Recent developments in Duchenne muscular dystrophy

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Objectives:

- 1. Brief discussion about DMD
- 2. Changing landscape as well emerging therapies in DMD
- 3. Biomarkers in DMD

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DMD

- Most common MD, ~ 1 in 3500-5000 live male births
- Xp21, one of the largest genes with 79 exons
- Clinical features:
  - Onset: 2-5 yr; Gross motor delays, calf hypertrophy; CK>10-100 times
  - Neurodevelopmental problems; Cardiac & Respiratory muscle involvement after first decade
  - Wheelchair confinement: 11-13 yr; Death: 15-30 yr (improved with advanced care)
- Pathology:
  - Severe dystrophic changes; Complete/almost total absence by immunohistochemistry; 0-5% by Western blot
- Genetics (out of frame mutations):
  - Large del/dup of one or more exons: > 2/3rd
  - Small deletions, point mutations, insertions, or splicing mutations: ~1/3rd
  - Genetic testing is of paramount importance to stratify patients who could be amenable to newer therapies

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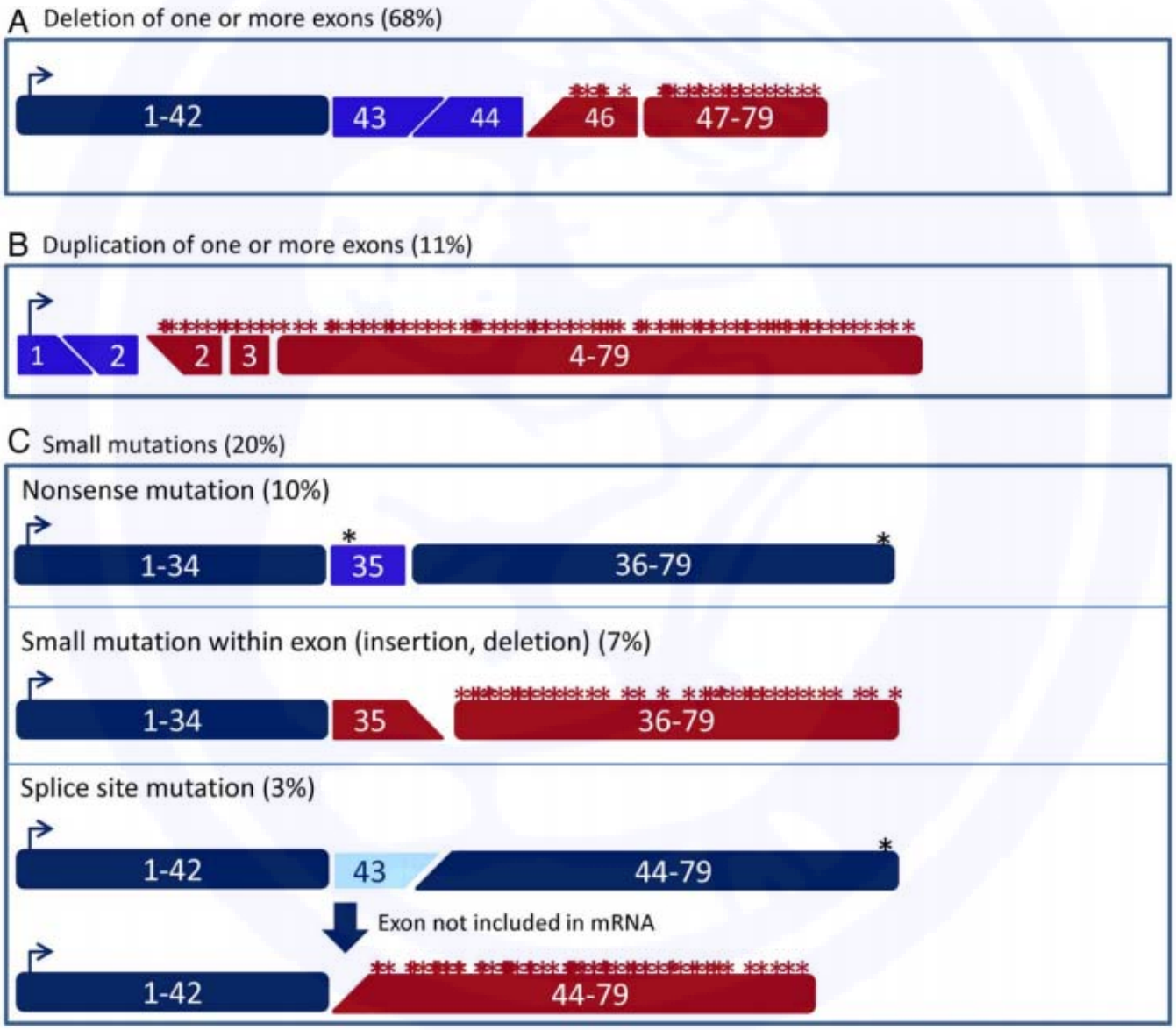
Genetics of DMD



Aartsma-Rus A, et al. J Med Genet 2016;53:145–151.

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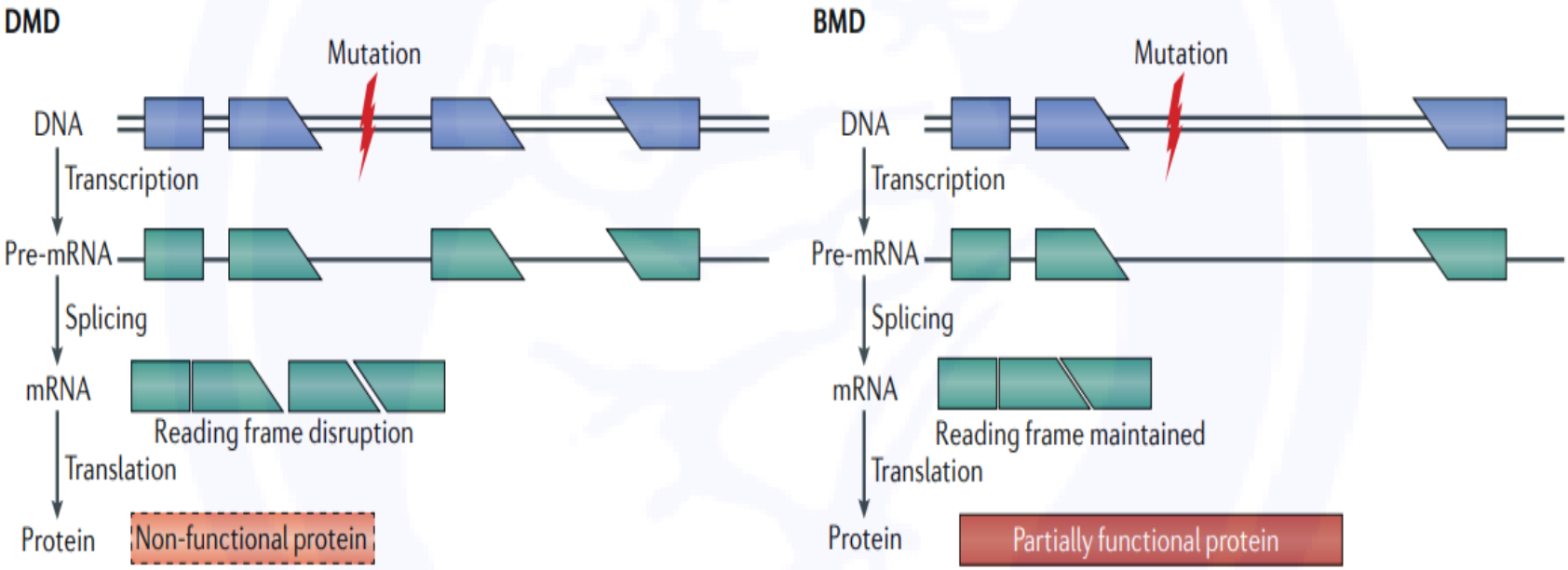
Reading Frame Hypothesis



Verhaart IEC, et al. Therapeutic developments for Duchenne muscular dystrophy. Nat Rev Neurol 2019;15:373-386.

