Neuromuscular Disorders 39 (2024) 5–9

Contents lists available at ScienceDirect

Neuromuscular Disorders

journal homepage: www.elsevier.com/locate/nmd

Long-term clinical follow-up of a family with Becker muscular dystrophy associated with a large deletion in the *DMD* gene

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ARTICLE INFO

Article history:
Received 8 March 2024
Revised 3 April 2024
Accepted 13 April 2024

Keywords:
Duchenne muscular dystrophy
Dystrophin
Gene therapy

ABSTRACT

Duchenne muscular dystrophy is a neuromuscular disease caused by *DMD* gene mutations that result in an absence of functional dystrophin protein. Patients with Duchenne experience progressive muscle weakness, are typically wheelchair dependent by their early teens, and develop respiratory and cardiac complications that lead to death in their twenties or thirties.

Becker muscular dystrophy is also caused by *DMD* gene mutations, but symptoms are less severe and progression is slower compared with Duchenne. We describe a case study of a patient with Becker muscular dystrophy who was still ambulant at age 61 years and had a milder phenotype than Duchenne, despite 46% of his *DMD* gene being missing. His affected relatives had similarly mild phenotypes and clinical courses. These data guided the understanding of the criticality of various regions of dystrophin and informed the development of micro-dystrophin constructs to compensate for the absence of functional dystrophin in Duchenne.

1. Introduction

Duchenne muscular dystrophy (DMD) is an X-linked, progressive neuromuscular disease caused by mutations in the *DMD* gene that lead to an absence of functional dystrophin protein [1,2]. DMD affects 1 in 3600 to 6000 male neonates worldwide [3,4]. Patients with DMD typically experience motor delays and muscle weakness between 2 and 7 years of age and often have decreased upper limb function, lose ambulation, and become wheelchair-dependent in their early teens [5,6]. Most people with DMD die of cardiac or respiratory failure in their twenties or thirties [1,7,8].

Currently, there is no cure for DMD, but corticosteroid therapy is beneficial in delaying disease progression. However, long-term corticosteroid use can cause serious adverse effects, such as bone fractures, infections, and gastrointestinal bleeding [9]. Other therapeutic approaches, including exon skipping and stop codon readthrough therapies, aim to promote the production of internally truncated dystrophin [10]. However, many patients with DMD have mutations that are not amenable to these treatments. Thus, there is an unmet need for more broadly effective therapies that can address the underlying cause of DMD by compensating for the absence of functional dystrophin [11].

The *DMD* gene, which contains 79 exons and encodes a large (427-kDa) cytoskeletal protein called dystrophin, is the largest protein-coding gene in the human genome [5,6]. Dystrophin interacts with a complex network of proteins known as the dystrophin-associated protein complex (DAPC) and maintains an important structural link between the intracellular cytoskeleton and the extracellular matrix (Fig. 1) [5,8,12]. Dystrophin protein consists of the N-terminal (NT) domain, which contains the binding sites for F-actin; the central rod domain, which contains 24 spectrin-like repeats (R1–R24); four hinges (H1–H4); the cysteine-rich (CR) domain; and the C-terminal domain [4,5,8,12].

Mutations in the *DMD* gene can lead to different severities of muscular dystrophy, with DMD being the most severe progressive form and Becker muscular dystrophy (BMD) showing a much milder clinical course [5]. DMD is associated with gene mutations that disrupt the reading frame or generate premature stop codons, causing deficiencies in DAPC assembly or F-actin binding and preventing the production of functional dystrophin [5,8]. However, BMD is associated with deletions that retain the reading frame, enabling the production of shortened dystrophin that retains the key functional protein domains [8,13].

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https://doi.org/10.1016/j.nmd.2024.04.004

