Review Article

A Comprehensive Review of Duchenne Muscular Dystrophy: Genetics, Clinical Presentation, Diagnosis, and Treatment

ABSTRACT

Duchenne Muscular Dystrophy (DMD) is a genetic disorder involving progressive muscle deterioration leading to loss of mobility, cardiomyopathy, and respiratory complications leading to an early death by the fourth decade of life. Males are affected more often as DMD results from a mutation in the dystrophin gene residing on the X chromosome. The DMD genetic mutation results in a complete functional lack of dystrophin, which culminates as an inadequate connection between the intracellular actin filaments and the extracellular skeleton of muscle. Boys affected by DMD clinically present with muscle weakness before age five, are often wheelchair-bound by age 12, and rarely survive beyond the third decade of life.

Traditional treatment strategies have focused primarily on quality-of-life improvement and have included the use of glucocorticoids and physical therapy. No cure currently exists, however many novel treatments for DMD are currently being explored. Some of these involve gene therapy, exon skipping, stop codon skipping, CRISPR technology interventions, and the use of a retinal dystrophin isoform. In this comprehensive review, we recapitulate the literature findings to summarize the history, epidemiology, genetics, clinical presentation, diagnosis, and current and future strategies for the treatment of Duchenne Muscular Dystrophy.

Keywords: Duchenne Muscular Dystrophy; Dystrophin; Retinal Dystrophin; Antisense Oligonucleotide; Treatments; Diagnosis; Symptoms; Genetics

INTRODUCTION

Duchenne Muscular Dystrophy (DMD) is an X-linked recessive disease caused by a mutation in the *DMD* gene encoding for the dystrophin protein. DMD is characterized pathologically as a complete absence of the cytoskeletal protein [1]. DMD is characterized clinically by progressive muscle weakness with the distribution of predominant muscle fragility in the proximal extremities, neck, and chest [2]. DMD is the most common of the muscular dystrophies, and one of the most common fatal neuromuscular disorders, affecting 1 in 3,500 newborn boys [3]. The clinical presentation begins early in childhood with progressive muscle wasting and weakness, eventually leading to death. The protein defect is present at birth but is not typically clinically observed and diagnosed until the second or third year of life. This disease eventually leads to the inability to walk with an associated wheelchair requirement around age 12, severe scoliosis secondary to muscle weakness,

and eventual death due to cardiac and/or respiratory failure by the mid-twenties, especially in those patients not choosing ventilator support [2].

Edward Meryon was the first physician to link a disorder of muscle to the symptomology of DMD in 1851 [4]. DMD was later comprehensively described by Guillaume-Benjamin-Amand Duchenne in 1868 [5]. Over 100 years later, the dystrophin gene and protein were uncovered in 1986 and 1987, respectively [6–8]. By linking the clinical picture of the disease with its molecular basis, researchers were able to piece together the pathophysiology of DMD. The human *DMD* gene resides at locus Xp21.2 and produces a rod-shaped cytoplasmic structural protein primarily in skeletal muscle with isoforms in cardiac muscle, smooth muscle, brain nerve cells, and the retina [9–11]. The *DMD* gene is 2.3 Mb [12] in humans with 79 exons [13], producing a 14 kb RNA and 427 kDa protein [10].

One-third of DMD cases are due to a *de novo* mutation, and two-thirds of cases are of familial origin, usually from a female carrier [14]. With the common introduction of the disease through a carrier mother, the pedigree would indicate 50% of male children being affected with DMD and 50% of female children being unaffected carriers of DMD. Becker Muscular Dystrophy (BMD) is a less severe form of muscular dystrophy in which similar symptoms appear but with a slower and less severe progression [15]. Statistical analysis found the global prevalence of DMD to be three times higher than the rate of BMD [16]. Global DMD prevalence is around 7.1 per 100,000 males and 2.8 per 100,000 of the general population. DMD incidence was found to be 19.8 per 100,000 live male births [17]. It was found that from 2006 to 2015 in the U.S., 3,256 deaths were due to DMD in the male population under 40 years of age, with 71% of these mortalities being confined within the age range of 15 to 29 years of age [18].

Though it is unusual for X-linked disorders to demonstrate different frequencies across different racial groups, muscular dystrophies (MD) have drastically different affected racial populations. It appears MD are statistically higher in the Caucasian male population than males in other racial groups. Caucasian American males experience an average annual death rate of 0.43 per 100,000 from MD, compared to that of African American males, which have a rate of 0.28 per 100,000 [18]. Native Americans and Alaska Natives are found to have 0.20 deaths per 100,000 and Asian and Pacific Islanders with 0.21 deaths per 100,000. Hispanic white and non-Hispanic whites also had different rates of MD incidences. Hispanic whites have a death rate from MD of 0.31 per 100,000, as compared to non-Hispanic whites with a rate of death of 0.46 per 100,000 [18].

Children born with DMD are of normal height and weight at birth but often fall below the expected growth curves during early childhood. Boys born to families without a past medical history of DMD are normally diagnosed during the fourth year of life. Dystrophic myopathy presents with rapid, progressive muscle degeneration and a loss of functional skeletal muscle over time. Motor delays or abnormal gait are the most common presenting symptoms [19]. Other presentations may include communication disorders such as language delays, or overall global developmental delays [19]. Incidental lab findings may also lead to the diagnosis of DMD, such as elevated serum creatine kinase or transaminase levels [19].

MOLECULAR PATHOLOGY OF DMD DISEASE

The *DMD* gene is located at Xp21 which affects the sarcolemma protein dystrophin [20]. The immense dystrophin gene is constructed of 79 exons and spans more than 2,200 kb, making up 0.1% of the entire human genome [21]. Full-length dystrophin is a 427 kDa structural protein in muscle tissue, responsible for the integrity of muscle cells. This muscle isoform links integral membrane proteins to the actin cytoskeleton and is thought to stabilize the sarcolemma during muscle activity [22]. The dystrophin protein contains four functional

domains: an N-terminus actin-binding domain (ABD1), a long spectrin-like repeat domain, a cysteine-rich domain, and a C-terminus domain [10,22]. The ABD1 domain binds to F-actin filament and cytokeratin 19 (K-19) in muscle [23–25]. The long spectrin-like repeat is the flexible cytoskeletal central rod portion of the dystrophin protein, which also contains a second actin-binding site, namely an actin-binding motif (ABD2) providing further actin-binding ability [23,26]. The cysteine-rich domain interacts with syntrophin proteins [27,28] and the C-terminus interacts with the dystrophin-associated glycoprotein complex which includes sarcoglycans and dystroglycans, dystrobrevin, and syntrophin [27,29–31] (Figure 1). In addition, dystrophin binds to neuronal nitric oxide synthetase (nNOS) in the sarcolemma [32]. nNOS produces nitric oxide which mediates vasodilation of muscle blood supply, playing a role in muscle fatigue [33].

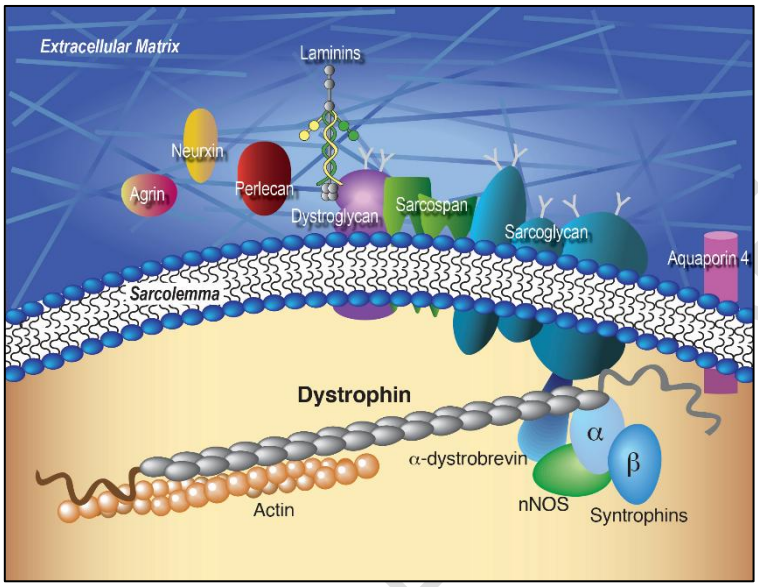

Figure 1. Dystrophin-Glycoprotein Complex. Dystrophin interacts with numerous proteins of the cell including actin, dystrobrevin-alpha, syntrophins. The glycoprotein complex also includes sarcoglycans and dystroglycans, and nitric oxide synthetase (nNOS). This complex provides membrane stabilization in muscle cells.

