). In addition, we also found that the nuclei labelled for nesprin-1 showed an altered protein location, an increased number of deformed nuclei and an increase number of nuclear inclusions (Figure 8B-8E). A previous study, using myoblasts and myotubes from patients with DM1, although they are completely separate cell lines from ours, demonstrated this tendency for nesprins -1 and -2 to decrease with an increase in the number of CTG repeat length [22]. The reduction in intracellular protein levels of nesprin -1 and -2, can also occur in cells of patients with EDMD and CDM that have mutations in the *SYNE-1* and/or *SYNE-2* gene [103]. These two pathologies usually have abnormal nuclear morphology, micronuclei and fragmented nuclei [15,103], similar to what we observed in our results. These changes are usually due to an incorrect location of the LINC complex proteins (nesprins and SUN 1/2) or their interactors (lamin A/C) [15,104,105]. The low intracellular protein levels of nesprins in the cells result in a defective interaction of the proteins of the LINC complex and / or proteins associated with the complex (e.g. emerin and lamin A/C) with the nuclear actin. With this function impaired the nuclear positioning, the architecture of the NE, the gene expression and maintenance of muscle fibers in patients with muscle diseases is affected [102]. Therefore, the decrease in intracellular protein levels of nesprin-1 is related to the structural changes in the NE (deformed nuclei and nuclear inclusions) that we observed in our results.

In summary, our results demonstrated that DM1 patients-derived fibroblasts had different levels of intracellular NE protein than control fibroblasts, and that these changes are also associated with structural differences of the nuclei (deformed nuclei, increased nuclear area and increased nuclear inclusions). Our have made the hypothesis that changes in the nuclear envelope are a hallmark of DM1 more robust.

5. Bibliographic references

[1] M. De Antonio *et al.*, “Unravelling the myotonic dystrophy type 1 clinical spectrum: A systematic registry-based study with implications for disease classification,” *Rev. Neurol. (Paris).*, vol. 172, no. 10, pp. 572–580, 2016, doi: 10.1016/j.neurol.2016.08.003.

[2] K. Yum, E. T. Wang, and A. Kalsotra, “Myotonic dystrophy: disease repeat range, penetrance, age of onset, and relationship between repeat size and phenotypes,” *Curr. Opin. Genet. Dev.*, vol. 44, pp. 30–37, 2017, doi: 10.1016/j.gde.2017.01.007.

[3] S. Tomé and G. Gourdon, “DM1 phenotype variability and triplet repeat instability:

Challenges in the development of new therapies," *Int. J. Mol. Sci.*, vol. 21, no. 2, pp. 1–10, 2020, doi: 10.3390/ijms21020457.

[4] J. D. Brook *et al.*, "Molecular basis of myotonic dystrophy: Expansion of a trinucleotide (CTG) repeat at the 3′ end of a transcript encoding a protein kinase family member," *Cell*, vol. 68, no. 4, pp. 799–808, 1992, doi: 10.1016/0092-8674(92)90154-5.

[5] L. P. W. Ranum and J. W. Day, "Myotonic Dystrophy: RNA Pathogenesis Comes into Focus," *Am. J. Hum. Genet.*, vol. 74, no. 5, pp. 793–804, 2004, doi: 10.1086/383590.

[6] M. Mahadevan *et al.*, "Myotonic dystrophy mutation: An unstable CTG repeat in the 3′ untranslated region of the gene," *Science (80-.).*, vol. 255, no. 5049, pp. 1253–1255, 1992, doi: 10.1126/science.1546325.

[7] R. E. M. A. van Herpen *et al.*, "Divergent Mitochondrial and Endoplasmic Reticulum Association of DMPK Splice Isoforms Depends on Unique Sequence Arrangements in Tail Anchors," *Mol. Cell. Biol.*, vol. 25, no. 4, pp. 1402–1414, 2005, doi: 10.1128/mcb.25.4.1402-1414.2005.

[8] D. G. Wansink, R. E. M. A. van Herpen, M. M. Coerwinkel-Driessen, P. J. T. A. Groenen, B. A. Hemmings, and B. Wieringa, "Alternative Splicing Controls Myotonic Dystrophy Protein Kinase Structure, Enzymatic Activity, and Subcellular Localization," *Mol. Cell. Biol.*, vol. 23, no. 16, pp. 5489–5501, 2003, doi: 10.1128/mcb.23.16.5489-5501.2003.

[9] T. Mateus, F. Martins, A. Nunes, T. Herdeiro, and S. Rebelo, "Metabolic alterations in Myotonic Dystrophy type 1 and their correlation with Lipin function," Int. J.Environ. Res. Public Health, vol.18, no.4, 2021, doi: 10.3390/ijerph18041794

[10] A. Philips, T. Lubov, T. Timchenko, and E. Al, "Disruption of Splicing Regulated by a CUG-Binding Protein in Myotonic Dystrophy," *Science (80-.).*, vol. 180, pp. 737–740, 1998.

[11] M. Alwazzan, E. Newman, M. G. Hamshere, and J. D. Brook, "Myotonic dystrophy is associated with a reduced level of RNA from the DMWD allele adjacent to the expanded repeat," vol. 8, no. 8, pp. 1491–1497, 1999.

[12] T. A. Lee, Johanna E; Cooper, "Pathogenic mechanisms of myotonic dystrophy," *Biochem Soc Trans*, vol. 37, no. 0 6, 2009, doi: 10.1042/BST0371281.Pathogenic.