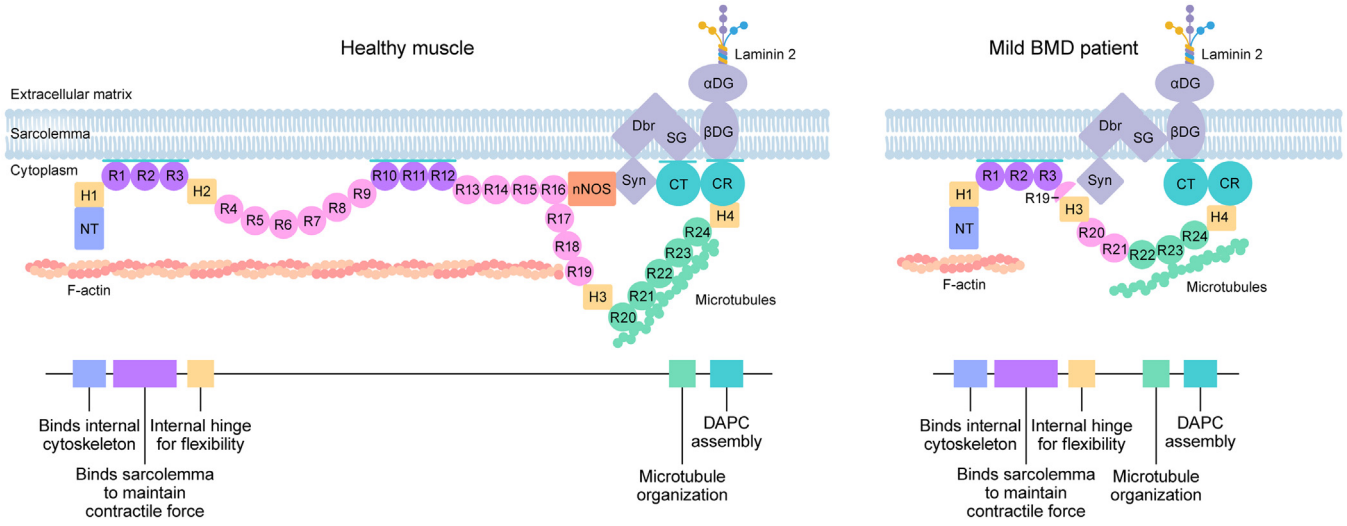

Fig. 1. Wild-type dystrophin in healthy muscle and shortened dystrophin in BMD.
αDG, alpha-dystroglycan; βDG, beta-dystroglycan; BMD, Becker muscular dystrophy; CR, cysteine rich; CT, C-terminus; DAPC, dystrophin-associated protein complex; Dbr, dystrobrevin; H, hinge; nNOS, neuronal nitric oxide synthase; NT, N-terminus; R, spectrin-like repeat; SG, sarcoglycan; Syn, syntrophin. Adapted from Zhao J, Kodippili K, Yue Y, et al. *Hum Mol Genet*. 2016;25[17]:3647–3653, by permission of Oxford University Press and the European Society for Medical Oncology.

A promising approach to treating DMD is gene transfer therapy designed to restore dystrophin expression using a non-replicating, non-pathogenic viral vector called an adeno-associated virus (AAV). While AAVs possess desirable qualities for use in gene therapies for muscle diseases due to their transduction efficiency and high tropism for target tissues, the virus can only package genomes and transgenes of limited size (≈4.7 and ≈4.5 kb, respectively) [2,14].

Studies investigating the clinical phenotypes of patients with different deletions in the *DMD* gene have informed the development of minigenes, which can accommodate the packaging limitations of AAV vectors. The minigene concept was based on a 1990 case study by England et al. describing a family with BMD [13]. One member of this family (Patient 324) was still ambulant at age 61 years, even though 46% of his *DMD* gene was missing [8,13].

We report the mutation and the long-term clinical courses of BMD in patient 324 and his affected relatives, who shared a shortened but functional dystrophin protein that informed the development of micro-dystrophins for the treatment of DMD.

As the patient is deceased, written informed consent was obtained from the patient's relative for publication of this case report and any accompanying images.

2. Case report

2.1. Patient 324

Patient 324 was missing 46% of his *DMD* gene coding sequence due to an in-frame deletion in exons 17–48, which encode part of the central rod domain (Fig. 1) [8,13]. The deletion of exon 17 encompassed the hinge 2 region, spectrin-like repeats 4 to 18, and a portion of spectrin-like repeat 19 [5]. The reading frame was maintained, as is typical in patients with a BMD phenotype [5].

Photographs taken before the patient was in his thirties did not reveal signs of pseudohypertrophy of the calves (Fig. 2). He did not present with neurologic symptoms until age 35 years, when he fell while running to catch a bus. He was diagnosed with BMD at age 38 years, after visiting a neurologist. At diagnosis, he presented with very little weakness in his arms, but deficits in strength were detected in his hip flexors, extensors, quadriceps, and femoris. The hip abductor muscles and adductors were mildly affected. A clinical geneticist reported that, for a patient with BMD, Patient 324 was very fit. At age 61 years, he could crawl up stairs, drive a car, and walk short distances with a walking aid (Fig. 2). He died in his seventies, at which point he had been using a wheelchair full-time. The patient's BMD likely contributed to his loss of mobility over time.

