High-Yield Muscular Dystrophy Pathology

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AMERICAN ASSOCIATION OF NEUROPATHOLOGISTS

THE UNIVERSITY OF IOWA

Disclosures

- I have the following relevant financial relationships to disclose
  - Consultant
    - Audentes Therapeutics/Astellas Gene Therapies

      (Work not related to the content of this talk)

Learning Objectives

- Examine common proteins involved in muscular dystrophies and recognize dystrophic histopathologic features
- Compare and contrast immunostaining patterns in Duchenne versus Becker muscular dystrophies
- Summarize the molecular genetic basis of dystroglycanopathies and the tools helpful for diagnosis
- Identify unique histopathologic findings of dysferlinopathies

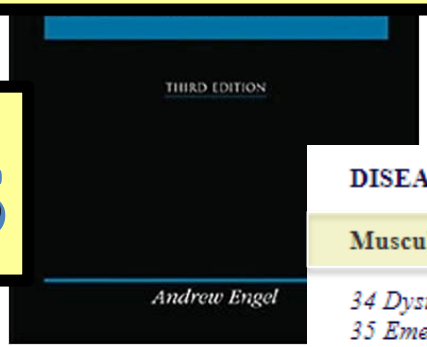
Muscular dystrophies in 1 hour?

Common

Pitfalls to avoid

Tips for diagnosis

Unique



DISEASES OF …

Muscular Dystr…

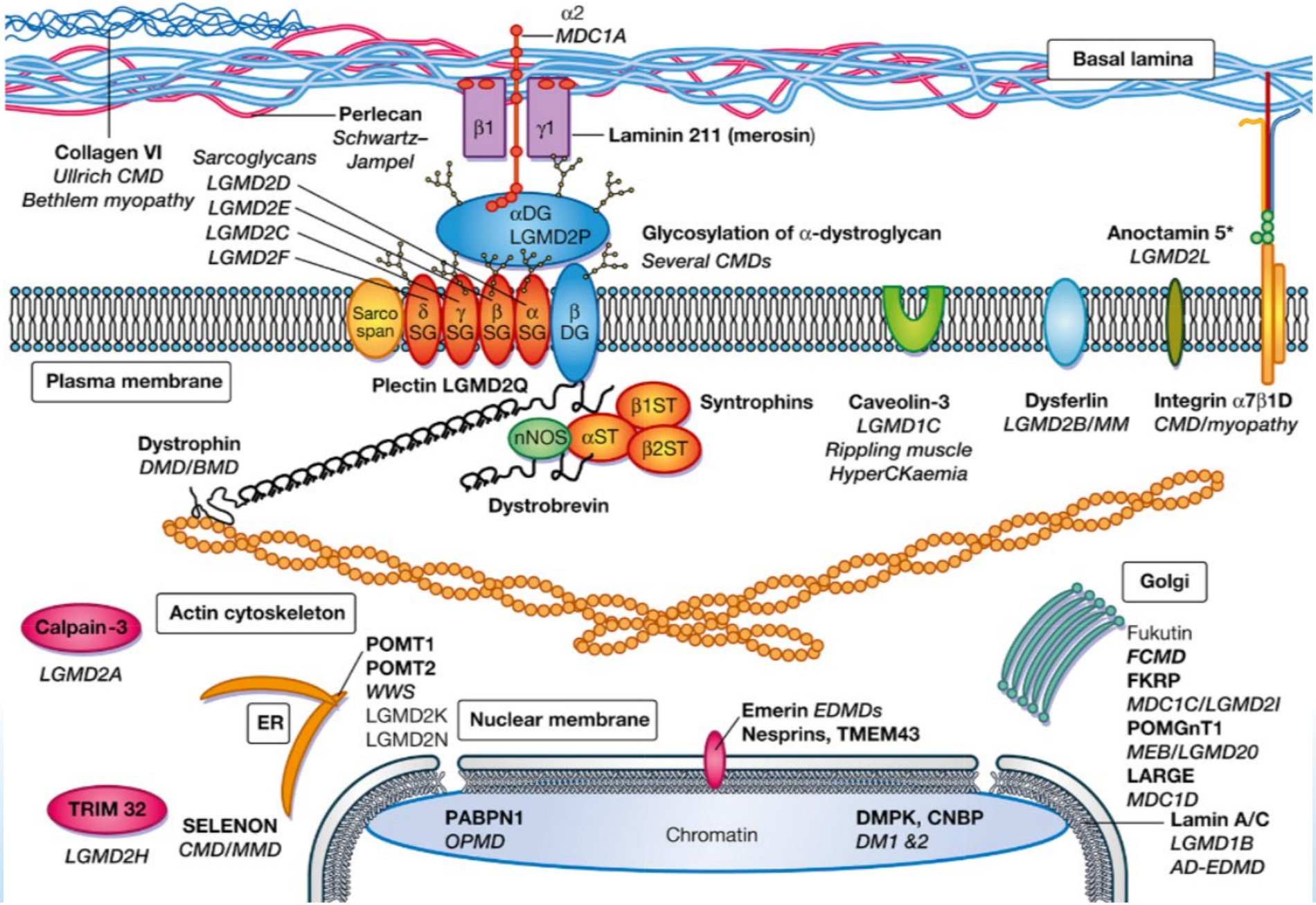
DYSTROPHY

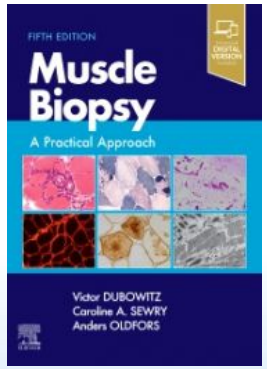
dys = bad/faulty
trophe/trophia = nourishment

a wasting of body tissues, of genetic origin (as we now know), or due to inadequate or defective nutrition (what was originally postulated)

What are muscular dystrophies?

- Myopathies = disease characterized clinically by muscle weakness
  - Muscular dystrophies are a subset of myopathies characterized physiologically/pathologically by repeated cycles of myonecrosis and regeneration
- Genetic (mostly inherited) muscle disorders
- Gene alterations → protein alterations → muscle disease
- Proteins located in many myofiber compartments are involved in muscular dystrophies
  - Reticular lamina and basal lamina (a.k.a. basement membrane), sarcolemma (a.k.a. plasma membrane), subsarcolemma, cytoskeleton, myofibrils, nuclei, Golgi, lysosomes, etc.

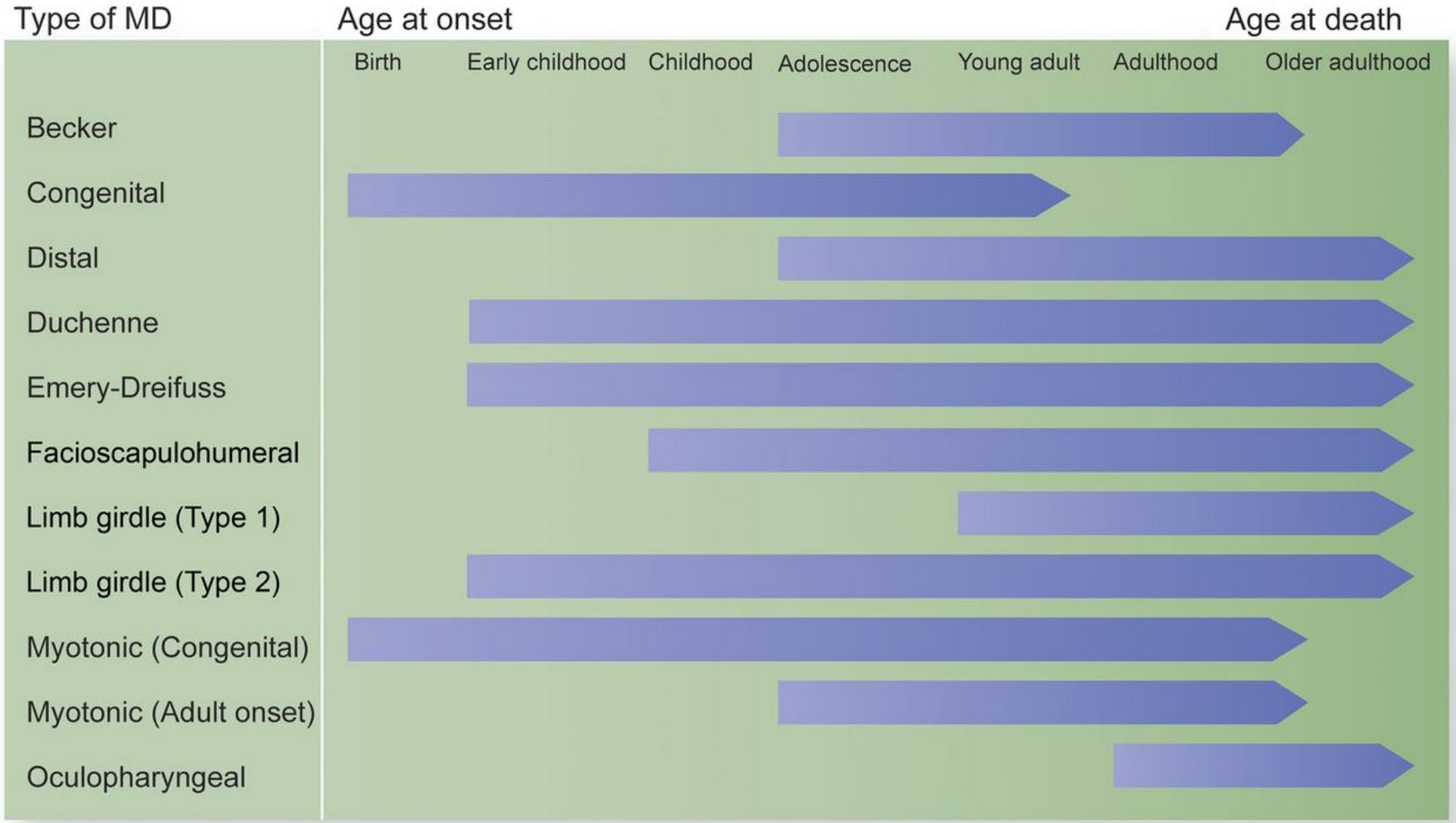




Dubowitz, Sewry, and Oldfors. Muscle Biopsy: A Practical Approach. 5th edition. 2021. Elsevier

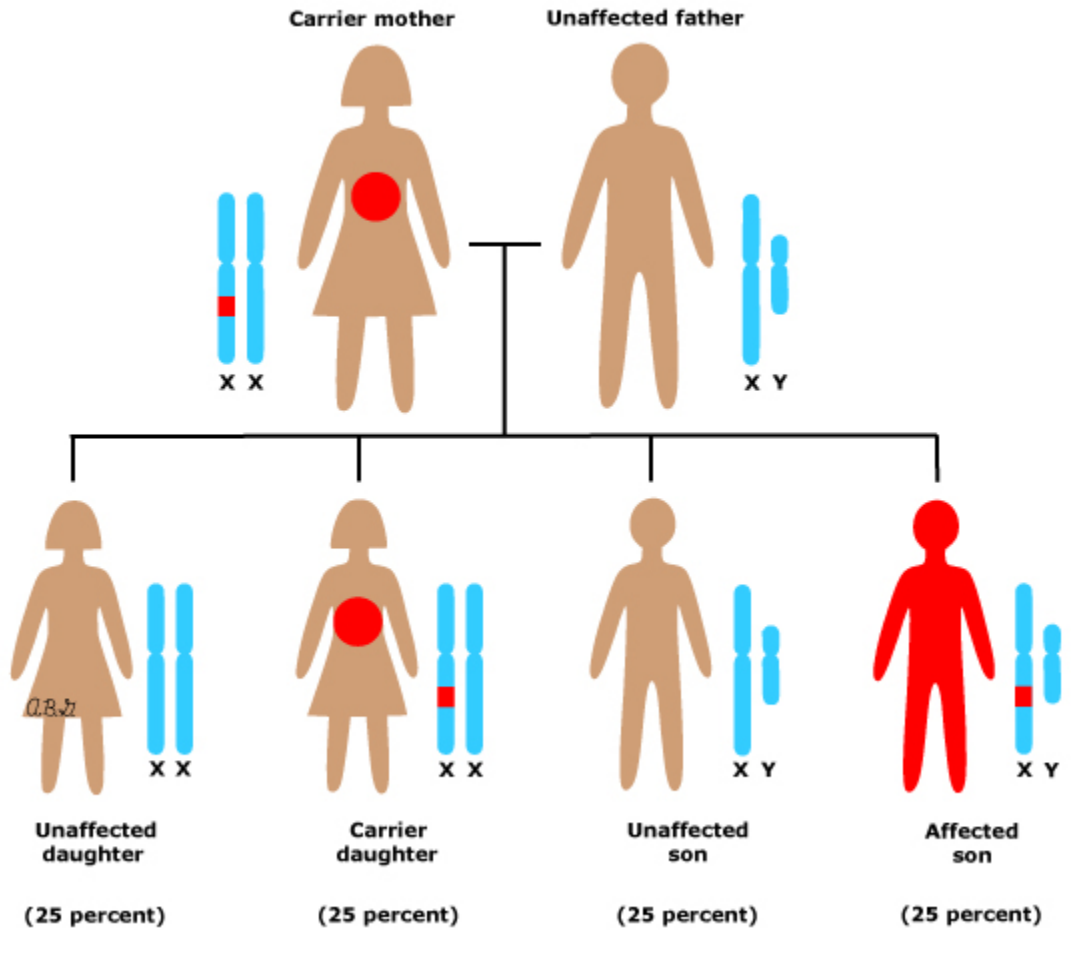
How do we classify (and keep straight!) muscular dystrophies?