The *DMD* gene includes at least five independent promotors that transcribe specific alternative first exons for each promoter. Three of these promotors express full-length dystrophin proteins while the remaining two promotors occur near the coding sequence for the C-terminus, containing the last domain [22]. Several surrogate forms arise from alternative splicing of six exons of the C-terminus. This great structural diversity of dystrophin suggests this protein may serve several distinct anatomical, genetic, and biochemical functions. The *DMD* gene produces several diverse isoforms driven by different promoters including Dp427 (dystrophin protein 427 KDA) in muscle, lymphoblasts, brain, Purkinje cells of the cerebellum, Dp260 in the retina, Dp140 in the brain and kidney, Dp116 in Schwann cells, Dp71 in brain and retina, and Dp40 in the brain [34–39].

In muscle cells, the dystrophin complex localizes at the cell membrane and connects the intracellular cytoskeleton to the extracellular matrix. It has been hypothesized that dystrophin acts as a membrane stabilizer during muscle contraction to help prevent contraction-induced damage [21]. Dystrophin functions to provide stability and structure between the actin filaments and the sarcolemma of muscle, this structure also being involved in cellular

signaling [40–42]. This primary function provides mechanical reinforcement to the sarcolemma and helps protect the cell membrane from the stress established during cycles of muscle contraction and relaxation [41]. These interactions between various proteins could induce conformational changes in calcium channels, resulting in increased calcium channel activity. Enhanced calcium influx can cause oxidative stress in muscle cells, which can progress to mitochondrial dysfunction and result in cell apoptosis [43]. Due to this increased calcium influx in DMD, measurements of heightened intracellular calcium are an important initial clinical finding in DMD [2]. Therefore, it can be concluded that interactions between sarcolemma-associated proteins, like dystrophin, and calcium channels are relevant to the pathophysiology of muscular dystrophy. In DMD, the functional loss of dystrophin results in the destabilization of the entire dystrophin-glycoprotein complex. There has been additional new evidence that an error in the assembly of the nuclear lamina could be a shared feature of muscular dystrophy and that in addition to dystrophin's structural role, the dystrophin complex could play a role in cellular signaling for activities such as mechanical force transduction and cell adhesion [21]. In the absence of dystrophin, severe muscle dysfunction, and wasting occur, as seen in DMD [44]. With only reduced dystrophin, as in BMD, similar symptoms appear with a slower and less severe progression [45].

DMD GENETIC MUTATIONS

The loss of dystrophin protein in DMD occurs via various genetic mechanisms affecting dystrophin expression. Several exons have been described as deletion hotspots for muscular dystrophy diseases, including exons 3-19 and 42-60 [46]. The possible genetic mutations that can cause DMD is vast. These include deletions, duplications, insertions, and nucleotide changes. However, 50-70% of DMD mutations are large deletions, of which most are deletions that span one or multiple exons [46,47]. Next most prevalent (~20%) genetic changes are small deletions, insertions, and nucleotide point mutation changes that result in stop codons. The next category of genetic mutations in DMD is large duplications, making up ~12% of diagnosed cases. Any left-over genetic mutations fall into the division of subexonic insertions, deletions, splice mutations, and missense mutations [47]. There appears to be no correlation between the size of the gene deletions in DMD and the severity of the disease [48].

It was noticed early in muscular dystrophy studies that patients could present with larger or overlapping deletions in BMD patients, but express milder phenotypes than DMD patients [22]. This discovery led to the reading frame hypothesis which explained this phenomenon. When patients possess a dystrophin interstitial deletion that preserves the reading frame, the resulting transcription produces a shortened protein that expresses relatively normal function [49]. This contrasts with small deletions that shift the reading frame and result in a short protein with no anatomical function due to a premature stop codon. The deletion of any set of exons has been shown to result in a diverse range of clinical symptoms. However, different phenotypes have been associated with deletions in distinct dystrophin domains, creating a pathofunctional map of the dystrophin protein [22].

Large N terminus deletions typically lead to variable but serious muscular dystrophy phenotypes. Proximal rod domain deletions result in mild or atypical muscular dystrophy phenotypes, possibly proving this domain relatively expendable. Distal rod deletions are usually present with BMD phenotypes. However, DMD usually results from deletions of the C terminus domain [50]. DMD patients' western blots display quantitative evidence of little or no dystrophin protein present in their cells. This complete lack of dystrophin contrasts with patients that have moderately reduced dystrophin levels and result in phenotypes with milder forms of muscular dystrophin diseases such as BMD or mild atypical myopathy [22]. The difference in phenotype between DMD and BMD appears to be due to reading frame disruption [51,52]. An out-of-frame deletion of *DMD* typically produces an unstable mRNA

and shortened dystrophin, which is eventually destroyed. This destruction leads to the DMD phenotype and complete lack of dystrophin [51]. The BMD phenotype is the result of in-frame deletions, maintaining a functional but less-effective dystrophin which is smaller in size than normal dystrophin and is usually expressed in lower amounts [51]. The mutated protein transcribed from these frameshift mutations is vulnerable to the protective mechanism of nonsense-mediated mRNA decay. This surveillance pathway serves to reduce gene expression errors caused by a premature stop codon. The products of the transcription that surpass this mechanism are unstable due to the carboxy-terminal truncated protein and are commonly degraded, leaving little or no dystrophin protein produced in the cell [46].

These phenotypes suggest that not only is the location of deletion important for determining the severity of muscular dystrophy diseases, but the overall levels of dystrophin expression and function also play a crucial role. This explains not only the broad range of dystrophin isoforms but also the extremely diverse clinical phenotypes associated with muscular dystrophin diseases.

Almost two-thirds of DMD mutations are genetic deletions that span one or multiple exons [46]. As previously discussed, these deletions tend to group around two mutational hotspots. The most common deletion is exon 45 to exon 55, representing up to 74% of deletions, which removes a central portion of the rod domain [52]. The second most common deletion hotspot accounts for around 15% of deletions and is located at exon 3 to exon 19, which removes all or part of the actin-binding domain and a portion of the rod domain [46]. Possible deletions in DMD patients are quite heterogeneous, with the top 21 most common deletions accounting for only 45% of deletions expressed in DMD patients [53].

The distribution of deletion breakpoints is greatly disseminated in the proximal region deletion hotspot and the distal deletion hotspot, which only contain a few introns. This proximal deletion hotspot distribution accounts for the high diversity in possible deletion events in the dystrophin protein. There have been 30 observed breakpoint sites, but the most frequent deletion breakpoints occur at intron 44 and account for 20% of these deletion breakpoints [52]. Intron 47 is the second most common site, making up ten percent of these starting breakpoints. Intron 50 is the third most common breakpoint site and comprises only eight percent of the deletion breakpoints [53].

Small deletions are the second most frequent type of mutation, accounting for around 22% of all mutations. Of the total number of small deletions, 40% result in a nonsense mutation, 32% result in frameshift mutations, 27% result in splice site mutations, and 1% to 2% result in missense mutations [52]. The clinical severity of these small deletions depends on the occurrence of reading frame disruptions and the point of premature termination of dystrophin protein synthesis.

Nonsense mutations make up 40% of small deletions, which translates to around 9% of all mutations. Transitions are the most common mutational event in nonsense mutations and lead to premature stop codons, with transversions being the second most common. The most typical codon substitution is T instead of C, resulting in the change of arginine (CGA codon) to the stop codon TGA. Due to its ability to undergo oxidative deamination, the CpG dinucleotide is one of the most common hotspots for mutations in the human population [52]. Nearly 44% of these substitutions involve the CpG dinucleotide, with the most common being located at exon 66.

The second most common independent small deletion is a frameshift mutation. However, frameshift mutations can result from small deletions, insertions, or duplications of several nucleotides. The most common frameshift mutation patterns involve one to four nucleotides.

Splice mutations are the third most common small deletion. However, splice mutations rarely affect sites other than the two highly conserved “AG” and “GT” dinucleotides in DMD patients [52]. As stated earlier, missense mutations are extremely rare in DMD patients. However, when missense mutations do occur, they cause severe phenotypes regardless of the retention of some dystrophin protein and dystrophin-associated proteins.