[13] E. S. Folker and M. K. Baylies, "Nuclear positioning in muscle development and disease," *Front. Physiol.*, vol. 4 DEC, no. December, pp. 1–10, 2013, doi: 10.3389/fphys.2013.00363.

[14] M. A. Wheeler *et al.*, "Distinct functional domains in nesprin-1 α and nesprin-2 β bind directly to emerin and both interactions are disrupted in X-linked Emery – Dreifuss muscular dystrophy," vol. 3, 2007, doi: 10.1016/j.yexcr.2007.03.025.

[15] Q. Zhang *et al.*, “Nesprin-1 and -2 are involved in the pathogenesis of Emery - Dreifuss muscular dystrophy and are critical for nuclear envelope integrity,” *Hum. Mol. Genet.*, vol. 16, no. 23, pp. 2816–2833, 2007, doi: 10.1093/hmg/ddm238.

[16] T. Zu *et al.*, “Non-ATG-initiated translation directed by microsatellite expansions,” *Proc. Natl. Acad. Sci. U. S. A.*, vol. 108, no. 1, pp. 260–265, 2011, doi: 10.1073/pnas.1013343108.

[17] L. Gueneau *et al.*, “Mutations of the FHL1 Gene Cause Emery-Dreifuss Muscular Dystrophy,” *Am. J. Hum. Genet.*, vol. 85, no. 3, pp. 338–353, 2009, doi: 10.1016/j.ajhg.2009.07.015.

[18] E. Hatch and M. Hetzer, “Breaching the nuclear envelope in development and disease,” *J. Cell Biol.*, vol. 205, no. 2, pp. 133–141, 2014, doi: 10.1083/jcb.201402003.

[19] L. W. Michael, “THE NUCLEAR ENVELOPE ITS STRUCTURE AND RELATION TO CYTOPLASMIC MEMBRANES,” *J. Biophys. Biochem. Cytol.*, vol. I, no. 3, pp. 257–270, 1955, [Online]. Available: http://www.ncbi.nlm.nih.gov/pubmed/13242591.

[20] R. Rodríguez, O. Hernández-Hernández, J. J. Magaña, R. González-Ramírez, E. S. García-López, and B. Cisneros, “Altered nuclear structure in myotonic dystrophy type 1-derived fibroblasts,” *Mol. Biol. Rep.*, vol. 42, no. 2, pp. 479–488, 2015, doi: 10.1007/s11033-014-3791-4.

[21] P. Meinke, S. Hintze, S. Limmer, and B. Schoser, “Myotonic dystrophy-A progeroid disease?,” *Front. Neurol.*, vol. 9, no. JUL, pp. 1–12, 2018, doi: 10.3389/fneur.2018.00601.

[22] S. Hintze, L. Knaier, S. Limmer, B. Schoser, and P. Meinke, “Nuclear envelope transmembrane proteins in myotonic dystrophy type,” *Front. Physiol.*, vol. 9, no. OCT, pp. 1–7, 2018, doi: 10.3389/fphys.2018.01532.

[23] A. C. M. Basílio, “Characterization of the nuclear envelope alterations in Myotonic Dystrophy Type 1,” Universidade de Aveiro, 2019.

[24] R. E. Goodchild and W. T. Dauer, “The AAA+ protein torsinA interacts with a conserved domain present in LAP1 and a novel ER protein,” *J. Cell Biol.*, vol. 168, no. 6, pp. 855–862, 2005, doi: 10.1083/jcb.200411026.

[25] Y. H. Chi, K. Haller, J. M. Peloponese, and K. T. Jeang, “Histone acetyltransferase hALP and nuclear membrane protein hsSUN1 function in de-condensation of mitotic chromosomes,” *J. Biol. Chem.*, vol. 282, no. 37, pp. 27447–27458, 2007, doi: 10.1074/jbc.M703098200.

[26] A. R. Pinho *et al.*, “In Vitro Cytotoxicity Effects of Zinc Oxide Nanoparticles on Spermatogonia Cells,” *Cells*, vol. 9, no. 5, 2020, doi: 10.3390/cells9051081.

[27] C. D. Pereira, F. Martins, M. Santos, T. Müeller, O. A. B. da Cruz E Silva, and S.

Rebelo, *Nuclear Accumulation of LAP1:TRF2 Complex during DNA Damage Response Uncovers a Novel Role for LAP1*, vol. 9, no. 8. 2020.

[28] Y. H. Fu *et al.*, “An unstable triplet repeat in a gene related to myotonic muscular dystrophy,” *Science (80-.).*, vol. 255, no. 5049, pp. 1256–1258, 1992, doi: 10.1126/science.1546326.

[29] R. H. G. and KENNETH J. PIENTA and D. S. COFFEY, “CHARACTERIZATION OF NUCLEAR MORPHOLOGY AND NUCLEAR MATRICES IN AGEING HUMAN FIBROBLASTS,” *Mech. Ageing Dev.*, vol. 62, pp. 13–24, 1992.

[30] Y. Kondo *et al.*, “Molecular cloning of one isotype of human lamina-associated polypeptide 1s and a topological analysis using its deletion mutants,” *Biochem. Biophys. Res. Commun.*, vol. 294, no. 4, pp. 770–778, 2002, doi: 10.1016/S0006-291X(02)00563-6.