1. Age



How do we classify (and keep straight!) muscular dystrophies?
2. Pattern of inheritance

X-linked inheritance



X-linked muscular dystrophies:
- Duchenne and Becker MD
- Emery-Dreifuss MD

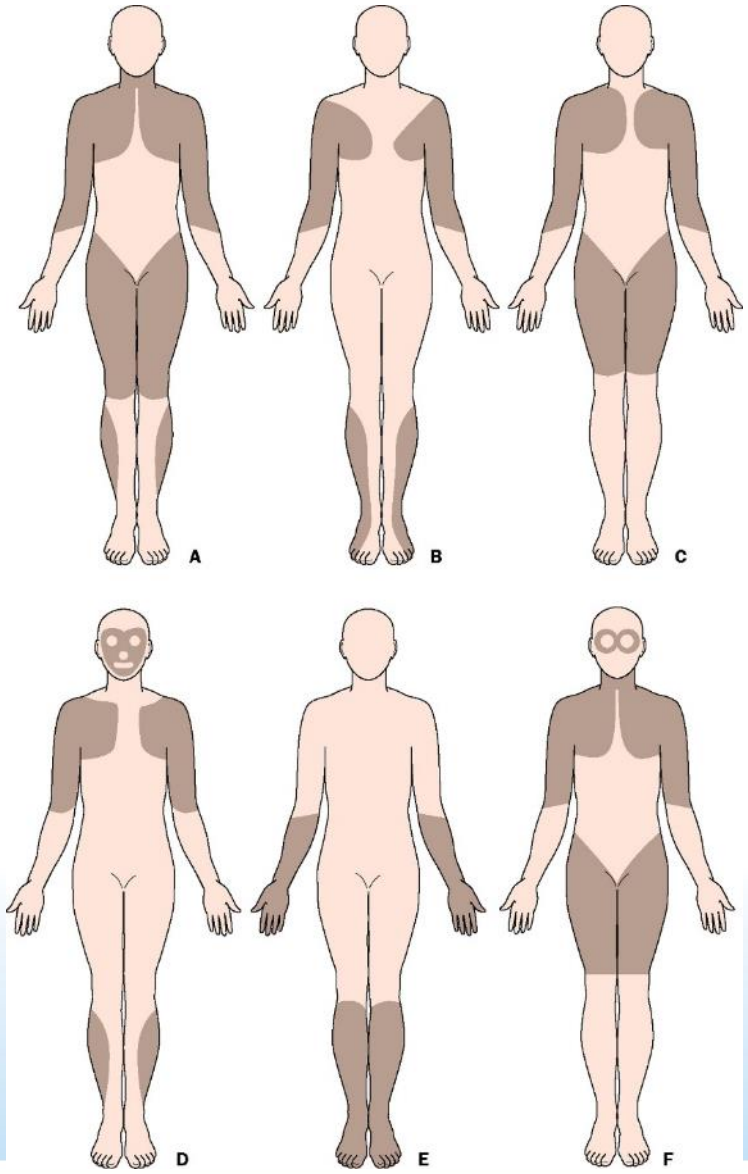
Autosomal recessive muscular dystrophies:
- LGMD
- Congenital MD
- Distal MD

Autosomal dominant muscular dystrophies:
- LGMD
- Facioscapulohumeral MD
- Distal MD
- Myotonic dystrophy
- Oculopharyngeal MD

https://www.uptodate.com/contents/overview-of-muscular-dystrophies-beyond-the-basics

How do we classify (and keep straight!) muscular dystrophies?

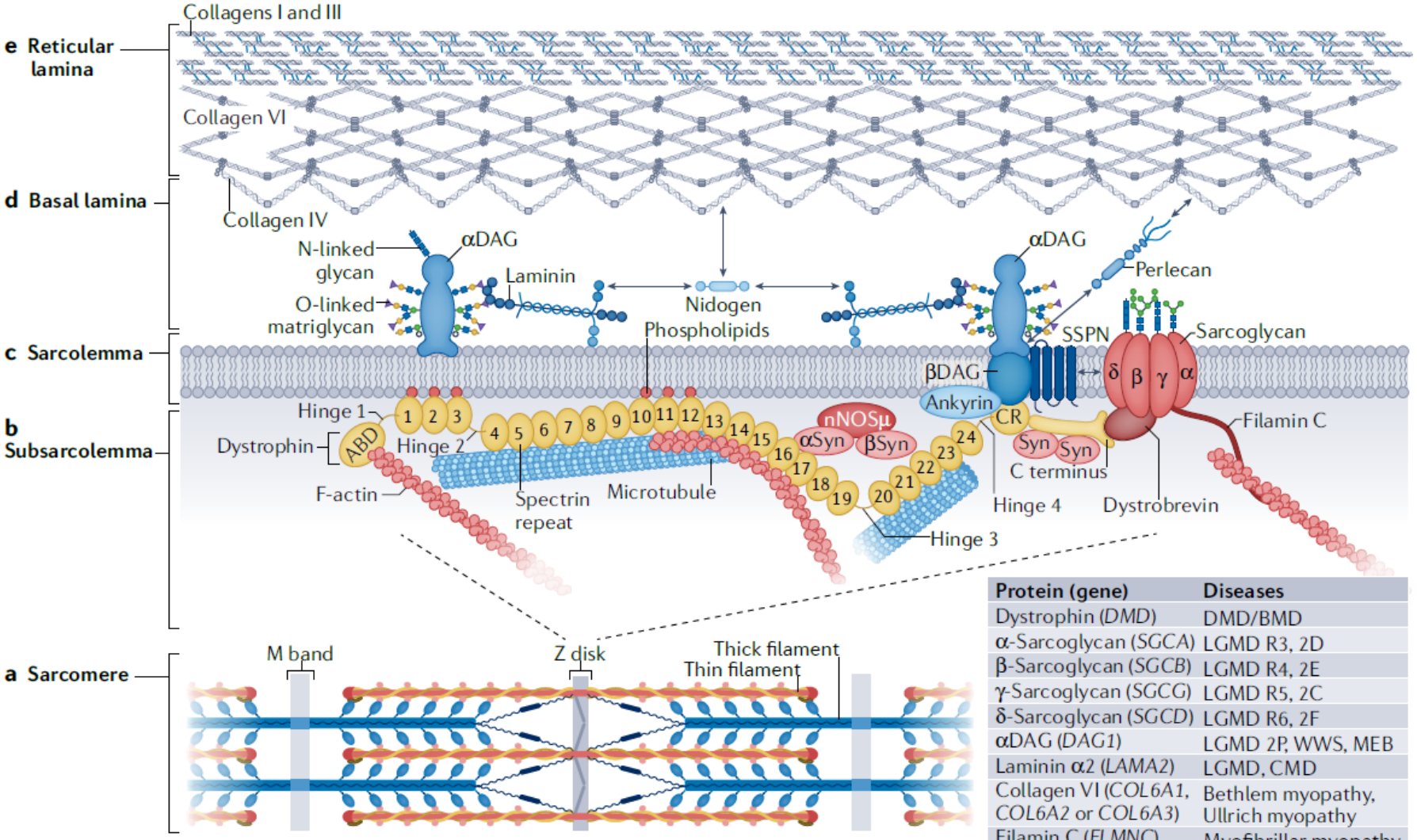
3. Muscle groups



A. Duchenne and Becker MD
B. Emery-Dreifuss MD
C. Limb girdle MD
D. Facioscapulohumeral MD
E. Distal MD
F. Oculopharyngeal MD

Emery. The Lancet. 2002. PMID: 11879882

How do we classify (and keep straight!) muscular dystrophies? 4. Genes/proteins and 5. Compartment of muscle involved



Protein (gene)	Diseases
Dystrophin (*DMD*)	DMD/BMD
α-Sarcoglycan (*SGCA*)	LGMD R3, 2D
β-Sarcoglycan (*SGCB*)	LGMD R4, 2E
γ-Sarcoglycan (*SGCG*)	LGMD R5, 2C
δ-Sarcoglycan (*SGCD*)	LGMD R6, 2F
αDAG (*DAG1*)	LGMD 2P, WWS, MEB
Laminin α2 (*LAMA2*)	LGMD, CMD
Collagen VI (*COL6A1*, *COL6A2* or *COL6A3*)	Bethlem myopathy, Ullrich myopathy
Filamin C (*FLMNC*)	Myofibrillar myopathy

Dowling et al. Nat Rev Mol Cell Biol. 2021. PMID: 34257452

A note on nomenclature

- LGMD nomenclature recently changed – European consensus
- Helpful to use the protein's name:
  - e.g. dystroglycanopathies, dystrophinopathies, sarcoglycanopathies, dysferlinopathy, etc

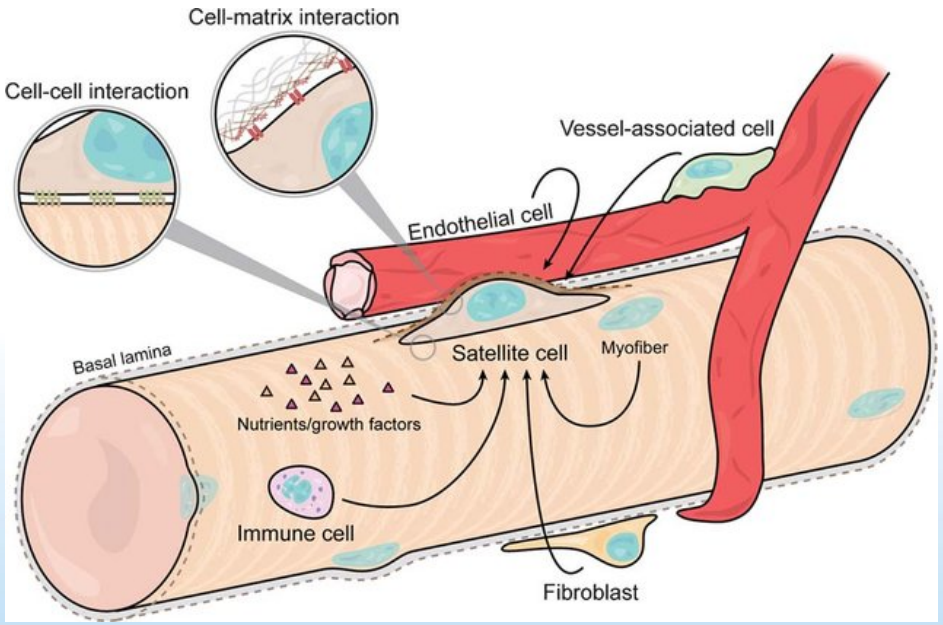
Old name	Gene	Proposed new nomenclature	Reason for exclusion
LGMD 1A	*Myot*	Myofibrillar myopathy	Distal weakness
LGMD 1B	*LMNA*	Emery–Dreifuss muscular dystrophy (EDMD)	High risk of cardiac arrhythmias; EDMD phenotype
LGMD 1C	*CAV3*	Rippling muscle disease	Main clinical features rippling muscle disease and myalgia
LGMD 1D	*DNAJB6*	LGMD D1 DNAJB6-related	
LGMD 1E	*DES*	Myofibrillar myopathy	Primarily false linkage; distal weakness and cardiomyopathy
LGMD 1F	*TNP03*	LGMD D2 TNP03-related	
LGMD 1G	*HNRNPDL*	LGMD D3 HNRNPDL-related	
LGMD 1H	?	Not confirmed	False linkage
LGMD 1I	*CAPN*	LGMD D4 calpain3-related	
LGMD 2A	*CAPN*	LGMD R1 calpain3-related	
LGMD 2B	*DYSF*	LGMD R2 dysferlin-related	
LGMD 2C	*SGCG*	LGMD R5 γ-sarcoglycan-related[a]	
LGMD 2D	*SGCA*	LGMD R3 α-sarcoglycan-related	
LGMD 2E	*SGCB*	LGMD R4 β-sarcoglycan-related	
LGMD 2F	*SGCD*	LGMD R6 δ-sarcoglycan-related	
LGMD 2G	*TCAP*	LGMD R7 telethonin-related	
LGMD 2H	*TRIM32*	LGMD R8 TRIM 32-related	
LGMD 2I	*FKRP*	LGMD R9 FKRP-related	
LGMD 2J	*TTN*	LGMD R10 titin-related	
LGMD 2K	*POMT1*	LGMD R11 POMT1-related	
LGMD 2L	*ANO5*	LGMD R12 anoctamin5-related	
LGMD 2M	*FKTN*	LGMD R13 Fukutin-related	
LGMD 2N	*POMT2*	LGMD R14 POMT2-related	
LGMD 2O	*POMGnT1*	LGMD R15 POMGnT1-related	
LGMD 2P	*DAG1*	LGMD R16 α-dystroglycan-related	
LGMD 2Q	*PLEC*	LGMD R17 plectin-related	
LGMD 2R	*DES*	myofibrillar myopathy	Distal weakness
LGMD 2S	*TRAPPC11*	LGMD R18 TRAPPC11-related	
LGMD 2T	*GMPPB*	LGMD R19 GMPPB-related	
LGMD 2U	*ISPD*	LGMD R20 ISPD-related	
LGMD 2V	*GAA*	Pompe disease	Known disease entity, histological changes
LGMD 2W	*PINCH2*	PINCH-2 related myopathy	Reported in one family
LGMD 2X	*BVES*	BVES related myopathy	Reported in one family
LGMD 2Y	*TOR1AIP1*	TOR1AIP1 related myopathy	Reported in one family
LGMD 2Z	*POGLUT1*	LGMD R21 POGLUT1-related	
Bethlem myopathy recessive	*COL6A1, COL6A2, COL6A3*	LGMD R22 collagen 6-related	
Bethlem myopathy dominant	*COL6A1, COL6A2, COL6A3*	LGMD D5 collagen 6-related	
Laminin α2-related muscular dystrophy	*LAMA2*	LGMD R23 laminin α2-related	
POMGNT2-related muscular dystrophy	*POMGNT2*	LGMD R24 POMGNT2-related	

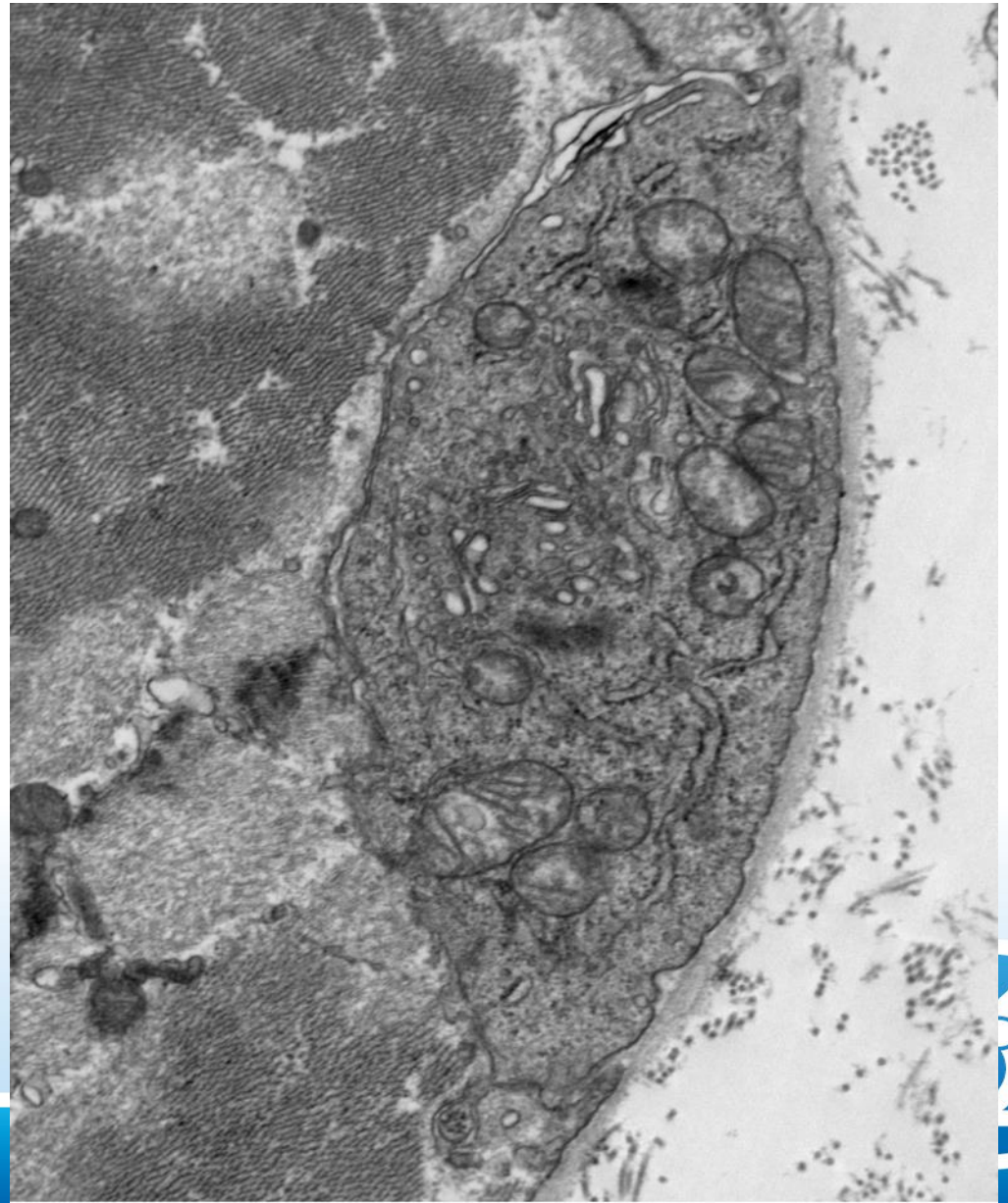
Straub et al. Neuromuscul Disord. 2018. PMID: 30055862

What do all muscular dystrophies have in common?