Gene duplications in DMD patients express even greater diversity than that of DMD deletions. Nearly 120 distinct large duplications have been identified and reported in DMD patients, with 70% of those having only been detected once [52]. The most common duplication is located at exon 2. This duplication is noted exclusively in DMD patients and while being the most common, accounts for only 9.8% of discovered duplications in DMD. Of the identified duplications, 98% of starting duplication breakpoints occur at intron 1 [52]. However, in contrast to deletions, there were no discrepancies in duplication sizes identified according to their location within the gene.

The reading frame rule holds true for 96% of DMD patient mutations. The 4% exception to this rule consists mainly of in-frame deletions starting at exon 3 in the actin-binding domain or in-frame duplications in the cysteine-rich domain [51]. This rule also explains the differences in skeletal versus cardiac muscle phenotypes due to differences in mRNA processing or loss of domains that have specific functions in each of these tissues.

The correlations between DMD patient phenotype and molecular deletion imply that the two exons directly following the deletion mutation are spliced together, resulting in the synthesis of modified mRNA and hence, the mutated dystrophin protein [54]. Deletions of both the cysteine-rich domain and the C terminus domain result in the DMD phenotype. In addition, both the cysteine-rich domain and C terminus domain have been highly conserved in evolution, suggesting the importance of these domains for proper dystrophin function [52]. The pathofunctional map formulated from DMD deletion analysis emphasizes the importance of the cysteine-rich and C terminus domain and illustrates that mutations of these domains are far more detrimental to the function of the dystrophin protein than are the N terminus and rod domain mutations.

Female carriers of a mutated *DMD* gene may show some symptoms related to carrier status, a status that can arise from a variety of genetic mechanisms including normal karyotype with skewed X chromosome-inactivation resulting in lower dystrophin levels, 45X, or Turner mosaic karyotypes, balanced X-autosome translocations with breakpoints in Xp21 within *DMD*, and uniparental disomy of the X chromosome [55–57]. Manifesting female carriers can exhibit minor muscle weakness and cardiac issues due to the process noted [58].

Animal models have uncovered some important cellular findings in the study of the etiology of DMD. The *mdx* mouse model confers a mild muscular dystrophy phenotype due to the loss of myofibril membrane stability [41]. It is thought that a mouse-specific dystrophin homolog, utrophin, compensates for the lack of dystrophin in *mdx* mice [59]. Indeed, double-knockout mice of dystrophin and utrophin have a more severe muscular dystrophy, which can be rescued via transgene expression of utrophin [60]. Moreover, *mdx* mice lose muscle nNOS, leading to muscle fatigue after exercise [61–63]. Damage of the muscle membrane might lead to increased calcium entry into muscle cells, exacerbating intracellular damage. Also, increased inflammation following muscle damage might increase inducible nitric oxide synthase (iNOS) expression, destabilizing ryanodine receptors and altering calcium homeostasis [64,65]. These mechanisms lead to increased levels of calcium from the extracellular space and the sarcoplasmic reticulum, respectively, leading to the activation of calpains and proteolysis [66,67].

Various research has elucidated the potential roles of epigenetic mechanisms in DMD. Non-coding RNAs including miRNAs and lncRNAs are upregulated and downregulated in DMD and could be potential biomarkers of disease [68]. The silencing of the *klotho* gene in *mdx* mice has been shown to play a role in muscle degeneration and fibrosis [69,70]. It also appears that histone deacetylases and histone acetyltransferases may play a role in DMD pathogenesis in zebrafish and could be potential therapeutic targets [71]. Also, it has been shown that increased TNF-alpha and NF-kb in DMD inhibit the regenerative potential of muscle satellite cells by silencing Notch1 [72,73].

CLINICAL DISEASE PRESENTATION

DMD primarily affects young males due to the *DMD*'s gene location on the X chromosome [6]. The clinical presentation of DMD has features of muscle weakness, muscle wasting, and fatigue [74,75]. These symptoms are usually first clinically evident between ages two to six years old and progressively get worse over a patient's lifetime [76]. Five signs/symptoms classically identify the first symptoms of DMD. Gross motor delay is the most common presenting sign/symptom, found first in 42% of children with muscular dystrophies [19]. The presence of gait abnormalities such as toe-walking is also a common initial symptom, followed by delays in beginning to walk, learning problems, and speech delays. The characteristic proximal weakness of DMD affects the lower extremities earlier and more aggressively than the upper extremities, eventually requiring the use of a wheelchair (typically by age 12). Evaluation of neck flexors will usually reveal weakness, such as not being able to lift the head when supine. This finding is useful when distinguishing DMD from other milder forms of muscular dystrophies [77]. Because of progressive muscle weakness, changes in stature and movement continue throughout childhood. A waddling gait, difficulty walking and running, difficulty climbing stairs, and frequent falls are common. The clinical sign of Gowers maneuver can also be observed in patients with DMD, characterized by fulfilling the standing position from a seated or supine position on the floor by first moving to a position of all fours, raising the posterior, and "walking" their hands backward and then up their body to raise the upper half of their torso [78] (Figure 2).

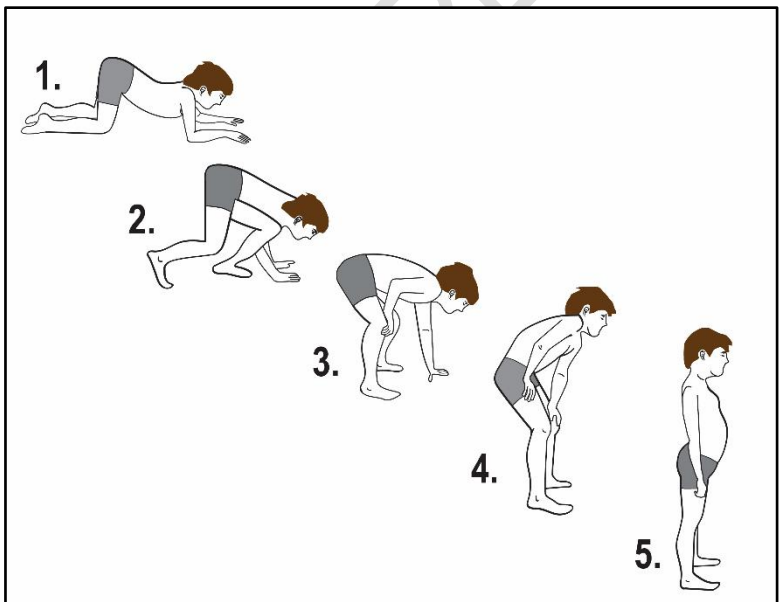

Figure 2. Gower's Sign. Gower's sign is a classic physical exam finding in DMD usually seen in children four to six years old. Weakness in lower extremity musculature forces patients to utilize their upper extremities to help arise from a prone position (1). The patient

uses their shoulders to initiate standing up (2) then uses their hands to support themselves on their legs (3). Finally, the patient moves their hands cephalad along their body to stand up (4, 5).

Spinal curvature disorders secondary to muscle weakness and inability to support the weight of the upper body are also prevalent. Scoliosis is the most common, but lordosis and kyphosis are also seen. Most patients with DMD will never be able to jump. Uniquely, DMD patients typically also present with enlarged calves, buttocks, shoulders, and tongue early in the disease course [79,80]. These enlargements begin as true tissue hypertrophy but later become pseudohypertrophy as muscle is replaced with connective and adipose tissue [19]. The movement of muscles controlled by cranial nerves is spared, with the exception of the sternocleidomastoid muscle of cranial nerve 11 [77].

The typical disease timeline of discovery by age four, wheelchair-bound by 12 years of age, and eventual death in the mid-late twenties due to cardiac or respiratory failure can be interrupted by a “honeymoon phase”. This phase takes place between the timeline of three- to six years old and provides some evidence of ephemeral improvement due to the development of normal motor functions outpacing the evolution of the disease [77]. Unfortunately, this period is short-lived and ends with the continuation of relentless deterioration.

There are several overall disease ramifications in DMD, one of which is sleep disorders. Obstructive sleep apnea is the prevailing sleep disorder during the first decade of life in one-third of patients with DMD [81]. During the second decade of life, boys with DMD develop hypoventilation during sleep, causing respiratory acidosis. There are four described stages of hypercapnic chronic respiratory failure [81]. Stage one is described as a sleep disorder without hypercapnia present. Stage two is a sleep disorder with hypercapnia that is only present in rapid eye movement (REM) sleep. Stage three is characterized as hypercapnia taking place in both REM and non-REM sleep. The last stage, stage four, occurs when hypercapnia is present during both awake and sleeping hours. The mean survival, unless respiratory support is provided, is less than 12 months after the occurrence of stage four [81].

