The role of genetics in Duchenne Muscular-Dystrophy(DMD) and treatment strategies

Professor: Dr. Eslami

Present by: Sara Najafi

Introduction

- General Information

➢ The term "muscular dystrophy" incorporates an assortment of hereditary disorders that lead to progressive, generalized disease of the muscle prompted by inadequate or missing glycoproteins in the muscle cell plasma membrane. Muscular dystrophy is a non-communicable disorder with abundant variations. Each has its pattern of inheritance, onset period, and the rate at which muscle is lost. Alterations in specific genes cause different representations of this disease.

- Anatomy

➢ Research has established that the gracilis, semimembranosus, semitendinosus, and sartorius muscles can be affected in patients with muscular dystrophy. Feet can exhibit an equinovarus deformity. The pelvis can tilt. There may be contractures throughout the body. Spinal deformities may produce lordosis or scoliosis. The eye can exhibit cataracts and bilateral ptosis.

Natural History

Onset usually occurs in the third to fourth decades. But, it may reveal in infancy or undergo accelerated deterioration near the age of onsetParents of affected individuals may present concern that their child is not walking as well as other children their age. The child may have trouble kicking a ball due to weakness. Pseudo-drop events caused by weakness of quadriceps muscle may also be present. Both parents could be healthy. On physical examination, the affected individual will have massive calf muscles plus lower limb proximal muscle weakness. This condition will make affected individuals want to utilize their arms and hands to aid in rising from a seated position (Fig.1). Other complaints can involve a history of delayed ambulation, toe walking, calf hypertrophy, and proximal hip girdle muscle instability.

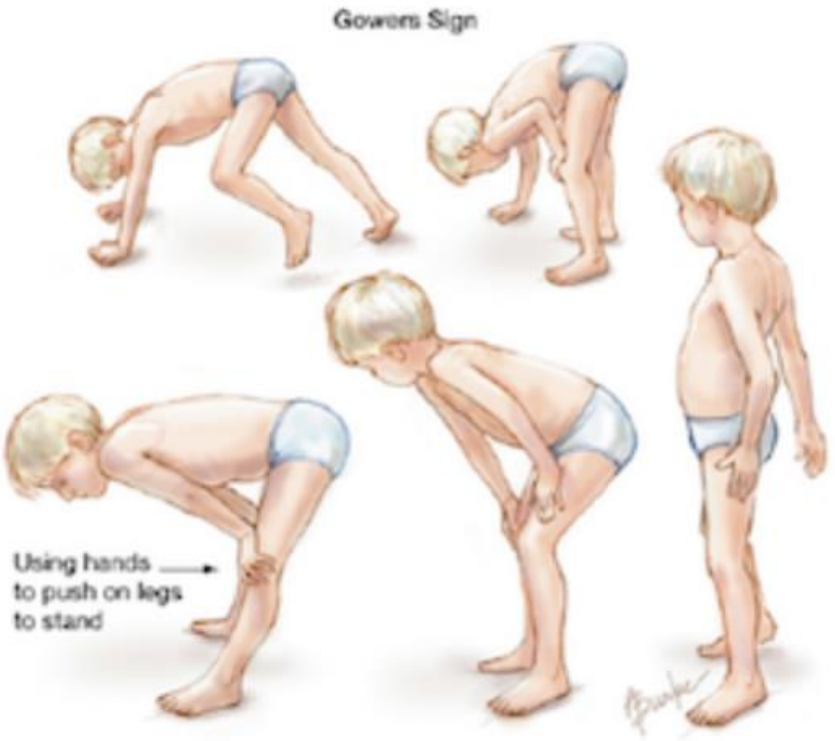


Fig.1: DMD sign

X-LINKED RECESSIVE INHERITANCE

- Presentations may also incorporate asymptomatic elevation of serum creatine kinase (CK), exertion intolerance, dilated cardiomyopathy, malignant hyperthermia, quadriceps myopathy, language delay, and Turner syndrome (Duchenne in X chromosome monozygotic females). For some with subclinical muscular dystrophy, the diagnosis is initially suspected by family history or the appearance of raised liver enzymes, the basis for which is unclear.These enzymes may include alanine aminotransferase and aspartate aminotransferase.

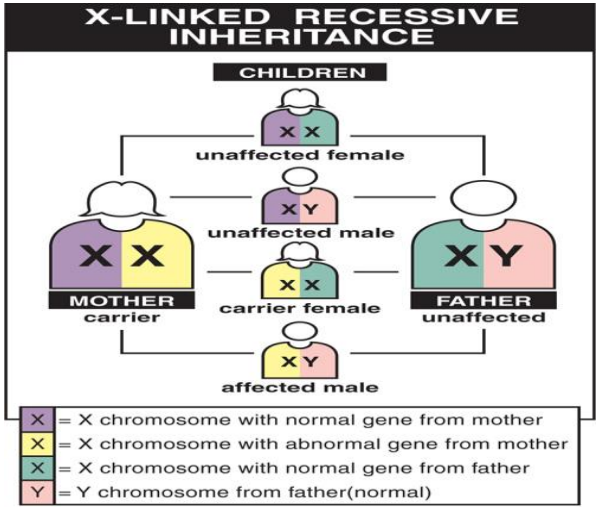


Fig.2 When a girl inherits a flawed dystrophin gene from one parent, she usually also gets a healthy dystrophin gene from her other parent, giving her enough of the protein to protect her from the disease. Males who inherit the mutation get the disease because they have no second dystrophin gene to make up for the faulty one.

X-LINKED RECESSIVE INHERITANCE

Since the inheritance of muscular dystrophy can be X-linked, the overwhelming majority of patients are male. Symptomatic disease in daughters is explainable by Turner syndrome, skewed X chromosome inactivation, translocation of the mutated gene to an autosome, or uniparental disomy (both copies of a chromosome set originated of one parent).Usually, symptomatic females present in infancy with proximal muscle weakness.Reports exist of increased weakness in adulthood, myalgias, spasms, and lethargy as initial manifestations.Scoliosis and sustained alveolar hypoventilation can cause severe problems for every child with muscular dystrophy.

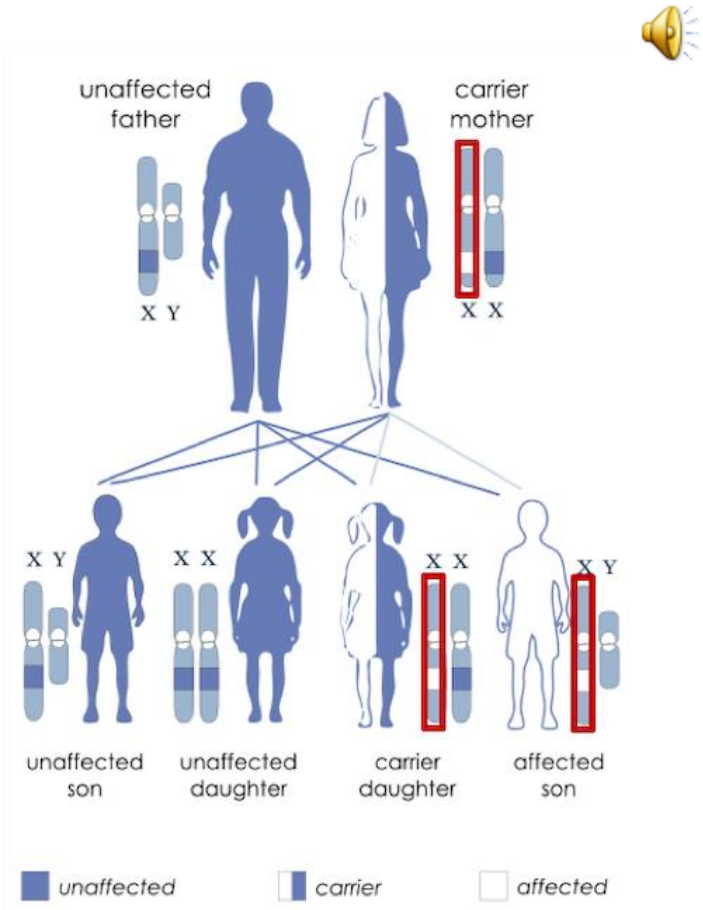


Fig.3 Boys who inherit one broken *DMD* gene will develop muscular dystrophy, while girls who inherit one broken gene will be carriers.

