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Review

Duchenne Muscular Dystrophy: A Molecular Approach

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Abstract: Duchenne Muscular Dystrophy (DMD) is an X-linked recessive congenital myopathy, with a low prevalence but a high mortality rate. It is due to different mutations of the DMD gene, the largest gene of the human genome, and leads to the production of a non-functional dystrophin, a fundamental protein responsible for muscle integrity. DMD is transmitted by female carriers and is characterized by a generalized progressive muscular dystrophy and weakness in males, being fatal before the age of 20 years due to cardiorespiratory failure. The aim of this study was to perform an integrative systematic review about the effect of interventions on DMD mutations of the information available in randomized studies with non-randomized studies. A research protocol was designed following the PICO methodology. Bibliography eligibility criteria were defined to answer the PICO's question. Three independent searches were performed, namely in Cochrane, PubMed and Google. The data collection methodology used eligibility criteria. To extract information, five variables have been parameterized. Fifty-four papers were included in this integrative systematic review. Because scientists have succeeded in locating DNA errors and mutations with increasingly precise technologies, we are now able to develop new therapies that make it possible to avoid, bypass or edit the mutated DNA. There is information in non-randomized studies on the effect of interventions on DMD mutations. There are few therapies available for DMD mutations. Combining information from non-randomized studies with information available from randomized studies on the effect of interventions on DMD mutations, DNA analyses and advances in biomolecular science are confirmed and represent the basis of therapies with a direct impact on the predictability of disease progression.

Keywords: Duchenne muscular dystrophy; X-linked disease; dystrophin; dystrophin-associated protein complex

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Introduction

Duchenne Muscular Dystrophy (DMD) and Becker Muscular Dystrophy (BMD) are X-linked recessive congenital myopathies due to mutations in the gene that encodes for dystrophin. The difference between both diseases is that DMD has more serious consequences for patients than BMD, as the mutations in the first case can disrupt the reading frame, leading to more serious molecular consequences on dystrophin synthesis. Dystrophin is a structural protein whose function is to stabilize the myocyte membrane to ensure the stability of the muscle cell. DMD is transmitted by female carriers, characterized by a generalized progressive muscular dystrophy and weakness in males, and fatal before the age of 20 years due to cardiorespiratory failure [1].

Giovanni Semmola was probably the first, in 1834, to describe this severe and progressive muscular dystrophy in two boy patients [2]. Later in 1852, Dr Edward Meryon mentioned an "early degeneration of the muscles with fatty infiltration", which did not affect patients' nervous systems. He studied six families to find a genetic correlation between the patients. His conclusions were the following: (1) it was a familial disease that affected only boys and was transmitted by the female gender; (2) it was essentially a muscle disease that did not affect the nervous system; (3) the pathology was the consequence of the rupture of the sarcolemma, a cellular structure that constitutes the cell membrane of muscle cells [3]. About ten years later, in France, Guillaume Benjamin Armand Duchenne published a detailed case description [4] and an album of photographs of several affected patients [5]. He became the first to take a biopsy to obtain

tissue from a living patient for histological examination, which allowed him to conclude with certainty that the disease was muscular, and so he gave it his name [6].

The prevalence of DMD has been a topic of debate, although it is considered a rare disease. Its worldwide prevalence is estimated to vary between 2.8 and 4.8 cases per 100,000 inhabitants ($p \leq 0,05$) [7,8]. The prevalence of births with DMD was estimated as 19.8/100,000 male births ($p \leq 0,05$) [7]. Mortality is very low between zero and ten years of age and increases with age (five-year mortality rates: 0.394/1,000). The average recorded survival is twenty-two years, but survival rates have increased over time, with a survival of 28.1 years ($p \leq 0,05$) for patients born after 1990 [9].

The main signs and symptoms include muscle weakness and pseudohypertrophy caused by the degeneration of muscle fibres and the accumulation of fibrous and adipose tissue [10]. Gowers' sign is one of the first warning signs and is manifested by the need for the child to use the arms to stand up, due to the muscular weakness of the legs [11]. This general weakness leads to inevitable bone weakening, scoliosis (severe spinal deformity), loss of reflexes, frequent falls, and changes in general posture. Children with DMD usually lose the ability to walk by early adolescence. Without intensive and targeted follow-up, patients usually die in late adolescence or in the early third decade of life due to progressive heart muscle weakness, respiratory complications, and infections [12].

DMD screening is initially based on clinical signs and symptoms, with genetic testing forming the basis of the final diagnosis. A significant increase in serum muscle enzymes, such as serum creatine kinase (CK), serum alanine transaminase (ALT) and serum aspartate transaminase (AST), can be seen in both sexes [13]. Through muscle biopsy, the presence of muscle fibre degeneration, necrosis, presence of small, regenerated fibres and mononuclear infiltrates can be verified [14,15].

Specialists recognize the importance of signs and clinical symptoms and of performing diagnostic procedures as early as possible. The DMD patient's management is based on a multidisciplinary perspective and involves a symptomatic and rehabilitation approach [13]. Corticotherapy is the only pharmacological strategy that delays the symptoms and complications of the disease. The level of chronic and ongoing lesions observed in DMD is one of the targets of glucocorticoids [16,17]. Prednisone and prednisolone are the most used corticosteroids at a dose of 0.75 mg/kg/day [13,14,18]. Surveillance and treatment of respiratory, cardiac, orthopaedic, nutritional, psychological and bone functions are fundamental to reduce health decline and expected complications [18].