2.2. Maternal uncle of patient 324 (relative II-2 in pedigree [Fig. 3])

The maternal uncle of Patient 324 had the same mutation as Patient 324 and showed signs of muscle weakness. Prior to his death at age 52 years due to cerebral hemorrhage, he was having difficulties walking and needed a walking aid. He showed no other neurologic symptoms. As his parents did not receive confirmatory genetic testing and clinical/pedigree information from earlier generations is not available, it is unclear whether one of the parents was a carrier or a germline mosaic due to a new mutation.

2.3. Patient 1353 (first cousin once removed of patient 324, relative IV-5 in pedigree [Fig. 3])

Patient 1353, whose parent was a carrier of BMD due to inheritance from the maternal uncle of Patient 324, had a deletion in the *DMD* gene identical to that of Patient 324 and first showed muscle weakness at age 21 years, when he experienced difficulties rising from squatting and one of his legs gave way while walking. He had a muscular build, with large quadriceps and calf muscles.

Based on clinical assessment, family history, and creatine kinase levels of 3318 U/L, he was diagnosed with BMD at age 21 years and experienced very little disease progression following diagnosis. Immunologic analysis of a muscle biopsy from this patient showed that the mutation resulted in the production of a shortened dystrophin localized correctly in muscle cells [13].

At age 25 years, Patient 1353 was a bodybuilder and did not notice muscle weakness in his arms. However, he noticed weakness when picking things up from the floor as well as changes when climbing the stairs or walking on uneven ground—which often led to falls that required external support to get up from, as he could no longer use his thighs for support. He could walk an average of 1 mile on a flat surface. He worked full-time as a metal polisher but was unable to complete certain tasks that required carrying items up the stairs. His job required him to stand most of the time, and he felt fatigued at the end of the workday.

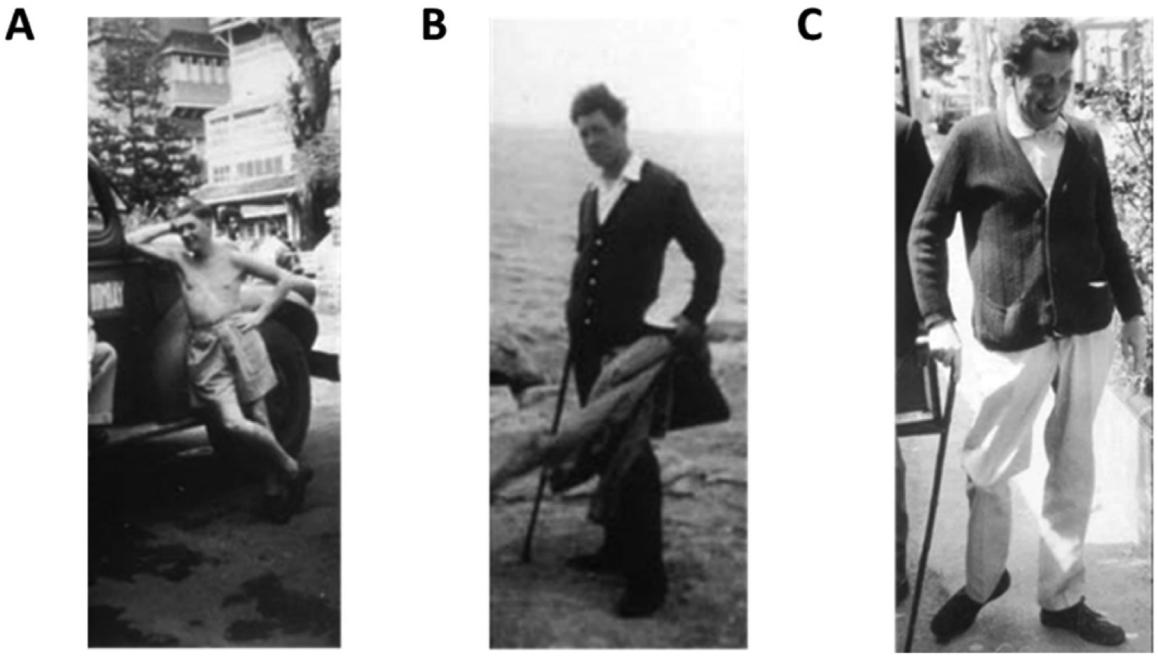

Fig. 2. Photographs of Patient 324.
(a) Age 20′s Patient 324 was in the army. (b) Age 45 years: Patient 324 had just climbed to the top of a cliff. (c) Age 60 years: Patient 324 was walking with a walking stick and had a waddling gait.