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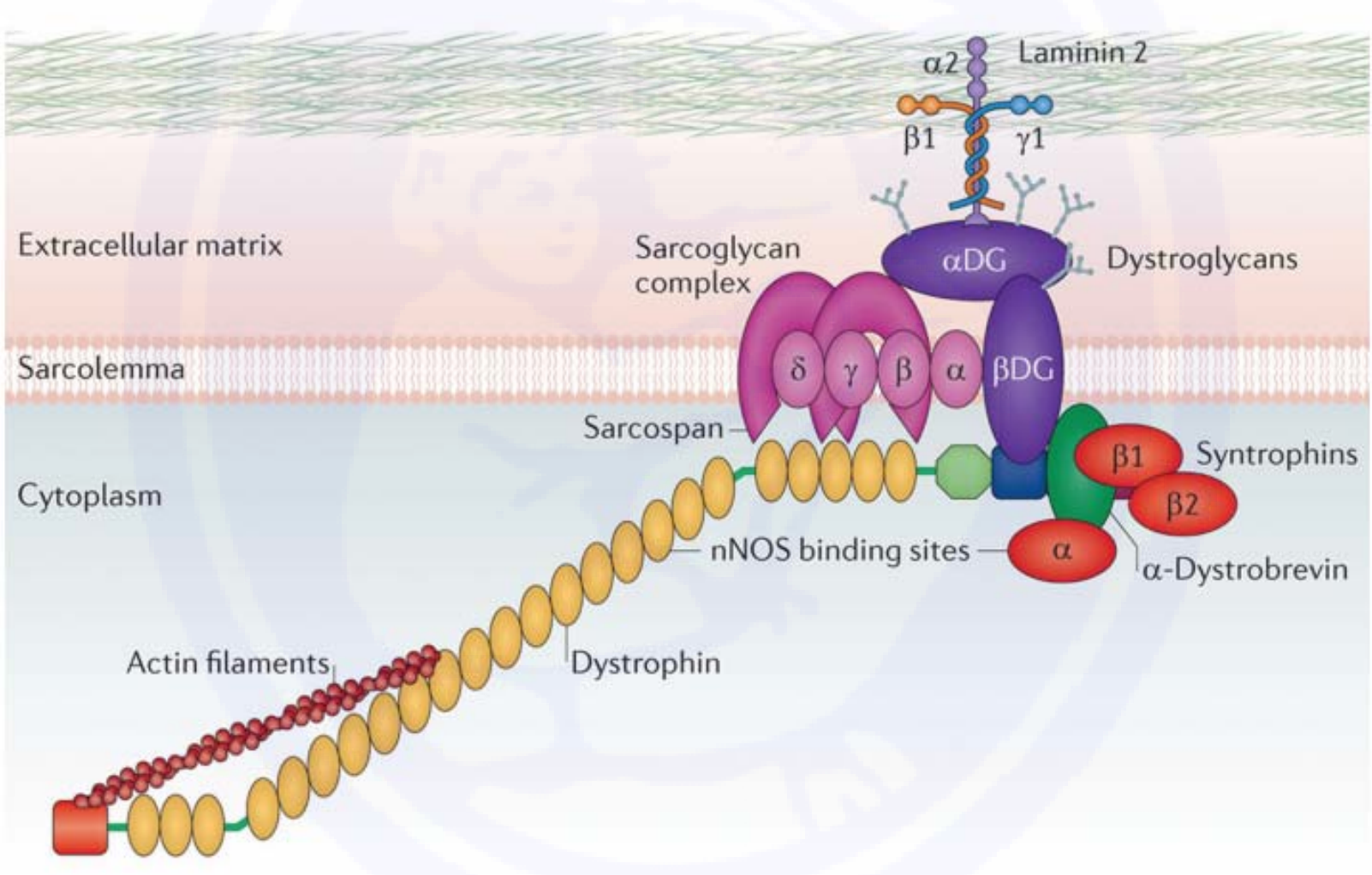
Schematic Diagram of Dystrophin



Thangarajh M. The Dystrophinopathies. Continuum (Minneap Minn). 2019;25:1619-1639.

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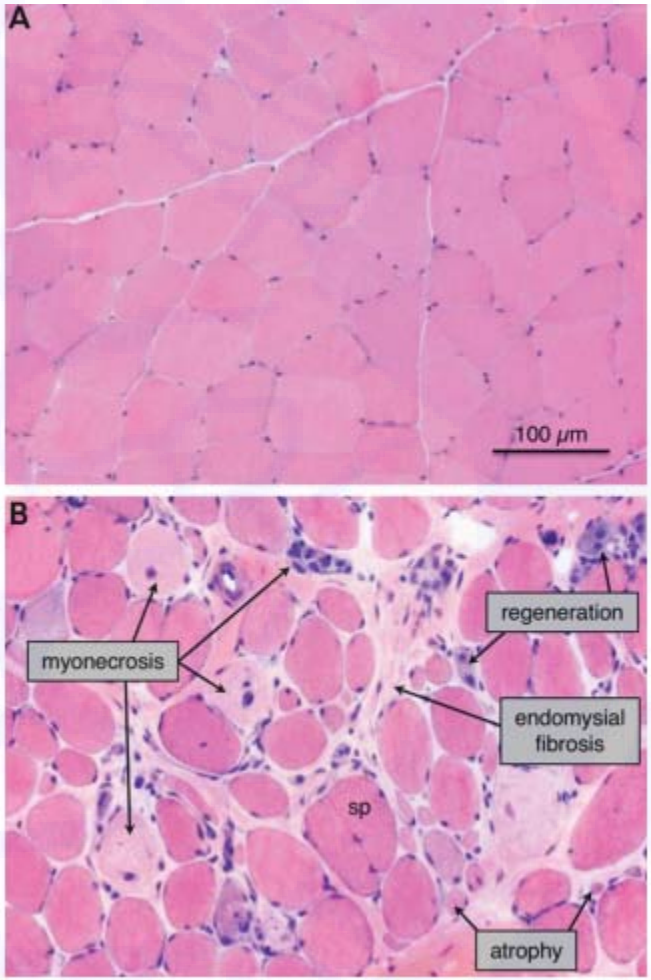
Muscle Biopsy in DMD

A: Normal muscle

B: DMD



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Other dystrophinopathy types

- BMD (inframe mutations)
  - Onset: 5-20 yr; Myalgias, cramps
  - More benign course; WC confinement: after 16 yr
  - Patchy staining by immunohisto; ~ 20% by WB
- Intermediate type:
  - Mild DMD/ severe BMD phenotype
- Isolated Cardiomyopathy
- Carriers of DMD/BMD:
  - 15-20% LV dilation, 8% dilated cardiomyopathy in DMD carrier