[31] M. Santos *et al.*, “Identification of a novel human LAP1 isoform that is regulated by protein phosphorylation,” *PLoS One*, vol. 9, no. 12, pp. 1–32, 2014, doi: 10.1371/journal.pone.0113732.

[32] J. B. Serrano, O. A. B. da Cruz e Silva, and S. Rebelo, “Lamina associated polypeptide 1 (LAP1) interactome and its functional features,” *Membranes (Basel).*, vol. 6, no. 1, pp. 1–19, 2016, doi: 10.3390/membranes6010008.

[33] N. A. Timchenko, Z. J. Cai, A. L. Welm, S. Reddy, T. Ashizawa, and L. T. Timchenko, “RNA CUG Repeats Sequester CUGBP1 and Alter Protein Levels and Activity of CUGBP1,” *J. Biol. Chem.*, vol. 276, no. 11, pp. 7820–7826, 2001, doi: 10.1074/jbc.M005960200.

[34] A. Theadom *et al.*, “Prevalence of muscular dystrophies: A systematic literature review,” *Neuroepidemiology*, vol. 43, no. 3–4, pp. 259–268, 2014, doi: 10.1159/000369343.

[35] D. Furling, D. Lemieux, K. Taneja, and J. Puymirat, “Decreased levels of myotonic dystrophy protein kinase (DMPK) and delayed differentiation in human myotonic dystrophy myoblasts,” *Neuromuscul. Disord.*, vol. 11, no. 8, pp. 728–735, 2001, doi: 10.1016/S0960-8966(01)00226-7.

[36] T. Sullivan *et al.*, “Loss of A-type lamin expression compromises nuclear envelope integrity leading to muscular dystrophy,” *J. Cell Biol.*, vol. 147, no. 5, pp. 913–919, 1999, doi: 10.1083/jcb.147.5.913.

[37] A. D. Stephens *et al.*, “Chromatin histone modifications and rigidity affect nuclear morphology independent of lamins,” *Mol. Biol. Cell*, vol. 29, no. 2, pp. 220–233, 2018, doi: 10.1091/mbc.E17-06-0410.

[38] J. Lammerding, J. Hsiao, P. C. Schulze, S. Kozlov, C. L. Stewart, and R. T. Lee, “Abnormal nuclear shape and impaired mechanotransduction in emerin-deficient

cells," *J. Cell Biol.*, vol. 170, no. 5, pp. 781–791, 2005, doi: 10.1083/jcb.200502148.

[39] J. R. Brouwer, A. Huguet, A. Nicole, A. Munnich, and G. Gourdon, "Transcriptionally repressive chromatin remodelling and CpG methylation in the presence of expanded CTG-repeats at the DM1 locus," *J. Nucleic Acids*, vol. 2013, 2013, doi: 10.1155/2013/567435.

[40] N. Tomita, R. Fujita, D. Kurihara, H. Shindo, R. D. Wells, and M. Shimizu, "Effects of triplet repeat sequences on nucleosome positioning and gene expression in yeast minichromosomes.," *Nucleic Acids Res. Suppl.*, no. 2, pp. 231–232, 2002, doi: 10.1093/nass/2.1.231.

[41] Y. H. Wang, S. Amirhaeri, S. Kang, R. D. Wells, and J. D. Griffith, "Preferential nucleosome assembly at DNA triplet repeats from the myotonic dystrophy gene," *Science (80-.).*, vol. 265, no. 5172, pp. 669–671, 1994, doi: 10.1126/science.8036515.

[42] Y. H. Wang and J. Griffith, "Expanded CTG triplet blocks from the myotonic dystrophy gene create the strongest known natural nucleosome positioning elements," *Genomics*, vol. 25, no. 2, pp. 570–573, 1995, doi: 10.1016/0888-7543(95)80061-P.

[43] M. Polychronidou, A. Hellwig, and J. Grosshans, "Farnesylated nuclear proteins Kugelkern and lamin Dm0 affect nuclear morphology by directly interacting with the nuclear membrane," *Mol. Biol. Cell*, vol. 21, no. 19, pp. 3409–3420, 2010, doi: 10.1091/mbc.E10-03-0230.

[44] R. Ungricht and U. Kutay, "Mechanisms and functions of nuclear envelope remodelling," *Nat. Rev. Mol. Cell Biol.*, vol. 18, no. 4, pp. 229–245, 2017, doi: 10.1038/nrm.2016.153.

[45] M. S. Lee and R. Craigie, "Protection of retroviral DNA from autointegration: Involvement of a cellular factor," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 91, no. 21, pp. 9823–9827, 1994, doi: 10.1073/pnas.91.21.9823.

[46] K. Lee, T. Haraguchi, T. Koujin, Y. Hiraoka, and K. Wilson, "Distinct functional domains in emerin bind lamin A and DNA-briging protein BAF," *Cell*, pp. 4567–4573, 2001, [Online]. Available: https://pubmed.ncbi.nlm.nih.gov/11792821/.

[47] M. A. D'Angelo and M. W. Hetzer, "The role of the nuclear envelope in cellular organization," *Cell. Mol. Life Sci.*, vol. 63, no. 3, pp. 316–332, 2006, doi: 10.1007/s00018-005-5361-3.