Historically, this disease has enabled scientists to discover and describe the molecular mechanisms behind dystrophin and its importance in muscle integrity, allowing them to outline the best therapeutic intervention strategies. According to Cochrane recommendations, the expected benefits and some of the expected harms of a given intervention can often be assessed in randomized trials. However, many serious harms from an intervention are rare or do not arise during the follow-up period of randomized trials, preventing randomized trials from providing high-quality evidence on these effects, even when combined with a meta-analysis, which is one of the most important reasons for including non-randomized studies in a review to assess potential unexpected or rare harms of interventions [19]. We are not aware of evidence that risk of bias due to missing evidence affects randomized trials and non-randomized studies on intervention effects differentially. However, it is difficult to believe that publication bias could affect non-randomized studies on intervention effects less than randomized trials, given the increasing number of safeguards associated with carrying out and reporting randomized trials that act to prevent reporting biases [20]. These safeguards are much less applicable to non-randomized studies on intervention effects, which may not have been executed according to a pre-specified protocol, may not require explicit ethical approval, are unlikely to be registered, and do not always have a research sponsor or funder. The likely magnitude and determinants of publication bias for non-randomized studies on intervention effects are not known. In addition, secondary research can, and should, be used to study and justify the need for further primary research [19]. Thus, the aim of this study is to perform an integrative systematic review about the effect of interventions on DMD mutations of the information available in randomized studies with non-randomized studies.

Materials and Methods

A research protocol was designed. The mandatory research question was, considering the PICO methodology: "Is there published information on non-randomized studies that evaluates the effect of interventions on DMD mutations?". The PICO methodology is summarized in Table 1.

Table 1. PICO strategy.

P (Participants)	Patients with DMD mutations
I (Interventions)	Interventions on DMD mutations
C (Comparison)	Comparison of interventions in patients with DMD mutations
O (Outcome)	Effect of treatment of DMD mutations

Bibliography eligibility criteria were defined to answer the PICO's question. Three independent searches were performed, namely in Cochrane, PubMed and Google. The first search was performed in Cochrane for methodological support of the study design. The second search was performed in PubMed with the keywords "*Duchenne Muscular Dystrophy*", "*Dystrophin*", "*Dystrophin-Associated Protein Complex*", "*X-linked disease*", "*Molecular*", "*Genetic*". An advanced MeSH Terms search was performed with the expression *((Duchenne Muscular Dystrophy) OR (Dystrophin) OR (Dystrophin-Associated Protein Complex)) AND (X-linked disease) AND ((Molecular) OR (Genetic)).* The eligibility criteria applied the PubMed filters "Review", "Clinical trial", "Books and documents", "Meta-Analysis", "Systematic Review" and "Randomized controlled trials" without time limit. In both situations, the results obtained were screened in the first step by title and abstract and, in the second step, by full reading. An independent Google search was performed for "*Genetic and Molecular Database*" and "*Duchenne Guidelines*" and the results obtained were scrutinized by full reading. The data collection methodology used eligibility criteria. To extract information, five variables have been parameterized, namely "Author / Year", "Study design", "Epidemiology" (Epi), "Diagnosis criteria" (D), "Biomolecular mechanisms" (MOL), "Genetic mechanisms and aetiology" (Gen) and "Therapeutic strategies" (Th). The eligibility criteria and data extraction were checked by the two Authors.

Results

The research strategy included in this integrative systematic review was carried out according to the research protocol. The selection process and results are explained by the PRISMA flowchart (Fig. 1).

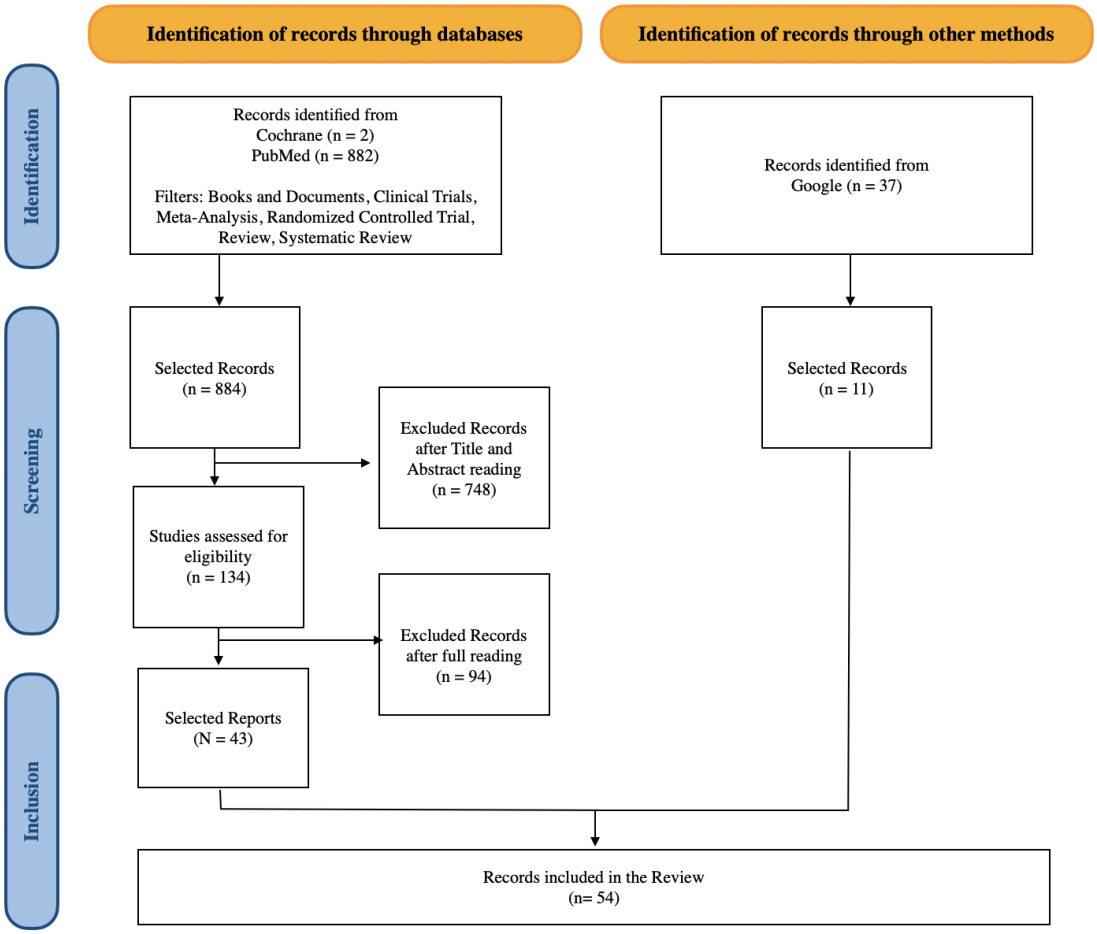

Figure 1. PRISMA Flowchart.