While the patient initially managed with one walking stick, over time he required the aid of two walking sticks. His difficulties with walking and falling gradually increased, and by age 40 years, he was unable to walk on raised surfaces, such as slopes and stairs. His upper arm muscles also grew weaker, and he became wheelchair-dependent in his mid-forties. He slowly lost additional muscle and experienced loss of upper and lower body strength in the chest, arms, and both legs.

Patient 1353 is currently 59 years old and has contractures in his hips, knees, and feet. His shoulder mobility is limited, he is unable to straighten his elbows, and his wrist movements and grip are weak. He is wheelchair-bound, but to preserve his independence as much as possible, he has implemented adaptations, including the use of hoists for transfers and adapted cutlery that enables him to feed himself by resting his elbows on a high surface and dipping his head toward his hands. In the last decade, he has lost the ability to turn the steering wheel of his adapted car and is awaiting a vehicle with an electronic joystick. He occasionally has leg cramps, which he experienced regularly during his twenties and thirties.

The patient has had no issues with chewing, swallowing, or recurrent chest infections. However, over the last decade, he has had recurrent urinary infections and bladder control issues, which may have been exacerbated by an accident that resulted in a severed urethra. He has occasionally experienced periods of constipation requiring laxatives. His hearing is normal, but he has noted vision changes, such as blurred vision and the need for brighter light while reading, despite normal findings from an optician's assessment.

3. Discussion

The mild phenotypes of patients with BMD have informed our understanding of the criticality of various domains of the dystrophin protein [14,15]. Key functional domains highlighted in these case studies as important to the function of the shortened dystrophin are the NT domain, a combination of hinge domains, sections of the central rod domain (spectrin-like repeats), and the CR domain [5].

The NT region binds to the intracellular actin cytoskeleton and is essential for dystrophin function [15]. The hinge domains of dystrophin allow for flexibility by providing the structural mechanism for dystrophin to function as a shock absorber [16]. In addition, select spectrin-like repeats bind to the sarcolemma (R1–R3) to maintain contractile force or influence microtubule organization [17,18]. The CR region recruits for the assembly of the DAPC, which plays a critical role in linking the cytoskeleton to the extracellular matrix [19]. Detailed functional analyses of shortened dystrophin constructs in animal models have demonstrated that the correct phasing of repeats, paired arrays of repeats, and the presence of a hinge domain are all important for the function of the modified dystrophin [20]. Importantly, the exon 17–48 deletion removes approximately two-thirds of the spectrin-like repeat 19 coding region (R19) [21]. In micro-dystrophin constructs, precise phasing of R19 spectrin-like repeats through removal of the remaining approximately one-third of R19 improved the functionality of the protein [20].

Notably, while the C-terminus region was also preserved in these patients, research has shown that this region is dispensable and not required for the assembly of the DAPC [22]. Additionally, the absence of the neuronal nitric oxide synthase (nNOS) binding domain (R16–R17) [18] in the patients in the pedigree reported here (Fig. 3) indicates that nNOS is not critical for cardiac function, at least in patients younger than 60 years, as none of the patients were reported to have cardiomyopathy. Notably, most NOS found in the healthy heart is the endothelial isoform (eNOS) and, in cardiomyocytes, nNOS is primarily localized to the sarcoplasmic reticulum [23].

Clinical data from Patient 324, an ambulant patient with BMD and a deletion of almost half the *DMD* gene (exons 17–48), and his affected relatives formed the clinical basis for the construction of micro-dystrophin (Fig. 1). The family members all had the same genotype and manifested mild clinical phenotypes, suggesting that the mild clinical courses were linked to the specific genotype rather than another compensating gene. However, differences in