- DYSTROPHIC PATHOLOGY!
  - Myonecrosis and regeneration

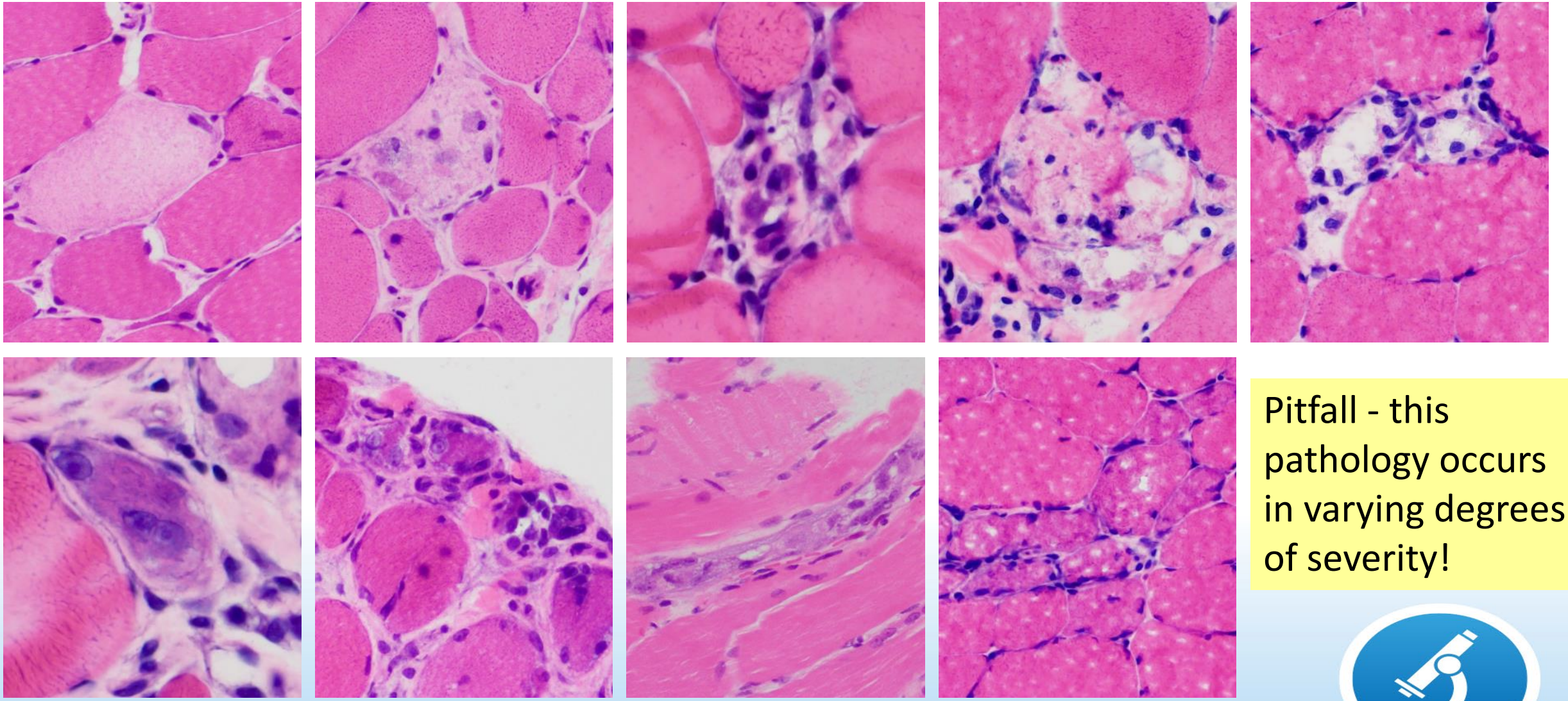
- Satellite cells are important



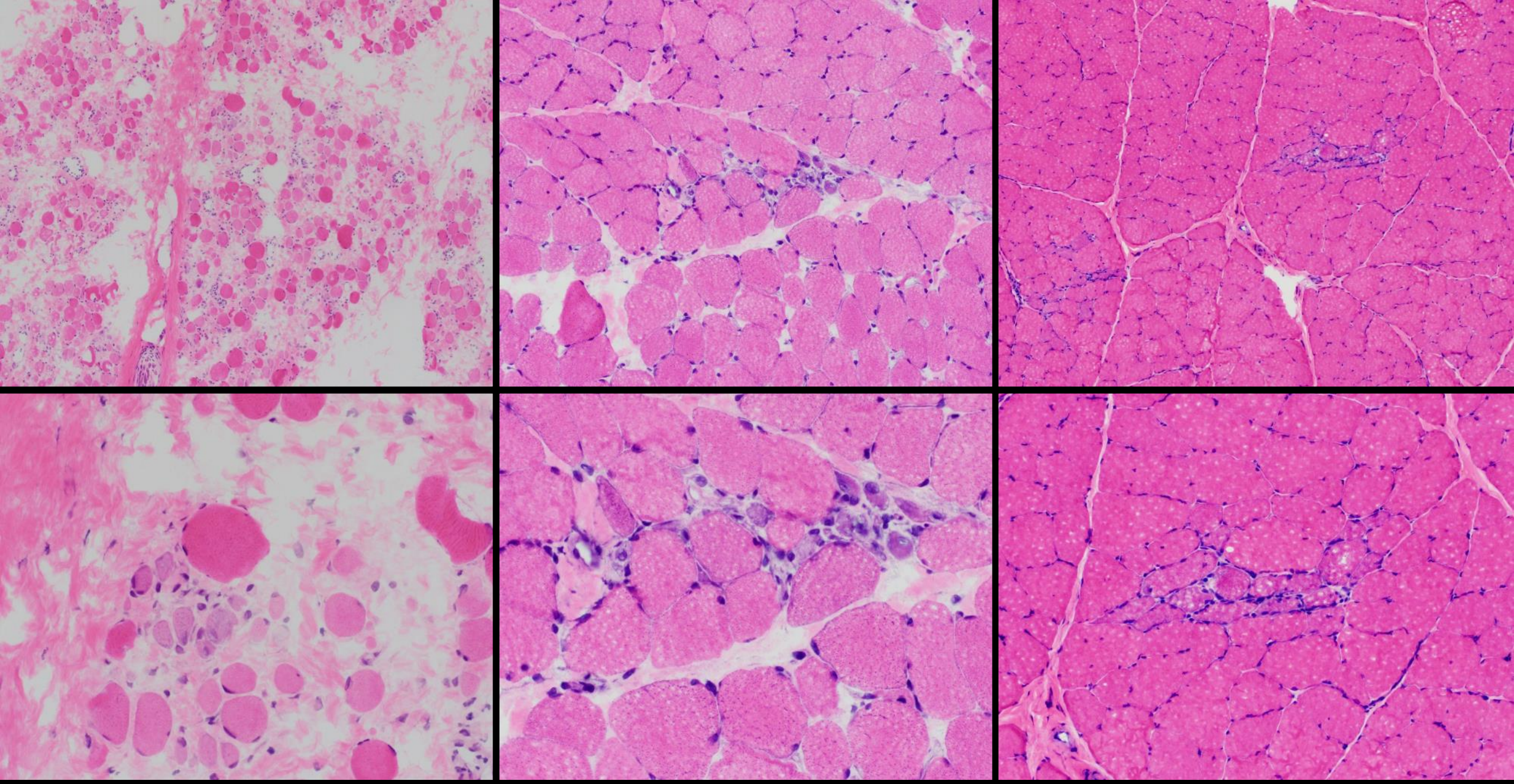


Bentzinger et al. Bioessays. 2013. PMID: 22886714

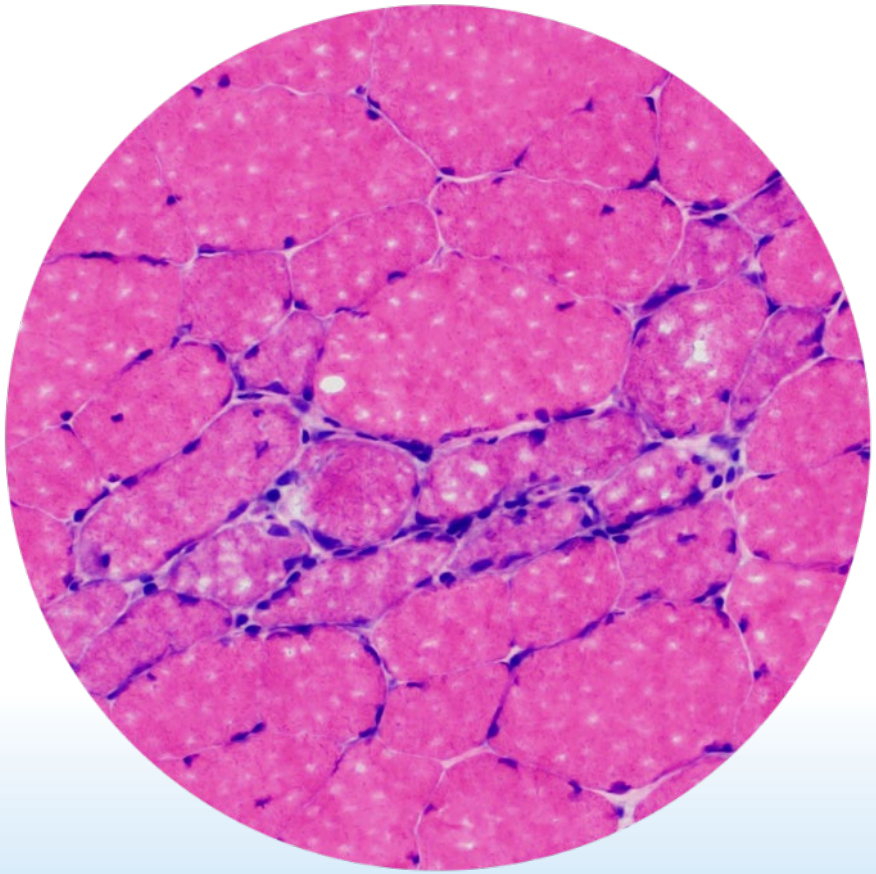
Dystrophic pathology = acute coagulative necrosis → myophagocytosis → satellite cell proliferation → regeneration



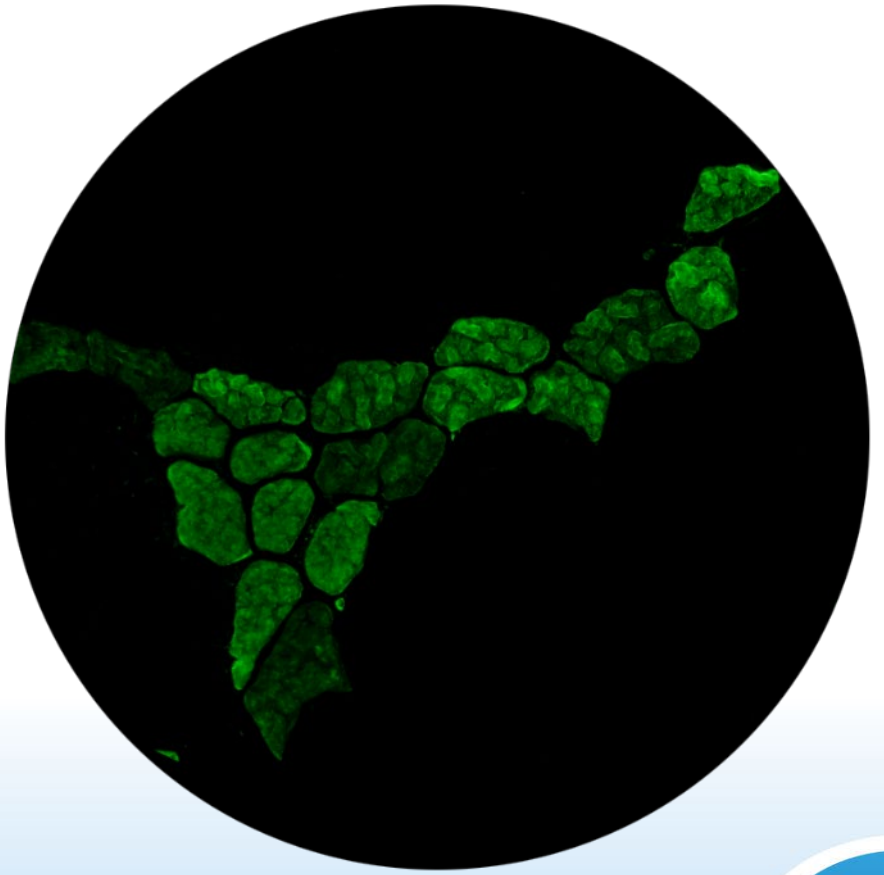
Pitfall - this pathology occurs in varying degrees of severity!