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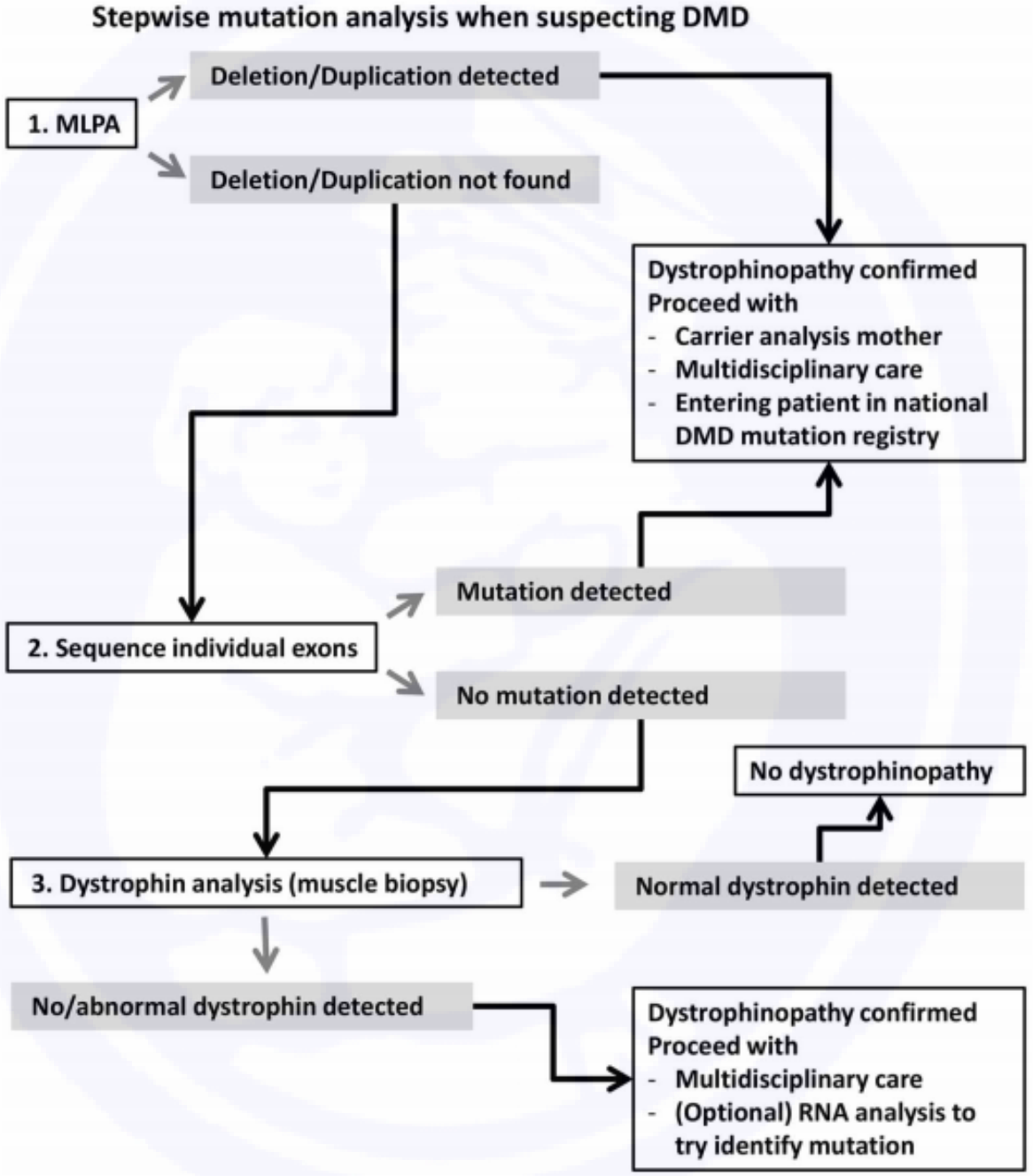

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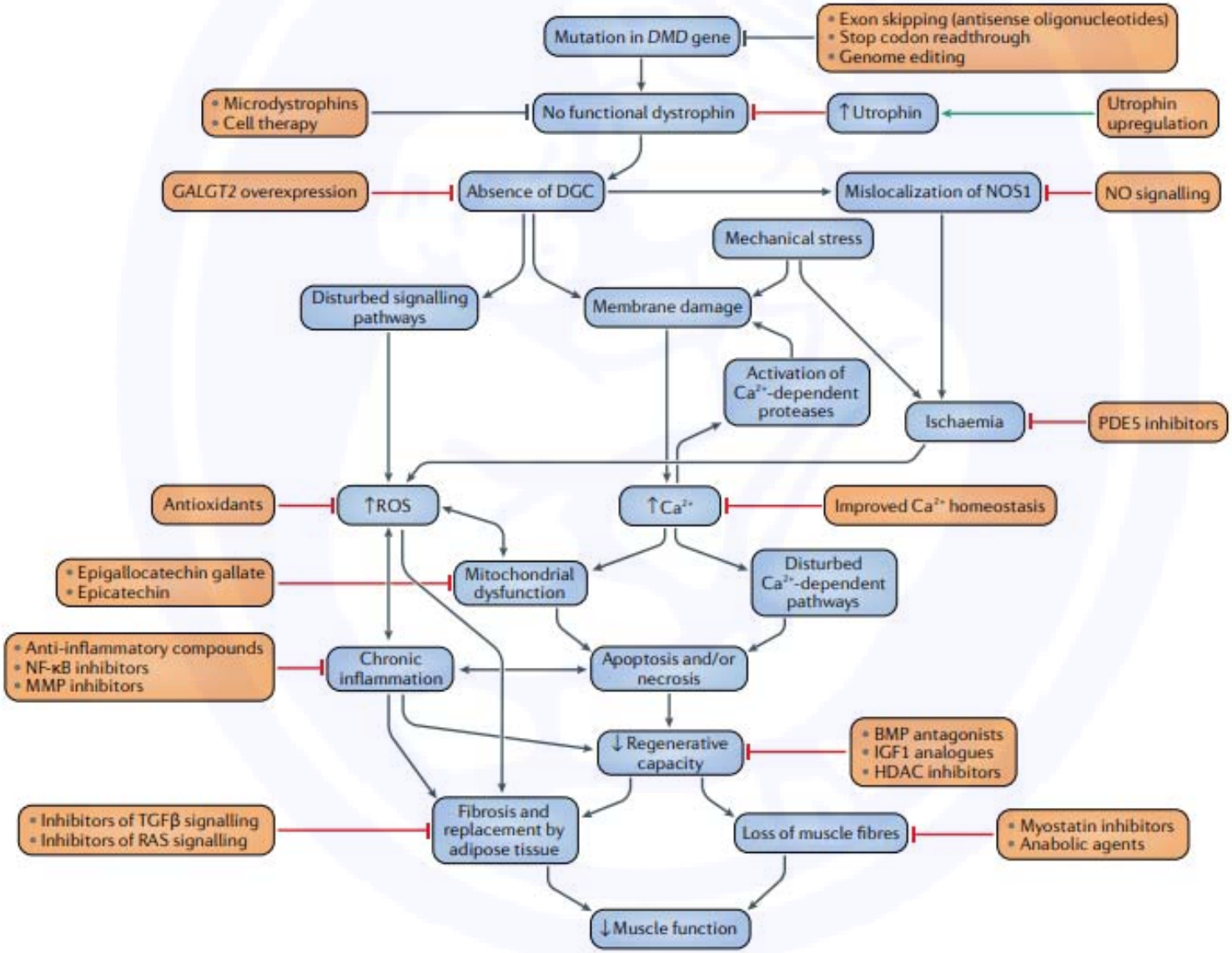
DMD Therapies