Patterns of Spread

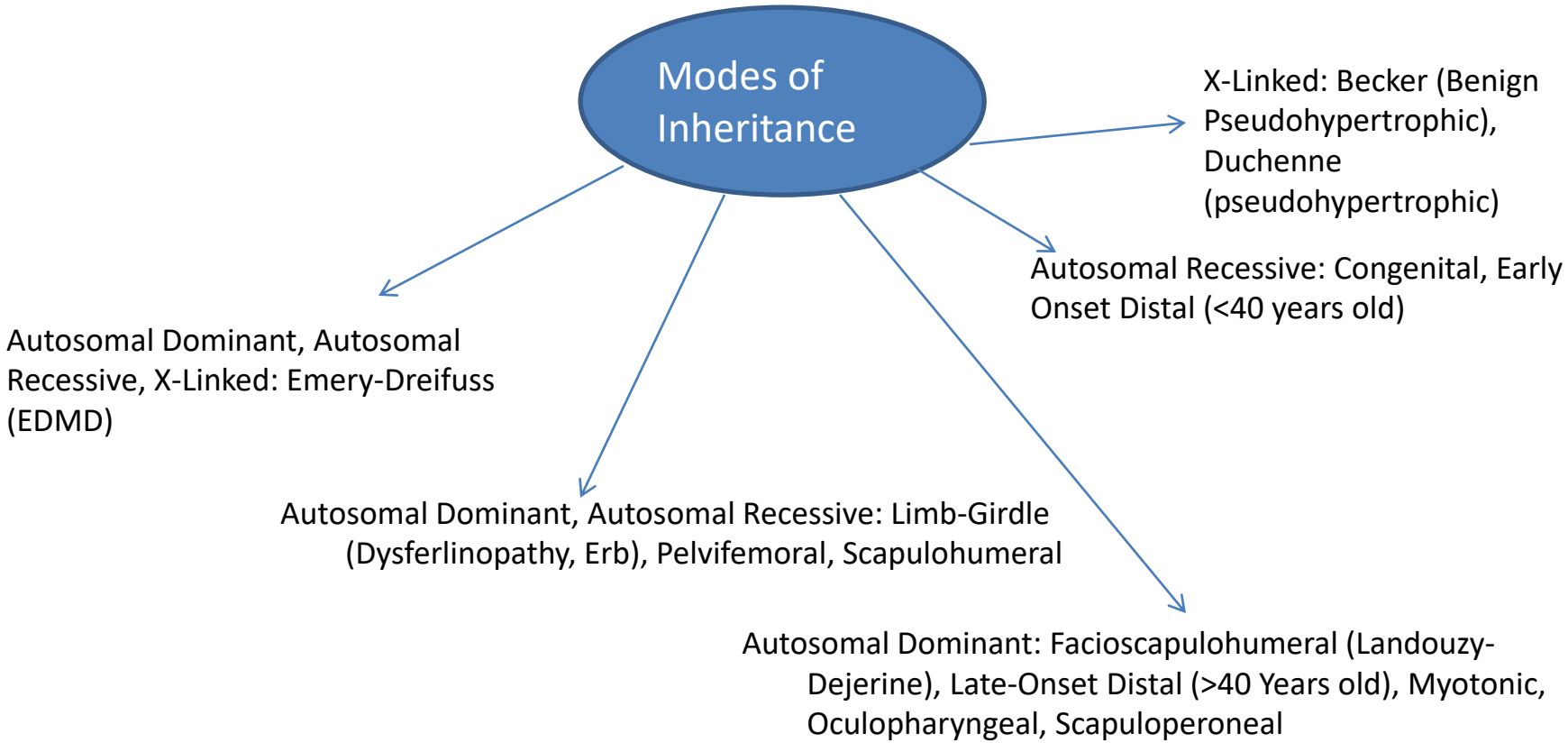
- Muscular dystrophy can be caused by mutations in numerous genes and can be transferred in an X-linked, autosomal dominant, or autosomal recessive fashion.Changes in the X-linked gene DMD, which encodes dystrophin, is the most frequent cause of muscular dystrophy. This why the phenotype is manifested in hemizygous males because they have only a single copy of the X chromosome. One should note that mutations in dystrophin also create allelic heterogeneity.Mutations in the DMD gene, for example, may cause muscular dystrophy of both Duchenne or the less serious Becker, based on the extent of the lack of protein.

Etiology

Cause

- Muscular dystrophy most often results from defective or absent glycoproteins in the muscle membrane.[1] Each type of muscular dystrophy results from different gene deletions or mutations, causing various enzymatic or metabolic defects. The dystrophin gene is the largest in the human genome, with 79 exons.The dystrophin gene is subject to a high rate of spontaneous mutations because of its enormous size (>2 × 106 bases).

Effect


Modes of Inheritance
X-Linked: Becker (Benign Pseudohypertrophic), Duchenne (pseudohypertrophic)
Autosomal Recessive: Congenital, Early Onset Distal (<40 years old)
Autosomal Dominant, Autosomal Recessive, X-Linked: Emery-Dreifuss (EDMD)
Autosomal Dominant, Autosomal Recessive: Limb-Girdle (Dysferlinopathy, Erb), Pelvifemoral, Scapulohumeral
Autosomal Dominant: Facioscapulohumeral (Landouzy-Dejerine), Late-Onset Distal (>40 Years old), Myotonic, Oculopharyngeal, Scapuloperoneal

mutation

- Duchenne Muscular Dystrophy

➢ Caused: mutation of the dystrophin gene

- A spontaneous mutation (a third of cases)
- X-linked recessive maternal-fetal transmission(two-thirds of cases.)

➢ Location: small arm (p) of the X chromosome at the Xp21

➢ Effect result: a non functional dystrophin protein, which causes similar effects as to that seen in Becker muscular dystrophy.

➢ Age: approximately 3 to 5 years of age

- Female Duchenne muscular dystrophy

➢ Cause: an error in female somatic cells whereby one X-chromosome becomes inactivated at an early stage, creating a mosaic representation of heterozygous X-linked genes. The frequency of germline mosaicism in oocytes or sperms varies per individual but can be up to 14%27

Epidemiology

- Most Common Childhood Muscular Dystrophy: Duchenne
- Most Common Adult Muscular Dystrophy: Myotonic
- Prevalence Of Muscular Dystrophy (General Population): 16 to 25.1 per 100,000.
- Frequency Of Muscular Dystrophy (General Population): 1 per 3,000 to 8,000.
- Incidence Of Muscular Dystrophy (Male Births): 1 per 5,000 or 200 per 1,000,000.

Becker Muscular Dystrophy

- Prevalence (General Population): 4.78 per 100,00
- Frequency (Live Male Births): 1 per 18,000.
- Incidence (Live Male Births): 1 per 5618.

Congenital Muscular Dystrophy

- Prevalence (General Population): 0.99 per 100,000.
- Prevalence (Children): 0.82 Per 100,000.[71]
- Prevalence (General Population-Italy): 1 per 16,000.
- Incidence (General Population-Italy): 0.563 per 100,000.

Duchenne Muscular Dystrophy

- Incidence (Live Male Births): 1 per 5,136.
- Prevalence (General Population): 4.78 per 100,000.
- Frequency (General Population): 13 to 33 per 100,000.
- Frequency (Males): 1 per 3,500.

Emerry-Dreifuss Muscular Dystrophy

- Prevalence (General Population): 0.39 per 100,000.[71]
- Prevalence (Children): 0.22 per 100,000.[71]

Facioscapulo humeral Muscular Dystrophy

- Prevalence (General Population): 3.95 per 100,000.
- Prevalence (Children): 0.29 per 100,000.

Limb-Girdle (Erb) Muscular Dystrophy

- Prevalence (General Population): 1.63 per 100,000.
- Prevalence (Children): 0.48 per 100,000.

Myotonic Muscular Dystrophy

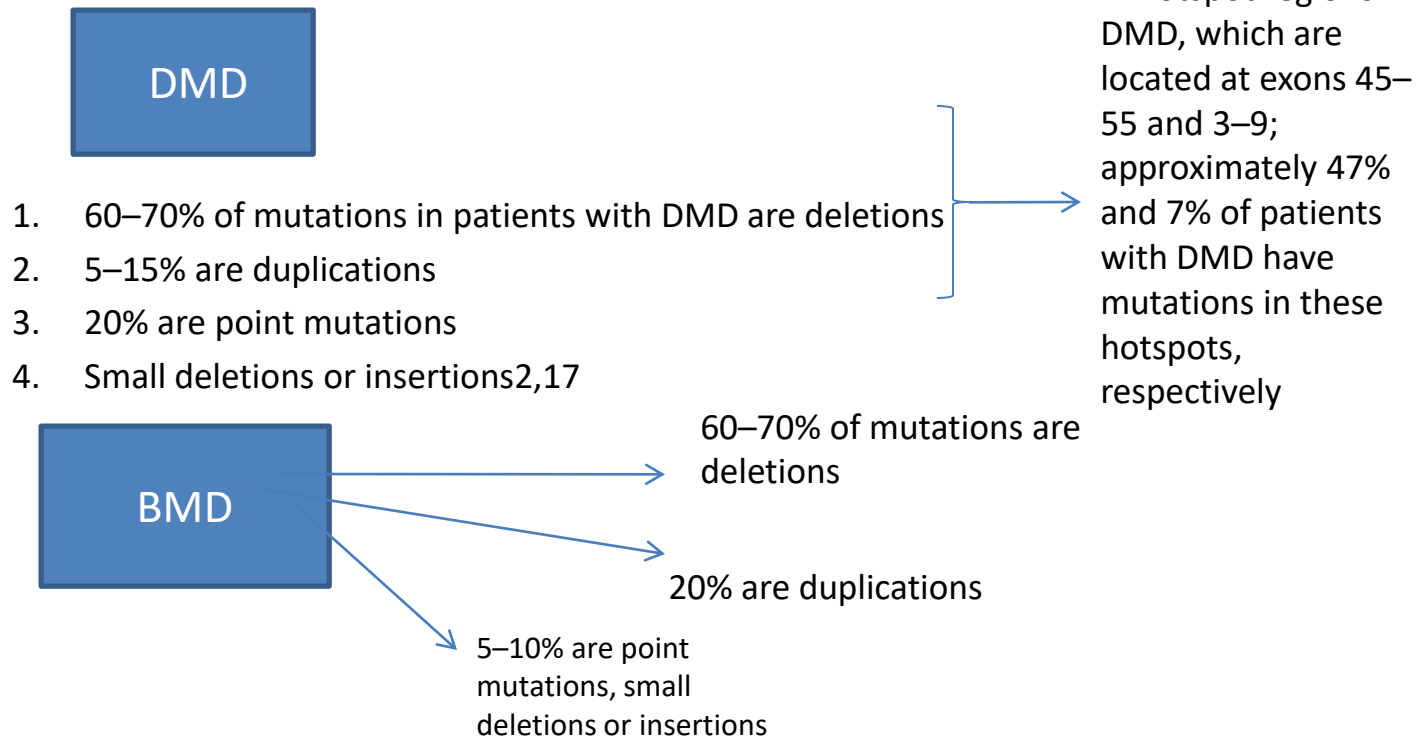
- Prevalence (General Population): 8.26 per 100,000.
- Prevalence (Children): 1.41 per 100,000.