Data extraction

The final sample of this integrative systematic review included fifty-four publications. Each of the publications was analysed and the variables defined in the data collection methodology were recorded, according to the eligibility criteria, which were part of the study design. The extracted information was included in the discussion of the integrative systematic review. The procedure was checked by the two Authors and the results are presented in Table 2.

Table 2. Data extraction ("Epidemiologic data" (Epi), "Diagnosis criteria" (D), "Biomolecular mechanisms" (Mol), "Genetic mechanisms and aetiology" (Gen), "Therapeutic strategies" (Th)).

Author / Year	Study design	Epi	D	Mol	Gen	Th
Allen D.G. *et al.* 2016 [26]	Review		✓	✓		
Anderson J.E. *et al.* 1996 [17]	Clinical Trial					✓
Berget S.M. 1995 [39]	Review				✓	✓
Birnkrant D.J. *et al.* 2018 [13]	Review of Literature		✓			✓
Bladen C.L. *et al.* 2015 [33]	Review	✓			✓	
Blake D.J. *et al.* 2002 [24]	Review			✓	✓	
Bowles D.E. *et al.* 2012 [51]	Clinical Trial			✓	✓	✓
Broomfield J. *et al.* 2021 [9]	Systematic Review	✓	✓			
Bushby K. *et al.* 2010 [14]	Review of Literature		✓			✓
Cooper G.M. *et al.* 2006 [27]	Book			✓		
Crisafulli S. *et al.* 2020 [7]	Systematic Review	✓				
Darras B.T. *et al.* 2015 [15]	Book	✓	✓	✓	✓	
Doorenweerd N. *et al.* 2017 [25]	Original Article		✓	✓		✓
Duan D. *et al.* 2021 [42]	Review	✓	✓	✓	✓	✓
Duchenne G.B. 1861 [4]	Historical Document		✓	✓	✓	
Duchenne G.B. 1862 [5]	Historical Document		✓	✓	✓	
Duchenne G.B. 1868 [10]	Historical Document		✓	✓	✓	
Emery A.E. *et al.* 2017 [3]	Meeting		✓			
Endo T. 2007 [29]	Review			✓		
Ervasti J.M. *et al.* 2008 [30]	Review					
European Medicines Agency 2015 [50]	Guideline	✓	✓			✓
Expasy – Swiss Institute of Bioinformatics 2022 [34]	Database			✓		
Gao Q.Q. *et al.* 2015 [28]	Review			✓		✓
Happi Mbakam C. *et al.* 2022 [36]	Review			✓		✓
Himič V. *et al.* 2021 [53]	Review			✓	✓	✓
Al Mosawi A.J. *et al.* 2019 [2]	Review of Literature		✓			
Jay V. *et al.* 2001 [6]	Case Report		✓			
Juan-Mateu J. *et al.* 2015 [35]	Clinical Trial		✓			
Jukes T.H. 1987 [39]	Review				✓	
Klug W.S. *et al.* 2014 [21]	Book		✓		✓	✓
Łoboda A. *et al.* 2020 [49]	Systematic Review		✓	✓	✓	✓
Lu X. *et al.* 2021 [47]	Case Series				✓	

Author / Year	Study design	Epi	D	Mol	Gen	Th
Mirovsky Y. *et al.* 2005 [11]	Case Series		✓			✓
Mosca N. *et al.* 2021 [45]	Review			✓		✓
National Center for Biotechnology Information 2023 [22]	Database	✓		✓	✓	
National Human Genome Research Institute 2023 [37]	Dictionary				✓	
National Institute of Neurological Disorders and Stroke 2013 [12]	Book	✓	✓	✓	✓	✓
NCI Dictionary of Genetics Terms 2022 [31]	Dictionary				✓	
Nussbaum R.L. *et al*. 2016 [41]	Book	✓	✓		✓	✓
Online Mendelian Inheritance in Man 2022 [1]	Database	✓	✓	✓	✓	
Pichavant C. *et al.* 2011 [54]	Review				✓	✓
Quattrocelli M. *et al.* 2021 [52]	Review			✓		✓
Salari N. *et al.* 2022 [8]	Systematic Review	✓				
The Human Protein Atlas 2022 [23]	Database	✓	✓	✓	✓	
Tidball J.G. 2011 [16]	Review		✓	✓		✓
Timpani C.A. *et al.* 2017 [43]	Systematic Review		✓	✓		✓
Trabelsi M. *et al.* 2014 [40]	Original Article		✓		✓	
Van Essen A.J. *et al.* 1997 [32]	Guideline	✓	✓	✓	✓	✓
Van Westering T. *et al*. 2015 [44]	Systematic Review			✓		✓
Verhaart I.E.C. *et al.* 2019 [46]	Review					✓
Wehling-Henricks M. *et al*. 2009 [48]	Original Article			✓		
Yiu E.M. *et al.* 2015 [18]	Review			✓		✓

Discussion

1. Key notions about the DMD gene and the dystrophin protein

The DMD gene. It is the largest known gene in the human genome [21]. It is located on the short arm (p) of the X chromosome, in two regions, Xp21.1 and Xp21.2. After transcription and splicing, its 79 exons encode for dystrophin (Dp427), containing 3,685 amino acids. The large size of the DMD gene makes it very vulnerable to mutations [1,22,23]. Depending on the cells in which this gene is located, the splicing carried out and the promoters with which it is associated, this single gene encodes for different proteins, called Dp427 isoforms, among which Dp427m, responsible for dystrophin in muscle tissues [24]. There are also other isoforms with expression in other tissues, such as (among others): Dp427p, expressed in Purkinje cells; Dp427c, predominantly expressed in neuronal cells of the cortex and hippocampus; Dp260, expressed in retinal cells; Dp116, expressed in peripheral neurons; Dp71, ubiquitously expressed, with higher levels in the central nervous system [24,25]. The presence of these isoforms in various tissues may be an explanation for the high incidence of learning delay, behavioural and neurodevelopmental disorders in patients with DMD [25].