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Anti-Inflammatory Therapies

- Oral corticosteroids: standard of care in early ambulatory phase
- Corticosteroids prolong ambulation, reduce decline in cardiopulmonary function and risk of scoliosis, and improve life expectancy
- Doses: Prednisone (0.75mg/kg/d) or Deflazacort (0.9 mg/g/d)
- Dosing regimens: Daily, 10 days on and off, high weekly doses
- Deflazacort (Emflaza): less weight gain, behavioral changes compared to prednisone
- A 5-year, randomized, double-blind study comparing daily deflazacort, daily prednisone, and intermittent prednisone has been completed; results are pending (FOR-DMD)

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Anti-Inflammatory Therapies

- Muscle fiber degeneration from inflammation due to chronic NF-κB activation
- Vamorolone - steroid analog with membrane-stabilizing and anti-inflammatory properties (including inhibition of NF-κB)
  - Daily vamorolone treatment suggested efficacy at doses of 2.0 and 6.0 mg/kg/d in an exploratory 24-week open-label study[a]
- Edasalonexent is an inhibitor of NF-κB
  - A phase 2 trial in DMD patients had shown promise→ just finished a phase 3 study in steroid-naïve DMD patients[b]

a: https://clinicaltrials.gov/ct2/show/NCT03439670

b: https://clinicaltrials.gov/ct2/show/NCT02439216

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Antioxidants

- Reactive oxygen species (ROS)→ muscle cells susceptible to injury via increased membrane permeability, protein degradation, activation of the inflammatory cascade, and mitochondrial
- Idebenone: synthetic short-chain benzoquinone and coenzyme Q10 derivative→ restores mitochondrial function through its role in mitochondrial electron transport chain
- A phase 3 study demonstrated improved pulmonary function in DMD patients not using corticosteroids
- A phase 3 study for DMD patients on oral corticosteroids is currently underway (SIDEROS)

Buyse GM, et al. Efficacy of idebenone on respiratory function in patients with Duchenne muscular dystrophy not using glucocorticoids (DELOS): a double-blind randomised placebo-controlled phase 3 trial. Lancet. 2015;385:1748–57.

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Anti-Fibrotic Agents

- Downregulation of TGF-β→ decrease fibrosis in preclinical DMD models
  - Losartan, a antihypertensive agent failed to show significant functional benefit
- Connective tissue growth factor (CTGF) promotes fibrosis and reduces muscle fiber regeneration
  - Pamrevlumab (GF- 3019) is a humanized monoclonal antibody against CTGF which is undergoing a phase 2, open-label study in nonambulatory DMD patients
- Histone deacetylase (HDAC) inhibitors have beneficial role in the mdx mouse model
  - Givinostat is an HDAC inhibitor that has anti-inflammatory, anti-fibrotic, and regenerative properties, currently in a phase 3 study in ambulatory DMD patients

Datta N, Ghosh PS. Update on Muscular Dystrophies with Focus on Novel Treatments and Biomarkers. Curr Neurol Neurosci Rep. 2020;20(6):14.

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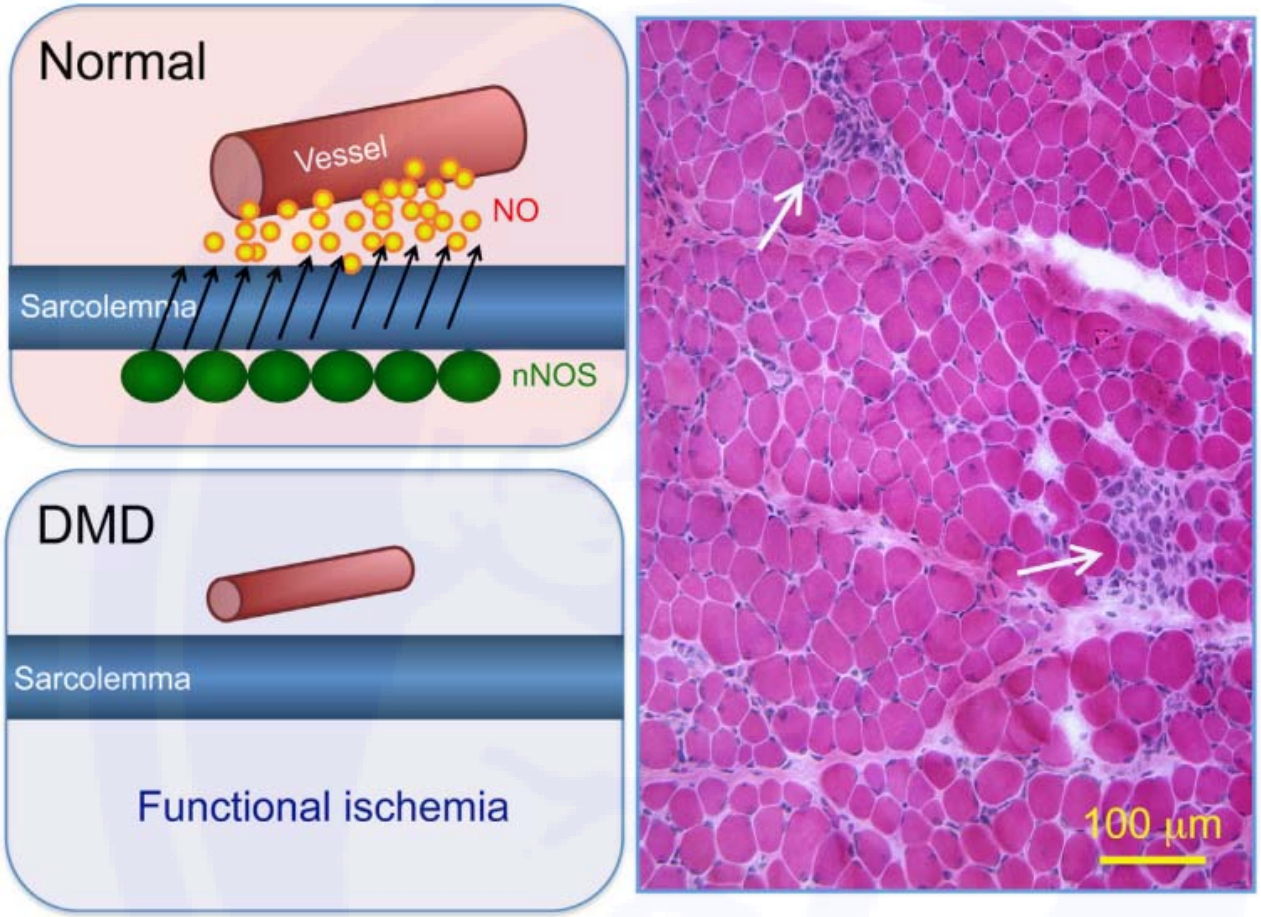

- In normal muscle, nNOS is localized at the sarcolemma→ allows immediate diffusion of NO to the vasculature and vasodilation in contracting muscle
- In DMD, the loss of sarcolemmal nNOS compromises this process and leads to functional ischemia. T
- he H&E-stained image illustrates focal ischemic lesions (arrow) as the first observable histological change in a 3-week-old affected dog. Despite the absence of dystrophin, histologically, the majority of myofibers appeared normal at this age.

Duan D. Systemic AAV Micro-dystrophin Gene Therapy for Duchenne Muscular Dystrophy Mol Ther. 2018;26:2337-2356.