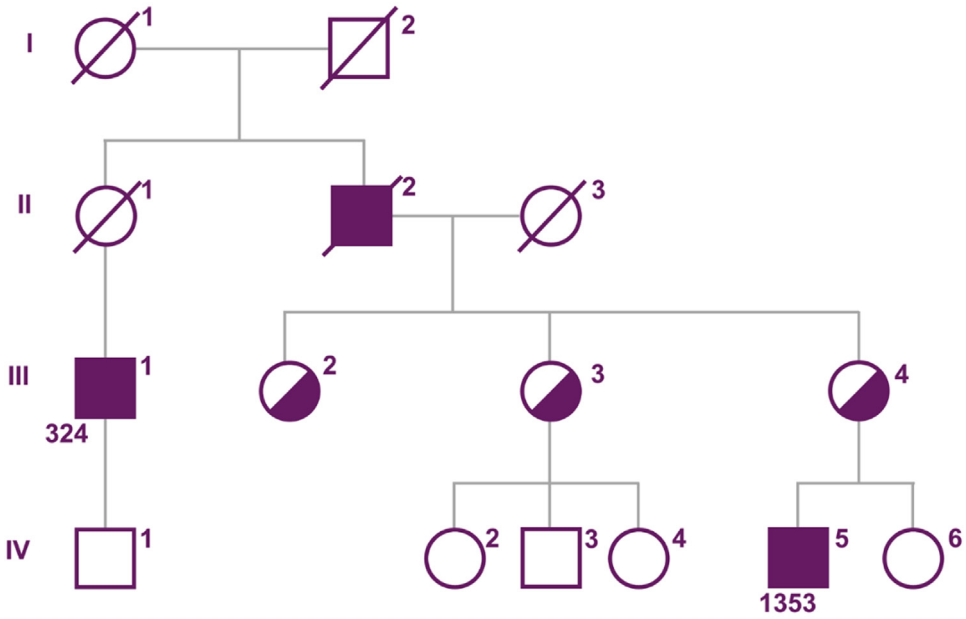

Fig. 3. The pedigree of Patient 324 suggests that a compensating gene does not explain the mild BMD phenotype.
The proband (IV-5; Patient 1353) in this family presented to a neurologist because of a family history of muscle disease. A male relative (III-1; Patient 324) presented with muscle weakness in his thirties, but his disease progression was slow, and he could still walk with a walking aid at age 61 years. A second male relative (II-2) could walk with a walking aid and died at age 52 years due to cerebral hemorrhage [13]. BMD, Becker muscular dystrophy. Adapted with permission by England SB, et al. *Nature*. 1990;343(6254):180–182. Copyright © 1990, Springer Nature Limited.

the disease course observed between Patient 1353 and Patient 324 highlight the variability of disease progression. While Patient 1353 experienced falls by age 25 years and contractures by age 59 years, Patient 324 did not present with neurologic symptoms until age 35 years and could still drive and walk short distances with a walking aid at age 61 years. Exploration of potential modifiers of disease trajectory that may account for some of these differences in severity is an interesting direction for future research.

The makeup of dystrophin in these patients with mild phenotypes may provide clues regarding which domains of dystrophin are critical to functionality. The dystrophin in Patient 324 included the R1 to R3 region, which directly interacts with the sarcolemma, and the essential anchor areas at the NT for actin and the CR domain for the DAPC [14]. This finding implies that massive in-frame deletions can be compensated for by the central rod domain of the dystrophin protein without having a clinical impact as severe as that seen in DMD [6]. In fact, a dystrophin micro-gene modeled on this exon 17 to 48 deletion and inserted into expression plasmids, retroviruses, and adenovirus vectors for in vivo delivery to muscle fibers functioned well [6,20]. Additionally, in transgenic *mdx* mice, this micro-dystrophin restored the normal muscle phenotype [24,25].

One gene therapy that was developed based on data from Patient 324 is delandistrogene moxeparvovec, which, as of March 2024, is approved in the United States, United Arab Emirates, Qatar, Kuwait, Bahrain, and Oman for the treatment of ambulant pediatric patients aged 4 through 5 years with DMD and a confirmed mutation in the *DMD* gene [26]. The delandistrogene moxeparvovec transgene is designed to retain key functional domains of the wild-type protein [14,27]. Additional gene therapies modelled after Patient 324, including SGT-001 and PF-06939926, are currently being investigated in phase 1/2 and 1 studies, respectively [28,29].

In conclusion, the mild phenotypes of patients with BMD guided scientific understanding of the criticality of various regions of dystrophin and informed the development of micro-dystrophin constructs to compensate for the absence of functional dystrophin in patients with DMD, with the potential for these patients to experience relatively mild disease similar to that observed in patients with BMD.

Declaration of competing interest

K.E.D. is a member of the Scientific Advisory Board for Sarepta Therapeutics.

J.V. declares no competing interests.

CRediT authorship contribution statement

Kay E Davies: Writing – review & editing, Data curation, Conceptualization. **Julie Vogt:** Writing – review & editing, Data curation.