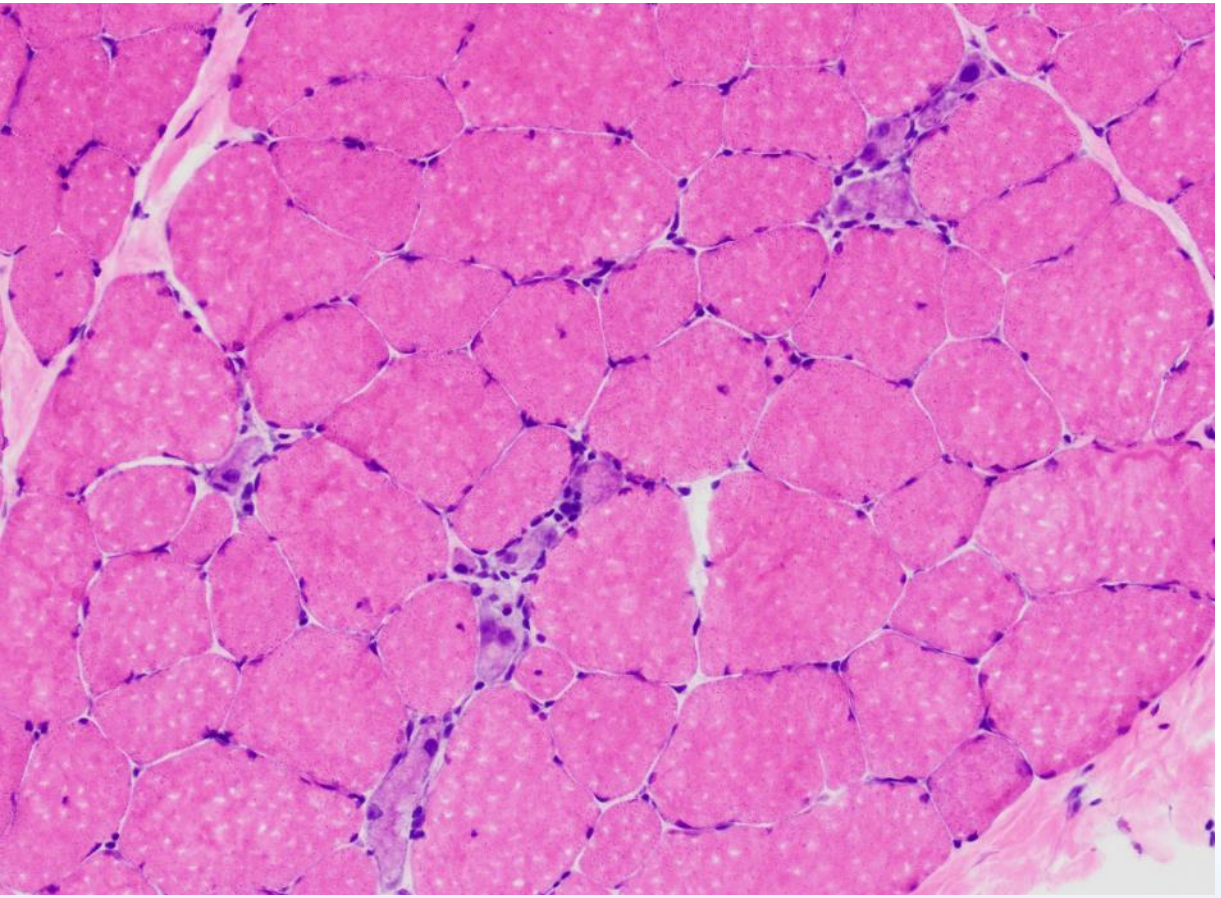
Basophilia = regeneration

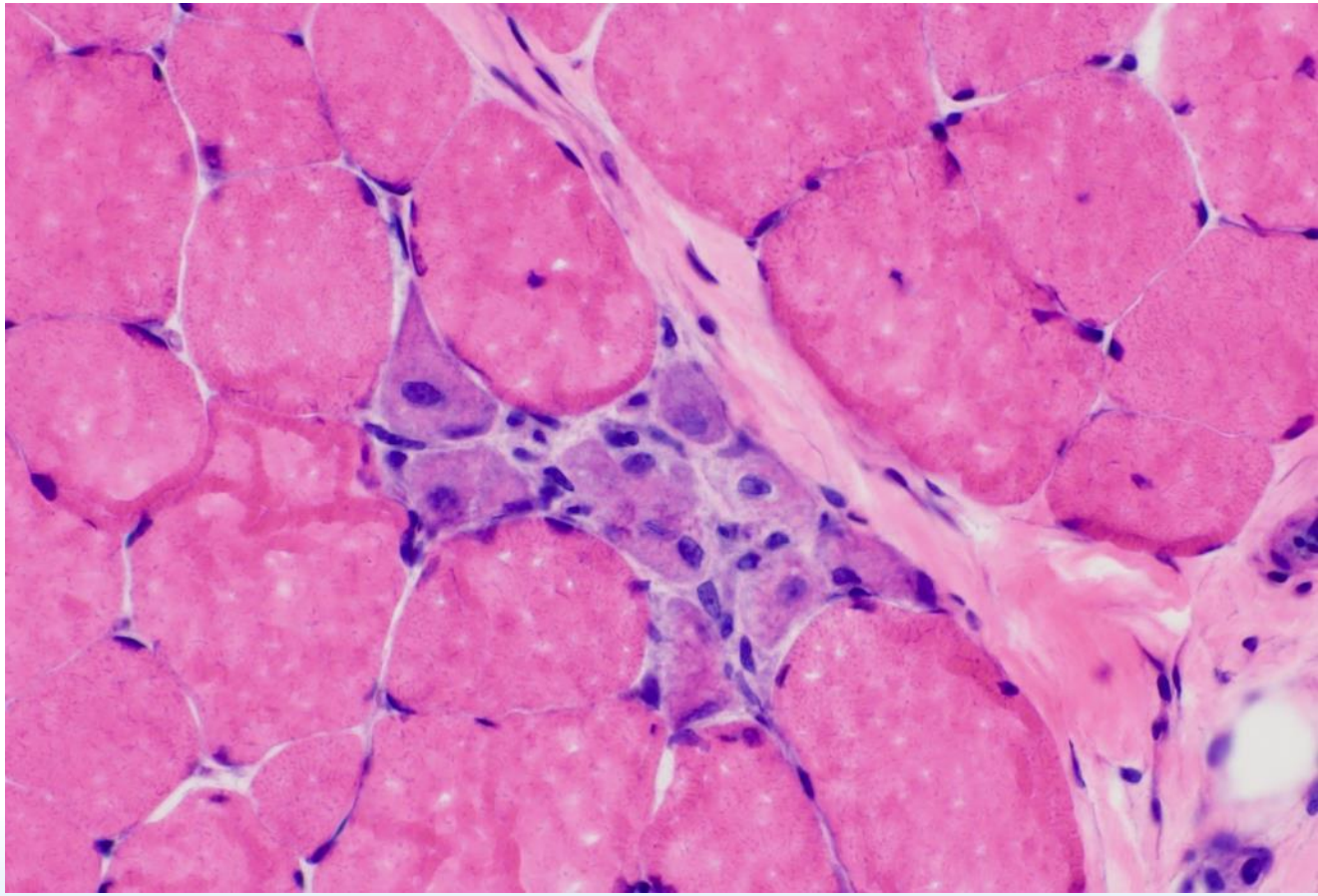


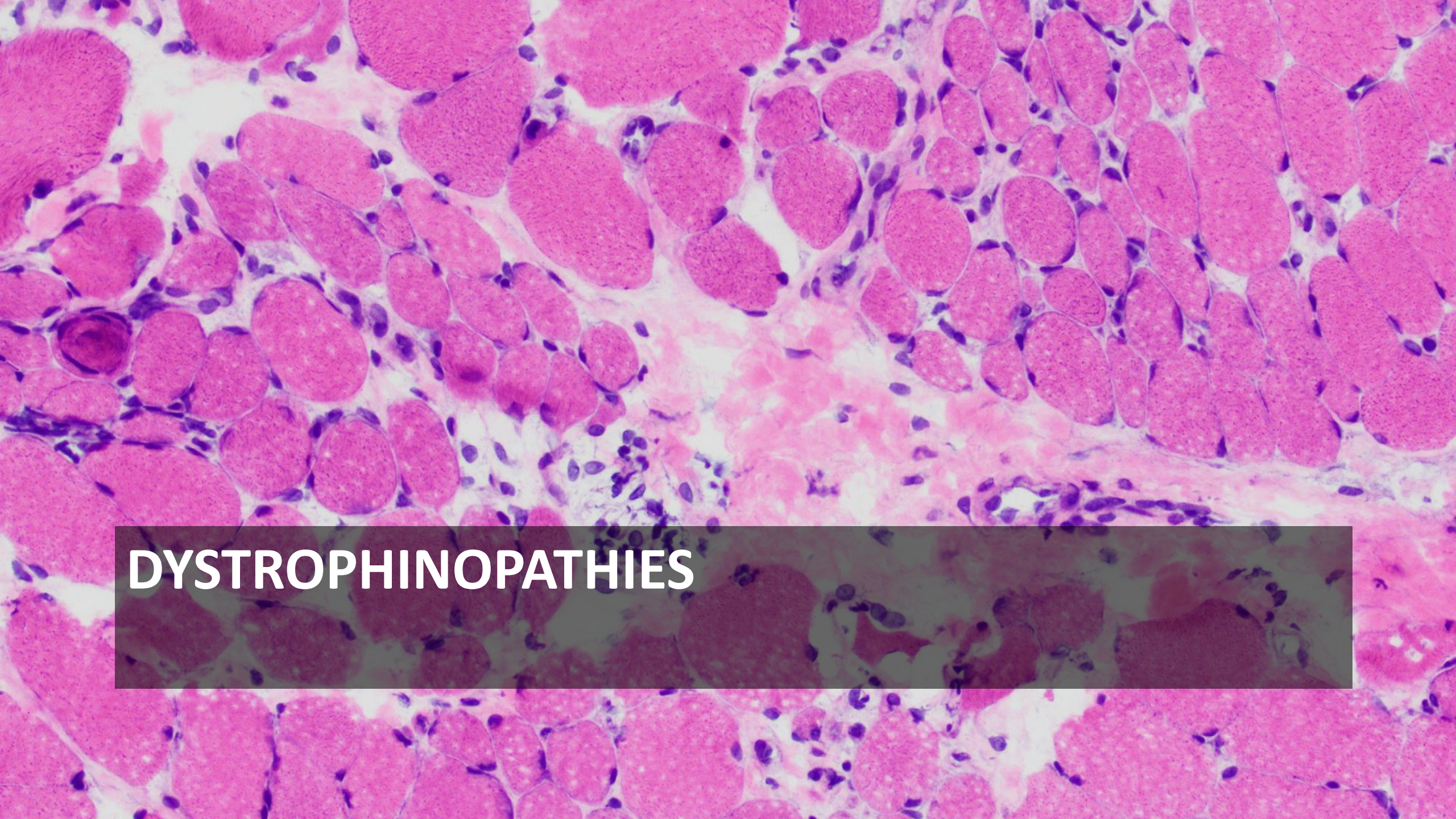
Embryonic myosin heavy chain



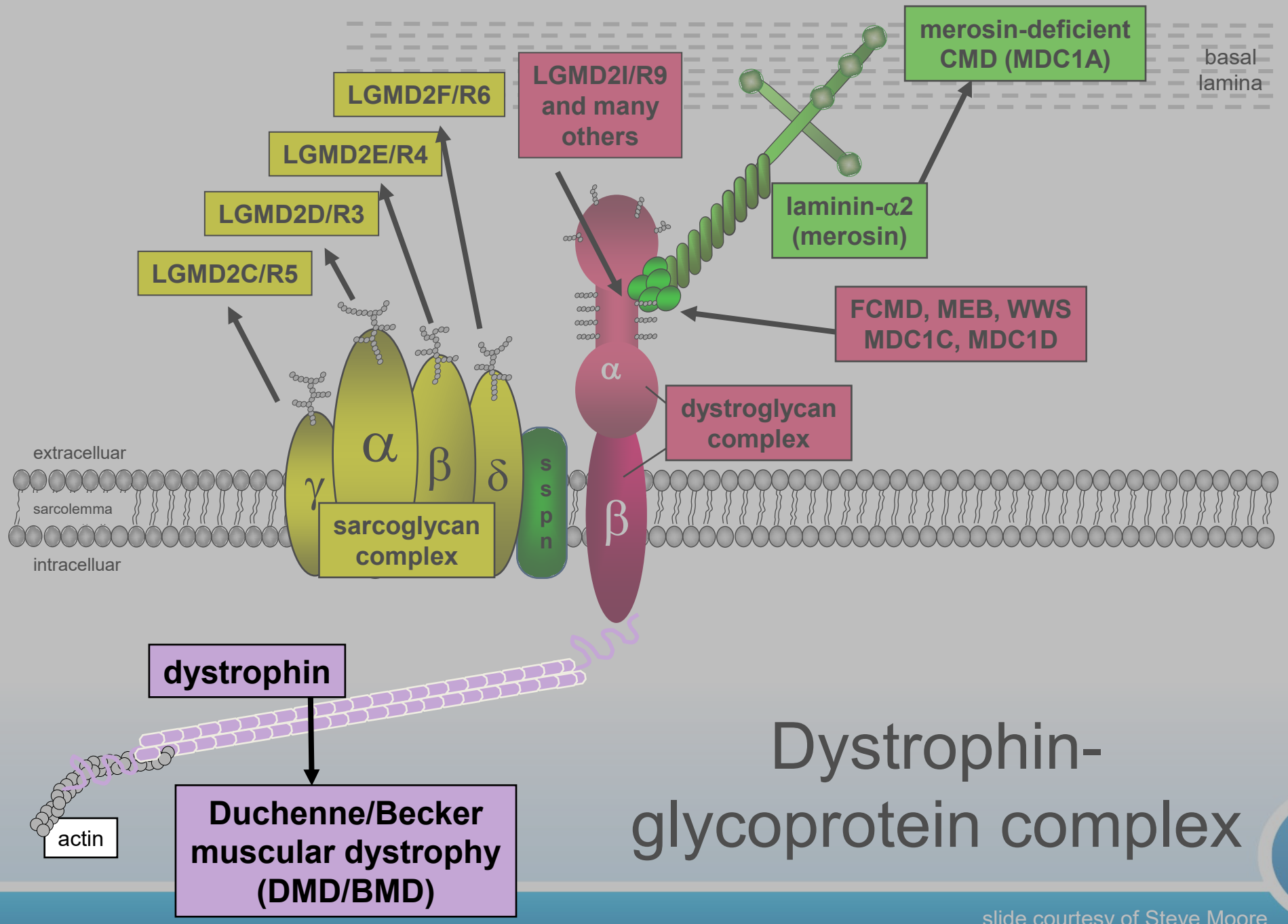
Grouped regeneration is a clue!