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Phosphodiesterase 5A Inhibition

- Lack of defective dystrophin→ reduced functional NO-generating enzymes→ poor muscle perfusion →muscle damage

- Phosphodiesterase inhibition (PDEi)→ improves muscular perfusion→ reducing muscular degeneration

- Sildenafil (short-acting PDEi) and tadalafil (long-acting PDEi) demonstrated reduced muscle damage with exercise in DMD animal models

- A phase 3 study found no significant effect of tadalafil on 6MWT

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Myostatin Inhibition

- Myostatin (growth differentiation factor 8, GDF8)→ limits skeletal muscle growth→ avoid abnormal hypertrophy by inhibition of myoblast maturation

- Myostatin inhibitors increase muscle mass and reduce fibrosis

  - ACE-031: myostatin inhibitor and is a fusion protein of activin receptor type IIB and IgG1-Fc which binds myostatin and related ligands.

  - A trend of maintained 6MWD versus the decline in placebo groups was noted, early-phase trials were stopped due to epistaxis and telangiectasias

Campbell C, et al. Myostatin inhibitor ACE-031 treatment of ambulatory boys with Duchenne muscular dystrophy: results of a randomized, placebo-controlled clinical trial. Muscle Nerve.2017;55:458–64.

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Utrophin Modulation

- Utrophin has significant homology to dystrophin; present during both fetal development and muscle regeneration and is expressed mostly at neuromuscular junctions
- Dystrophin-utrophin double mutants showed more severe muscle weakness than dystrophin-only mutant mice
- Ezutromid was a promising agent to increase utrophin expression in DMD; however, a phase 2 study in ambulatory DMD patients failed to demonstrate efficacy
- Another strategy of utrophin expression modulation is through adeno-associated virus (AAV)-mediated gene delivery of the GALGT2 gene to effect GALGT2 overexpression[a]
- GALGT2 acts at the synaptic regions to add the terminal GalNAc to an O-linked carbohydrate antigen on α-dystroglycan
- Overexpression of GALGT2 → ectopic expression of many synapse-associated proteins, including utrophin→ thereby ameliorating DMD phenotype

Gene transfer clinical trial to deliver rAAVrh74.MCK.GALGT2 for Duchenne muscular dystrophy. https://clinicaltrials.gov/ct2/show/NCT03333590.

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Improvement of Cardiac and Respiratory Function

- CAP-1002: allogenic cardiocyte precursor stem cells are administered directly to heart via catheterization of coronary arteries to treat cardiomyopathy
  - Currently, in phase 1/2 studies and some scar size reduction and inferior wall improvements have been seen
- Rimeporide, a sodium proton exchanger (NHE-1) inhibitor, initially developed for heart failure
  - Granted orphan drug status in Europe for a similar indication in DMD
- Carmeseal-MD/P-188 NF targets muscle membrane stabilization by its amphiphilic structure properties to improve cardiac, respiratory, and muscular weakness
  - Preclinical studies demonstrated reduction in respiratory function decline

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Dystrophin Protein Function Restoration

- Significant recent development in research and clinical studies
- 3 principal strategies:
  - A: Exon skipping (amenable to ~ 80% of mutations)
  - B: Nonsense Mediated Suppression (~10-15% mutations)
  - C: Gene therapy (irrespective of mutation type)

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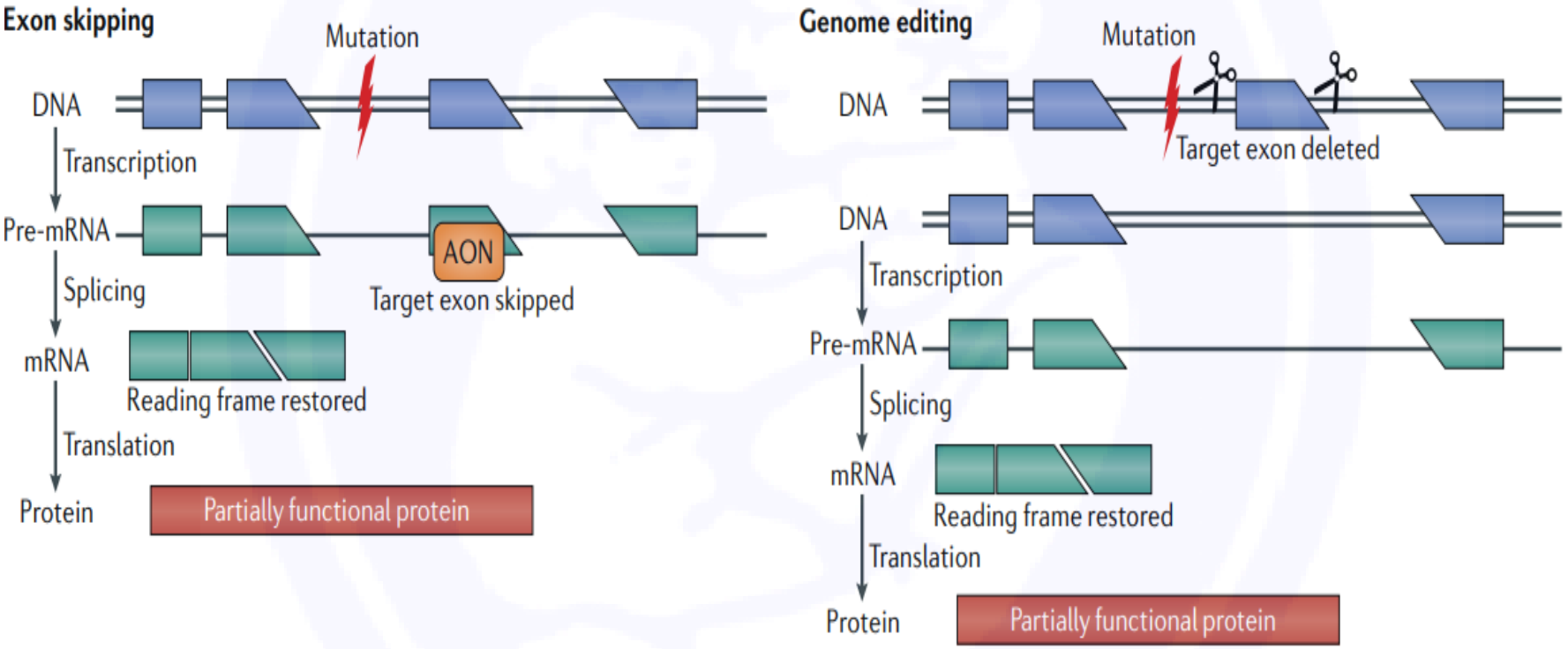

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Exon Skipping

- Functional protein product is salvaged by the use of synthetic antisense oligonucleotide (ASO) targeted mRNA level to skip out-of-frame mutations→ reading frame restoration
- First ASO clinical trials involved exon 51 skipping using the compounds drisapersen and eteplirsen; 13% are amenable to exon 51 skipping
  - Eteplirsen (Exondys 51) received accelerated FDA approval in 2016 based primarily on a small trial (n = 12) that demonstrated a 23% increase in dystrophin-positive muscle fibers and 6MWD improvement
  - First drug to receive FDA approval using dystrophin quantification as a surrogate outcome measure for DMD
  - Weekly iv infusions; generally well tolerated
  - Longer term studies showed some stabilization of motor and pulmonary functions