[48] A. J. Koch and J. M. Holaska, "Emerin in health and disease," *Semin. Cell Dev. Biol.*, vol. 29, pp. 95–106, 2014, doi: 10.1016/j.semcdb.2013.12.008.

[49] J. Beaudouin, D. Gerlich, N. Daigle, R. Eils, and J. Ellenberg, "Nuclear envelope breakdown proceeds by microtubule-induced tearing of the lamina," *Cell*, vol. 108, no. 1, pp. 83–96, 2002, doi: 10.1016/S0092-8674(01)00627-4.

[50] D. Salina, K. Bodoor, D. M. Eckley, T. A. Schroer, J. B. Rattner, and B. Burke, “Cytoplasmic dynein as a facilitator of nuclear envelope breakdown,” *Cell*, vol. 108, no. 1, pp. 97–107, 2002, doi: 10.1016/S0092-8674(01)00628-6.

[51] H. Cantwell and P. Nurse, “Unravelling nuclear size control,” *Curr. Genet.*, vol. 65, no. 6, pp. 1281–1285, 2019, doi: 10.1007/s00294-019-00999-3.

[52] L. Seaman, W. Meixner, J. Snyder, and I. Rajapakse, “Periodicity of nuclear morphology in human fibroblasts,” *Nucleus*, vol. 6, no. 5, pp. 408–416, 2015, doi: 10.1080/19491034.2015.1095432.

[53] A. Muchir and H. J. Worman, “The nuclear envelope and human disease,” *Physiology*, vol. 19, no. 5, pp. 309–314, 2004, doi: 10.1152/physiol.00022.2004.

[54] K. J. Roux and B. Burke, “Nuclear envelope defects in muscular dystrophy,” *Biochim. Biophys. Acta - Mol. Basis Dis.*, vol. 1772, no. 2, pp. 118–127, 2007, doi: 10.1016/j.bbadis.2006.06.001.

[55] B. M. Paschal and J. B. Kelley, “Nuclear Lamina,” *Encycl. Biol. Chem. Second Ed.*, pp. 310–313, 2013, doi: 10.1016/B978-0-12-378630-2.00477-1.

[56] J. Lammerding *et al.*, “Lamin A / C deficiency causes defective nuclear mechanics and mechanotransduction Find the latest version : Lamin A / C deficiency causes defective nuclear mechanics,” *J. Clin. Invest.*, vol. 113, no. 3, pp. 370–378, 2004, doi: 10.1172/JCI200419670.Introduction.

[57] E. Battey, M. J. Stroud, and J. Ochala, “Using nuclear envelope mutations to explore age-related skeletal muscle weakness,” *Clin. Sci.*, vol. 134, no. 16, pp. 2177–2187, 2020, doi: 10.1042/CS20190066.

[58] A. T. Bertrand *et al.*, “Lamin A/C Assembly Defects in LMNA-Congenital Muscular Dystrophy Is Responsible for the Increased Severity of the Disease Compared with Emery–Dreifuss Muscular Dystrophy,” *Cells*, vol. 9, no. 4, p. 844, 2020, doi: 10.3390/cells9040844.

[59] A. J. Earle *et al.*, “Mutant lamins cause nuclear envelope rupture and DNA damage in skeletal muscle cells,” *bioRxiv*, 2018, doi: 10.1101/364778.

[60] S. Kandert, M. Wehnert, C. R. Müller, B. Buendia, and M. C. Dabauvalle, “Impaired nuclear functions lead to increased senescence and inefficient differentiation in human myoblasts with a dominant p.R545C mutation in the LMNA gene,” *Eur. J. Cell Biol.*, vol. 88, no. 10, pp. 593–608, 2009, doi: 10.1016/j.ejcb.2009.06.002.

[61] A. Muchir *et al.*, “Nuclear envelope alterations in fibroblasts from patients with muscular dystrophy, cardiomyopathy, and partial lipodystrophy carrying lamin A/C gene mutations,” *Muscle and Nerve*, vol. 30, no. 4, pp. 444–450, 2004, doi: 10.1002/mus.20122.

[62] H. J. Worman and G. Bonne, “‘Laminopathies’: A wide spectrum of human diseases,”

Exp. Cell Res., vol. 313, no. 10, pp. 2121–2133, 2007, doi: 10.1016/j.yexcr.2007.03.028.

[63] D. Lourim and J. J. C. Lin, “Expression of nuclear lamin A and muscle-specific proteins in differentiating muscle cells in ovo and in vitro,” *J. Cell Biol.*, vol. 109, no. 2, pp. 495–504, 1989, doi: 10.1083/jcb.109.2.495.

[64] D. Lourim and J. J. C. Lin, “Expression of wild-type and nuclear localization-deficient human lamin A in chick myogenic cells,” *J. Cell Sci.*, vol. 103, no. 3, pp. 863–874, 1992.

[65] J. F. Collard, J. L. Senecal, and Y. Raymond, “Redistribution of nuclear lamin A is an early event associated with differentiation of human promyelocytic leukemia HL-60 cells,” *J. Cell Sci.*, vol. 101, no. 3, pp. 657–670, 1992.

[66] G. Lattanzi *et al.*, “Emerin expression at the early stages of myogenic differentiation,” *Differentiation*, vol. 66, no. 4–5, pp. 208–217, 2000, doi: 10.1046/j.1432-0436.2000.660407.x.