Dystrophin protein. It is a long cytoskeleton protein (110 nm) on the cytoplasmic side of the sarcolemma, or muscle cell membrane, that is part of the dystrophin protein complex (DPC). It is the fundamental binding protein between the subsarcolemmal actin and the extracellular matrix, by anchoring to a group of membrane proteins of the DPC [26]. The function of the DPC is to stabilize the myocyte membrane to ensure cell stability. The DPC confers structural stability to the sarcolemma and protects muscle fibres from mechanical stress during muscle contraction [27]. It may also play a role in cell signalling by interacting with proteins that send and receive chemical signals (neuronal nitric oxide synthase, nNOS). Dystrophin has four main functional domains, including an amino-terminal actin-binding domain, a central domain, a cysteine-rich domain, and a carboxyl-terminal [28] (Fig. 2).

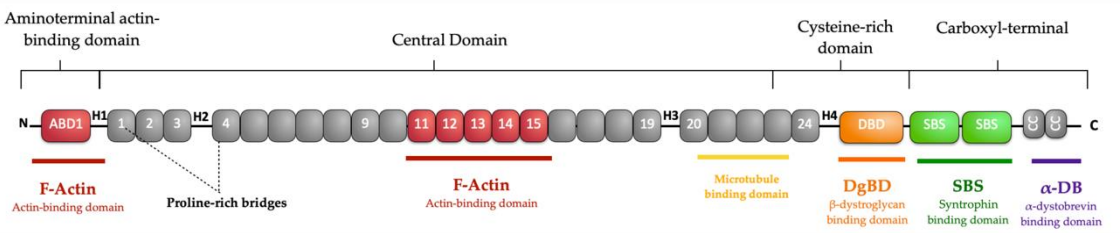

Figure 2. The dystrophin protein. Original figure by the Authors.

The amino-terminal actin-binding domain (ABD1) contains 2 calponin homologous domains (CH1 and CH2) whose function is to bind directly with F-actin to bind dystrophin and the DPC to the subsarcolemmal actin network. The central domain contains a second actin-binding site (ABD2) that collaborates with ABD1 to form a strong lateral association with actin filaments and a domain that mediates the interaction of dystrophin with microtubules. The cysteine-rich domain forms the binding site for β-dystroglycan (DgBD). The carboxyl terminus contains binding sites for syntrophins (SBD – SBS, syntrophin binding site), dystroglycan (DgBD – dystroglycan binding site) and α-dystrophin (CC – coiled-coiled domain). Dystrophin is therefore not an isolated, independent protein. It is linked to many proteins through specific binding sites. Thus, its malfunction can interfere with the functioning of the complex to which it belongs, the DPC [28].

Dystrophin protein complex. The DPC can be divided into three groups, based on their cellular location: extracellular (α-dystroglycan); transmembrane (β-dystroglycan, sarcoglycan, sarcospan); cytoplasmic (dystrophin, dystrobrevin, syntrophins, nNOS) (Fig. 3). Dystroglycan is a transmembrane binding protein with two subunits, α-dystroglycan in the extracellular matrix and β-dystroglycan inserted into the membrane. α-Dystroglycan binds strongly to extracellular matrix proteins containing laminin G domains [29].

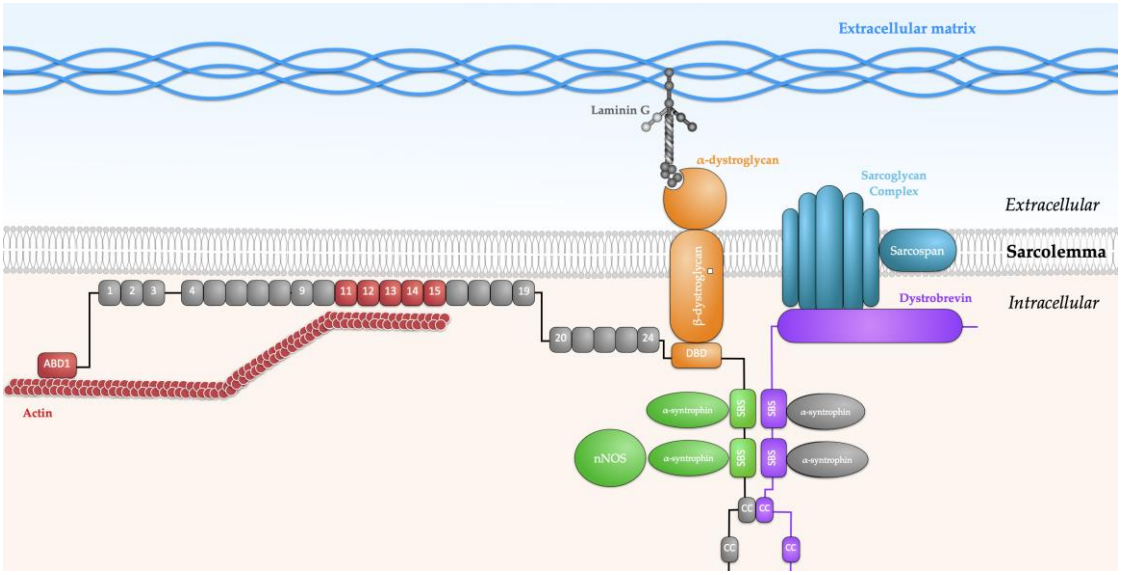

Figure 3. Dystrophin-associated protein complex. Original figure by the Authors.