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Exon Skipping

- Golodirsen (exon skipping 53 Vyondys- recently FDA approved): ~ 10% of DMD patients amenable to golodirsen; 2% of patients on golodirsen are amenable to eteplirsen, as deletion of exon 52 is amenable to either exon 51 or 53 skipping
- Casimersen (exon skipping 45: not yet FDA approved): ~ 9% amenable to casimersen
- Peptide-conjugated phosphorodiamidate morpholino oligomers (PPMO) are ASOs with neutral DNA analogs→ preclinical studies shown improved muscle cell penetration, favorable pharmacokinetics, and notable penetration into the heart and diaphragm
  - SRP-5051, a PPMO for exon 51 skipping studied in a phase 1 study
- Three other exon skipping programs underway:
  - NS-065 for exon 53 skipping; WVE-210201 for exon 51 skipping; DS-5141b for exon 45 skipping

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Nonsense Mediated Suppression

- Nonsense mutations → generate a stop codon→ truncated and nonfunctional proteins
- Read-through strategy results in suppression of the stop codon→ production of partially functional dystrophin
- Proof of efficacy initially demonstrated by aminoglycoside antibiotic-gentamicin
- Ataluren- (oral agent, better safety profile), promotes read-through of premature stop codon
  - A phase 2 trial revealed increased dystrophin expression on muscle biopsy, which led to conditional approval in Europe
  - A phase 3 study did not show improvement in 6MWD in all DMD patients and was not approved by the FDA
  - Further studies in ambulatory DMD patients are underway to address these concerns

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Gene Therapy

- Challenges of gene therapy in DMD:
  - Restoration of normal dystrophin gene would be ideal
  - Dystrophin gene's large size makes packaging inside viral vectors difficult
- Concept of micro- and minidystrophin:
  - A BMD patient was found to be ambulatory at age 61 with deletion of exons 17–48→ development of micro- and minidystrophin constructs small enough to be packaged in AAV → modify DMD phenotype severity

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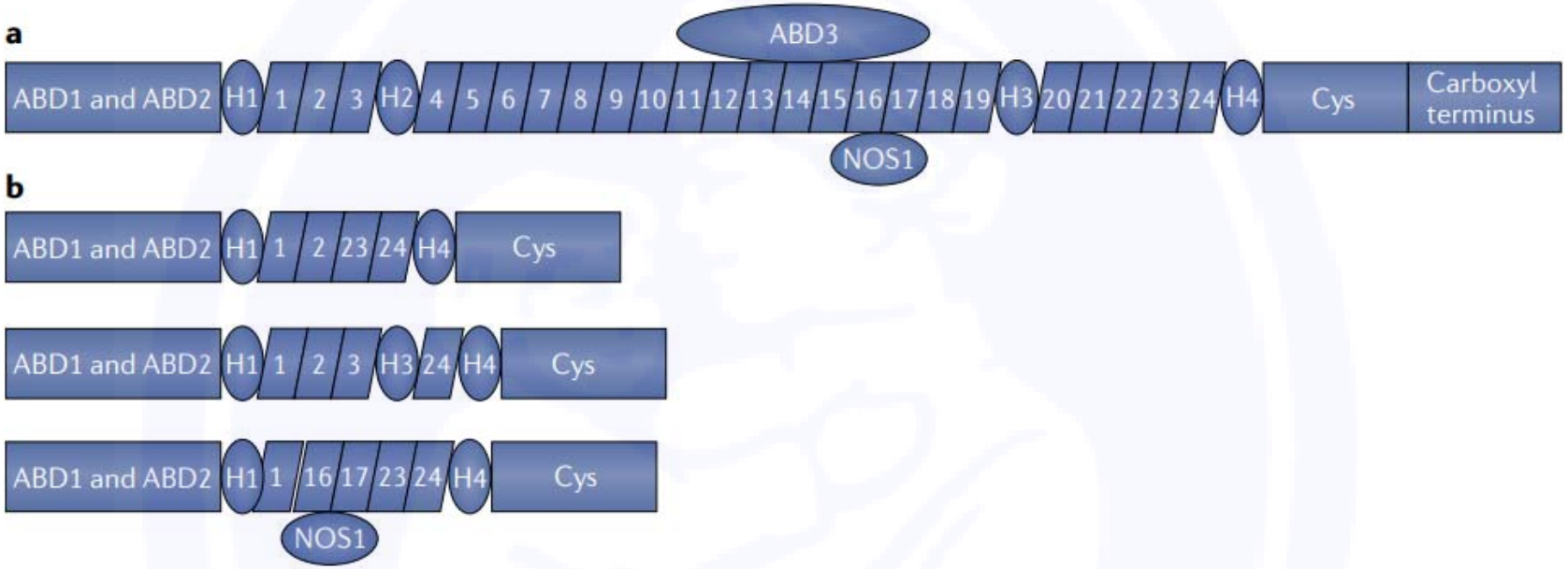
Gene Therapy



- Crucial domains of the dystrophin protein:
- NH3 terminal actin-binding domains (ABD1–ABD3) and the cysteine-rich (Cys) domain which binds to β-dystroglycan and connected to the extracellular matrix protein laminin
- Aminoterminal and Cys domains are interspersed by 24 spectrin-like repeats (numbered 1–24) and 4 hinge domains (H1–H4)
- Dystrophin also contains a binding domain for nitric oxide synthase (NOS1) located at spectrin-like repeats 16–17
- Carboxy-terminal domain binds to syntrophin

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Gene Therapy

- 3 programs are using different AAV vector serotypes and different promoters to augment expression of dystrophin in the muscle cells
- One-time intravenous infusions with systemic corticosteroids to dampen immunogenic response from viral vector infusions
  - AAVrh74 capsid with an MHCK7 promoter (Sarepta)
  - AAV9 capsid with a CK8 promoter (Solid Bioscieces)
  - AAV9 capsid with an unspecified muscle-specific promoter (Pfzier)
- Early results are promising with reduction of CK, significant expression of dystrophin on iummostaining and WB in muscle biopsies of DMD patients
- Long term concerns:
  - duration of the response
  - need for re-dosing,
  - side effects (hepatic dysfunction, complement activation syndromes)
  - integration in host genome