In addition to physical complications, patients with DMD are also noted to have intellectual disabilities. These include both learning difficulties and behavioral issues [82–84]. The dystrophin protein is not only present in skeletal and cardiac striated muscle but can also be observed as isoforms in the central nervous system and retina [85]. The lack of functional dystrophin in these crucial and distinct areas of the human body plays a complicated role in how the structure is ultimately related to its function. Thirty percent of children diagnosed with DMD suffer from an intellectual impairment, having an intellectual quotient (IQ) less than or equal to 85, which is one standard deviation below the accepted population average of 100 [86]. Verbal IQ is noted to be more impaired than performance IQ, explaining the common speech delay seen in these patients. There has been no correlation found between increased intellectual disability and the severity of generalized weakness. In addition, some patients appear to have a comorbid autism spectrum disorder, attention deficit hyperactivity disorder, or obsessive-compulsive disorder [87,88]. It is important to note, however, that unlike the muscular component of the disease, the intellectual component is not progressive [89]. These patients may require specialist teachers and/or neuropsychologists and access to these specialists should be made available for DMD patients at a young age to help assess the patients' possible needs and provide guidance.

Orthopedic complications are also commonplace in patients suffering from DMD. As stated earlier, scoliosis develops in most children with DMD. The presence of scoliosis is a key

factor in the decreased pulmonary vital capacity found in DMD patients [90]. The increased incidence of falling due to muscular dystrophy and the associated muscle weakness leads to more long bone fractures in this population than in the general population [91]. Fractures are also partly due to the development of osteoporosis in most children with DMD. Osteoporosis can occur due to the continuous treatment with glucocorticoids and/or lack of weight-bearing exercise due to muscle loss. This continual loss of bone minerals such as calcium and phosphate lead to decreased bone density, resulting in frequent fractures. Osteoporosis progression begins even during the ambulatory portion of this disease process and continues throughout life.

Cardiomyopathy is an expected development in the DMD disease evolution. Patients will show cardiac symptoms including increased creatine kinase and transaminase levels along with irregular heartbeat [92–96]. Cardiac fibrosis leads to a variety of cardiac diseases including, and most commonly, dilated cardiomyopathy, resulting in the disruption of heart rate, rhythm, and conduction [96]. Cardiomyopathy usually appears in DMD patients between the ages of 10-15 years of age and is the second most direct cause of death, yet some sources suggest it is becoming the leading cause of death as quality-of-life care and ventilator support has led to longer lifespans with more opportunity to develop cardiomyopathy [97]. Cardiac pathology can present as early as ten years of age and is present in all patients over 18 years old. Though cardiac involvement is prevalent among the DMD population, about three-fourths of patients under the age of 18 are asymptomatic due to the skeletal muscle disease involvement causing physical inactivity outpacing the cardiac muscle pathology [96].

Respiratory complications are eventually present in all patients with DMD and commonly are associated with the cause of death. Respiratory complications result from weakness in the diaphragm and accessory breathing muscles [98–100]. Restrictive lung disease is inevitable in all DMD patients and leads to chronic respiratory insufficiency [101]. Vital capacity (VC) is regularly evaluated in DMD patients to monitor respiratory health throughout their life. In boys with DMD, VC usually increases until around age ten years and then decreases at a rate of 8-12% per year [101]. Normal VC in healthy patients is between three to five liters. Once a DMD patient's VC reaches less than one liter, there is a substantial risk of death within the next two years [101]. Dysphagia is also present later in DMD [102–104].

DIAGNOSIS

Accurate diagnosis aims to allow for earlier diagnostic interventions and reduce the length and severity of the symptomatic portion of DMD. Muscular dystrophy diagnosis should be completed by a neuromuscular specialist who can investigate both clinical and diagnostic tests and conclusively interpret the results. Referral to a geneticist and genetic counselor after diagnosis is suggested to increase knowledge and support for the patient and family when moving forward with the disease reality [2].

The diagnosis of DMD is usually first suspected when a child exhibits muscle weakness, Gowers' sign (a clinical observation in which DMD patients 'walk up' their legs to attain a standing position) [78], scoliosis, difficulty walking, or other symptoms listed in the previous section. The diagnosis of DMD can be confirmed by many laboratory tests including blood tests for elevated creatine kinase levels [93], multiplex PCR [105,106], and high throughput DNA sequencing [107].

As innovative technologies emerge, genetic counseling and prenatal diagnosis are becoming utilized more often. Prior to delivery, almost all fetal muscular dystrophies can now be discovered using these technologies. Prenatal screening includes techniques such as amniocentesis and chorionic villus sampling [108–110] and relative haplotype dosage

(RHDO) analysis of maternal plasma to sequence the fetus genome from cell-free fetal DNA fragments in maternal plasma [111–113]. Preimplantation genetics can also be used by removing the blastomere following in-vitro fertilization, followed by DNA extraction and analysis [114,115].

One of the initial diagnostic tools used when DMD is suspected is a serum creatine kinase (CK) test [2]. Blood CK levels can be elevated ten times the normal values in newborns with a dystrophin disease [116]. Blood sample measurements of serum CK is a straightforward and inexpensive test that can be performed in most hospitals. DMD serum CK levels are elevated from birth and detection at this time will help provide early diagnostic intervention and improve patient quality of life over the course of the disease.

The use of electromyography (EMG) was once a traditionally popular method of diagnosing DMD. EMG measures skeletal muscle response to nerve stimulation by recording the electrical activity of target muscle cells. This diagnostic test helps establish the myopathic properties of the existing dystrophy and can exclude diseases with neurogenic causes and peripheral nerve disorders [2]. Patients with DMD EMG test results will show complex repetitive discharges. These complex repetitive discharges appear due to abnormal and spontaneous action potentials associated with cell membrane instability. The muscle membrane instability detected by EMG is indicative of muscle pathology and is a hallmark of dystrophinopathies [117]. However, due to the EMG test being an invasive technique, it is an unpopular analysis to perform on children and is not thought to be necessary for a definitive diagnosis.

Another common method to diagnose DMD is a muscle biopsy to observe skeletal muscle histology. Muscle biopsy and dystrophin analysis can be useful to confirm a DMD diagnosis when genetic testing is negative or a genetic variant of unknown significance is revealed [118]. All dystrophies share histological findings of abnormal variations in muscle fiber size and necrosis, presence of tissue macrophages, and degree of replacement of muscle mass by connective and adipose tissue depending on the stage of the disease [2]. An open muscle biopsy must be performed to ensure adequate amounts of muscle tissue are available to determine the type of muscular dystrophy. However, a needle biopsy may be appropriate if the differential diagnosis includes only DMD [119]. Once the muscle sample is obtained, the main analysis for DMD includes immunocytochemistry and immunoblotting for the dystrophin protein [120]. It is crucial to differentiate between the total or partial absence of the dystrophin protein to conclusively distinguish between DMD and other dystrophinopathy phenotypes. Patients with DMD will have no dystrophin on a western blot (or nearly none), while BMD patients will have reduced dystrophin that is smaller (80%), normal size (15%), or larger (five percent) [121]. In DMD patients, the finding of less than five percent of normal dystrophin is diagnostic [1,122]. In BMD patients, a finding of 20-100% of normal dystrophin levels are diagnostic [1,122]. Genetic testing is required after a positive muscle biopsy diagnosis of DMD.

Some providers and patients may find it appropriate to omit the muscle biopsy test in favor of continued clinical investigation with genetic testing due to the invasive and painful nature of the biopsy. If genetic testing is performed and no mutation is identified in a patient with elevated serum CK levels and DMD symptoms, the next diagnostic evaluation would be a muscle biopsy. Newer clinical techniques have also been explored for the diagnosis of DMD. The use of MRI (magnetic resonance imaging) and ultrasound to evaluate muscle size and progressive changes have been proposed as novel non-invasive techniques for DMD diagnosis [123,124].