β-Dystroglycan binds to dystrophin in the cysteine-rich domain. The sarcoglycan complex (CSG) is formed by combinations of several sarcoglycan units (in striated muscle, α-, β-, γ- and δ-sarcoglycan; in smooth muscle, ε-, β-, ζ- and δ-sarcoglycan) [28]. Sarcoglycan is associated with dystroglycan and sarcospan. Sarcoglycan forms a tight complex with sarcospan, strengthening the bond between α- and β-dystroglycan. In addition to its structural role, the sarcoglycan-sarcospan subcomplex is also involved in signal transduction and mechanoprotection, but its exact role is not fully known [28]. Dystrobrevin is

suggested to be involved in the structural integrity of muscle cells by interacting with cytoskeleton-binding proteins, and in signal transduction by interacting with syntrophins. Syntrophins anchor a variety of signalling molecules near their sites of action. nNOS has been shown to have a direct interaction with α-syntrophin [30]. nNOS is an enzyme generally located in the sarcolemma, bound to the DPC. Dystrophin plays an important structural role in the cell. By binding to the dystroglycan complex, it indirectly links the extracellular matrix and subsarcolemmal actin, and thus provides stability to the cell and prevents damage caused by muscle contraction.

2. Duchenne Muscular Dystrophy

Inheritance. DMD is inherited by an X-linked recessive pattern that is responsible for variants of the DMD gene located on the short-arm (p), at locus 21.1-21.2 [1]. It causes a recessive mutation in the female to become dominant in the male, although this condition does not exclude the existence of symptomatic carriers [31]. Females are considered carriers because they usually do not show signs and symptoms when they have mutations in the DMD gene. When a woman has mild signs and symptoms, the onset of her signs and symptoms depends on the pattern of X-chromosome inactivation, called mosaicism [8]. Some carriers show electrocardiographic abnormalities and mild to severe cardiomyopathy described in association with muscle weakness [1]. In addition, DMD can cause early death in these patients [32].

Hotspots of mutation. Two regions of the DMD gene are considered hotspots for mutation (Fig. 4), where these events occur at a higher rate, namely between exons 45 and 55, and between exon 3 and exon 10 [21]. Some exons are more frequently mutated, such as exons 51, 53, 45, 44, 43, 46, 50, 52, 55 and 8 [33]. In the study by Bladen *et al.*, the duplication of exon 2 was analysed as the most frequent, followed by that of exon 17 [33,34].

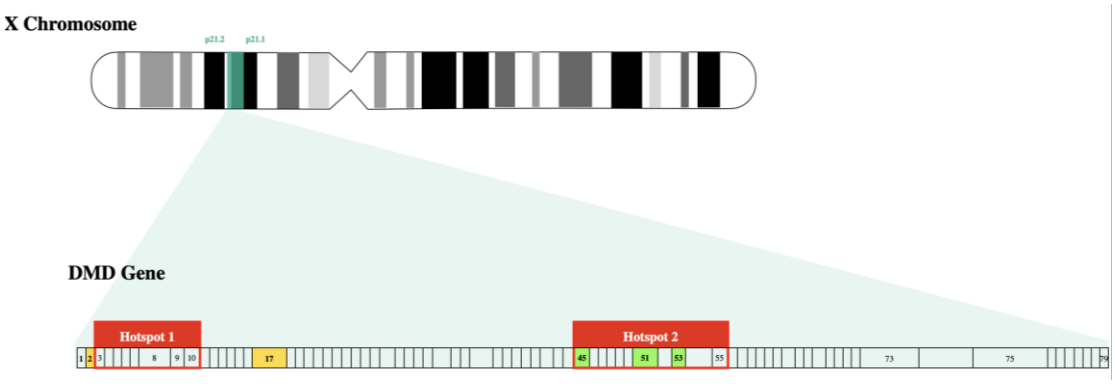

Figure 4. Distribution of the most common mutations in the DMD gene. In red, the two principal hotspots of mutations. In bright green, the exons most frequently affected by deletion. In yellow, the exons most frequently affected by duplication. Created by the Authors.

Mutation types. DMD is not caused by a single type of mutation (Fig. 5). In fact, any type of mutation, namely base substitutions, deletions, and duplications or insertions, at the 21.1-21.2 locus of the X chromosome, can cause the DMD phenotype or BMD, a phenotypic expression with less aggressiveness [33]. The clinical severity depends on whether the gene reading frame is preserved and/or whether the final protein remains functional [35].

Large exon deletions are the most common mutations responsible for DMD phenotypes and comprise about 65-70% of all mutations in the gene. Large exon duplications account for 11% of total DMD gene mutations, with single exon duplication being less frequent than deletion [33,36]. These large mutations can produce an alteration of the ribosome's reading frame, causing a frameshift mutation [27,34-37]. Frameshift mutations can also result in the production of a premature termination codon (PTC), which will cause translation to be interrupted (nonsense mutation). The resulting protein will be non-functional or dysfunctional, depending on the location of the PTC in the gene [21].

Point mutations are mutations in small portions of DNA and account for approximately 25-30% of the molecular pathology of the DMD gene [35]. Nonsense and missense mutations can be found in this category. Nonsense mutations represent about 10% of DMD mutations and occur by transition (70%) and transversion (30%) mutational events. Cytosine-to-thymine substitution is the most frequent (90%) [38]. Missense mutations are infrequent (less than 1%) among the mutations responsible for DMD [33,35].

More unusual mutations may exist, such as splicing site mutations and mid-intronic mutations. Splicing site mutations (<10 base pairs of the exon) account for about 3% of DMD gene mutations [33,39,40]. Mid-intronic mutations represent 0.3% of DMD gene mutations [33,40]. It is reported that 10-15% of exon/intron duplications and about 3% of small insertions/deletions responsible for DMD can also be included [33]. Some identified base substitution mutations that can cause DMD are part of the Expasy database [34].