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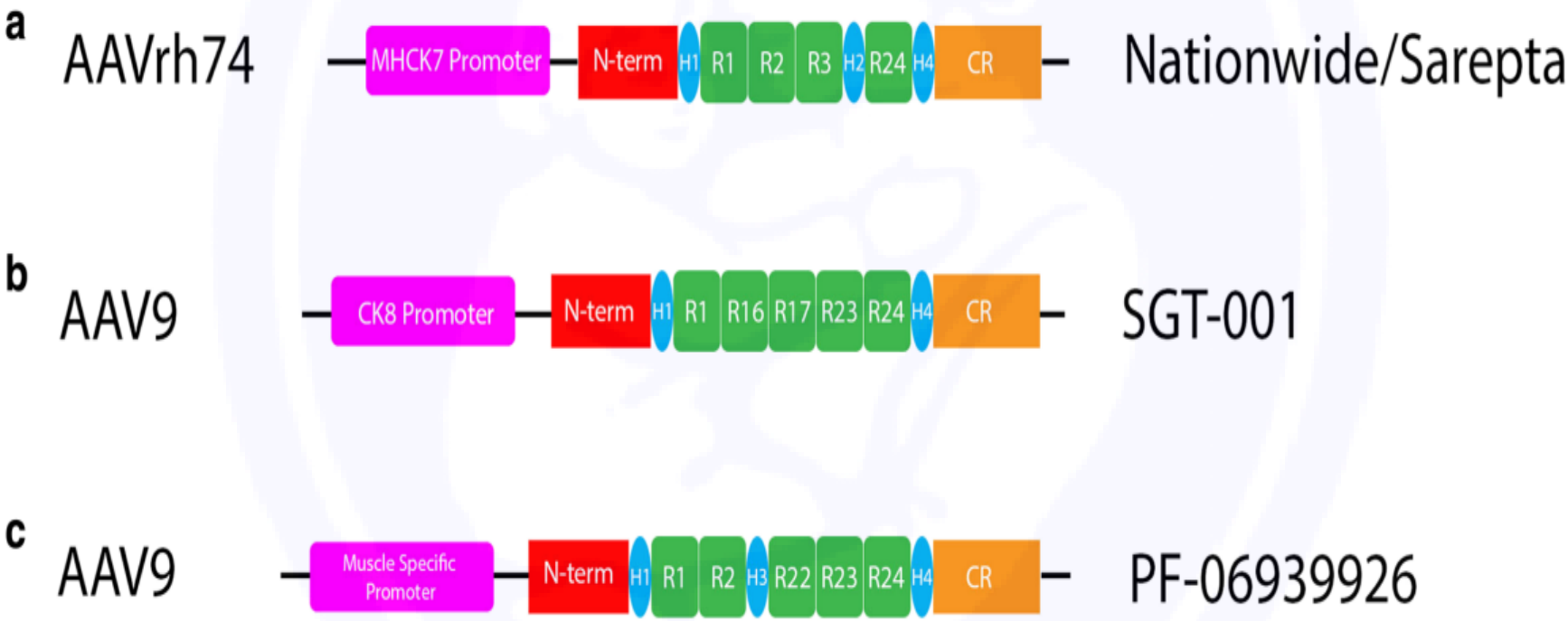
Microdystrophins



Shieh PB. Emerging Strategies in the Treatment of Duchenne Muscular Dystrophy. Neurotherapeutics (2018) 15:840–848.

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CRISPR/Cas9

- Recent discovery of clustered regularly interspaced short palindromic repeats (CRISPR) technology, in which an endonuclease called Cas9 can cleave the genome in a precise manner when coupled with a strand of guide RNA appears very promising

- Can be employed for a number of different mutations including patients with exon or multi-exon deletions

- Allow gene editing to be implemented in virtually all DMD mutations, providing tremendous potential for individualized treatment

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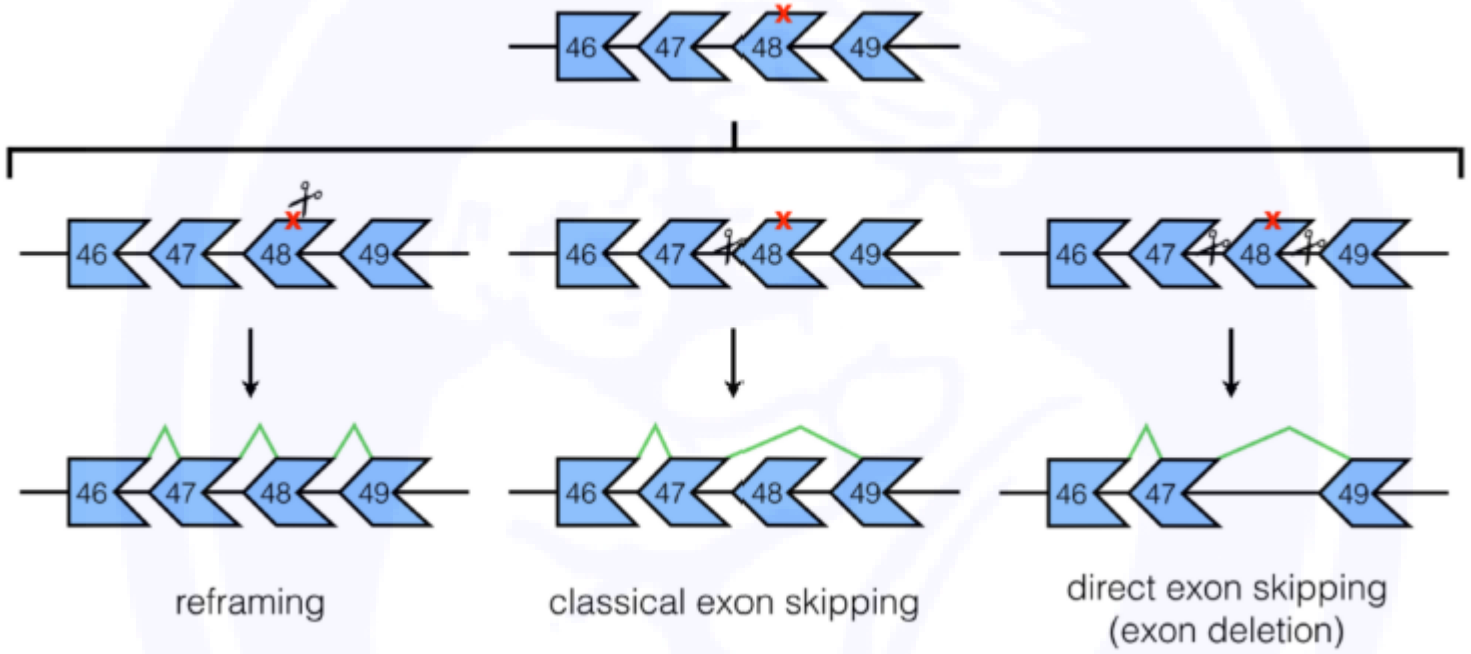
CRISPR/Cas9



- Non-homologous end joining (NHEJ) mediated gene correction by CRISPR/Cas9

- These approaches differ depending on which site is targeted by the gRNA (represented by scissors), as well as by the number of gRNAs used for treatment

J. Pers. Med. **2018**, 8, 38; doi:10.3390/jpm8040038

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Biomarkers

- Reliable DMD biomarker development lags behind novel therapeutic agents
- Most commonly used disease progression measures used in clinical trials are various functional scales:
  - 6 minute walk distance (6MWD)
  - Motor function measure (MFM)
  - North Star Ambulatory assessment (NSTAR) rating scales
  - These measures are subject to individual variation and patient motivation
- Most widely used diagnostic biomarker is serum CK
  - Useful for basic screening but is a weak pharmacodynamic biomarker and surrogate end point
  - CK in dried spot test can be used in newborn screening for early detection of DMD
- Dystrophin analysis via immunohistochemical staining (IHC) or western blot (WB) in muscle biopsy specimens are rarely used for diagnostic purposes now
  - With advent of gene-based therapies to restore dystrophin protein, these measures are used as surrogate endpoints for efficacy
  - Correlation between functional measures and expression of dystrophin in biopsies still weak

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Biomarkers

- MRI and MRS are useful noninvasive techniques to assess replacement of muscle tissue by fibrous and fatty replacement

- Pros:
  - Assess muscle tissue and thus disease progression
  - Offers reliable reproducible quantification across centers in clinical trials
  - Does not depend on patient motivation

- Cons:
  - Discomfort associated with immobilization
  - Difficult to perform in smaller children
  - Costs can be prohibitive outside of the clinical trials