Oculopharyngeal Muscular Dystrophy

- Prevalence (General Population): 0.13 per 100,000.
- *US, England, Australia, & Canada unless otherwise specified

Genetics

- Thousands of different mutations in DMD have been found in patients have been found in patients with DMD or BMD2:



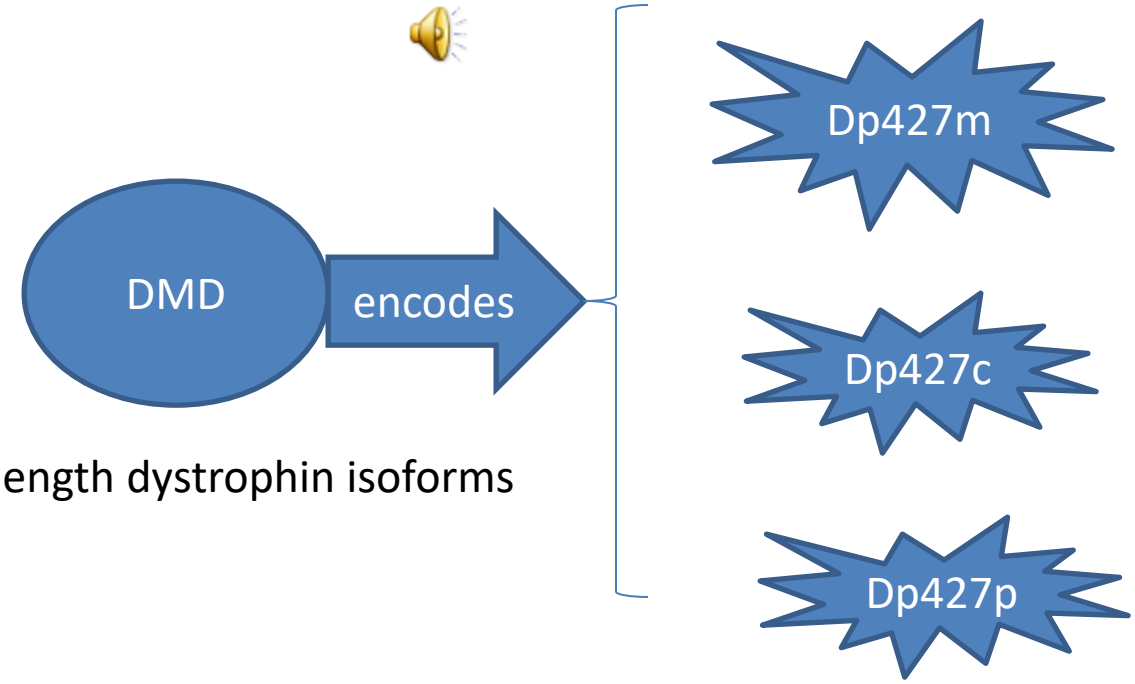
Mechanisms/pathophysiology
DMD and dystrophin

- DMD is primarily a disease of muscle degeneration and necrosis ,although the mechanisms underlying muscle death have been elusive. Several hypotheses of muscle death were debated before the discovery of dystrophin, including:

1) Muscle ischaemia
2) Motor neuron abnormality
3) Nutritional deficiency
4) Metabolic defects
5) Aberrant calcium regulation
6) Sarcolemmal damage(the most popularity as it seems to explain many clinical findings of DMD)

❖ Sarcolemmal hypothesis

❑ DMD is caused by structural and/or functional defects of a sarcolemmal protein owing to mutations in the encoding gene29; subsequent investigations identified dystrophin and DMD as the causative protein and gene, respectively, although in contrast to the initial hypothesis, dystrophin is a subsarcolemmal rather than a sarcolemmal protein.



full-length dystrophin isoforms

These isoforms are expressed in cortical neurons and cerebellar Purkinje cells, respectively. The Dp427p isoform was identified in mice and research suggests that Dp427p expression in human cerebellum is very low during embryonic development and postnatally

In addition to the full-length dystrophin isoforms, full-length dystrophin isoforms are produced by four internal promoters. These isoforms are primarily expressed throughout different tissues:

- Dp260 is expressed primarily in the retina
- Dp140 is expressed in the central nervous system, kidney36 and at high levels in embryonic brain
- Dp116 is primarily expressed in peripheral nerves and Schwann cells
- Dp71 is expressed ubiquitously but at higher levels in neuronal cells than in other cell types
- Dp40, arises from the same promoter as Dp71 but is polyadenylated in intron

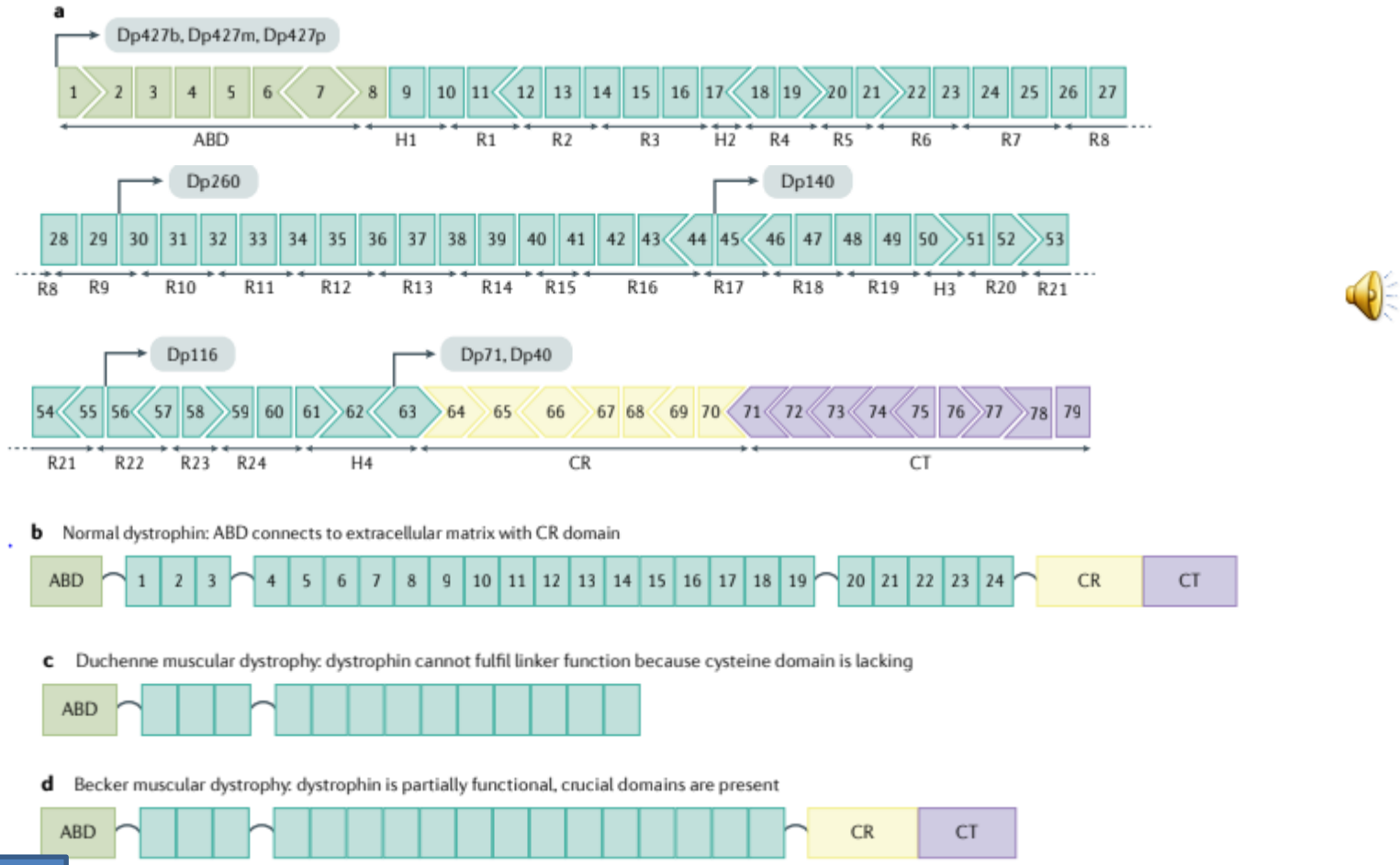


Fig.4

| Schematic depiction of *DMD* and dystrophin protein. a | The ~2.4 Mb full-length *DMD* gene contains eight promoters and 79 exons. Three upstream promoters (Dp427b, Dp427m and Dp427p) produce the ~11.4 kb full-length cDNA and the 427 kDa full-length dystrophin protein. Four internal promoters (Dp260, Dp140, Dp116 and Dp71) generate N-terminal truncated non-muscle isoforms of dystrophin. Alternative splicing at the 3'-end and alternative polyadenylation (the addition of a poly(A) tail to RNA) yield additional isoforms of dystrophin such as Dp40. The full-length protein generated from Dp427m is the primary muscle isoform. **b** | Full-length dystrophin can be divided into four major domains, including the N-terminal F-actin-binding domain (ABD; encoded by exons 1–8), rod (R; encoded by exons 8–64), cysteine-rich (CR; encoded by exons 64–70) and C-terminal (CT; encoded by exons 71–79) domains. The rod domain can be further divided into 24 spectrin-like repeats and four interspersed hinges. **c** | In patients with Duchenne muscular dystrophy (DMD), protein production is prematurely truncated and the resulting protein is not functional. This leads to the loss of connections between the cytoskeleton and the extracellular matrix. **d** | By contrast, in patients with Becker muscular dystrophy, a partially functional dystrophin is produced that contains the crucial domains required to connect to F-actin and the extracellular matrix.