DYSTROPHINOPATHIES

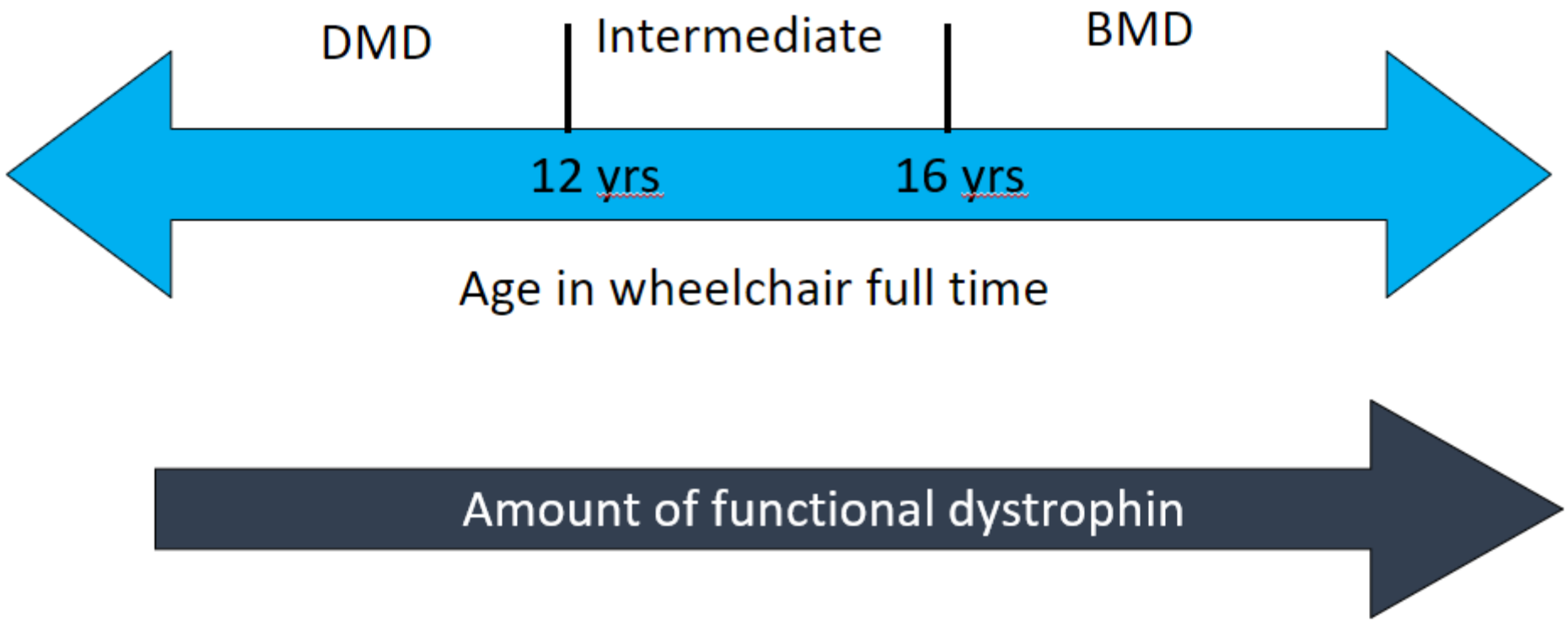


Dystrophin-glycoprotein complex

slide courtesy of Steve Moore

Duchenne and Becker muscular dystrophies – *DMD* gene – X-linked recessive disorders

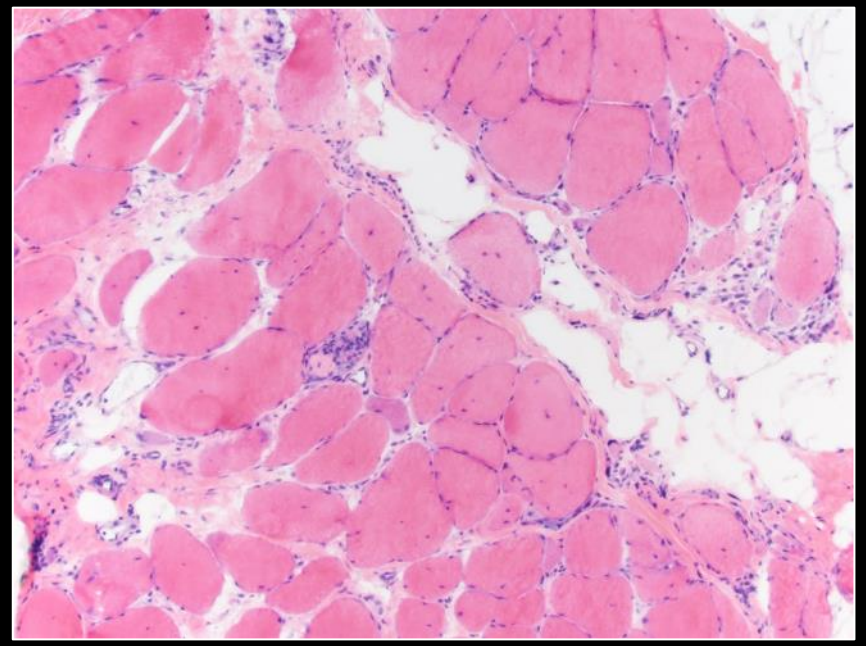
- Duchenne MD
  - Most common and prototypical MD
  - Translational reading frame lost
    - Out-of-frame deletions, duplications, mutations, rearrangements
    - *no* dystrophin protein expressed
  - Lose the ability to ambulate independently before the age of ~14
  - CK 10-50x normal
  - Proximal muscle weakness (LG pattern)
- Becker MD
  - Milder allelic variant of DMD
  - Translational reading frame maintained
    - In-frame deletions, duplications, or mutations
    - Reduction in amount, alteration in size, change in expression of protein
  - Maintain independent ambulation longer than DMD patients, but there is a continuum

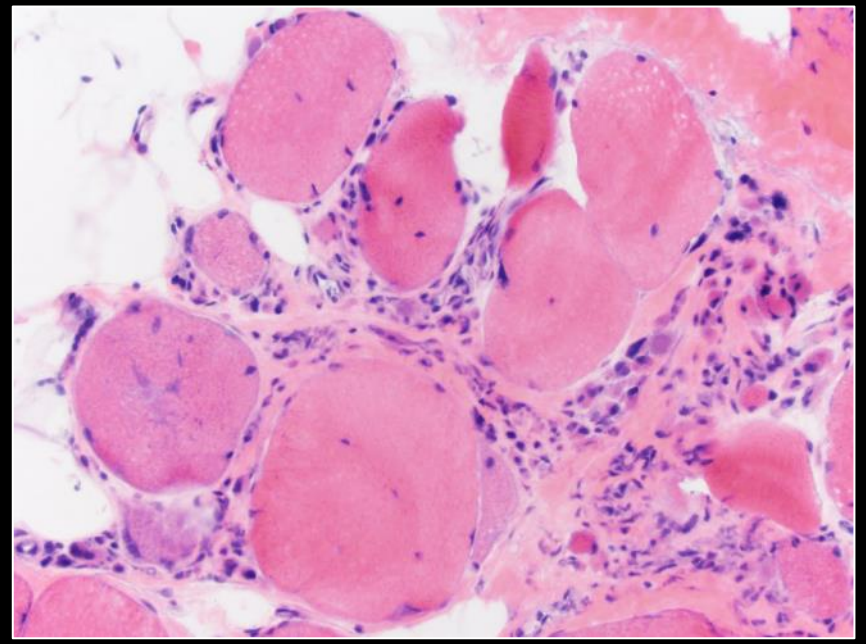

DMD
Intermediate
BMD
12 yrs
16 yrs
Age in wheelchair full time
Amount of functional dystrophin

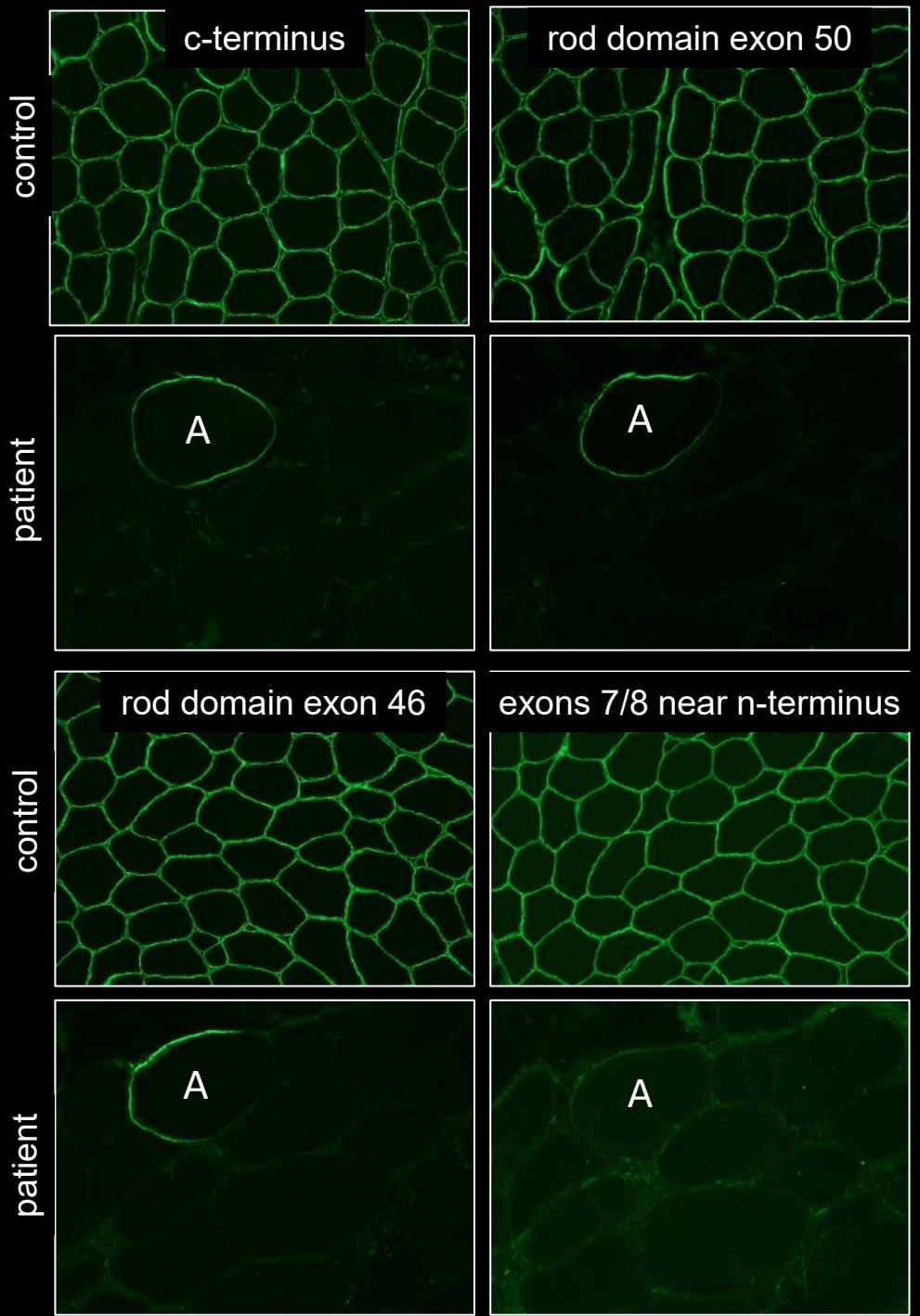
When are muscle biopsies done for the diagnosis of DMD/BMD?

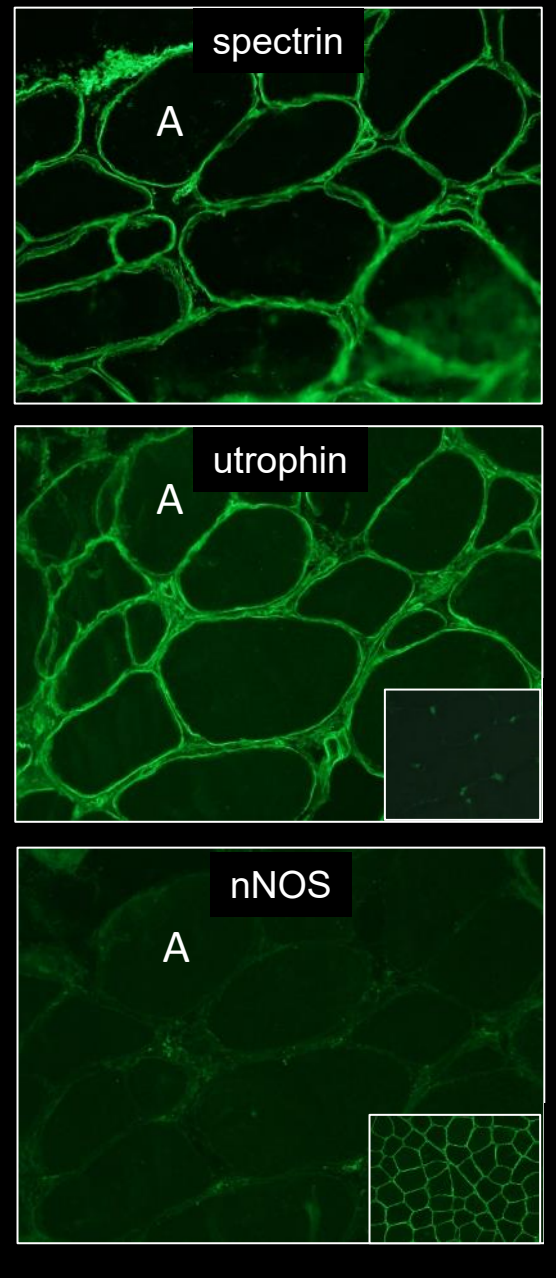
- ~95% of cases are diagnosed by genetic testing
  - Flanigan, et al, Am J Hum Genet. 2003 Apr;72(4):931-9
- BUT
  - Sometimes genetic testing isn't diagnostic
    - If only deletion/duplication testing is performed and not sequencing
    - If a variant of uncertain significance (VUS) is called in the *DMD* gene
  - Sometimes DMD/BMD isn't suspected clinically
    - Older patients and manifesting female carriers
  - Sometimes the clinical presentation doesn't line up with the suggested molecular changes
    - *DMD* variant predicted to give a severe DMD phenotype, but patient's clinical presentation is more concerning for BMD