Genetic diagnostic testing is designed to perform a conclusive quantitative analysis of dystrophin genes to identify most mutations resulting in the DMD phenotype, including common deletions and duplications. Testing for large deletions or duplications should be done first since they are more common (70-80% of cases) than point mutations (20-30% of cases) [125,126]. If the minimal genetic diagnostic testing reveals nothing, it will be followed by a full gene sequencing qualitative approach [49]. This qualitative analysis is completed by sequencing the entire coding region of the dystrophin gene with the aim of detecting small mutations such as small deletions, single base substitutions, and splicing mutations. Common genetic diagnostic tests include identifying dystrophin mutations via multiplex PCR, multiplex ligation-dependent probe amplification, comparative genomic hybridization, and next-generation sequencing.

Multiplex PCR amplifies fragments of the dystrophin gene and analyzes them simultaneously. The benefits of using the multiplex PCR diagnostic tool are its wide availability and lack of expense. However, this method only detects deletions, so can only be used as a first step diagnostic tool [120]. If the result is negative, another quantitative diagnostic technique will have to be performed. Multiplex PCR also does not cover the whole dystrophin gene, but rather can only identify up to ten different exons in a single reaction. The targeted exons are usually located at hotspots but regardless, characterization of the mutation may not be a complete picture. However, endpoints of many deletions can be determined, which provides insight into the possible phenotype and severity of the disease due to endpoint effects on the translational reading frame [127].

Multiplex ligation-dependent probe amplification (MLPA) is currently one of the most widely used quantitative methods for identifying DMD [128]. MLPA can effectively genetically screen both symptomatic DMD patients as well as carrier females with MLPA or array-MLPA [129]. MLPA simultaneously tests all 79 *DMD* exons of the dystrophin gene and analyzes the gene for the structural modification and the presence of copy number variations through a multiplex polymerase chain reaction event. These identified copy number variations signify the presence of deletions or duplications that indicate disease.

Comparative genomic hybridization (CGH) scans the full dystrophin gene while searching for copy number variations using the oligonucleotide-based array [128]. Many array probes are used to help eliminate the possibility of false positives due to single nucleotide polymorphism. CGH can be used specifically if the diagnostic physician believes that the mutation is in an unusual location. CGH allows for the use of custom arrays that target the area of interest on the dystrophin gene but still maintain the appropriate resolution for conclusive results. The full view of the dystrophin gene allowed by the CGH diagnostic technique allows for the investigation of regions not routinely characterized such as the intron and 3’ and 5’ regions. This allows for CGH to detect unusually complex mutation arrangements and accurately define the mutation breakpoints [130]. CGH is highly valued for its dual ability to detect DMD molecular diagnosis and specific DMD gene mutations. The CGH technique is not only useful for clinical diagnosis but can also be readily used in DMD research to investigate non-coding regions for the etiopathogenesis of mutations for diagnostic implications [130]. CGH can also complete the complex task of defining breakpoints of large rearrangements to assist in the understanding and correlation of genotype versus phenotype in DMD.

Next-generation sequencing (NGS) includes a vast variety of technologies, each of which has its own unique biochemical strategy. NGS sequences millions of copies of DNA fragments simultaneously, allowing for a quick and accurate turnaround of DNA sequencing output for genetic diagnosis. This modern technology allows for the possibility of creating a diagnostic program that can identify both copy number variations and single nucleotide

variations at once. A full evaluation of the *DMD* gene requires not only a molecular quantitative analysis to detect copy number variations, but also genomic sequencing, both of which NGS can help provide. NGS also allows the remaining rare mutations that are not identified by the two previously stated methods to be diagnosed by more specific transcript analysis. One study showed NGS could accurately diagnose up to 92% of DMD/BMD patients [107]. Moreover, next-generation high throughput DNA sequencing techniques can be combined with MLPA to increase the accuracy of results [107,131,132].

Fluorescence *in situ* hybridization (FISH) can be used for large deletions and duplications but is not common [133]. Other genetic techniques used in diagnosing *DMD* deletions and duplications include qPCR, and chromosomal microarray analyses (CMA) [134–139]. If a large mutation is not detected, DMD can be genetically diagnosed via scanning for point mutations. Small point mutation detection is often done via denaturing high-performance liquid chromatography and direct sequencing [140,141].

If an analysis by one of these listed techniques uncovers and identifies the full characterization of the dystrophin mutation, then no further testing through other diagnostic techniques is advised. However, if the deletion or duplication test is negative, further sequencing should be completed on the dystrophin gene to pinpoint any small deletions or point mutations [49]. A full and complete characterization of the dystrophin mutation should be performed, locating all deletion endpoints and point mutations. This allows all the information gathered to be implemented in concluding how the patient's specific mutation will affect the dystrophin protein gene's reading frame. This reading frame correlation is the main key to determining the patient's phenotype from all the possible variations in dystrophinopathies. Furthermore, this genetic understanding may allow the patient to participate in mutation-specific gene therapies currently on trial [120].

TRADITIONAL TREATMENT STRATEGIES FOR DMD

Unfortunately, there are no current medical treatments that will significantly change the course of DMD. The only treatments currently available aim to temporarily maintain the patient's general overall health, manage symptoms, and improve the patient's quality of life. Corticosteroids, specifically glucocorticoids, have traditionally been used to slow the decline in muscle strength of DMD patients. It is believed glucocorticoid therapy helps DMD patients by reducing muscle inflammation [142]. Improvements can be measured in decreased Gowers' time, distance walking time, stair climbing time, increased weight workouts, leg function grade, and forced vital capacity volume [143]. Regrettably, the effectiveness of corticosteroids only lasts from 6-24 months and does not alter the overall mortality of DMD [143].

There are two main steroid treatments regularly recommended for DMD patients, prednisolone and deflazacort. It is suggested these steroid treatments begin at the point of complete motor development before any muscle deterioration begins [120]. Prednisolone and deflazacort are thought to be equally proficient at preserving muscle integrity, however, they each exhibit their own distinct side effects, with varying degrees of severity. Studies have found the most effective regime to be 0.75 mg/kg of prednisolone given daily [143].
The addition of a regular physical therapy regime is also important in maintaining physical functioning. Physical therapy should begin in early childhood and continue throughout life. The overarching goal of physical therapy is to help DMD patients gain and maintain muscle strength and function. Physical therapy is also thought to help prevent muscle contractures common in DMD patients [89].

Respiratory failure is a common cause of death in patients with DMD, and respiratory status should be monitored closely throughout the patient's life. Pulmonary function tests should be

conducted regularly, starting at DMD diagnosis. Each respiratory function evaluation should include oxyhemoglobin saturation and spirometry testing. Spirometry should include forced vital capacity (FVC), first-second forced expiratory volume (FEV1), maximal mid-expiratory flow rate, maximum inspiratory and expiratory pressures, and peak cough flow [144]. To avoid and monitor hypercapnia, awake carbon dioxide tension should be evaluated yearly through capnography. Patients should also be evaluated for other non-pulmonary respiratory disorders such as obstructive sleep apnea, oropharyngeal aspiration, gastroesophageal reflux, dysphagia, and asthma [144]. Patients confined to a wheelchair should also receive annual laboratory studies of complete blood counts, serum bicarbonate concentration, and chest radiography [144].

Once respiratory insufficiency is found, it can be treated with intermittent noninvasive positive-pressure ventilation (NIPPV) therapy [145]. Many patients choose elective tracheostomy when more invasive ventilatory support becomes necessary, to help ensure sufficient lung function to assist in reaching the third decade of life [2]. Antibiotics should also be prescribed at any sight of infections of the respiratory tract, to cease any further lung function deterioration. DMD patients should also expect to eventually receive ventilatory support to aid in treating hypercapnia symptoms, improve quality of life, lengthen life span, and diminish the need for emergency hospital visits [144].

Cardiac ailments are also common in DMD disease processes. Complete cardiac evaluations should be performed on children as soon as a DMD diagnosis is made and should continue throughout their lifetimes, at the minimum biannually. Commonly performed tests are electrocardiography and echocardiography. It is common for DMD patients to be late in the disease process when cardiac signs and symptoms are first noted, due to cardiac symptoms being masked for a prolonged period by physical inactivity. Symptoms of cardiac distress should be treated with a diuretic, angiotensin-converting enzyme (ACE) inhibitor, digitalis glycosides, or beta-blocker depending on the individual situation [146]. Immediate detection of cardiomyopathy in DMD is crucial for the best patient outcomes.

Any form of surgery is not recommended in the early course of the DMD disease process [147–149]. Possible detrimental outcomes from surgeries could arise from an anesthetic risk or extended periods of bed rest. Eventually, disease contractures might require treatment with surgical correction procedures to help maintain extremity ambulation. Scoliosis surgical correction, specifically through the Luque technique, is also becoming more accepted in DMD patients to improve sitting comfort and maintain full vital capacity lung volume [2]. Other surgeries may also help maintain lung function, adding an additional few extra years onto the life expectancy of DMD patients.