[67] M. Kulashreshtha, I. S. Mehta, P. Kumar, and B. J. Rao, “Chromosome territory relocation during DNA repair requires nuclear myosin 1 recruitment to chromatin mediated by γ-H2AX signaling,” *Nucleic Acids Res.*, vol. 44, no. 17, pp. 8272–8291, 2016, doi: 10.1093/nar/gkw573.

[68] K. C. Toh, N. M. Ramdas, and G. V. Shivashankar, “Actin cytoskeleton differentially alters the dynamics of lamin A, HP1α and H2B core histone proteins to remodel chromatin condensation state in living cells,” *Integr. Biol. (United Kingdom)*, vol. 7, no. 10, pp. 1309–1317, 2015, doi: 10.1039/c5ib00027k.

[69] N. M. Ramdas and G. V. Shivashankar, “Cytoskeletal control of nuclear morphology and chromatin o1rganization,” *J. Mol. Biol.*, vol. 427, no. 3, pp. 695–706, 2015, doi: 10.1016/j.jmb.2014.09.008.

[70] E. B. Harmon, M. L. Harmon, T. D. Larsen, J. Yang, J. W. Glasford, and M. B. Perryman, “Myotonic dystrophy protein kinase is critical for nuclear envelope integrity,” *J. Biol. Chem.*, vol. 286, no. 46, pp. 40296–40306, 2011, doi: 10.1074/jbc.M111.241455.

[71] T. Kuilman, C. Michaloglou, W. J. Mooi, and D. S. Peeper, “The essence of senescence,” pp. 1–17, 2010, doi: 10.1101/gad.1971610.

[72] Joseph R. Tran, H. Chen, X. Zheng, and Y. Zheng, “Lamin in inflammation and aging,” *Curr Opin Cell Biol*, no. 40, pp. 124–130, 2016, doi: 10.1016/j.ceb.2016.03.004.

[73] T. Shimi *et al.*, “The role of nuclear lamin B1 in cell proliferation and senescence,” *Genes Dev.*, vol. 25, no. 24, pp. 2579–2593, 2011, doi: 10.1101/gad.179515.111.

[74] P. Scaffidi and T. Misteli, “Reversal of the cellular phenotype in the premature aging

disease Hutchinson-Gilford progeria syndrome," *Nat. Med.*, vol. 11, no. 4, pp. 440–445, 2005, doi: 10.1038/nm1204.

[75] O. Dreesen *et al.*, "Lamin B1 fluctuations have differential effects on cellular proliferation and senescence," *J. Cell Biol.*, vol. 200, no. 5, pp. 605–617, 2013, doi: 10.1083/jcb.201206121.

[76] A. Barascu *et al.*, "Oxidative stress induces an ATM-independent senescence pathway through p38 MAPK-mediated lamin B1 accumulation," *EMBO J.*, vol. 31, no. 5, pp. 1080–1094, 2012, doi: 10.1038/emboj.2011.492.

[77] F. R. Gotzmann J, "Lamins and Emerin in Muscular Dystrophy: The Nuclear Envelope Connection," *Madame Curie Bioscience Database*. https://www.ncbi.nlm.nih.gov/books/NBK6513/#_NBK6513_pubdet_ (accessed Nov. 27, 2020).

[78] G. F. Brady, R. Kwan, J. Bragazzi Cunha, J. S. Elenbaas, and M. B. Omary, "Lamins and Lamin-Associated Proteins in Gastrointestinal Health and Disease," *Gastroenterology*, vol. 154, no. 6, pp. 1602-1619.e1, 2018, doi: 10.1053/j.gastro.2018.03.026.

[79] J. M. Holaska and K. L. Wilson, "Multiple roles for emerin: Implications for Emery-Dreifuss muscular dystrophy," *Anat. Rec. - Part A Discov. Mol. Cell. Evol. Biol.*, vol. 288, no. 7, pp. 676–680, 2006, doi: 10.1002/ar.a.20334.

[80] S. Rebelo, E. F. Da Cruz e Silva, and O. A. B. Da Cruz e Silva, "Genetic mutations strengthen functional association of LAP1 with DYT1 dystonia and muscular dystrophy," *Mutat. Res. - Rev. Mutat. Res.*, vol. 766, pp. 42–47, 2015, doi: 10.1016/j.mrrev.2015.07.004.

[81] J. Shin *et al.*, "Lamina-associated Polypeptide-1 Interacts with the Muscular Dystrophy Protein Emerin and is Essential for Skeletal Muscle Maintenance," vol. 26, no. 6, pp. 591–603, 2013, doi: 10.1016/j.devcel.2013.08.012.Lamina-associated.

[82] H. J. Shin, Ji-Yeon; Dauer, William T.; Worman, "Lamina-associated Polypeptide 1: protein interactions and tissue-selective functions," *Bone*, pp. 164–168, 2014, doi: 10.1016/j.semcdb.2014.01.010.

[83] I. Dorboz *et al.*, "Severe dystonia, cerebellar atrophy, and cardiomyopathy likely caused by a missense mutation in TOR1AIP1," *Orphanet J. Rare Dis.*, vol. 9, p. 174, 2014, doi: 10.1186/s13023-014-0174-9.

[84] R. Ghaoui *et al.*, "TOR1AIP1 as a cause of cardiac failure and recessive limb-girdle muscular dystrophy," *Neuromuscul. Disord.*, vol. 26, no. 8, pp. 500–503, 2016, doi: 10.1016/j.nmd.2016.05.013.