Classic DMD





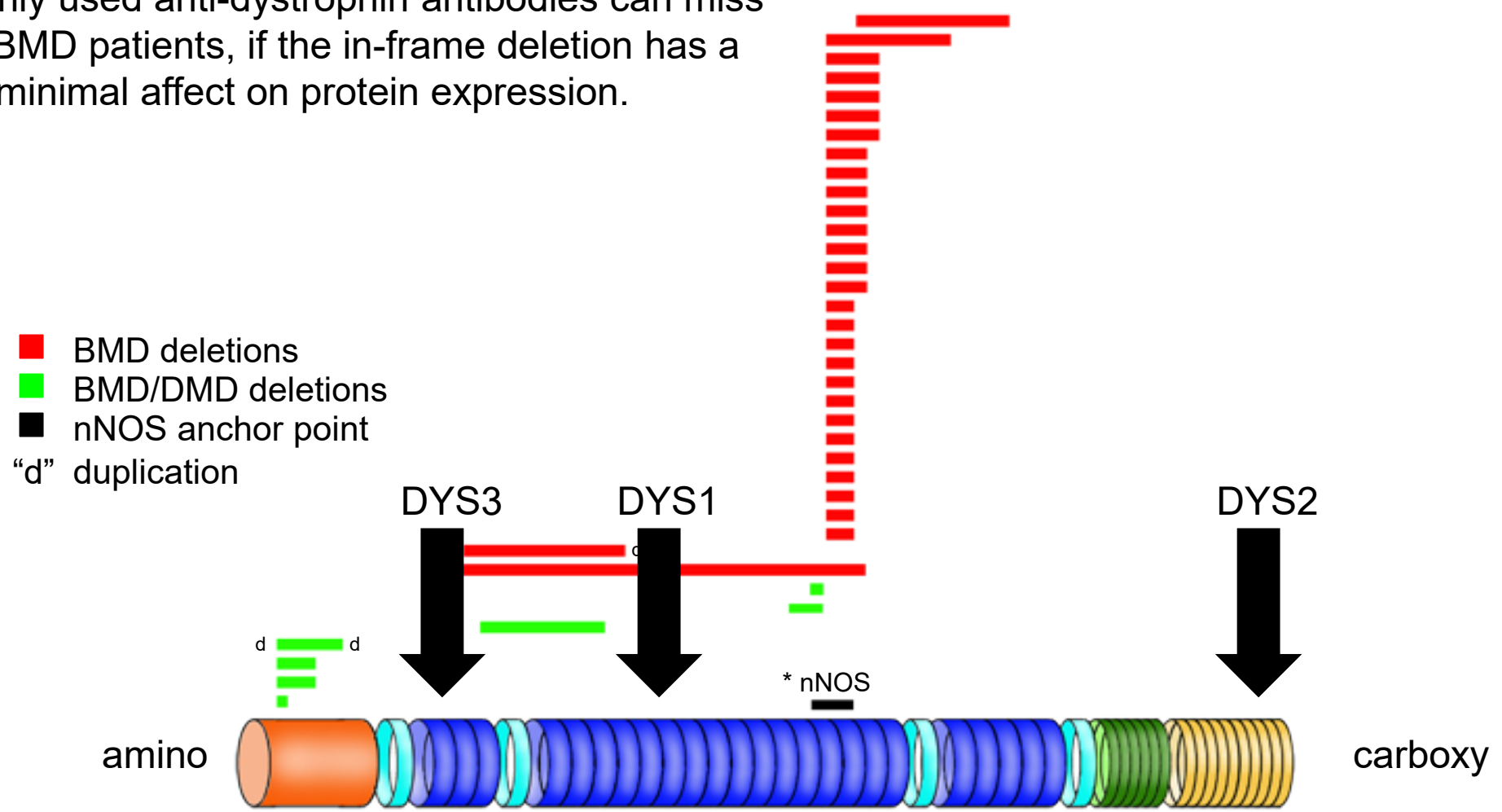




Images courtesy of Steve Moore

Immunostaining for diagnosis of BMD

Commonly used anti-dystrophin antibodies can miss some BMD patients, if the in-frame deletion has a minimal affect on protein expression.



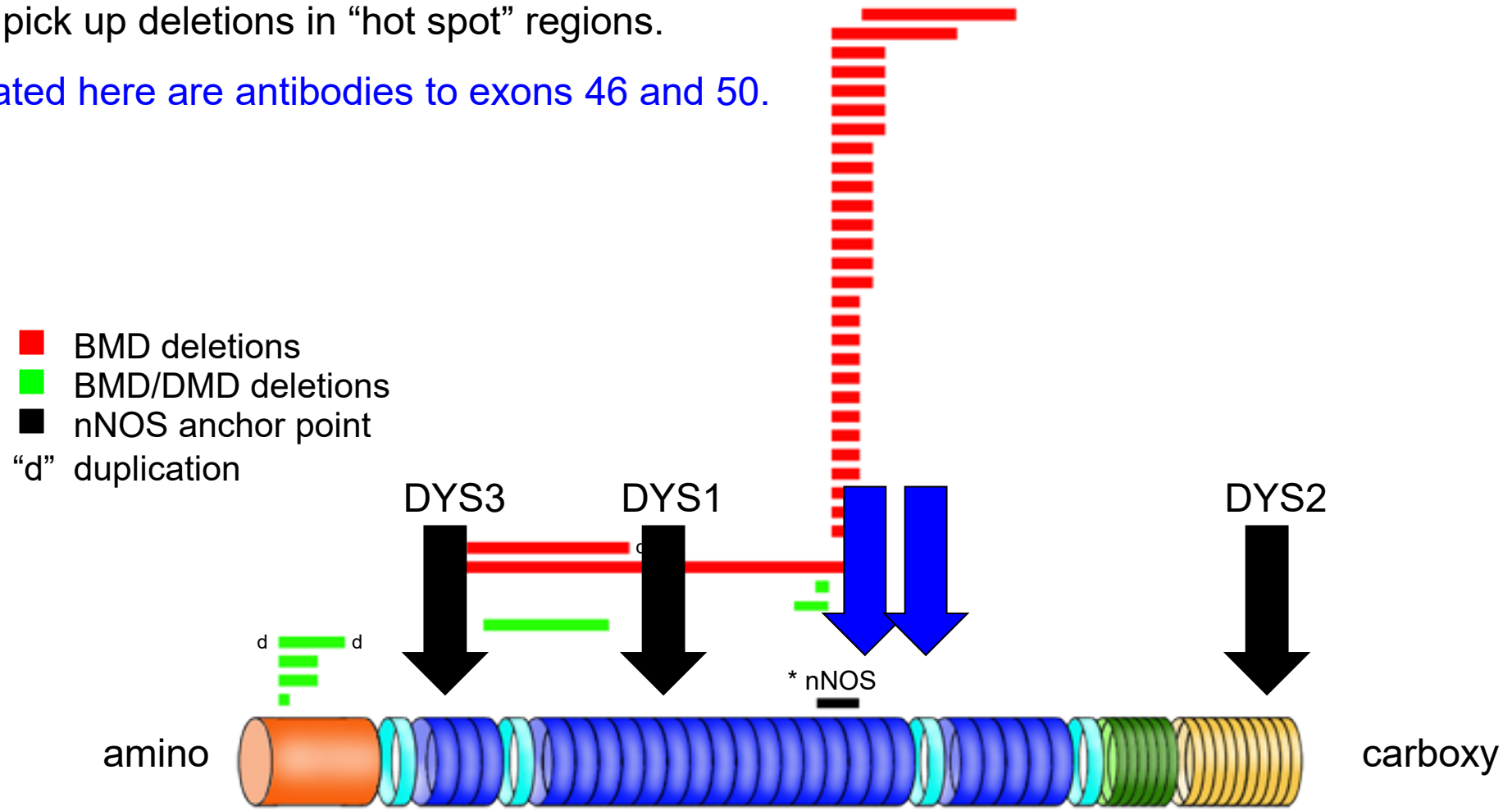
Adapted from Anderson "Dystrophinopathies" (2002) by Yvonne Kobayashi

* nNOS anchor point Lai Y et al (2009) J Clin Invest 119:624

Immunostaining for diagnosis of BMD

Other anti-dystrophin antibodies can be added to pick up deletions in “hot spot” regions.

Illustrated here are antibodies to exons 46 and 50.

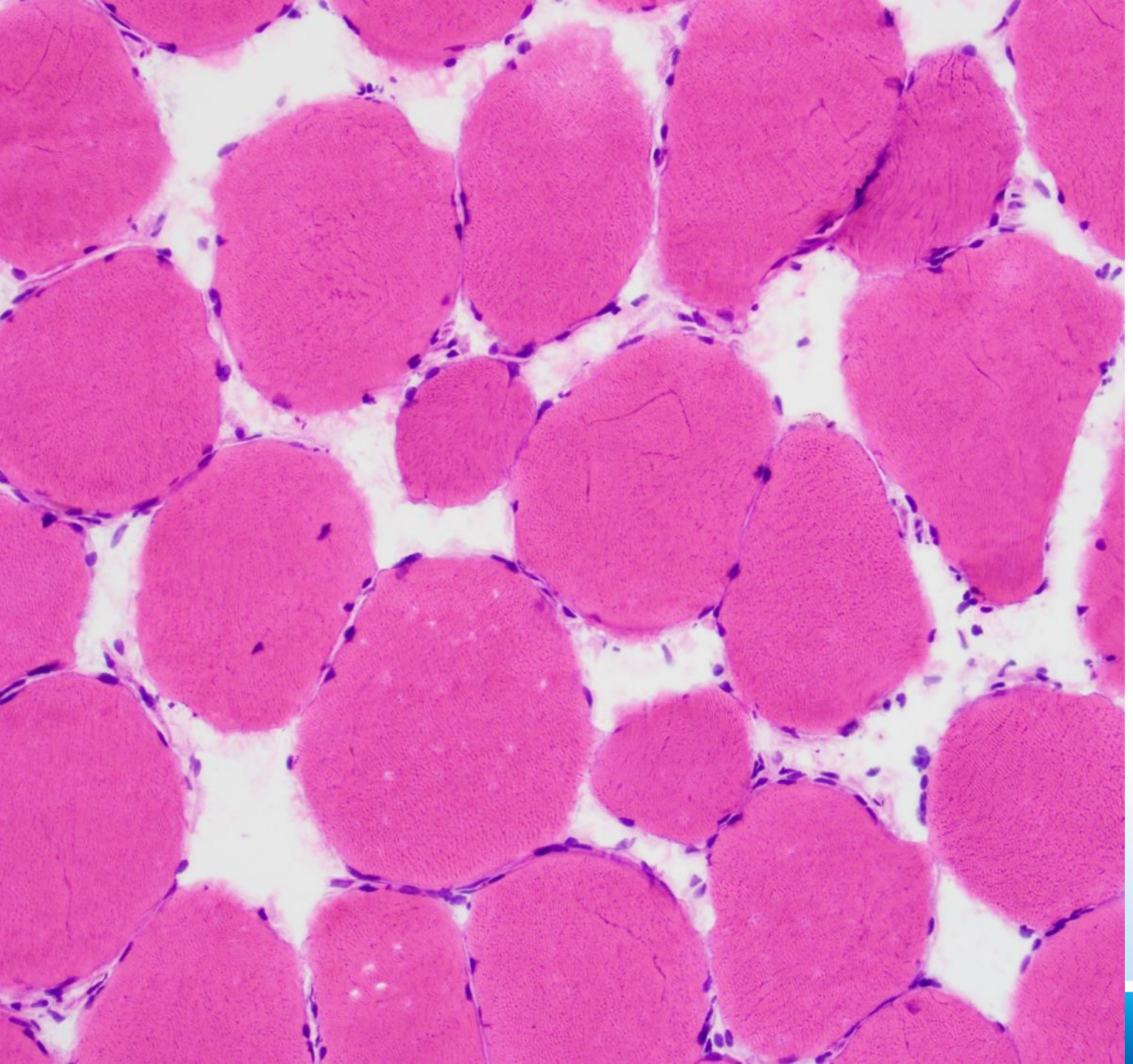


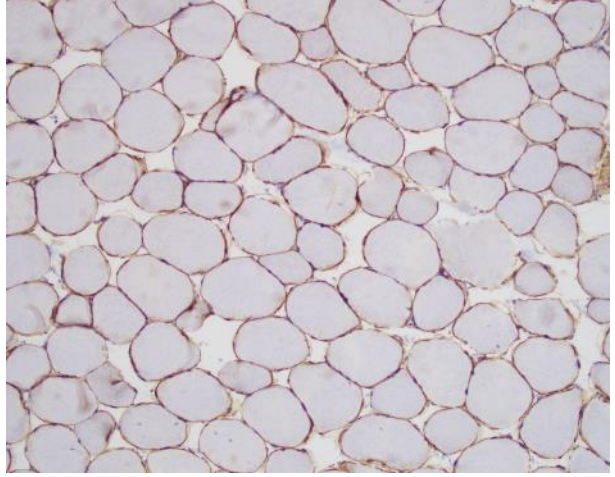
Adapted from Anderson “Dystrophinopathies” (2002) by Yvonne Kobayashi

* nNOS anchor point, Lai Y et al (2009) J Clin Invest 119:624

Pitfall - when DYS1, DYS2, and DYS3 are not enough

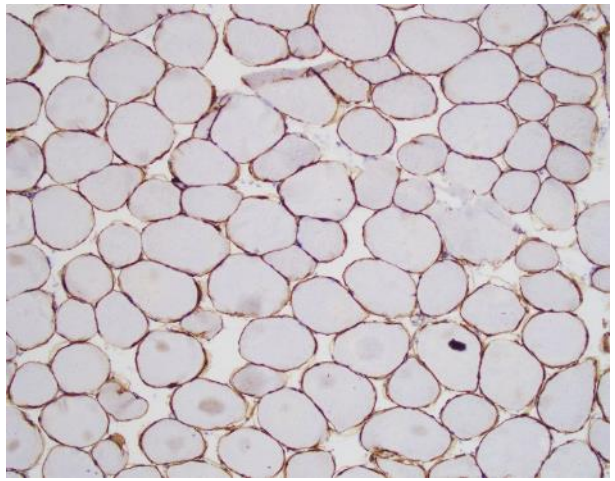
- 55-year-old man with mild neck flexion and extension weakness and cardiac arrhythmias that began in his early 40s
- Clinical concern for myotonic dystrophy type 2
- Gene DX cardiomyopathy panel:
  - *DMD* VUS that over the course of being worked up was changed to a benign variant
- Whole exome sequencing:
  - *SYNE1* heterozygous VUS (AR or AD; Emery-Dreifuss muscular dystrophy, spinocerebellar ataxia)
  - *TTN* heterozygous VUS (AR and AD; LGMDR10/2J, myofibrillar myopathy-HMERF, tibial muscular dystrophy, core myopathy, centronuclear myopathy)