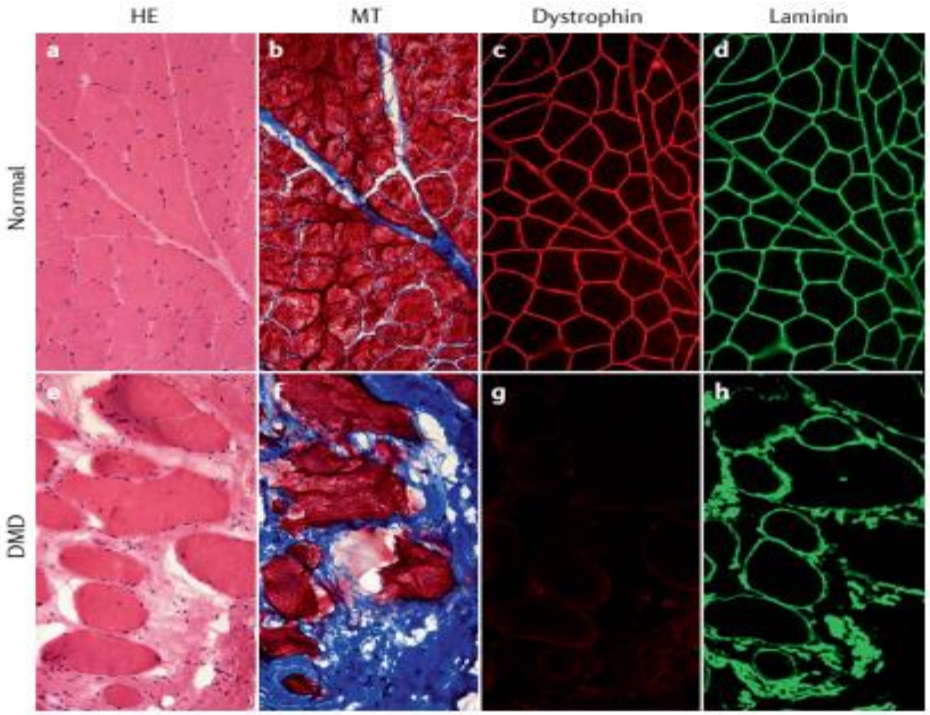
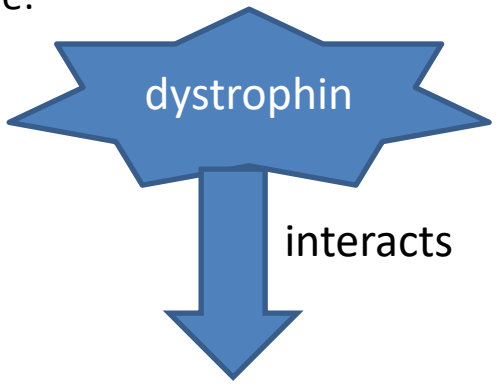


Fig.5

Healthy muscle and DMD muscle histology. Cross-sectional staining of healthy muscle (panels **a**–**d**) and skeletal muscle from a patient with Duchenne muscular dystrophy (DMD; panels **e**–**h**). Haematoxylin and eosin (HE) staining shows centrally nucleated myofibers, inflammatory cell infiltration, variable myofiber size, and endomysium and perimysium connective tissue deposition (panels **a** and **e**). Masson trichrome (MT) staining shows increased fibrosis (blue staining) in a patient with DMD compared with healthy muscle (panels **b** and **f**). Immunofluorescence labelling of dystrophin and laminin shows a lack of dystrophin in a patient with DMD compared with healthy muscle (panels **c** and **g**) and variation in myofiber size in DMD muscle (panels **d** and **h**).

Dystrophin-associated protein complex

- The elucidation of dystrophin-binding partners involved a combination of :
- ➢ Studies in human cell models+ animal models+ study of biopsy and autopsy tissues from patients with muscular dystrophy
- In striated muscle:



Sarcolemma, cytoskeleton (actin microfilaments, intermediate filaments, microtubules and other related structural proteins), channel proteins, and signalling or scaffolding proteins either directly or indirectly.

Dystrophin-associated protein complex

- Collectively, dystrophin and its binding partners form the dystrophin- associated protein complex (DAPC). The traditional DAPC was discovered in the early 1990s and was called the dystrophin glycoprotein complex (DGC) as several components are glycoproteins. The DGC contains 11 proteins: dystrophin, the dystroglycan subcom plex (α-dystroglycan and β-dystroglycan), the sarco glycan subcomplex (α-sarcoglycan, β-sarcoglycan, γ-sarcoglycan and δ-sarcoglycan), sarcospan, syntro phin, dystrobrevin and neuronal nitric oxide synthase (nNOS)41. Since the discovery of the DGC, our understanding on dystrophin-interacting partners has improved. More binding partners have been identified and the interaction domains of many of these proteins have been mapped. In addition, the dynamic nature of the DAPC is appreciated; different DAPCs exist in different tissues or cells and even in different regions of the same myocyte. Collectively, these data have improved our understanding of DMD pathogenesis.

Dystrophin-associated protein complex

Dystrophin interacts with the sarcolemma through four binding domains located in spectrin-like repeats R1–3 and R10–12 and in the cysteine rich (CR) and C-terminal (CT) domains42 (Fig. 6). Dystrophin–actin binding (mainly with filamentous γ-actin in the cytoplasm) occurs via the actin-binding domain (ABD) (by two calponin-homolog motifs) and R11–15 (by electrostatic interaction)43. The microtubule–dystrophin interaction occurs via R4–15 and R20–23 (refs44,45).

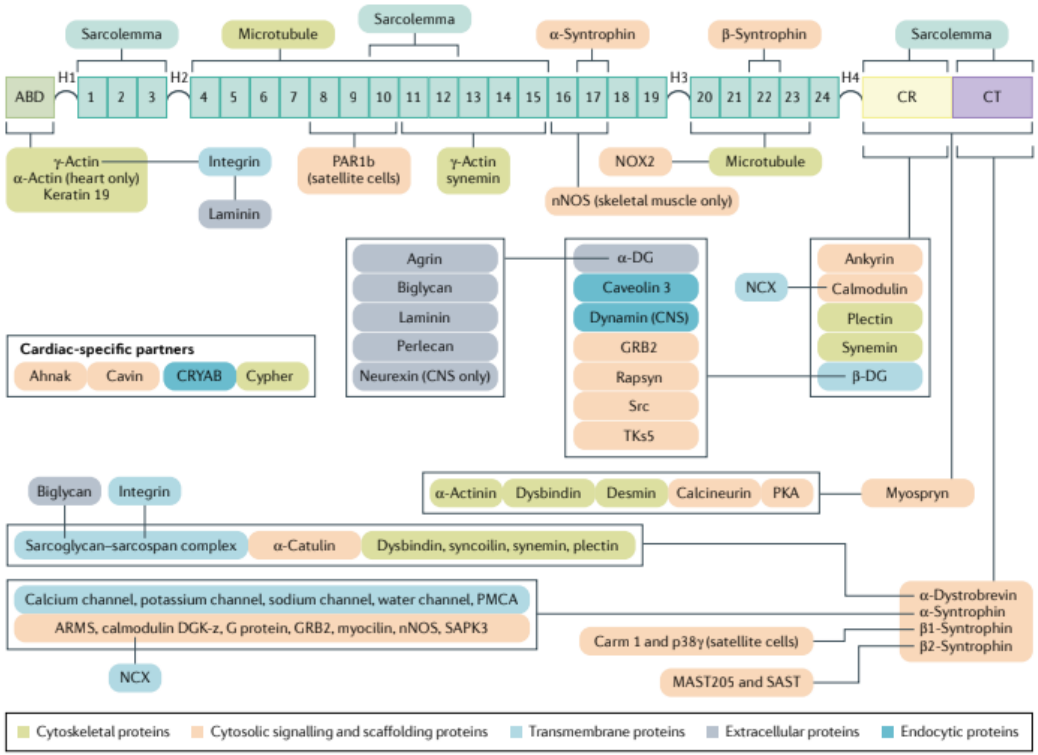


Fig.6

Dystrophin and binding partners. Dystrophin binding partners can be categorized as cytoskeletal proteins, transmembrane proteins, extracellular proteins, cytosolic signalling and scaffolding proteins, endocytic proteins, and cardiac-specific interacting proteins. ABD, actin-binding domain; CNS, central nervous system; CR, cysteine-rich domain; CT, C-terminal domain; DG, dystroglycan; NCX, sodium-calcium exchanger; nNOS, neuronal nitric oxide synthase; NOX2, NADPH oxidase 2; PMCA, plasma membrane calcium ATPase.

Dystrophin-associated protein complex

In addition, dystrophin directly associates with two intermediate filament proteins:

1. Proteins, keratin (via the N-terminal ABD)
2. Synemin (via R11–15 and the CR domain)

Interacts with the transmembrane protein β-dystroglycan, membrane docking protein ankyrin49 and the intermediate filament-associated protein plectin

- The connection between dystrophin and signalling and scaffolding proteins is more complex as it involves direct and indirect connections:

Dystrophin

Directly binds to PAR1b , α-syntrophin, β1-syntrophin, α1-dystrobrevin, α2-dystrobrevin and α3-dystrobrevin, calmodulin and myospryn

Dystrophin-associated protein complex

- α-Syntrophin brings several other proteins to the DAPC:

✓ Plasma membrane calcium ATPase (PMCA)
✓ Channel proteins (sodium channels Nav1.4 and Nav1.5)
✓ Potassium channels Kiv2 and Kiv4.1
✓ Calcium channels TRPC1 and TRPC4
✓ Water channel aquaporin 4

signalling proteins

✓ Calmodulin
✓ G proteins
✓ Grb2
✓ Myocilin
✓ Diacylglycerol kinase-ζ
✓ SAPK3 (also known as MAPK12 or p38γ MAPK)
✓ ARMS (also known as KIDINS220)

α-Syntrophin also works with R16/17 to jointly anchor nNOS to the sarcolemma in skeletal muscle

Dystrophin-associated protein complex

β1-syntrophin

- Engages the signalling and scaffold proteins MAST205 and SAST59. α-Dystrobrevin links intermediate filament-associated protein plectin, intermediate filament protein syncoilin and synemin, coiled-coil protein dysbindin, signalling protein α-catulin, and sarcoglycan–sarcospan complex to the DAPC55,56. Myospryn interacts with dysbindin, microfilament protein α-actinin, intermediate filament protein desmin, and signalling protein calcineurin and protein kinase A55.