[85] G. Kayman-Kurekci *et al.*, "Mutation in TOR1AIP1 encoding LAP1B in a form of muscular dystrophy: A novel gene related to nuclear envelopathies," *Neuromuscul.*

Disord., vol. 24, no. 7, pp. 624–633, 2014, doi: 10.1016/j.nmd.2014.04.007.

[86] G. Melcon *et al.*, “Loss of emerin at the nuclear envelope disrupts the Rb1/E2F and MyoD pathways during muscle regeneration,” *Hum. Mol. Genet.*, vol. 15, no. 4, pp. 637–651, 2006, doi: 10.1093/hmg/ddi479.

[87] R. Ozawa *et al.*, “Emerin-lacking mice show minimal motor and cardiac dysfunctions with nuclear-associated vacuoles,” *Am. J. Pathol.*, vol. 168, no. 3, pp. 907–917, 2006, doi: 10.2353/ajpath.2006.050564.

[88] D. A. Starr and L. S. Rose, “TorsinA regulates the LINC to moving nuclei,” *J. Cell Biol.*, vol. 216, no. 3, pp. 543–545, 2017, doi: 10.1083/jcb.201701054.

[89] B. Fichtman *et al.*, “Combined loss of LAP1B and LAP1C results in an early onset multisystemic nuclear envelopathy,” *Nat. Commun.*, vol. 10, no. 1, 2019, doi: 10.1038/s41467-019-08493-7.

[90] Cosmo A. Saunders .W. Gant, “LINCing defective nuclear-cytoskeletal coupling and DYT1 dystonia,” *Physiol. Behav.*, vol. 9, no. 2, pp. 207–216, 2016, doi: 10.1007/s12195-016-0432-0.

[91] F. C. Nery *et al.*, “tTorsinA binds the KASH domain of nesprins and participates in linkage between nuclear envelope and cytoskeleton,” *Biochim. Biophys. Acta - Mol. Basis Dis.*, pp. 3476–3486, 2008, doi: 10.1242/jcs.029454.

[92] B. Burke, “The nuclear envelope: Filling in gaps,” *Nat. Cell Biol.*, vol. 3, no. 12, 2001, doi: 10.1038/ncb1201-e273.

[93] N. K. Gill *et al.*, “DYT1 dystonia patient-derived fibroblasts have increased deformability and susceptibility to damage by mechanical forces,” *Front. Cell Dev. Biol.*, vol. 7, no. JUN, pp. 1–15, 2019, doi: 10.3389/fcell.2019.00103.

[94] M. Crisp *et al.*, “Coupling of the nucleus and cytoplasm: Role of the LINC complex,” *J. Cell Biol.*, vol. 172, no. 1, pp. 41–53, 2006, doi: 10.1083/jcb.200509124.

[95] Z. Jahed, D. Fadavi, U. T. Vu, E. Asgari, G. W. G. Luxton, and M. R. K. Mofrad, “Molecular Insights into the Mechanisms of SUN1 Oligomerization in the Nuclear Envelope,” *Biophys. J.*, vol. 114, no. 5, pp. 1190–1203, 2018, doi: 10.1016/j.bpj.2018.01.015.

[96] A. Méjat and T. Misteli, “LINC complexes in health and disease,” *Nucleus*, vol. 1, no. 1, pp. 40–52, 2010, doi: 10.4161/nucl.1.1.10530.

[97] P. Meinke *et al.*, “Muscular Dystrophy-Associated SUN1 and SUN2 Variants Disrupt Nuclear-Cytoskeletal Connections and Myonuclear Organization,” *PLoS Genet.*, vol. 10, no. 9, 2014, doi: 10.1371/journal.pgen.1004605.

[98] F. Haque *et al.*, “Mammalian SUN protein interaction networks at the inner nuclear membrane and their role in laminopathy disease processes,” *J. Biol. Chem.*, vol. 285, no. 5, pp. 3487–3498, 2010, doi: 10.1074/jbc.M109.071910.

[99] A. Janin and V. Gache, “Nesprins and lamins in health and diseases of cardiac and skeletal muscles,” *Front. Physiol.*, vol. 9, no. SEP, pp. 1–12, 2018, doi: 10.3389/fphys.2018.01277.

[100] T. Metzger *et al.*, “MAP and Kinesin dependent nuclear positioning is required for skeletal muscle function,” *Nature*, pp. 120–124, 2012, doi: 10.1038/nature10914.

[101] W. Roman *et al.*, “Myofibril contraction and crosslinking drive nuclear movement to the periphery of skeletal muscle,” *Nat. Cell Biol.*, vol. 19, no. 10, pp. 1189–1201, 2017, doi: 10.1038/ncb3605.

[102] S. Taranum *et al.*, “LINC complex alterations in DMD and EDMD/CMT fibroblasts,” *Eur. J. Cell Biol.*, vol. 91, no. 8, pp. 614–628, 2012, doi: 10.1016/j.ejcb.2012.03.003.

[103] G. T. Haskell *et al.*, “Whole Exome Sequencing Identifies Truncating Variants in Nuclear Envelope Genes in Patients with Cardiovascular Disease,” *Circ Cardiovas Genet.*, vol. 10, no. 3, doi: 10.1161/CIRCGENETICS.116.001443.Whole.