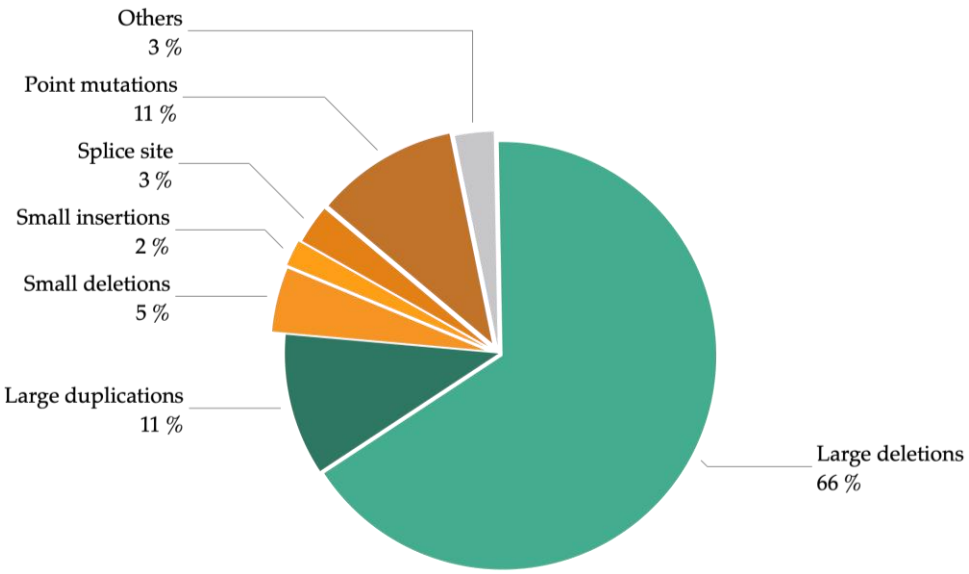

Figure 5. Type and frequency of mutations causing the DMD phenotype. Created by the Authors, adapted from [33].

Mutations of the DMD gene can affect protein production and function by chemical alterations and reading frame variations. These mutations will have direct impact on the physiological function of the sarcolemma, but also in the whole nervous system of DMD patients [41].

Biomolecular cascade. In DMD, dystrophin is extremely short or non-existent, often lacking the dystroglycan-binding end, making it dysfunctional. Therefore, whenever the muscle contracts, the sarcolemma tears. These rips lead to the uncontrolled diffusion of various molecules into and out of the myocyte, namely Ca^{2+} and CK (Fig. 6) [42].
Calcium ions, found abundantly outside the myocyte in physiological conditions, enter through the rips of the sarcolemma. It is the most important substance involved in muscle damage. The role of calcium-dependent proteases is to destroy dysfunctional and/or old proteins. But when the intracellular calcium increases excessively, these proteases become overactive and end up destroying functional and essential proteins [43]. Increased Ca^{2+} is believed to induce a sudden increase in mitochondrial membrane permeability through the mitochondrial permeability transition pore (PTP). The PTP is a voltage-sensitive channel and an increase in Ca^{2+} concentration induces the opening of this channel. The short-term opening of these channels is beneficial because mitochondria can regulate the intracellular Ca^{2+} and reactive oxygen species (ROS) balance. However, in the presence of high Ca^{2+} levels, as in DMD, the opening of these channels is prolonged, which is harmful to the cell [44]. Indeed, ROS are signalling molecules, produced moderately and continuously by skeletal muscles because of their contractile activity and high mitochondrial O_2 consumption. Excessive ROS release and increased ROS concentration in the cytoplasm induce myocyte necrosis by oxidative stress [45]. The increased membrane permeability, by the tears in the sarcolemma, also allows larger proteins, such as CK, to extravasate from the cell and eventually go into the blood. An elevated CK level in the blood is a finding often used to diagnose DMD. CK is an enzyme that stores energy for myocytes to use during contraction. With less CK, less energy storage occurs, which also weakens the muscles [44].
The absence of dystrophin leads to a secondary reduction in nNOS. The loss of nNOS from the sarcolemma reduces the overall nNOS content in dystrophic muscle, resulting in a decrease in its activity of nitric oxide (NO) production, which is a key signalling molecule involved in the regulation of muscle function and mass and blood flow [43]. In addition, NO interacts with phosphofructokinase (PFK), being a regulator of this glycolytic enzyme. Thus, reducing this interaction by the unavailability of NO will reduce energy production capacity [46].