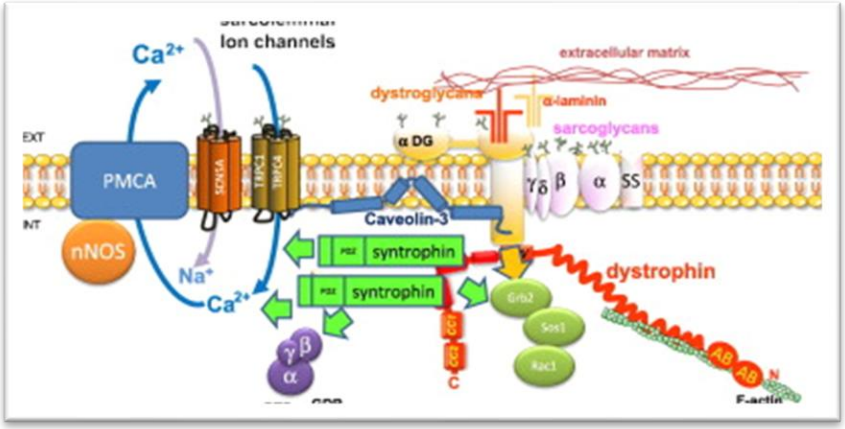


Fig.7 Cross talk between dystrophin-associated protein complex and signalling pathway involving sodium and calcium channels, Nitric oxyde synthase, Plasma membrane Calcium ATPase, as well as intracellular pathway depending on trimeric G proteins and Rac1 small GTPases.

Dystrophin-associated protein complex

- In addition to these proteins, the dystroglycan subcomplex and sarcoglycan–sarcospan complex bring in additional tertiary interacting proteins to the DAPC. These proteins include extracellular matrix proteins (such as laminin, agrin, perlecan and neurexin61), the extracellular signalling or structural protein biglycan, the transmembrane signalling or structural protein integrin, signalling proteins (such as Src, Grb2, Tks5 and rapsyn), and endocytic proteins (caveolin 3 and dynamin 1).

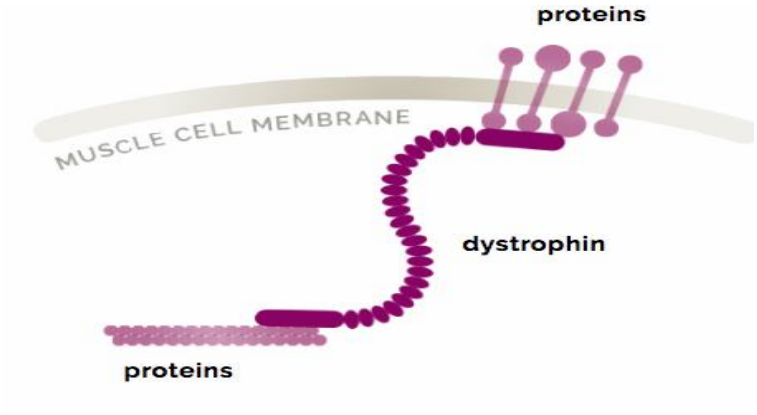


Fig.8 Schematic of dystrophin connecting the actin network to the transmembrane components of the DGC. In individuals with DMD, dystrophin is missing or made in very small amounts, which damages muscle tissue and ultimately leads to death by early adulthood.

Diagnosis, screening and prevention

- Obtaining an accurate genetic diagnosis

➢ DMD should be suspected in young males (aged 2–4 years old) who present with:

1. Delayed motor milestones
2. Muscle weakness
3. Hypertrophic calves
4. Gowers sign
5. 30% of patients with DMD have cognitive impairment at presentation and speech delay is common

❖ Exome sequencing or gene sequencing will likely be routine for all patients with suspected inherited diseases in the future, at present, it is more economical to perform a genetic diagnosis for dystrophinopathies using a step-wise approach

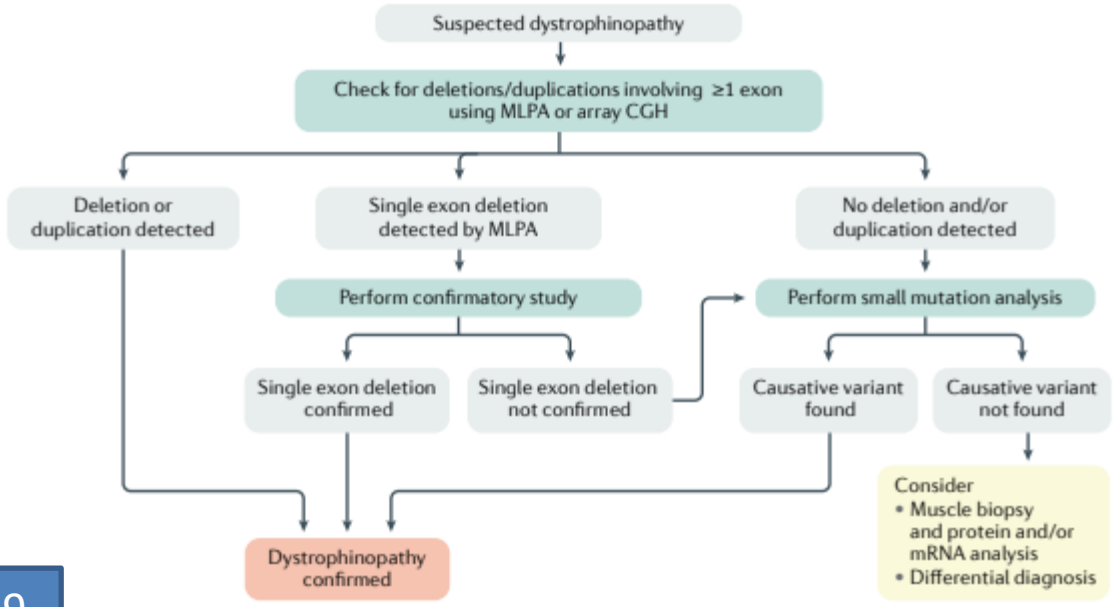


Fig.9

Diagnostic decision tree to confirm the genetic diagnosis of dystrophinopathies. Boys with typical symptoms of a dystrophinopathy and increased plasma creatine kinase (CK) levels should undergo genetic testing to confirm the diagnosis. Initially, multiplex ligation-dependent probe amplification (MLPA) or array comparative genome hybridization (CGH) should be used to identify causative *DMD* variants; however, if a causative variant is not identified (which occurs in ~25% of patients), small mutation analysis should be used. Boys without an identified causative variant after small mutation analysis should be referred for muscle biopsy and protein or mRNA analysis.

Diagnosis

Newborn screening

- The measurement of plasma CK levels:
- ~20% of positive results(were due to non-DMD disorders, including BMD and limb girdle and congenital muscular dystrophies) CK levels are generally substantially increased in patients with DMD from birth, with levels of >20,000U/L not uncommon.
- The rate of false negatives using this screening method was low (20 per 1,800,000)
- Plasma aspartate transaminase (AST)
- Alanine transaminase (ALT) levels

Prenatal screening for DMD

- Routine prenatal care of non-invasive prenatal screening based on the analysis of cell-free DNA in the maternal serum This technique detects fetal aneuploidies:

1. Trisomy 21,13,18
2. Maternal copy-number variations (CNVs) and also CNVs allows DMD to be distinguished from BMD with ~90% accuracy

Treatment

Glucocorticosteroid treatment

- The most recent guidelines strongly recommend the use of the glucocorticosteroids prednisone or deflazacort in boys with DMD when their motor development stops or starts to decline and to continue treatment throughout life.

Age:

- Between patients but should not be before the patient reaches 2 years of age and is generally around 4–5 years of age.

- There is no clear indication on which glucocorticosteroid should be used as both have evidence of improving strength and motor function and can delay loss of ambulation and pulmonary function, reducing the need for scoliosis surgery and delaying the onset of cardiomy opathy. Following earlier evidence that prednisone was more often associated with increased weight gain and deflazacort with cataracts, recent studies have reported a possible better role of daily deflazacort compared with daily prednisolone in delaying loss of ambulation and in increasing survival.

Glucocorticosteroid treatment

- The exact mechanism by which glucocorticoster-oids delay disease progression in DMD are not fully elucidated, as glucocorticoid receptor activation has pleiotropic effects. However, it has been suggested that glucocorticosteroids decrease inflammation and increase total muscle mass and strength in patients with DMD through the stimulation of insulin-like growth factors, decreased cytokine production, decreased lymphocyte reaction, enhanced myoblast proliferation and upregulation of synergistic molecules . Furthermore, glucocorticosteroids like prednisone and deflazacort also display mineralocorticoid activity (that is, they activate mineralocorticoid receptors in addition to the glucocorticoid receptors), which may play a role in a wide range of adverse effects associated with this treatment, including hypertension, fluid retention, weight gain and skin atrophy. Whether these adverse effects can be limited using a dissociative steroidal drug with some mineralocorticoid antagonistic activity (vamorolone) is being evaluated in a clinical trial for DMD.