[104] W. Chen, Y. Wang, Y. Abe, L. Cheney, B. Udd, and Y. P. Li, “Haploinsuffciency for Znf9 in Znf9+/- Mice Is Associated with Multiorgan Abnormalities Resembling Myotonic Dystrophy,” *J. Mol. Biol.*, vol. 368, no. 1, pp. 8–17, 2007, doi: 10.1016/j.jmb.2007.01.088.

[105] C. Zhou, L. Rao, C. M. Shanahan, and Q. Zhang, “Nesprin-1/2: Roles in nuclear envelope organisation, myogenesis and muscle disease,” *Biochem. Soc. Trans.*, vol. 46, no. 2, pp. 311–320, 2018, doi: 10.1042/BST20170149.

[106] H. Cantwell and P. Nurse, “Unravelling nuclear size control,” *Curr. Genet.*, vol. 65, no. 6, pp. 1281–1285, 2019, doi: 10.1007/s00294-019-00999-3.

CHAPTER IV: Concluding remarks and perpectives future

4.1 Concluding remarks

Myotonic dystrophy type 1 (DM1) belongs to the group of muscular dystrophies and is an autosomal dominant multisystemic disease that is essentially characterized by progressive distal muscle weakness and myotonia. DM1 is caused by an abnormal expansion of the CTG trinucleotide in the 3 'untranslated (3'UTR) region of the *Myotonic dystrophy protein kinase (DMPK)* gene. To date, the molecular mechanisms involved in this pathology are supported by three hypotheses, namely the RNA toxic gain-of-function, the rearrangement of the DM1 locus and the DMPK haploinsufficiency. However, these hypotheses do not explain the multisystemic signs and symptoms of DM1. Thus, the mechanisms underlying this pathology are not yet fully understood, and further studies are needed to understand them.

In recent years, studies carried out to unveil these mechanisms underlying DM1 have focused on changes in the nuclear architecture through studying the contribution of NE observed in cells of patients with DM1 (fibroblasts, myoblast and myotubes) namely, changes in the intracellular levels of the nuclear lamina (lamin A/C and lamin B1), of proteins in the inner nuclear membrane (SUN2) and of outer nuclear membrane proteins (nesprins -1 and -2), as well as nuclear deformations. These changes in the NE may potentially may be related with changes in DMPK levels, given the latter is central protein of this pathology and playing an important role in the muscle, particularly during myogenesis.

Therefore, the main objectives of this dissertation were to summarize the NE changes associated with DM1 previously reported in the literature, to determine the intracellular levels of some NE proteins and to determine their subcellular distribution and to evaluate the nuclear profile in DM1 patients-derived fibroblasts and control. Our results indicate that

Previous studies:

1. The alterations of NE and NE proteins reported in the literature in DM1 patients-derived myoblasts were, presence of nuclear invaginations, increased intracellular levels of the TMEM38a protein and decreased intracellular levels of lamin A, lamin B1, SUN2 in DM1-derived myoblasts and decreased nesprin-1 protein levels (giant isoform and N1-α2 isoform) in DM1-derived myotubes.;

2. The alterations of NE and NE proteins reported in the literature in DM1-patients derived fibroblasts were increased immunostaining of NE and nucleoplasm,

augment nuclear size, presence of deformed nuclei and nuclear invaginations for nuclei marked for lamin A/C, lamin B1 and emerin;

Present study:

3. A decreased DMPK protein levels in DM1 patients-derived fibroblasts regardless of the number of CTG repetitions tested;
4. The characterization of DM1 patients-derived fibroblast and nuclei was achieved. Essentially, DM1-derived fibroblasts nuclei present identical circularity to the control fibroblasts; increased crossed diameter ratio and increased nuclear area was observed in DM1-derived fibroblasts when compared to control fibroblasts (Figure 1);