Although the physical characteristics of dystrophy diseases are most frequently the focus, it is important not to forget the mental hardships brought on by this disease. Four quality-of-life domains of DMD patients have been recognized and studied. These include physical health, social relationships, physiological status, and well-being [150]. The physical domain includes pain in its analysis. It has been found that patients with DMD have impaired quality of life in the physical domain that is relative and reactive to the tangible progression of DMD disease status, including the decline in ambulation over time. Though it may seem apparent, arm function has also been associated with several of the quality-of-life domains, so it is essential to keep upper limb function for as long as medically possible [150]. In addition, the social and physiological domains are also found to be more impaired than healthy children of the same age. Unfortunately, the well-being domain quality of life is also found to be lower than the general population and usually underestimated by caregivers and guardians [150]. It is suggested that along with medical interventions, patients should also be encouraged to participate in social activities to improve their quality of life. There should also be

predetermined preparation for transitioning DMD patients from pediatric medical care and educational programs into adult care to limit stress from this change.

PROGNOSIS

Over the years, advancements in science and technology have had significant effects on patients with DMD. Historically DMD patients died in their adolescent years or early twenties, however, DMD patients are now living into their thirties and occasionally into their forties [151]. This change in statistical trends increases the need for accurate and wide-scope information on outcomes for current DMD patients to better evaluate novel treatments. Eventually, death is caused most often by cardio-respiratory failure in patients that survive into their thirties and beyond, but some unexpected deaths do still occur in young patients [152]. The increasing lifespan of DMD patients has also changed the classification of this disease. Now with patients surviving well into adulthood, DMD is also considered an adulthood disease [97].

NOVEL TREATMENT STRATEGIES

Continued research has drastically changed the quality of life and outcomes of DMD patients. Many new treatments have been and are currently being investigated for novel, more efficacious approaches to treating DMD. Several areas of genetic research are currently under investigation, hopefully leading to improved treatment of DMD patients and potential cures for this harsh disease. A variety of different genetic pathways potentially leading to treatments for DMD are under examination. Novel treatment strategies include exon skipping, nonsense codon read-through, myostatin inhibitors, gene therapy through compensating protein upregulation, dystrophin transgene, microdystrophin, stem cell therapy, and clustered regularly interspaces short palindromic repeat (CRISPR). Each of these research categories provides both unique advantages and disadvantages that complicate an innovative breakthrough.

Exon skipping is an area of active research that involves inducing the skipping of specific exons to restore the reading frame of the dystrophin protein to increase dystrophin expression in DMD patients [153]. This can, in theory, lead to a truncated but potentially functional version of dystrophin. An approach being investigated is the use of antisense oligonucleotides to produce exon skipping (Figure 3). One such novel drug, eteplirsen, is an uncharged phosphorodiamidate morpholino oligomer (PMO) used to skip exon 51 in DMD patients [154]. Eteplirsen is suitable to treat DMD patients with deletions ending at exon 50 and starting at exon 52, which accounts for ~20.5% of DMD deletion mutations and ~14% of DMD patients overall [47]. Eteplirsen has been shown to increase dystrophin-positive muscle fibers in DMD patients but has had inconclusive results about its effectiveness in restoring ambulation [154–156]. The FDA granted accelerated approval for eteplirsen in 2016, and more clinical trials are underway to confirm clinical benefit [157].

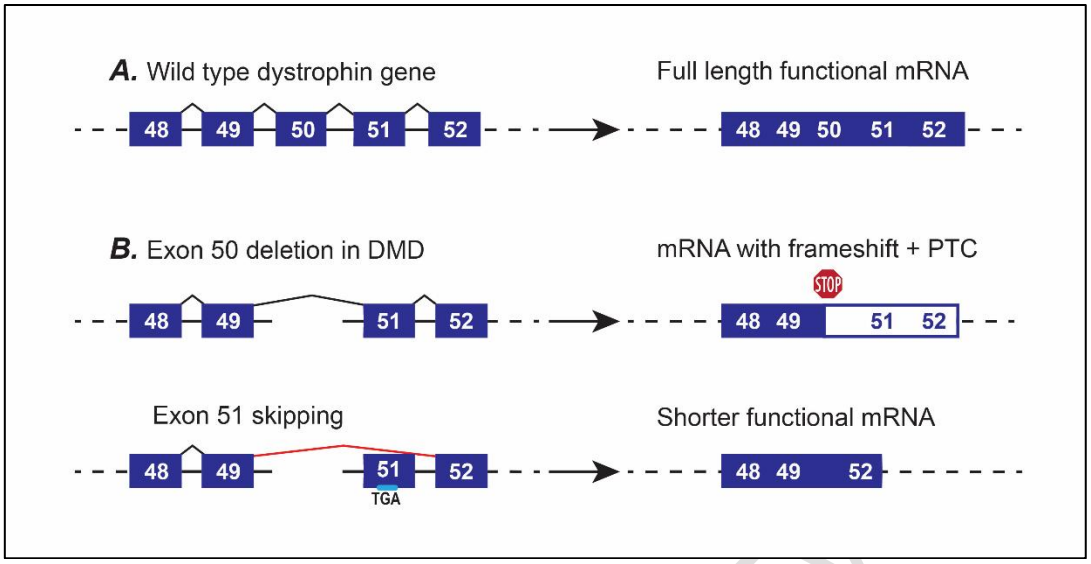

Figure 3. Exon Skipping. (A) Wild type dystrophin gene splicing with all normal functional exons. (B) Gene splicing in DMD with an exon 50 deletion of dystrophin. This results in mRNA with a premature termination codon (PTC) and non-functional dystrophin protein. Shown below is an exon therapy strategy resulting in mRNA without mutated exon 50 and PTC, yielding a shorter functional dystrophin.

Similar to eteplirsen, golodirsen is an antisense oligonucleotide of the phosphorodiamidate morpholino oligomer (PMO) that is used to induce skipping of exon 53 in dystrophin [158]. The PMO golodirsen is useful in about 7.7% of DMD patients [159] and has been shown to increase dystrophin in DMD patients, however, the results regarding improved or prolonged ambulation are inconclusive [158,160]. In 2019, the FDA granted golodirsen accelerated approval [161]. Viltolarsen is another exon 53 skipping antisense oligonucleotide currently undergoing clinical trials [162]. Likewise, casimersen is an antisense oligonucleotide for exon 45 of dystrophin that is still being investigated (Clinical Trials NCT04179409, NCT03532542). In February of 2021, casimersen was approved in the U.S.A. under the FDA Accelerated Approval Program for exon 45 skipping in DMD as it was shown to increase the amount of dystrophin in muscle. WVE-210201 (suvodirsen) is being investigated for skipping exon 51 of dystrophin [163–165]. Continued approval for this indication, as is the case with other accelerated approvals, is contingent on the results of the aforementioned clinical trials [166]. The expected phenotype after accomplishing accurate exon skipping is expected to resemble BMD and would prove a much better quality of life for DMD patients. Through the generation of specific exon skipping during mRNA splicing, antisense compounds were shown to rectify the *DMD* gene's reading frame and revitalize the dystrophin protein expression [167]. Further research is necessary to confirm the functional clinical benefit of exon skipping. Unfortunately, exon skipping might induce an innate immune response, reactivating dystrophin-specific T-cells and potentially limiting the technique's therapeutic capability [168].

Suppression of nonsense codons for DMD treatment is another major area of interest to the scientific community. Premature stop codons are typically caused by single nucleotide substitution, having dire consequences. Several compounds are under investigation for the intention of reading through or suppressing stop codons (Figure 4). Oligonucleotides are being used to repair specific mutations, such as a single nucleotide substitution. Also, aminoglycoside antibiotics such as gentamicin are known to read through nonsense codons,

potentially treating DMD caused by such mutations [169]. Ataluren is a drug that is thought to promote read-through of stop codons by insertion of near-cognate tRNAs at the stop codon site [170]. Clinical trials are ongoing for the effectiveness of ataluren in DMD, with some improvement seen with a six-minute walk test [171]. Regrettably, less than nine percent of DMD mutations are due to stop codons, causing this treatment strategy to only potentially cure a small fraction of DMD patients [47]. Also, stop codon readthrough and suppression might also cause a dystrophin-specific T-cell response, thus creating an immunogenicity obstacle in this possible treatment option [172].