Clinical trials and approved therapies

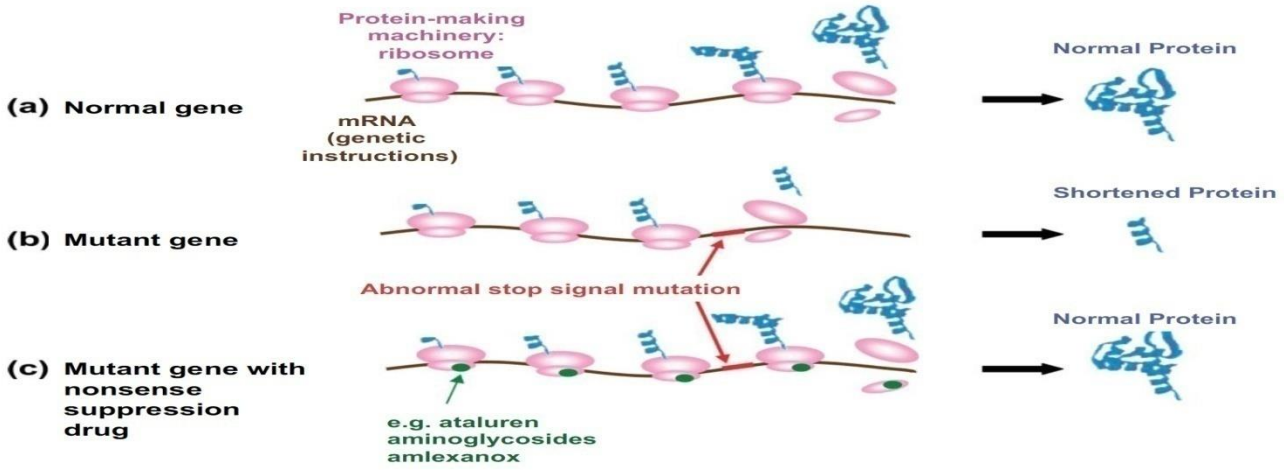
- Over the past two decades, several therapeutic approaches have focused on the different steps of DMD pathophysiology. These approaches can be broadly divided into:

1. Those targeting the restoration of dystrophin production
2. Those trying to reduce the secondary consequences of dystrophin deficiency

- Several of these approaches are being evaluated in clinical trials and are not available in clinical settings. However, some of these approaches have received regulatory approval in the USA, Europe and Japan.

❖ Small molecules targeting nonsense mutations

Fig.10 Mechanism of nonsense suppression therapy



Clinical trials and approved therapies

- Antisense oligonucleotides for out-of-frame deletions

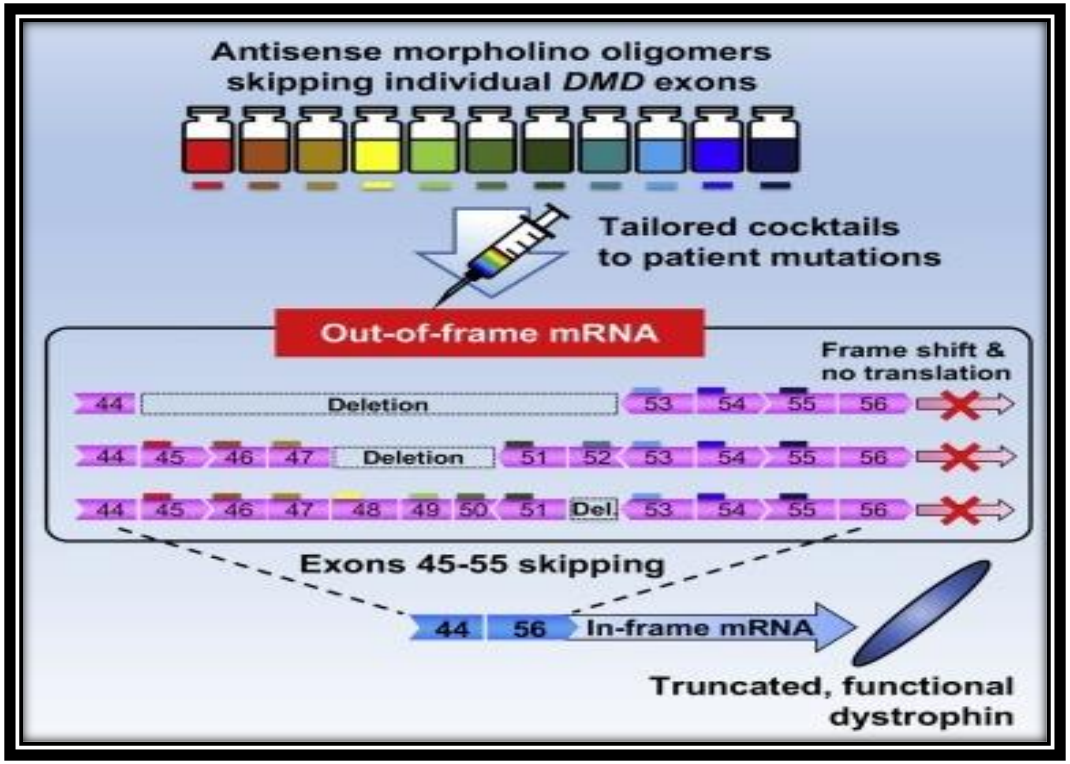


Fig.11 Exons 45–55 Skipping Using Mutation-Tailored Cocktails of Antisense Morpholinos in the *DMD* Gene

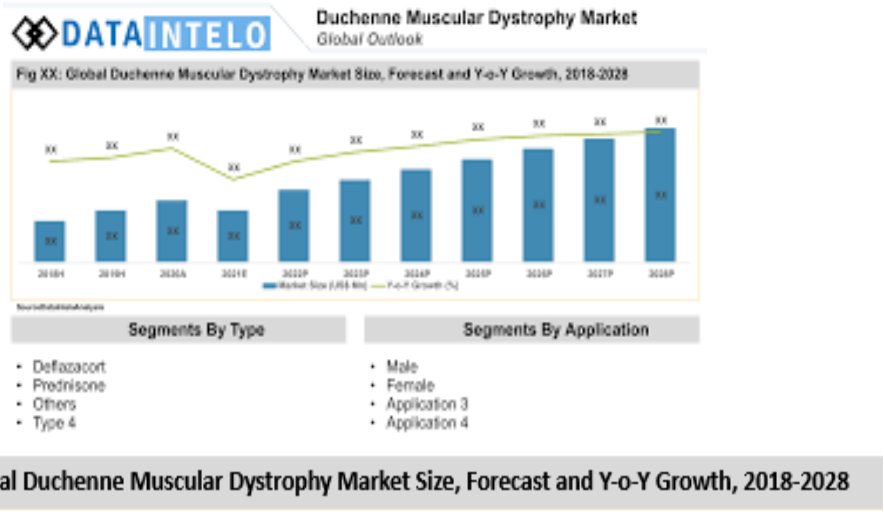
Quality of life

- DMD is a progressive, disabling and life-limiting disorder that will affect the lives of patients and their families. Some QOL studies in patients with DMD have reported a reduced QOL whereas others have found no differences compared with healthy individuals; these differences relate to the instruments used to assess QOL and the fact that health-related QOL and QOL are used interchangeably.
- ➢ Reduced physical ability and a greater disease severity main factors determining impaired QOL
- ➢ Neuromuscular disorder and this association has been confirmed in some studies
- QOL is a broader, multidimensional concept relating to one's fulfilment of individual needs, desires and expectations, encompassing more than mere functional abilities and physical health have the strongest correlation with life satisfaction like:
- ➢ Emotional well-being
- ➢ Self-assessed health
- ➢ Social life
- ➢ Family support
- ➢ Perceived control
- ➢ Recreational opportunities
- ➢ Sexual relations
- ❖ Despite the negative impact of muscle weakness, loss of function, and pain and fatigue, satisfaction with care and management, social interaction and support, adjusted expectations, and acceptance can help mitigate the negative effects of DMD on QOL Indeed, patients with DMD adapt to their disabilities and develop new goals, values and expectations.

Outlook

- Many therapeutic approaches for DMD are in development and can be split into approaches aiming to restore dystrophin and approaches targeting the downstream effects of loss of dystrophin to preserve muscle tissue for as long as possible. The therapeutic approaches for DMD do not restore muscle tissue and function that is lost; therefore, it is important to treat patients as early as possible. Furthermore, the efficacy of dystrophin-restoring therapies will rely on the quality of muscle, as only muscle will express dystrophin (transcript targeting) or benefit from dystrophin expression (genome editing and gene delivery). It is likely that a combination of approaches will be used in the future to restore dystrophin and maintain muscle quality and to slow down disease progression as much as possible.

Fig.12



: Global Duchenne Muscular Dystrophy Market Size, Forecast and Y-o-Y Growth, 2018-2028

Stem cell transplantation

What is this treatment?

- Of the dystrophin restoring strategies, stem cell tranplantation has the longest history. The rationale of stem cell transplantation for DMD is appealing as trans planted cells will contain a functional copy of DMD and will contribute to muscle repair upon transplantation. However, in practice, the abundance of muscle (>30% of body mass) and the number of individual skeletal muscles(>700) pose severe challenges to the development of an efficient stem cell therapy approach.

Canditate cells:

- Stem cell transplantation can be either allogenic (which requires immunosuppression in the recipient) or re-transplantation of corrected patient-derived cells. In addition, several types of stem cells can be used for transplantation, including satellite cells (myoblasts), bone marrow-derived cells, pericytes and mesenchymal stem cells. Satellite cells (myoblasts) are muscle stem cells that are quiescent and reside underneath the basal lamina (the layer of connective tissue that surrounds each muscle fibre). These cells are activated upon muscle damage to proliferate, self-renew and repair. Bone marrow-derived cells, pericytes and mesenchymal stem cells have the capacity to enter muscle and contribute to myogenesis and can also be used for transplantation. Of note, all these cell types have only a limited capacity to be expanded in vitro. Induced pluripotent stem cells differentiated into myogenic lineages offer another option that does not face this challenge; however, this approach has a risk for unlimited growth and teratoma formation.