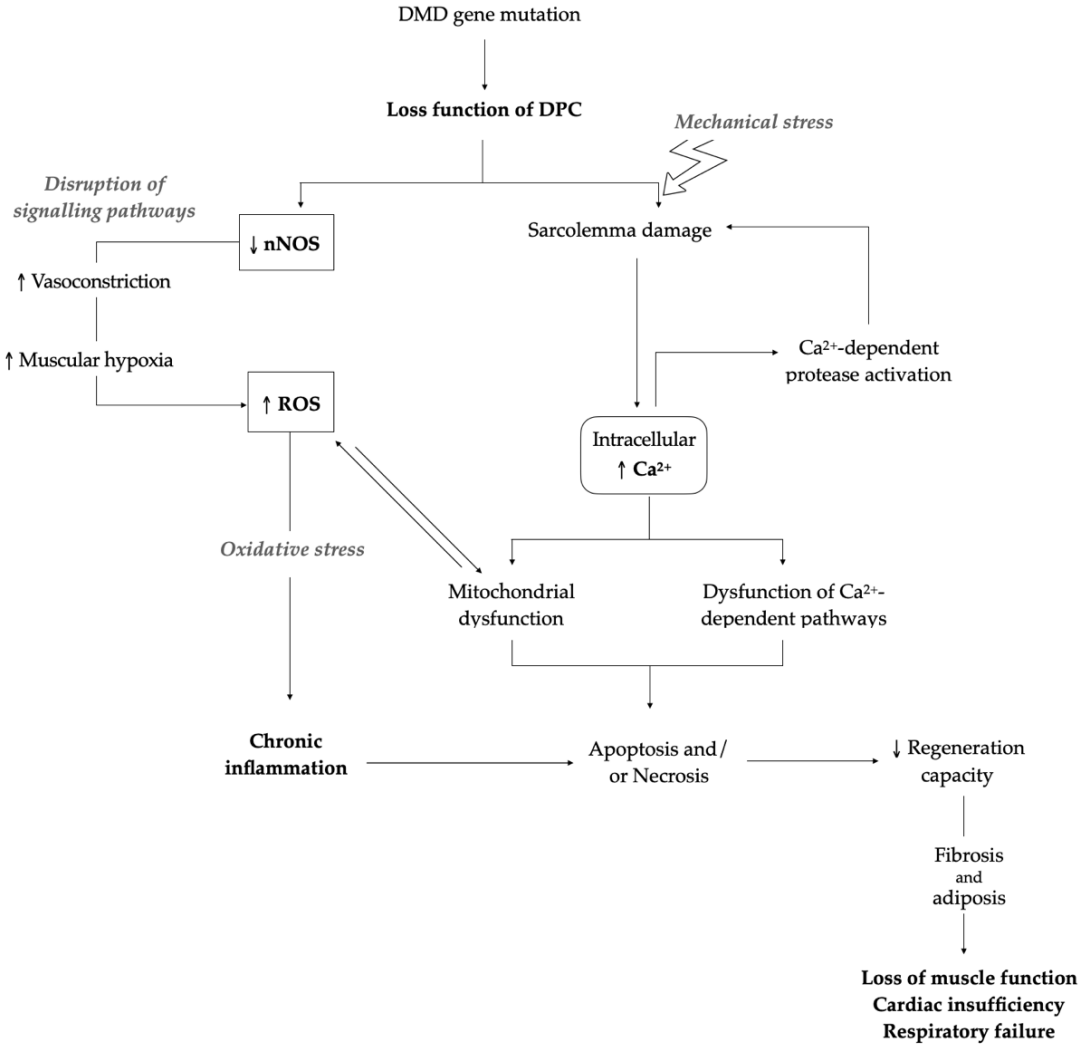

Figure 6. Biomolecular cascade resulting from DMD gene mutations. Created by the Authors, adapted from [47,48].

Muscle repair and regeneration can occur at younger ages. As patients age, muscles do not regenerate fast enough to keep up with the constant degeneration of myocytes. Instead, fibrous and adipose tissue begin to fill in the gaps. Because the fibrous and adipose tissues are unable to contract, the muscles become weaker over time [49].
Early diagnosis is a key factor for the medical practitioners because specific/targeted gene therapies can be chosen to treat the aetiology or molecular consequences, and not only the symptoms.

3. Development of targeted treatments

New approaches to DMD include therapies under investigation in the field of genetics and cell biology. The challenges to these new therapies are numerous and include appropriate clinical trial design, correct global approach aiming mutation specificity, safety and tolerability, and host immune responses [18].

Genetic-based therapies. Biotechnologies like readthrough agents, exon skipping and gene substitution or edition therapies are mutation-specific treatments. Their objective is to target the specific zone where the mutation occurs to revert it or ignore it.
Ataluren (PTC124) is, for instance, an agent that leads ribosomes to ignore a PTC or stop codon obtained in a nonsense mutation, allowing translation to continue and a functional protein to be produced (Fig. 7). They are only applicable to patients with such mutations [18]. Translarna® (ataluren) was designated an orphan medicine on 27 May 2005 by the European Medicines Agency [50].

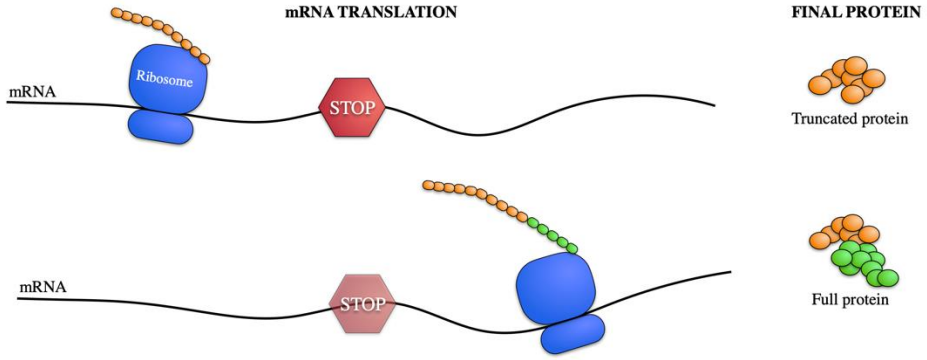

Figure 7. Mechanisms of action of readthrough agents. Created by the Authors.

Since exon deletions account for about 60-70% of total DMD cases, there are many ongoing clinical trials aiming to restore the normal dystrophin mRNA reading frame by skipping certain exons, which allows one or more pre-mRNA exons to be omitted to restore the dystrophin reading frame (Fig. 8). This approach uses antisense oligonucleotides (ASOs), which are designed to bind and modulate RNA splicing. ASOs can bind to mRNA and reduce the levels of toxic protein production by translational arrest due to blocked binding of ribosomal subunits [36].

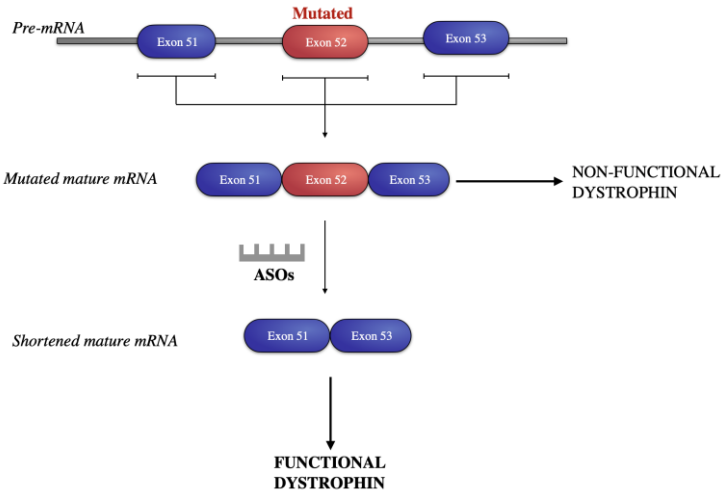

Figure 8. Exon skipping mechanism. Created by the Authors.