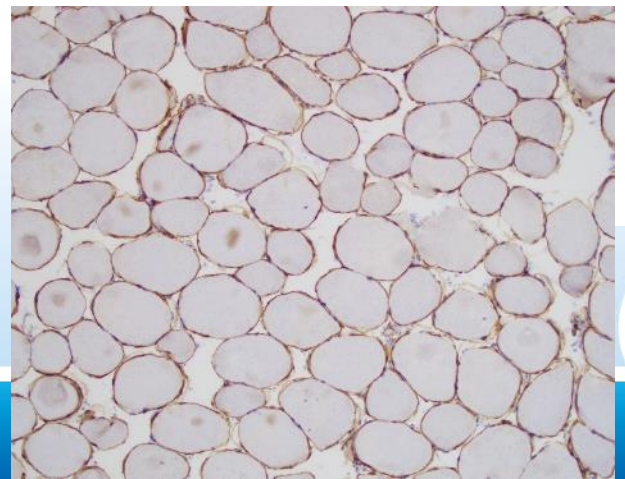
DYS 1

Exons 26-30



DYS 2

Carboxy terminus



DYS 3

Exons 10-12

DOVE: *DMD* Open-access Variant Explorer

c.7183G>A

Overview | *In Silico* Predictions | Therapies | Leiden Database | Sequences | myVariant.info | References

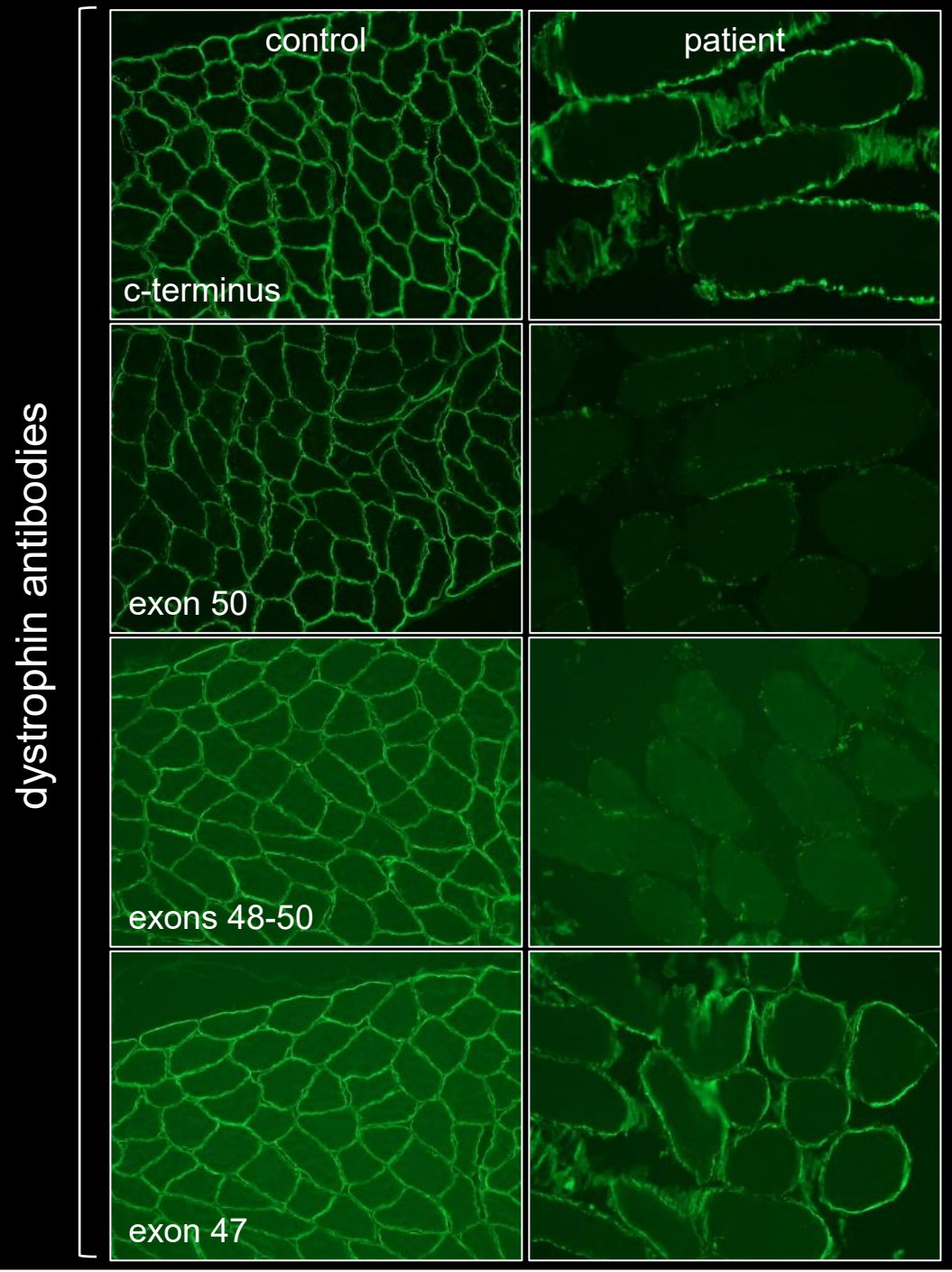
Summary

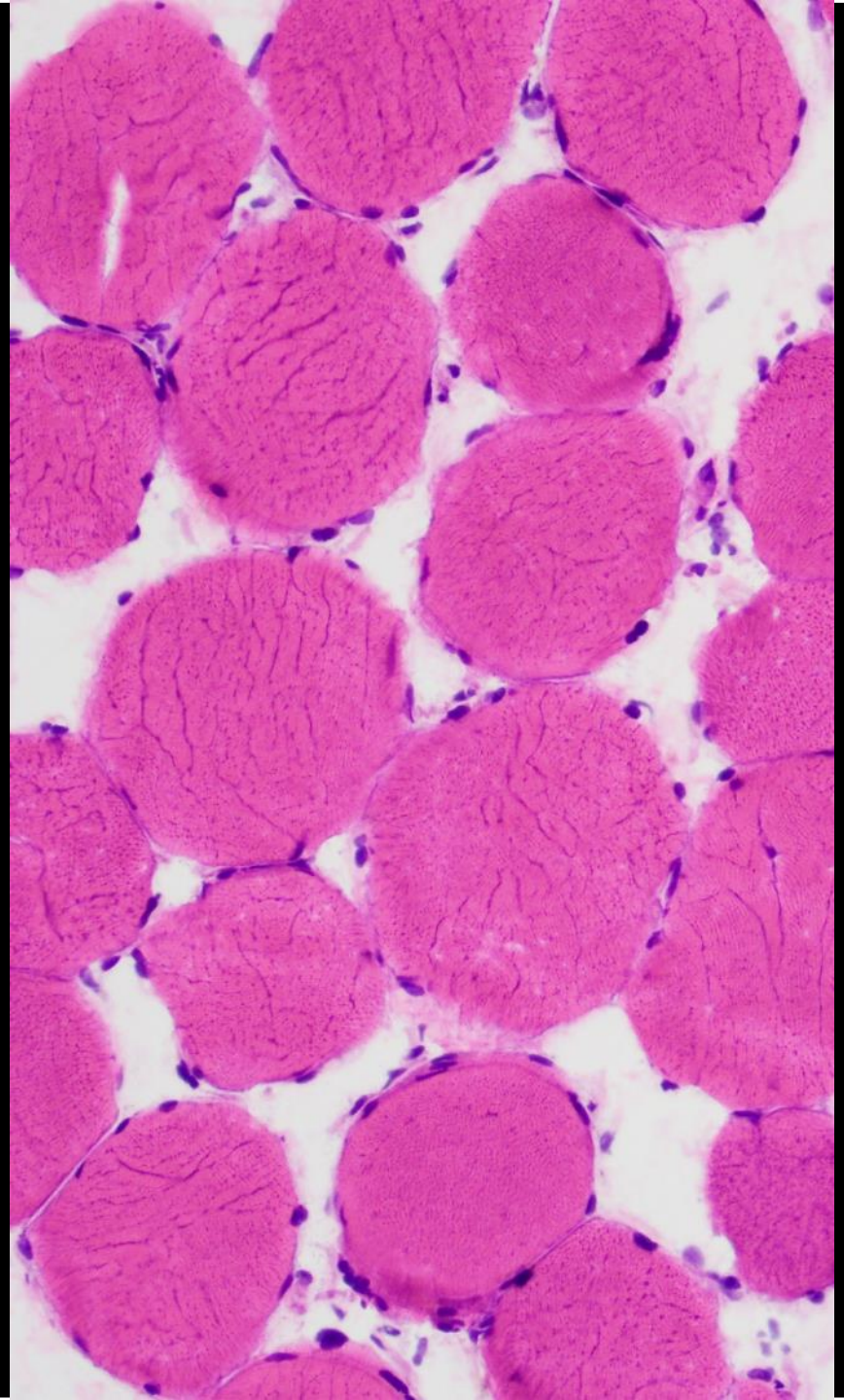
HGVS	**NM_004006.2:c.7183G>A** (Click here to check variant at Mutalyzer)
Genomic location (GRCh38)	**ChrX:31836735C>T**
Genomic location (GRCh37)	**ChrX:31854852C>T**
Mutation type	**Point mutation**
Exon number(s)	**49**
Domain(s)	**Central rod domain: Repeat 19**
Length of mutated sequence	**1** nucleotide(s)
Predicted consequence	**Missense** p.(Ala2395Thr)
Therapies Available or In Development	Not currently - Please see 'Therapies' tab
In Silico Predictions	**Changes to splice regulatory element(s) predicted** *Please check the 'In Silico Predictions' for more details*
ClinVar	Variant not found in ClinVar via myVariant.info, however, please click here to search ClinVar directly

Splicing Motifs

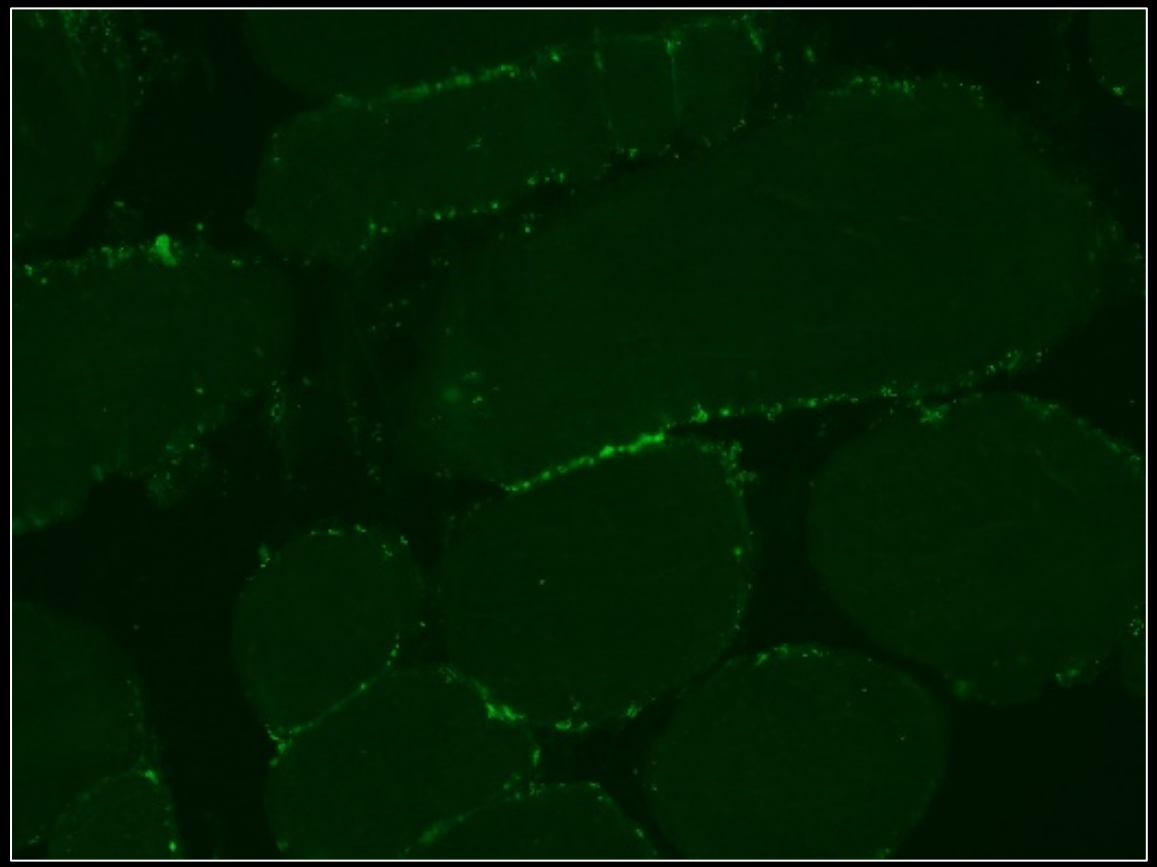
Motif	Scoring used	Type	Relative change
Exon Splice Enhancer (ESE)	Rescue-ESE	*NA*	Disruption of ESE
	ESEFinder	None	No motifs significantly changed
Exon Splice Silencer (ESS)	Fas-ESS Hexamers	*NA*	Mutation creates a novel ESS motif