Acknowledgements

This study was funded by Sarepta Therapeutics, Inc. Medical writing and editorial assistance were provided by Ayesha Babar and Jen Ciarochi of Nucleus Global in accordance with Good Publication Practice (GPP) 2022 guidelines (https://www.ismpp.org/gpp-2022). This article is dedicated to the late Dr. Sarah Bundey, who brought this family to our attention.

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Update

Neuromuscular Disorders
Volume 39, Issue , June 2024, Page 54

DOI: https://doi.org/10.1016/j.nmd.2024.05.010

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Contents lists available at ScienceDirect

Neuromuscular Disorders

journal homepage: www.elsevier.com/locate/nmd

Corrigendum

Corrigendum to "Long-term clinical follow-up of a family with Becker muscular dystrophy associated with a large deletion in the DMD gene" *Neuromuscular Disorders* 39 (2024) 5–9

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The authors regret that there was an error in the originally published version of Fig. 1. In the right panel ("Mild BMD patient"), the beta-dystroglycan should have been connected to the cysteine rich domain, not the C-terminus. Alpha-dystroglycan, sarcoglycan, dystrobrevin, and syntrophin also should have been shifted to the right. We have revised the figure accordingly.

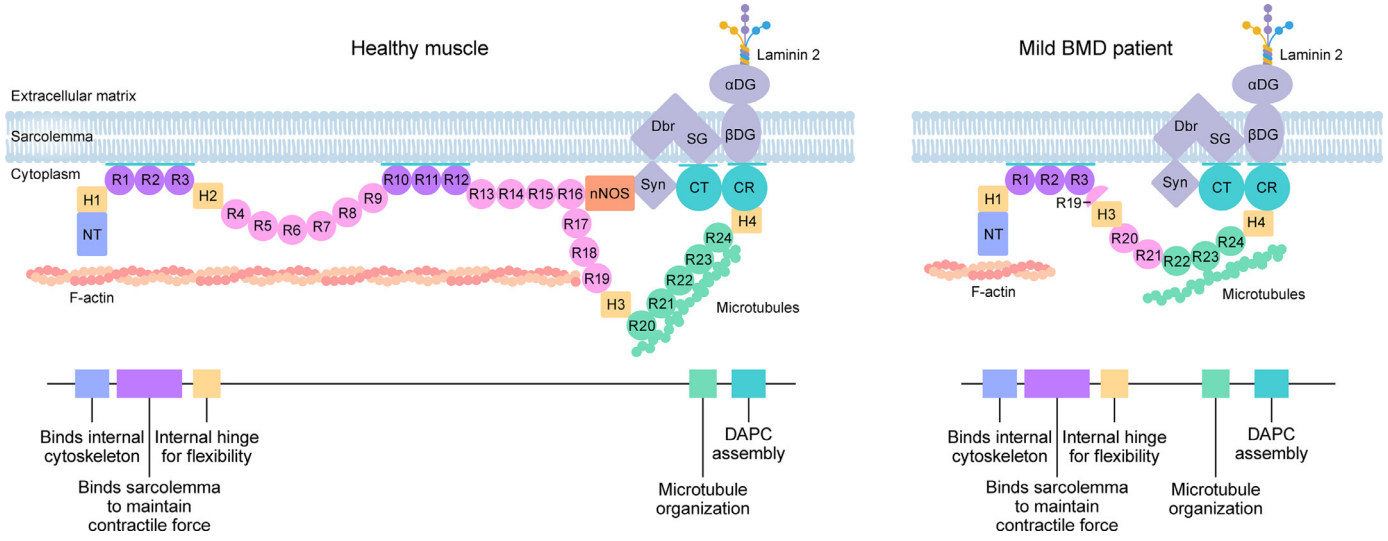

Fig. 1. Wild-type dystrophin in healthy muscle and shortened dystrophin in BMD. αDG, alpha-dystroglycan; βDG, beta-dystroglycan; BMD, Becker muscular dystrophy; CR, cysteine rich; CT, C-terminus; DAPC, dystrophin-associated protein complex; Dbr,dystrobrevin; H, hinge; nNOS, neuronal nitric oxide synthase; NT, N-terminus; R, spectrin-like repeat; SG, sarcoglycan; Syn, syntrophin. Adapted from Zhao J, Kodippili K, Yue Y, et al. *Hum Mol Genet.* 2016;25 [17] :3647–3653, by permission of Oxford University Press and the European Society for Medical Oncology.

The authors would like to apologise for any inconvenience caused.

DOI of original article: 10.1016/j.nmd.2024.04.004
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https://doi.org/10.1016/j.nmd.2024.05.010