Gene replacement therapy is also a studied alternative. It consists of transferring a functional copy of the dystrophin gene to restore muscle strength in DMD patients. However, the large size of the DMD gene makes it difficult to compact. In this context, the construction of mini- or micro-dystrophins is investigated to develop treatments for DMD [36] (Fig. 9). Some researchers study the safety and efficacy of the mini-dystrophin gene transferred into the biceps muscle for Duchenne muscular dystrophy patients using an adeno-associated virus (AAV) as a vector [51]. With the progress of virus-mediated gene therapies, it is likely that life expectancy, symptom development and treatment duration will change considerably for DMD patients [52].
Gene editing is a promising therapy, namely CRISPR-Cas9 gene editing technology, which enables precise and efficient DNA modification and has emerged as a potential therapeutic tool for the long-lasting correction of disease-causing variants [53]. The system includes three types of gene editing: deletion of the DNA fragment, i.e., base pair at the origin of the mutation; deletion of a larger DNA fragment using two gRNA targeting separate sites; gene correction, with the insertion of a new repair DNA sequence or a new gene with the CRISPR/Cas9 machinery, allows the cell to correct a gene, or even insert a new gene.

Cell biology-based therapies. Researchers focus their attention on therapies that aim to up-regulate an endogenous analogue of dystrophin and transplant stem cells, among other techniques.
Uthrophin is a protein that shares 80% sequence identity with dystrophin and is expressed in muscle during embryonic development [54], i.e., it is an endogenous analogue of dystrophin that can be upregulated to decrease the effects of the condition by improving skeletal muscle function. Some pharmacological agents have been found to upregulate utrophin expression and act in a protective manner in animal

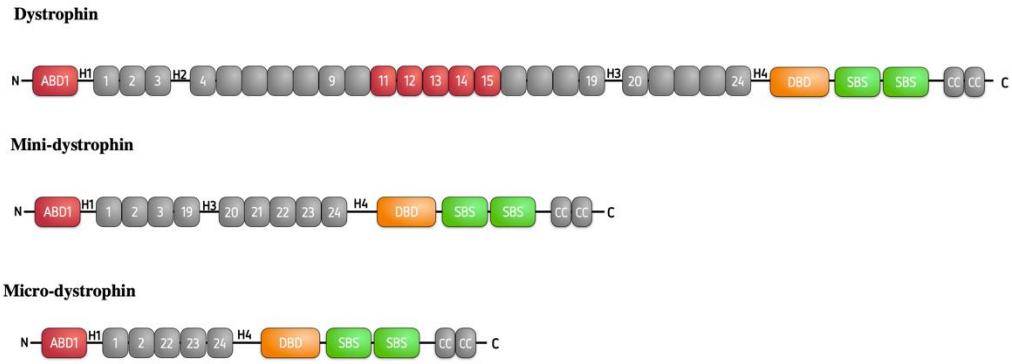

Figure 9. Dystrophin (11 kb) and its variations: mini-dystrophin (6 kb) with a deletion of H2-R18; micro-dystrophin (about 4 kb) with a deletion of R3-R21 and C-terminus. Created by the Authors.

models of DMD. One such utrophin modulator, Ezutromid (SMT C1100), has been shown to be safe for healthy adult men [49].
Stem cell transplantation can also be used to deliver a normal dystrophin (without the mutation) to dystrophic muscle. The transplanted cells must be able to proliferate, differentiate and integrate into the diseased host muscle [36] and aim to repair the damaged organ or to replenish the satellite cell group (precursor of myocytes) and allow dystrophin expression [49]. Cell types commonly transplanted are satellite cells, pluripotent stem cells, mesangioblasts, myoendothelial cells, and satellite cell-derived myoblasts. Cells can be delivered via multiple intramuscular injections, repeated cell delivery or systemic delivery [36].

There are many genetic and biomolecular therapies under investigation, some of them already used in humans, but few have reached the market. The results of these investigations have paved the way for therapies with direct consequences on the predictability of evolution, survival and quality of life of those affected. Some of the motion-capture technologies used in the production of director James Cameron's film, "Avatar 2" (which premiered in December 2022), are also part of the research, as explained in the article by Ricotti *et al.* (published in January 2023) [55], to enable improved diagnosis and assessment of the predictability of disease progression and monitoring of the effects of treatments currently being tested.

Conclusions

Duchenne Muscular Dystrophy is an X-linked recessive congenital disease that affects the DMD gene, the largest gene in the human genome, through several types of mutations responsible for the formation of an incomplete, amputated and non-functional dystrophin.
Dystrophin is a fundamental protein for the cytoskeleton of muscle tissues that provides stability to the cell and prevents damage caused by muscle contraction, being a bridge between the intracellular matrix of the myocyte through actin, and the extracellular matrix through the protein complex associated with dystrophin. When dystrophin is shortened and non-functional, it causes significant cellular damage that, initially, is compensated by cellular regeneration mechanisms, but which over time are unable to keep up with the degeneration of myocytes, causing the deposition of fibrous and fatty tissue in the sarcolemma.
It can be concluded that there is information in non-randomized studies about the effect of interventions on DMD mutations and that there are few therapies available for DMD mutations.
By combining information from non-randomized studies with information available from randomized studies on the effect of interventions on DMD mutations, DNA analyses and advances in biomolecular science are confirmed and represent the basis of therapies with a direct impact on the predictability of disease progression.
The importance of the topic suggests the need for future investigations that can provide greater life expectancy for those affected and a better quality of life in terms of useful life expectancy.

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Author Contributions

MM and PR conceptualized the work, defined the methodology, reviewed, and edited the draft. MM allocated resources, drafted the manuscript and was responsible for its visualization. PR supervised the work. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare no competing interests.

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