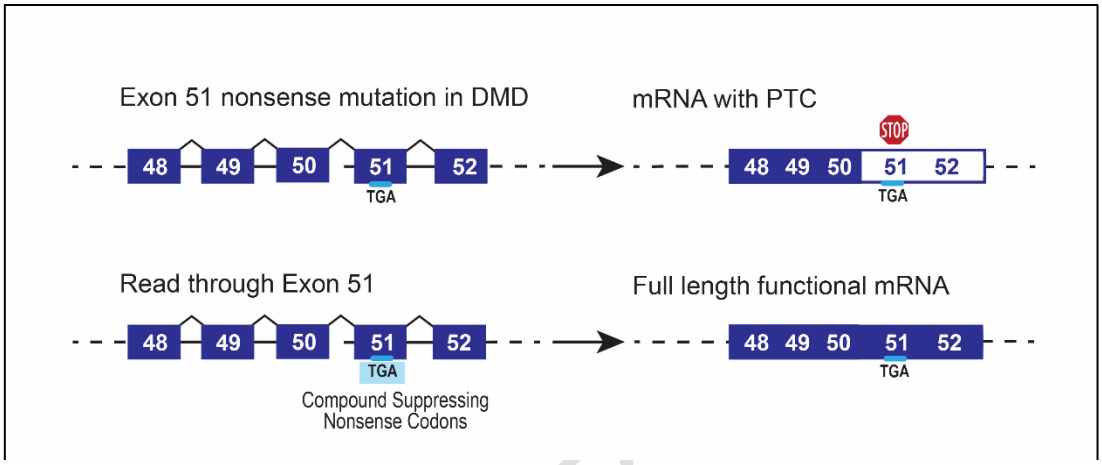

Figure 4. Stop Codon Read-Through. Gene splicing in DMD with a missense mutation in exon 51 of dystrophin results in premature termination codon (PTC) and nonfunctional dystrophin. Stop codon read-through therapy resulting in full-length functional dystrophin expression.

Targeting growth factors is another prominent area of research in DMD, specifically myostatin. Myostatin is a myokine (a protein created by myocytes in response to muscular contraction) that inhibits muscle growth and differentiation through an autocrine pathway [173]. Since DMD is a progressively degenerative process that transforms muscle fibers into necrotic tissue, mechanisms that regenerate muscle tissue could be a potential therapeutic route. Myostatin inhibition causes significant muscle hypertrophy and hyperplasia [174]. Therefore, inhibiting myostatin could potentially lead to an increase in muscle growth in DMD patients. Mice models have suggested the efficacy of this approach and current clinical trials are underway to investigate the usefulness of myostatin inhibitors in DMD patients [175–178]. The use of follistatin, an endogenous myostatin inhibitor, is also being investigated [179,180]. In addition, selective androgen receptor modulators (SARMs) provide a new potential DMD treatment through androgen-driven muscle growth [181–183]. Unfortunately, though myostatin inhibitors may very well increase muscle growth in DMD patients, the regenerated muscle growth still lacks dystrophin and creates defective DMD muscle.

Gene therapy has become a new and exciting field that could transform the way medicine is approached and cure many different diseases. There are three main trains of thought when using gene therapy to treat DMD patients. First, gene therapy could recreate full-length dystrophin through a transgene. Second, activating utrophin through gene therapy, utrophin serves a similar function to dystrophin but is not genetically located on the X chromosome. Lastly, using microdystrophin to create a BMD phenotype in DMD patients. Gene therapy can, unfortunately, cause an immune response in DMD patients because these patients lack naturally occurring functional dystrophin. Inducing transgene dystrophin is recognized by the

immune system as foreign and receives the complications of that ruling [184,185]. If dystrophin transgenes are used to treat DMD patients in the future, life-long immune suppression may be required, which carries its own consequences.

Using gene therapy to create a functional dystrophin transgene has been an area of much interest in treatment for DMD. Previous research has explored utilizing adeno-associated viruses (AAV) to deliver a functional version of dystrophin to DMD patients [184]. However, the dystrophin gene has 79 exons, which is much too large to fit into a recombinant adeno-associated virus (rAAV) [3]. To combat the size barrier of dystrophin, creating a functional but miniaturized dystrophin transgene has been sought as a solution and rAAV2.5-CMV-minidystrophin was engineered [186–189]. This miniaturized dystrophin transgene has been shown to restore partial muscle strength in murine models with DMD and more research may prove this model to be a helpful treatment for DMD [190]. So far there has been no detection of dystrophin-specific antibodies in patients, making humoral immunity unlikely to affect genetic therapy in DMD. However, cellular immunity may prove to be a barrier to this treatment option. Dystrophin-specific T-cells have been detected upon treatment with a dystrophin transgene [3]. T-cell immunity has also been identified in patients that have not received transgene treatment [3]. T-cell immunity against self and foreign dystrophin epitopes could be detrimental to the development of transgene treatment in DMD patients. It must also be ensured that rAAV vectors can be delivered to all targeted muscle groups in DMD, especially the heart in which a lack of dystrophin causes cardiomyopathy. This need for widespread delivery yields its own surplus of obstacles in treatment [191,192]. Other smaller “micro-dystrophin” proteins are currently under investigation, such as SRP-9001 (NCT03769116), SGT-001 (NCT03368742), and PF-06939926 (NCT03362502) [193,194]. The use of AAV for surrogate gene therapy with GALGT2 to help express dystrophin is also being investigated (NCT03333590) [195,196].

An alternative approach to DMD treatment is to attempt to strengthen DMD patients’ muscles through modulation of other homologous or associated proteins. The upregulation of a substitutable protein for dystrophin could potentially compensate for the lack of dystrophin in DMD patients. This idea stems from the upregulation of fetal hemoglobin to treat specific hematological conditions, such as sickle cell anemia [197–200]. One potential target includes the upregulation of utrophin, a protein only expressed during fetal development [201–203]. *Mdx* mouse models have been shown to alleviate some dystrophy symptoms through the production of utrophin [204]**.** Researchers hope to find a pharmacological agent that can produce utrophin in cells to treat DMD patients. Additionally, laminin-111, a protein necessary for fetal development, has shown promise in *mdx* mice models [205]. It has also been found that an enzyme hematopoietic prostaglandin D_2 synthase (HPGDS) may play a role in the necrosis of muscle tissue in DMD and is a target of inhibition [206–208]. Through inhibition of HPGDS, it is hypothesized that DMD progression could be slowed. Another therapeutic target is sarcospan (SSPN), a transmembrane protein that interacts with dystrophin and upregulates the utrophin-glycoprotein complex [209–211]. Furthermore, inhibition of sodium/proton type 1 exchanger (NHE-1) is being investigated for its role in sodium/calcium homeostasis in DMD muscle cells [212]. Rimeporide inhibits NHE-1 and is under investigation [213–215].

An additional therapeutic target for DMD is to reduce muscle fibrosis. Muscle fibrosis has been correlated with decreased muscle function in DMD patients [216]. Currently, a connective tissue growth factor (CTGF) inhibitor FG-3019 (pamrevlumab) suggested a benefit in mouse models and is undergoing clinical trials (NCT02606136, NCT04371666). Moreover, anti-inflammatory drugs such as vamorolone (a novel steroid) and CAT-1004 (an Nf-Kb inhibitor) are being investigated for efficacy in treating DMD [217,218]. This approach builds off the current mainstay treatment of glucocorticoids for DMD treatment. An antisense

oligonucleotide, ATL1102, is under investigation for its efficacy in reducing inflammation via inhibition of CD49d on T-cells (ACTRN12618000970246). In addition, CAP-1002 consists of cardiosphere-derived cells and is under investigation for its ability to reduce inflammation and treat DMD (NCT04428476) [219].

Another novel approach to treat DMD includes attempting to increase blood flow to muscles to help prevent fatigue. In DMD patients, neuronal nitric oxide synthase (nNOS) is deficient and may play a role in fatigue [220]. The use of phosphodiesterase inhibitors in DMD patients to restore muscle blood flow is underway and has shown benefit in *mdx* nNOS-deficient mice [221–223]. In addition, research is ongoing to investigate the use of nitric oxide and sodium nitrate (a nitric oxide donor) [224–226].