Evolving gene-therapeutic options

- Since murine, canine and porcine models of dystrophin mutations are available, novel therapies are intensely studied in the preclinical arena, such as gene replacement by mini- or micro-dystrophins. The deliberate shortening of dystrophin for therapeutic purposes rests on two elements:

1. Therapeutic vector with a high degree of myotropism and low toxicity and immunogenicity for systemic application, such as adeno-associated virus (AAV), which has been safely applied in clinical trials.
2. Due to the limited packaging capacity of AAV. a shortened form of dystrophin would be required.

- Example

- For example, minimal dystrophin version lacking spectrin-like repeats (R) from the rod domain of dystrophin, but retaining hinges 1 and 4 and the full N- and C-termini, or lacking or R2-15/R18-19/R20-23 and the C-terminus, can be packed into an AAV.
- In the mdx mouse model of DMD (a spontaneous mutation introducing a stop codon in exon , the former AAV9-based supplementary gene therapy improved cardiac morphology and function, as assessed by histology for fibrosis, by fractional shortening for pump function and by biomarkers indicating heart failure, such as brain natriuretic peptide. In this particular case, a heartspecific promoter was as efficient as an ubiquitously expressing CMV-promoter.

Example

- In a dog model of DMD (GRMD, point mutation in intron 6 splice acceptor leading to loss of exon 7), the latter approach (ΔR2-15/R18-19/R20-23/C) restored the dystrophin-associated complex, reduced inflammation and fibrosis [25]. More recently, the same GRMD model highlighted the efficacy of an AAV8-microdystrophin (ΔR4– 23/ΔCT) after locoregional (LR) or intravenous (iv) application. The LR application of the therapeutic vector achieved a high rate of transduction (43–59% of the targeted muscle cells), improving function, whereas the iv injection improved the clinical score of GRMD in a dose-dependent manner (with 1 × 1014 virus genomes approaching the gait quality of control dogs).

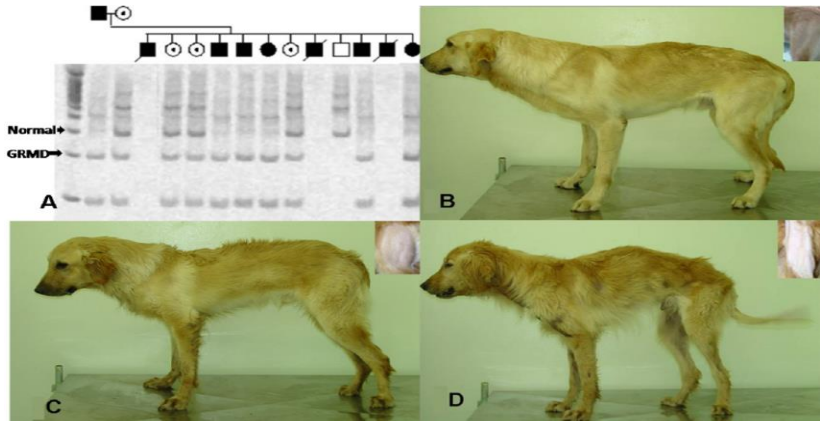


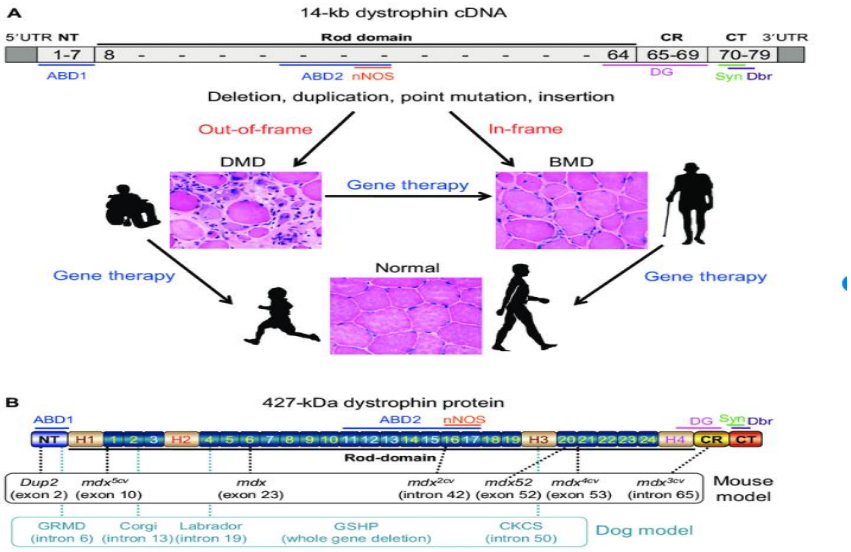
Fig.13

A. Duchenne muscular dystrophy genotyping of golden retriever muscular dystrophy (GRMD) puppies with X-linked muscular dystrophy that were genotyped within 48 h after birth. The genomic polymerase chain reaction product digested with Sau96I produces the wild-type band (310 bp) and the mutant band (150 bp) labeled with arrows. Squares = affected male. Filled circles = affected female. Open circles = carrier female. B. Grade I phenotype (Suflair dog). C. Grade II phenotype. D. Grade III phenotype showing general hypotrophy. Inset in B-D = thigh muscle mass.

Evolving gene-therapeutic options

Fig.14

- Meanwhile, three companies have developed AAVs encoding microdystrophins: Sarepta uses the ΔR4–23/ΔCT microdystrophin described above, whereas Solid Biosciences the ΔR2-5/Δ18-2/ΔCT form and Pfizer a ΔR3– 18/ΔCT construct, each under control of a muscle-specific promoter. While clinical trials are ongoing, promising clinical studies aiming at dystrophin expression are progressing, which have the potential to temporarily alter the course of the disease. Their results being eagerly awaited, novel options for gene correction have appeared.

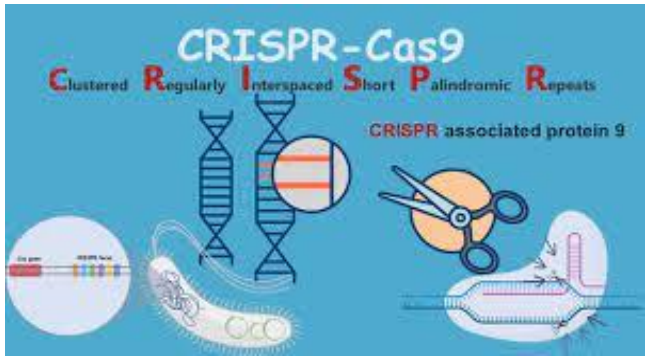


DMD gene therapy and dystrophin mutations in animal models. (A) The 14-kb dystrophin cDNA and the principle of DMD gene therapy. The numbers in the cDNA refers to exon number. The DNA sequence position of the main dystrophin domains and of the dystrophin-associated protein-binding sites (see Fig. 1) is also shown. Frameinterrupting (out-of-frame) mutation leads to severe DMD. In-frame mutation results in mild Becker muscular dystrophy (BMD). The primary goal of DMD gene therapy is to ameliorate muscle pathology and to improve muscle function. Gene therapy can convert the DMD phenotype to the benign BMD phenotype Gene therapy might also prevent or slow down the development of muscle disease if affected individuals are treated early enough. (B) Domain structure of dystrophin and location of the mutations in representative mou and dog models. ABD, actin-binding domain; CKCS, Cavalier King Charles spaniel; CR, cysteine-rich domain; CT, C-terminal domain; Dbr, dystrobrevin; DG, dystroglycan; GRMD, golden retriever muscular dystrophy; GSHP, German shorthaired pointer; nNOS, neuronal nitric oxide synthase; NT, Nterminal domain; Syn, syntrophin; UTR, untranslated region. See supplementary material Table S1 for a description on each model.

Evolving gene-therapeutic options

❖ CRISPR/Cas9

- With the development of genome editing systems, such as CRISPR/Cas9, it is now possible to make targeted modifications in the genome. CRISPR/Cas9 uses guide RNAs that hybridize to specific complementary regions in DNA and guide the Cas9 enzyme to induce a double stranded break at the targeted locations. These breaks are then repaired by the DNA repair systems. In dividing cells, error-free repair can occur via homologous recombination, thus providing a possibility to correct mutations. By contrast, in non-dividing cells, the error-prone, non-homologous end-joining system is used. As the most affected tissues in DMD are post-mitotic, the focus on genome editing therapy for DMD has been on using non-homologous end joining. Inspired by the exon-skipping approach, guide RNAs are designed such that they restore the reading frame. This can be achieved in multiple ways through, for example, deleting an exon, abolishing a splice site such that the exon is not included in the mRNA or reframing an exon (Fig. 15).