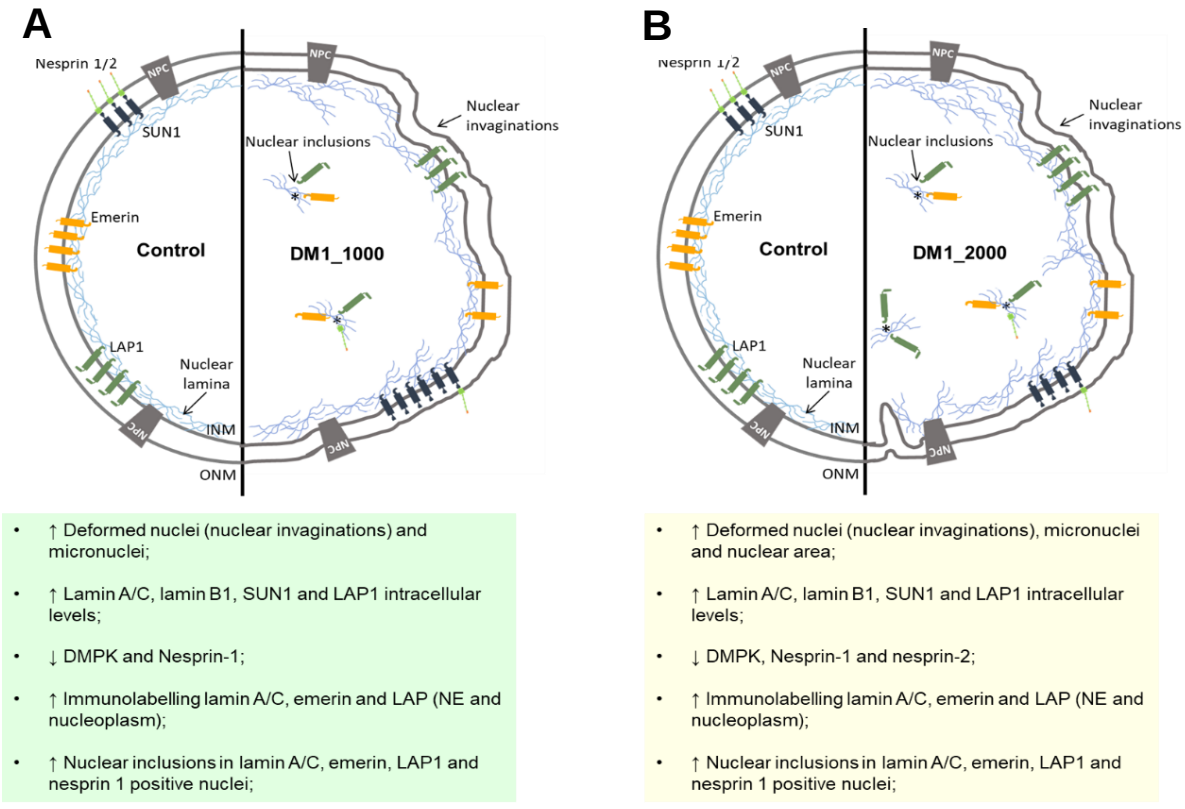

Figure 1: Schematic representation of the nuclear envelope and NE changes observed in DM1-derived and controls fibroblasts. The comparison was made between DM1_1000 (A) and DM1_2000 (B) patients derived fibroblasts reported in present study; DM1 – Myotonic dystrophy type 1; NE – Nuclear envelope; INM – Inner nuclear membrane; ONM – Outer nuclear membrane; SUN - Sad1/Unc-84; LAP1 – Lamin-associated with polypeptide 1; DMPK – Myotonic dystrophy protein kinase.

5. An increased intracellular protein levels of lamin A/C and LAP1 were observed and they were correlated with the increase of CTG repeat length; increased intracellular levels of lamin B1 and SUN1 proteins were detected regardless of the number of CTG repeat length (Figure 1);

6. In present study, a decreased in intracellular nesprin-1 protein levels accordingly the increased number of CTG repetitions; decrease in intracellular nesprin-2 protein levels only observed in DM1-derived fibroblasts (DM1_2000; Figure 1);

7. An increased number of nuclear inclusions in DM1-derived fibroblast positive for lamin A/C, emerin and nesprin-1 were observed; increased number of nuclei with 3 or more nuclear inclusions in positive nuclei for lamin A/C, emerin, LAP1 and nesprin-1 were detected, being the number of inclusions proportional to CTG repeat length;

8. The nuclear deformations were significantly increased in DM1 patients-derived fibroblast nuclei, the increase being proportional to CTG repeat length;

9. The DM1 patients-derived fibroblast nuclei present more mild and moderate nuclear invasions than control-derived fibroblasts, the increase being proportional to CTG repeat length;

10. The presence of increased number micronuclei was evident in DM1-derived fibroblasts, when compared with control ones.

In conclusion, the results presented indicate that there may be a relationship between changes in NE and changes in DMPK, the DM1 central protein in DM1. The technique western blotting analysis allowed the evaluation of changes in the intracellular levels of the proteins lamin A/C, lamin B1, LAP1, SUN1, nesprin-1 and nesprin-2 between DM1 patients-derived and control fibroblasts. In turn, the immunocytochemistry allowed the detection of increased structural changes in the NE, such as deformed nuclei, nuclear inclusions and an increase in the area of DM1 patients-derived fibroblasts compared to the control fibroblasts. Thus, it was possible to make more robust the data that already existed regarding the alterations of the NE in DM1.

4.2. Perspectives futures

Although our results are promising in relation to changes in NE and its proteins in patients with DM1, the need to understand the mechanisms behind these changes represent an important challenge, making them essential in further studies. To date, there are few studies related to the alterations of NE in DM1. In the future, it will be interesting to complement this study with the immunocytochemical evaluation of the following proteins, lamin B1, SUN1 and nesprin-2. In addition, it will be important to assess the intracellular levels of NE proteins and to assess their subcellular location in a relatively larger sample of patients and controls. As we know, patients diagnosed with DM1 can be categorized according to the severity of the disease (mild, adult and congenital) or according to the age of onset of the disease (late-onset, adult-onset, juvenile, infantile and congenital). In our study we included patients with approximately 1000 and 2000 CTG repetitions that can represent (but without certainty) the adult/classic and congenital/severe phenotype, respectively. Another point that will be interesting to find out is whether these changes in the NE allow to differentiate patients according to the phenotypes of the disease.

Another very important aspect is the cell model used, DM1 patients-derived fibroblasts, that are nor muscle cells. Although it is interesting to study the molecular mechanisms of DM1 in muscle cells, there is a relative difficulty in accessing muscle biopsies from dystrophic patients. On the other hand, the use of embryonic stem cells (hESCs) has raised ethical issues and has been the subject of controversy. Therefore, it would be interesting to validate the following data using fibroblasts-derived myoblast and also fibroblasts-derived myotubes.