Stem-cell therapy is also being examined as a prospect for DMD treatment. Some research on *mdx* mice has shown that a small number of hemopoietic stem cells can relocate from the bone marrow to affected muscle in these mice models and produce dystrophin [227]. The use of induced pluripotent stem cells (iPS) and other multipotent stem cells appear promising in mouse models, and research is ongoing in human patients [228–230]. Another approach is to use muscle satellite cells to regenerate DMD patients' skeletal muscle. Engraftment of satellite cells in *mdx* mice has proven effective and research is ongoing to use histone deacetylase (HDAC) inhibitors and WNT7a to induce satellite muscle cell differentiation and growth [231–234].

Finally, several studies have proven the efficacy of using CRISPR-Cas9 to restore high levels of dystrophin in iPSCs and murine, canine, and porcine models [235–240]. Various strategies have been employed to utilize CRISPR to correct DMD mutations. These include double-cut myoediting, single-cut myoediting, and nucleotide myoediting, each with their own potential benefits and consequences [240]. Double-cut myoediting utilizes two sgRNAs to flank a mutated exon for removal. Single-cut myoediting utilizes one sgRNA around the intron-exon junction of an exon and introduces insertions or deletions to destroy the splicing consensus sequence and cause exon skipping. Nucleotide myoediting uses base editors to repair a single point-mutation [240]. CRISPR technology has also created novel animal models for DMD [241–244]. One issue, however, is the inability to create cardiac phenotypes in animal models identical to those seen in DMD patients [244]. Creating phenotypes in animal models that match the real DMD patient phenotypes is crucial for the implementation of this technology. The creation of mosaics has also been a troubling outcome of CRISPR use in this research [245]. Additionally, immunogenicity to the AVV used to encode CRISPR presents a barrier to treatment [246]. Another area of concern is that each animal model's innate systems express certain restrictions, such as unique anatomy, physiology, and size, that cannot represent all tissue type aspects in DMD patients [247]. A possible remedy for this issue is to use different animal models to represent the different physiological stages in the progression of DMD in patients. For example, using murine models to represent the initial phase of the disease, canine models to represent DMD patients from five to ten years of age, and rabbit models for the presentation of cardiac symptoms in DMD [247]. Choosing the right animal model for each stage of the progression of DMD is important to reach clear therapeutic results.

In addition to targeting skeletal muscle dysfunction and loss, research into treating and preventing cardiomyopathy in DMD patients is underway. Clinical trials examining the effects of angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARBs), beta-blockers (NCT03779646), and diuretics in the prevention and treatment of cardiomyopathy in DMD are ongoing [248–252].

A novel pharmaceutical under investigation to treat the respiratory symptoms of DMD is idebenone, an antioxidant (NCT03603288), with some studies suggesting improvement [253–255].

ANALYSIS OF RETINAL DYSTROPHIN NOVEL THERAPY

The discovery of retinal dystrophin started with the observation that most boys with DMD have abnormal electroretinogram results [256–258]. These clinical findings led to the identification and cloning of the specific dystrophin isoform of 260 kDa. This Dp260 isoform was given the common name “retinal dystrophin” due to the natural occurrence of this protein in the plexiform layer of the retina. However, it is important to note that 20-30% of DMD patients have normal electroretinograms, signifying that these patients have a genetic mutation in their dystrophin gene upstream of the Dp260 isoform and therefore express retinal dystrophin in their eyes [259].

Retinal dystrophin is an isoform of the full-length dystrophin protein and is related to muscle dystrophin. Retinal dystrophin has four functional domains and is classified as mini dystrophin [260]. Interestingly, retinal dystrophin contains the equivalent of all of the protein binding sites found in normal muscle dystrophin [260]. These Dp260 binding sites include an actin side-binding site located in the rod domain, the dystroglycan-sarcoglycan complex site in the cysteine-rich domain, and the syntrophin sites in the C-terminal domain [260]. Dp260 is primarily expressed in the retina but has been detected in other tissues through PCR, including the brain and cardiac tissue of mice. However, it is not expressed in the muscle tissue of mice [260].

To overcome the immunogenicity barrier presented in AAV therapy, White et. al have undertaken efforts to investigate the potential therapeutic value of utilizing the retinal dystrophin isoform Dp260 to restore dystrophin in DMD patients [117]. Activating the expression of retinal dystrophin in skeletal and cardiac muscle in DMD patients is an exciting prospect in which the body might be able to use an endogenous protein to treat DMD.

The breakthrough of the creation of a retinal dystrophin transgene was accomplished through genetic engineering, in which the promotor for Dp260 was replaced by a muscle promoter [117]. This retinal dystrophin transgene was observed to be relatively stable in MM14 muscle cells, suggesting that the Dp260 transgene was able to bind securely to other cellular proteins and may have possibly replaced the Dp427 muscle dystrophin in these cells [117]. Overall, retinal dystrophin expression assists in a stable association between a cell’s cytoskeletal actin and sarcolemma construct and the dystrophin-glycoprotein complex and substantially slows the progression of muscle wasting in *mdx* mice [117].

Continuing research is being conducted on *mdx* and *mdx/utrn*-/- mice which express the retinal dystrophin transgene. The mice expressing the Dp260 transgene have been shown to display reduced amounts of inflammation and fibrosis in muscle tissue [117]. The *mdx/utrn*-/- Tg+ mice do experience some muscle degeneration, though greatly reduced from untreated *mdx/utrn*-/- mice [117]. The *mdx/utrn*-/- Tg+ mice experience attenuation of the skeletal and cardiac muscle phenotype common in DMD and survive the normal one-year life span, drastically increased from the *mdx/utrn*-/- lifespan of three months [117]. These results suggest that Dp260 presence in DMD diseased muscle tissue could prevent the severe consequences of this disease, resulting in a milder myopathy phenotype [261].

The retinal dystrophin transgene also seems to alleviate calcium channel dysfunction in DMD. Calcium dysregulation in DMD patients is hypothesized to cause cellular defects and possibly even cellular apoptosis in these already structurally unsound cells. Calcium channels have been observed to be defective due to the lack of dystrophin stabilization in

affected patients' cells. However, the observation of dystrophin-associated protein beta-dystroglycan co-localizing with voltage-gated calcium channels in the retina implies that retinal dystrophin may be able to bind the beta-dystroglycan in muscle cells and counteract the calcium channel defect in DMD [117]. This observation could be monumental in overcoming the calcium channel defect inflicted in DMD patients.

The medical and research communities are surrounded by different potential philosophies that will lead to the next treatment or cure. One such philosophy highlights the importance of how all the body's anatomy, physiology, immunology, etc. works together to perform high-functioning tasks. This thought highlights the potential retinal dystrophin treatment for DMD of using the body's naturally occurring resources and harnessing that creation to solve other complex issues experienced in the human body. The possibility of retinal dystrophin also possesses another clear advantage from other current research treatments for DMD. Retinal dystrophin is naturally occurring in nearly 30% of DMD patients that have an upstream mutation of the Dp260 region and will not be recognized as foreign by the immune system [117]. This innate protein in DMD patients is unlikely to cause an immune response. The probability of immunogenicity plagues other such potential DMD therapies and could cause even more obstacles for DMD patients to overcome after treatment.

Continuing research in retinal dystrophy for the treatment of DMD hopes to find a pharmaceutical able to induce Dp260 through a promotor in non-retinal cells. This retinal dystrophin expression in muscle cells might be able to attenuate the phenotype of DMD patients and allow these patients to experience a milder phenotype, as seen in other muscular dystrophies. By using naturally occurring structures in the body, treatments may be able to regenerate these complexes and unleash the brilliance of the complexity and sophistication of the human body's ability to cure itself.

CONCLUSION

DMD is a muscular disease with catastrophic and fatal consequences. DMD arises due to de novo and inherited genetic mutations causing a lack of functional dystrophin, a critical protein necessary for muscle structure and function. DMD patients lose muscle function over time, ultimately losing ambulation by age 12. Patients often die by 30-40 years of age due to respiratory failure or cardiomyopathy. Diagnosis of DMD involves prenatal screening, genetic testing, muscle biopsies, and clinical features. Currently accepted treatments include corticosteroids, physical therapy, and respiratory assistance, none of which are curative. Various novel treatment strategies are under investigation including exon skipping, nonsense codon read-through, myostatin inhibitors, stem cell therapy, CRISPR, and retinal dystrophin. Given the devastating symptoms and early fatality of DMD, new treatment options are necessary to improve the lives of these patients and more research is needed to hopefully find a cure.

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