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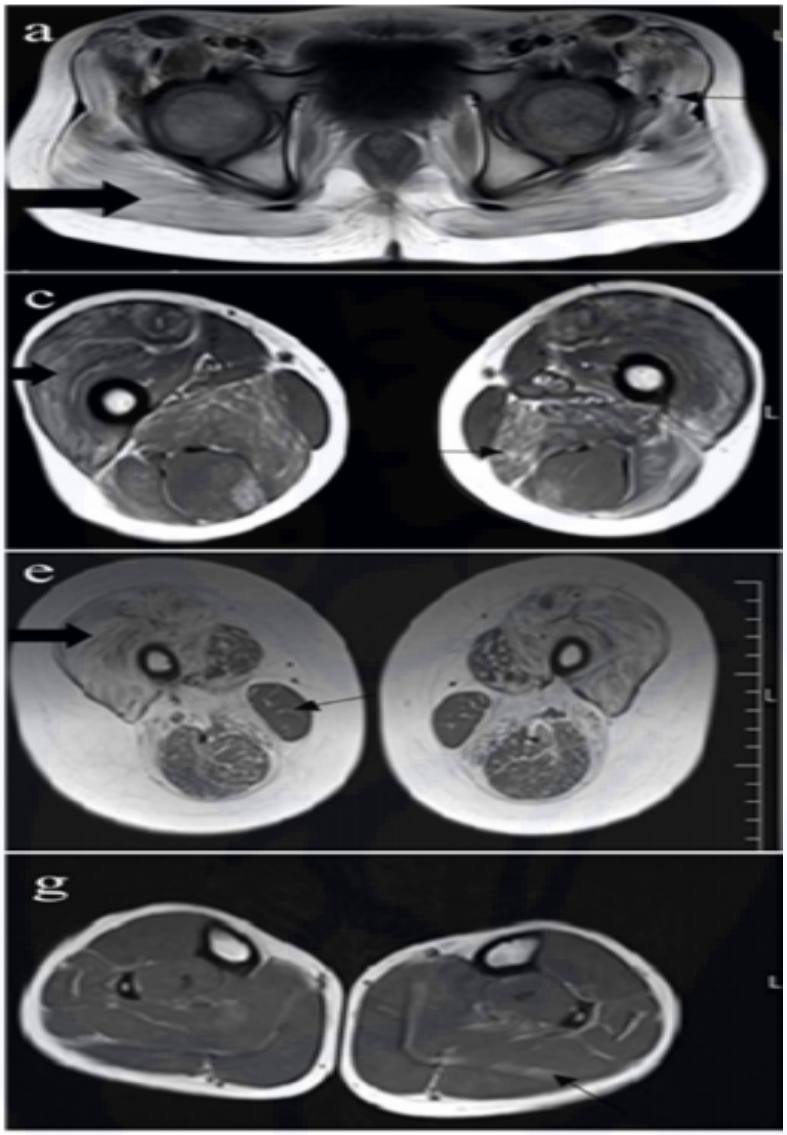

(a) T1 sequence at pelvis level shows gross fatty infiltration of Gluteus maximus (block arrow) and Gluteus medius (line arrow)

(c) T1 sequence at mid-thigh level shows fatty infiltration of Vastus lateralis (block arrow) and Adductor magnus (line arrow)

(e) T1 sequence at mid-thigh level in a different patient shows advanced fatty infiltration of Quadriceps (block arrow) with sparing and hypertrophy of Gracilis muscle (line arrow)

(g) T1 sequence at mid-leg level shows early fatty changes of Gastrocnemius and Soleus muscles (line arrow)

K. Polavarapu et al. / Neuromuscular Disorders 26 (2016) 768–774

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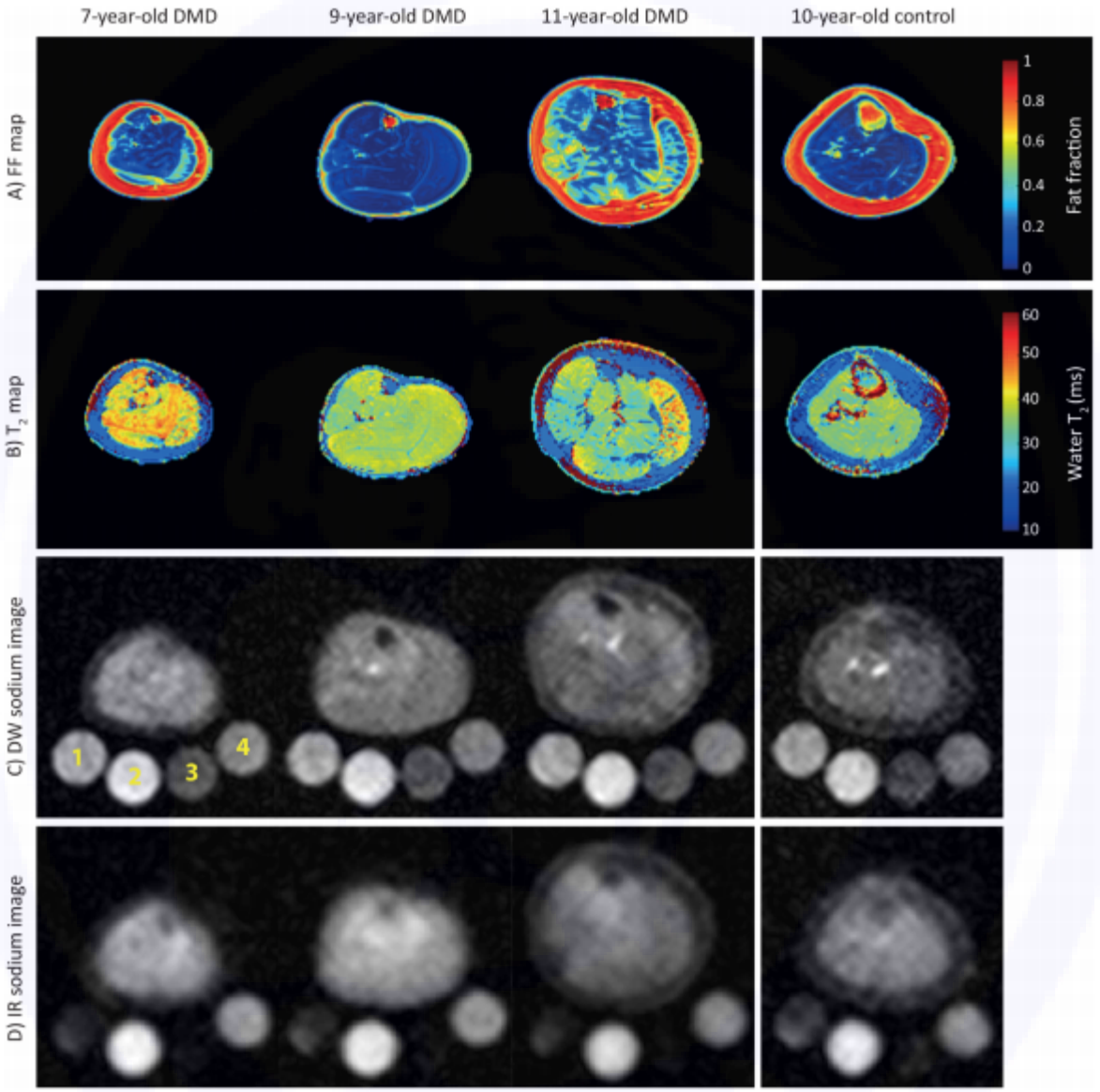

Illustration of (a) fat fraction maps, (b) water T2 maps, (c) DW 23Na images, and (d) IR 23Na images in the leg of a 7-yearold, 9-year-old , and 11-year-old DMD patients as well as a 10-year-old control

Gerhalter et al.: Sodium MRI Detects Early Dystrophic Changes. J. MAGN. RESON. IMAGING 2019;50:1103–1113

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Thank You

Questions and Comments

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