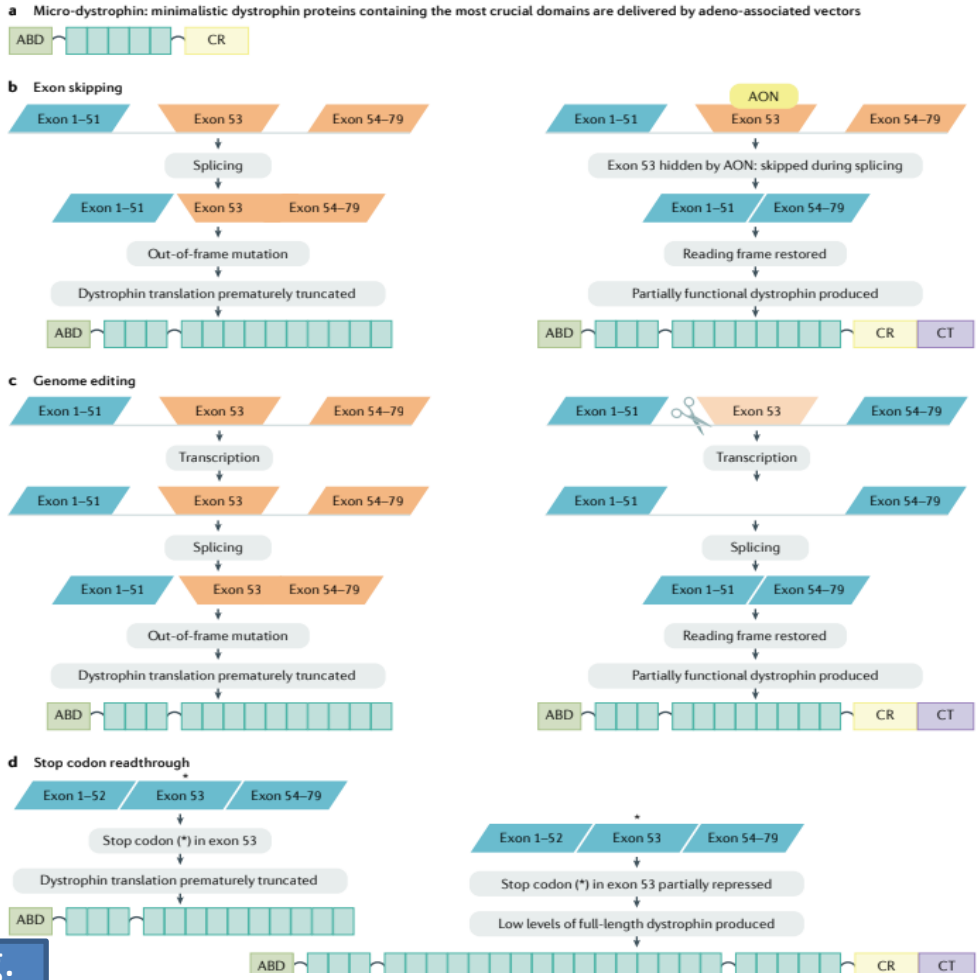


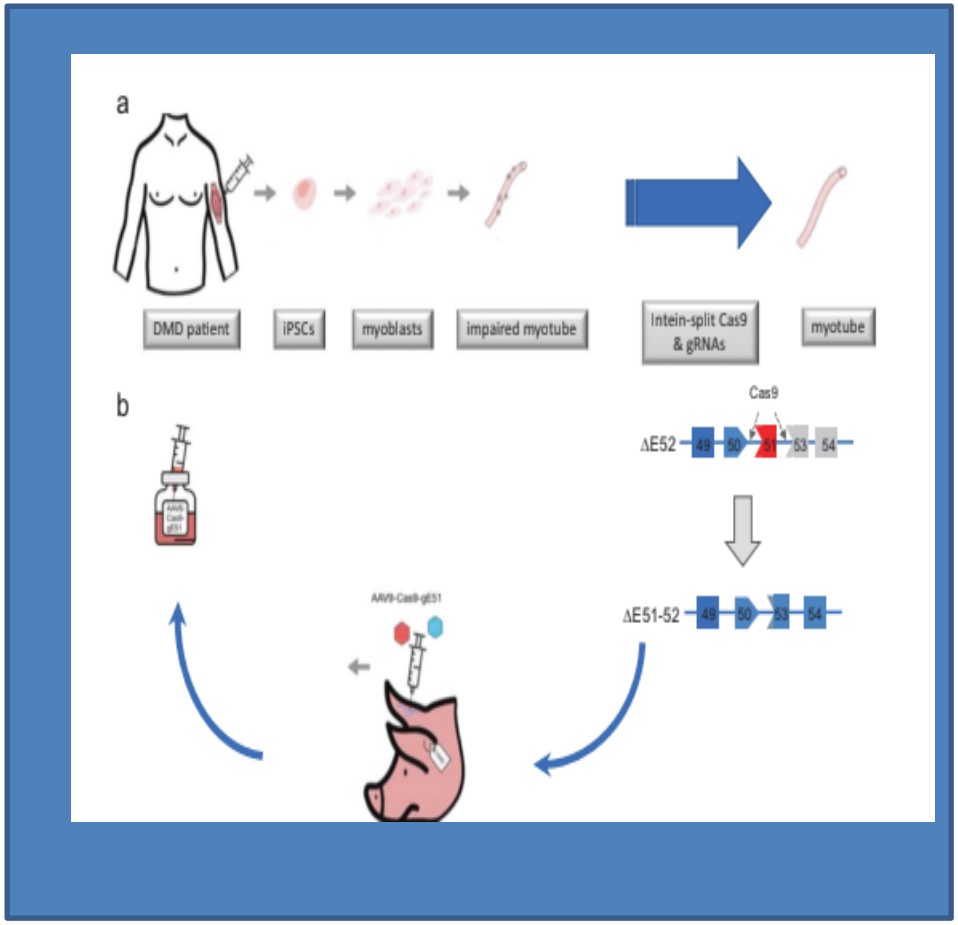
Fig. 15

| Dystrophin-restoring approaches. a | Micro-dystrophin gene therapy. To accommodate the limited capacity of adeno-associated viral vectors, cDNA constructs encoding only the most crucial parts of dystrophin have been generated to enable the expression of micro-dystrophins. **b |** Exon skipping. Antisense oligonucleotides (AONs) are used to target a specific exon during pre-mRNA splicing. The AON hides the targeted exon from the splicing machinery causing it to be skipped, thus restoring the reading frame, allowing the production of a Becker-like dystrophin. **c |** Genome editing. Guide RNAs are used to direct Cas9 to target regions in the DNA to delete an exon from the gene, thereby restoring the reading frame of mRNA produced from this gene, allowing the production of Becker-like dystrophin. **d |** Stop codon readthrough. For patients with nonsense mutations (indicated by an asterisk), compounds can suppress the premature stop codon use, facilitating instead the inclusion of an amino acid. Thus, a full-length dystrophin can be produced. ABD, actin-binding domain; CR, cysteine-rich domain; CT, C-terminal domain.

Evolving gene-therapeutic options

Sketch of the experimental workflow: Patient cells lacking DMD exon 52 were converted to iPS cells and subsequently to muscle cells. An intein-split version of Cas9 was encoded into two AAV vectors, together with two gRNAs excising exon 52. Demonstrating that the AAV-Cas9-gRNAs excising exon 51 (AAV-Cas9-gE51) enabled expression of a shortened but stable dystrophin (51-52, the same approach was successfully used in the porcine model of DMD (lacking exon 52), serving as basis for further development of a therapeutic agent.

- **Fig16. Sketch of the experimental workflow: Patient cells lacking DMD exon 52 were converted to iPS cells and subsequently to muscle cells.**



Summary

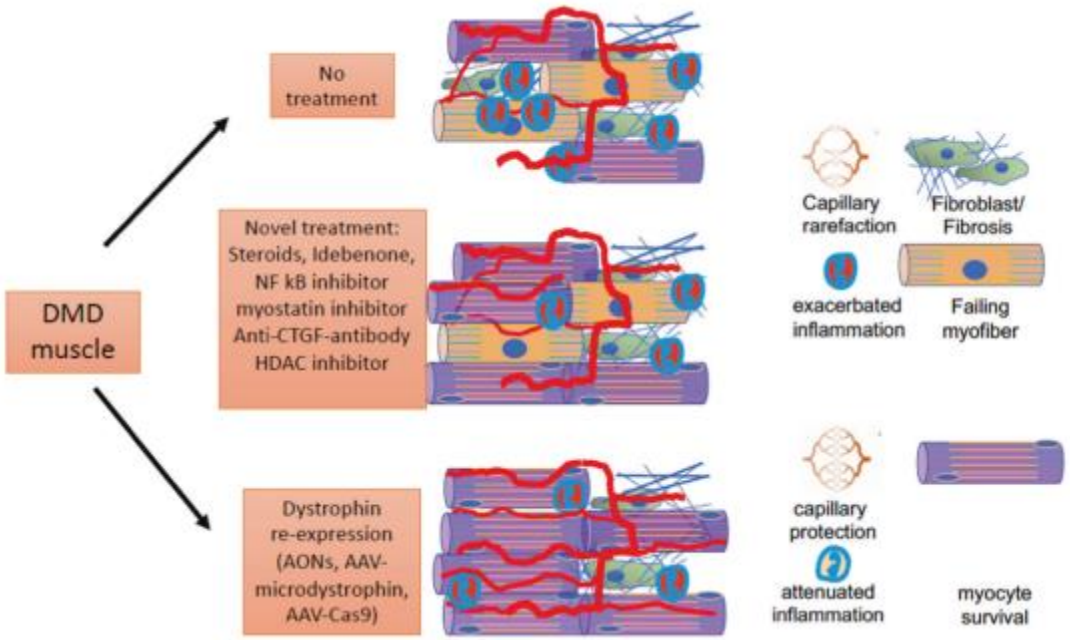


Fig.17 Effects of DMD treatment: DMD muscle fibres without treatment display hallmarks of muscle decay, such as centralized nuclei, fibrosis, capillary rarefaction and continuous inflammation(no tratment). Upon current and novel pharmacologic treatment**,** inflammation and muscle fibre decay may be decelerated, without alteration of the underlying mechanical strain leading to cell death, e.g., by necroptosis. In contrast, therapeutic strategies aiming at dystrophin re-expression (AONs = antisense oligonucleotides targeting exon skipping) correct the disease-causing deficit of dystrophin, either temporally (AONs), or for prolonged intervals (AAV-micro-dystrophin) or permanently (AAV-Cas9).

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3. Genome editing for Duchenne muscular dystrophy: a glimpse of the future?; Christian Kupatt , Alina Windisch, Alessandra Moretti, Eckhard Wolf, Wolfgang Wurst, Maggie C. Walter; Received: 8 July 2020 / Revised: 1 December 2020 / Accepted: 15 January 2021 / Published online: 2 February 2021 The Author(s) 2021. This article is published with open access