https://www.dmd.nl/DOVE

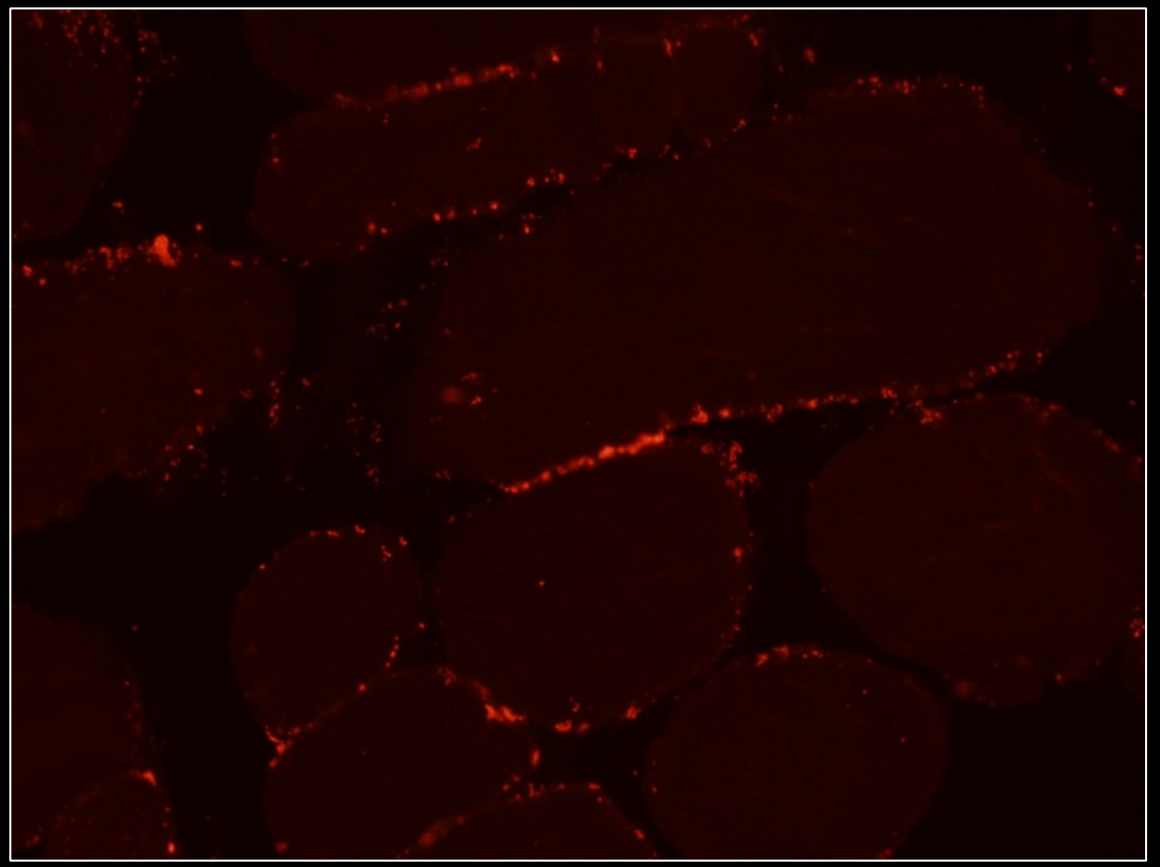

dystrophin antibodies
control
patient
c-terminus
exon 50
exons 48-50
exon 47

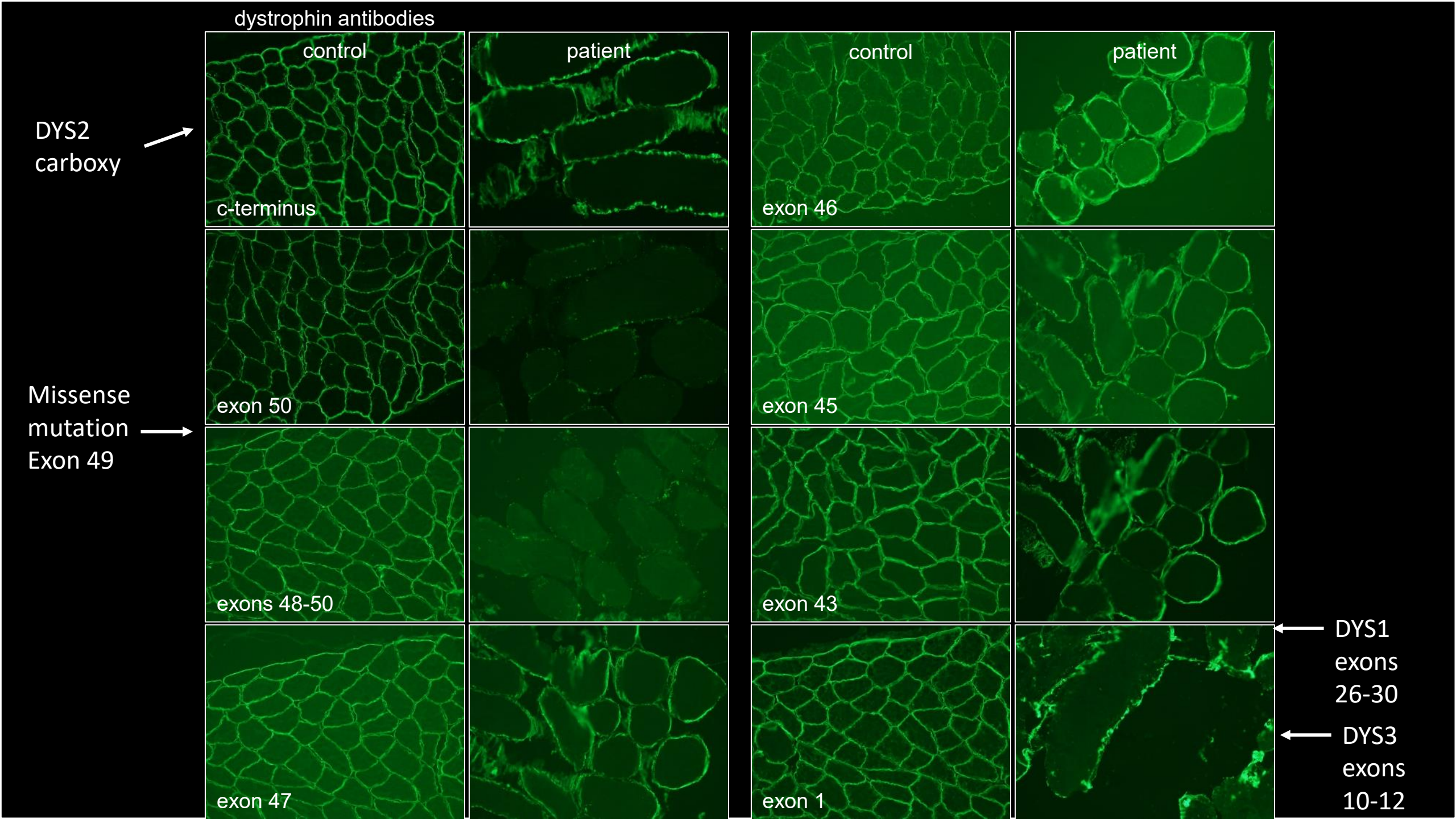


Dystrophin exon 50



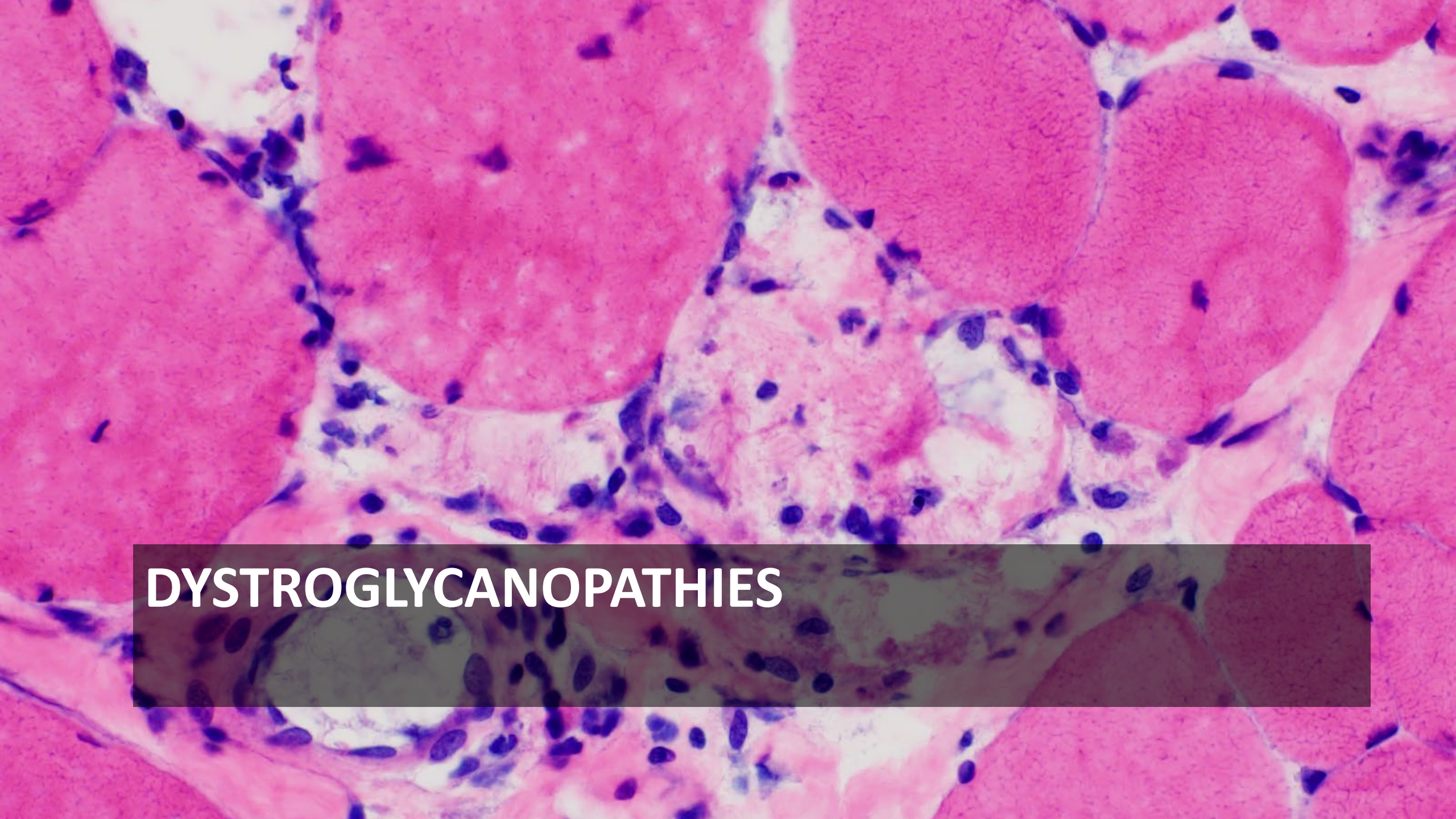
Autofluorescence (lipofuscin)



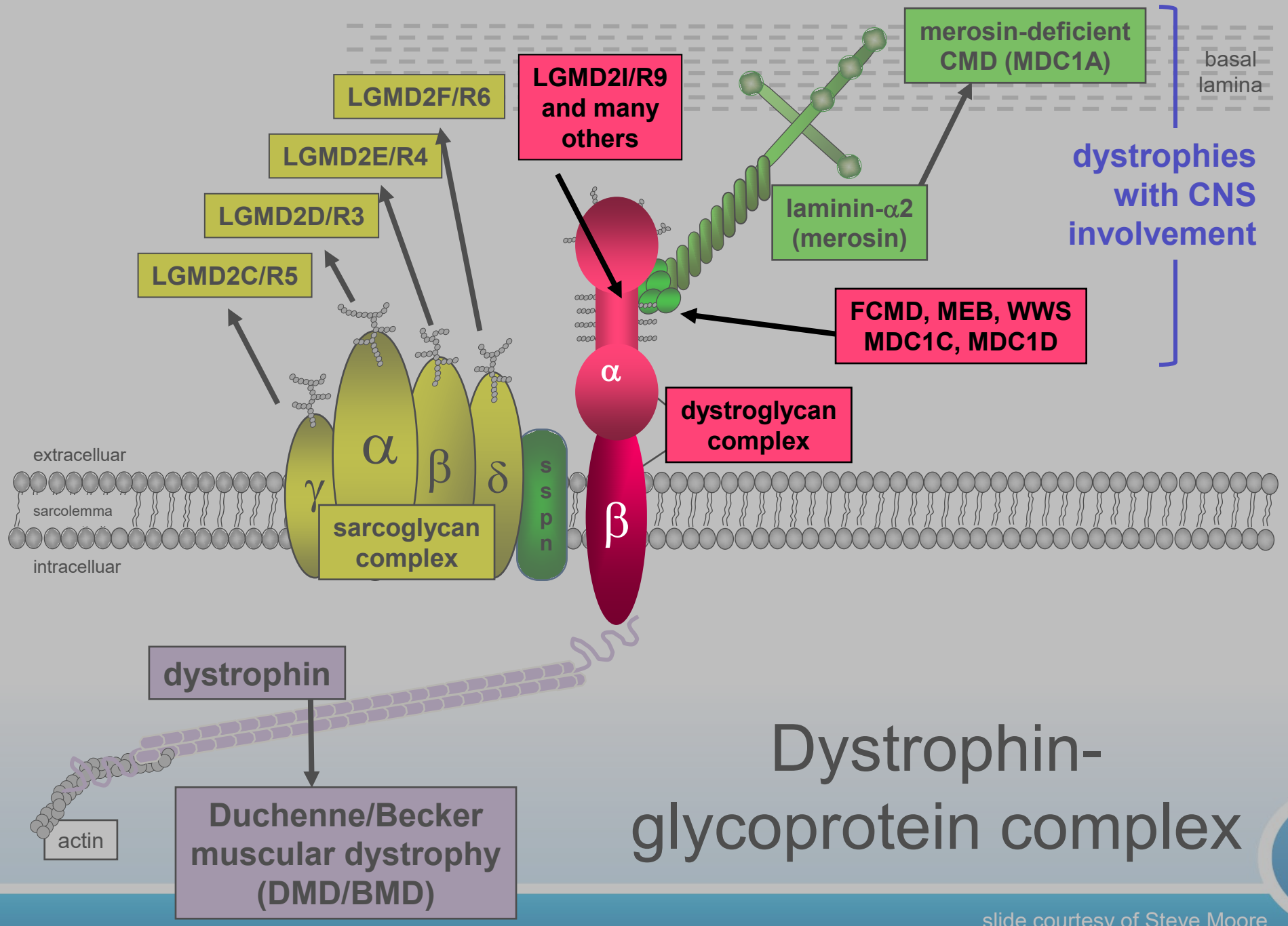

dystrophin antibodies
control
patient
control
patient
DYS2
carboxy
c-terminus
exon 46
exon 50
exon 45
Missense
mutation
Exon 49
exons 48-50
exon 43
DYS1
exons
26-30
DYS3
exons
10-12
exon 47
exon 1

Dystrophinopathy take-home points

- Very common
- You will see unexpected dystrophinopathy biopsies despite advances in genetic testing
  - Can include much older patients and female manifesting carriers
- Classic DMD characterized by total loss of dystrophin protein expression with revertant fibers, utrophin expression, and nNOS loss
- While DYS1, DYS2, and DYS3 are good screening antibodies, you will miss a high percentage of BMD cases



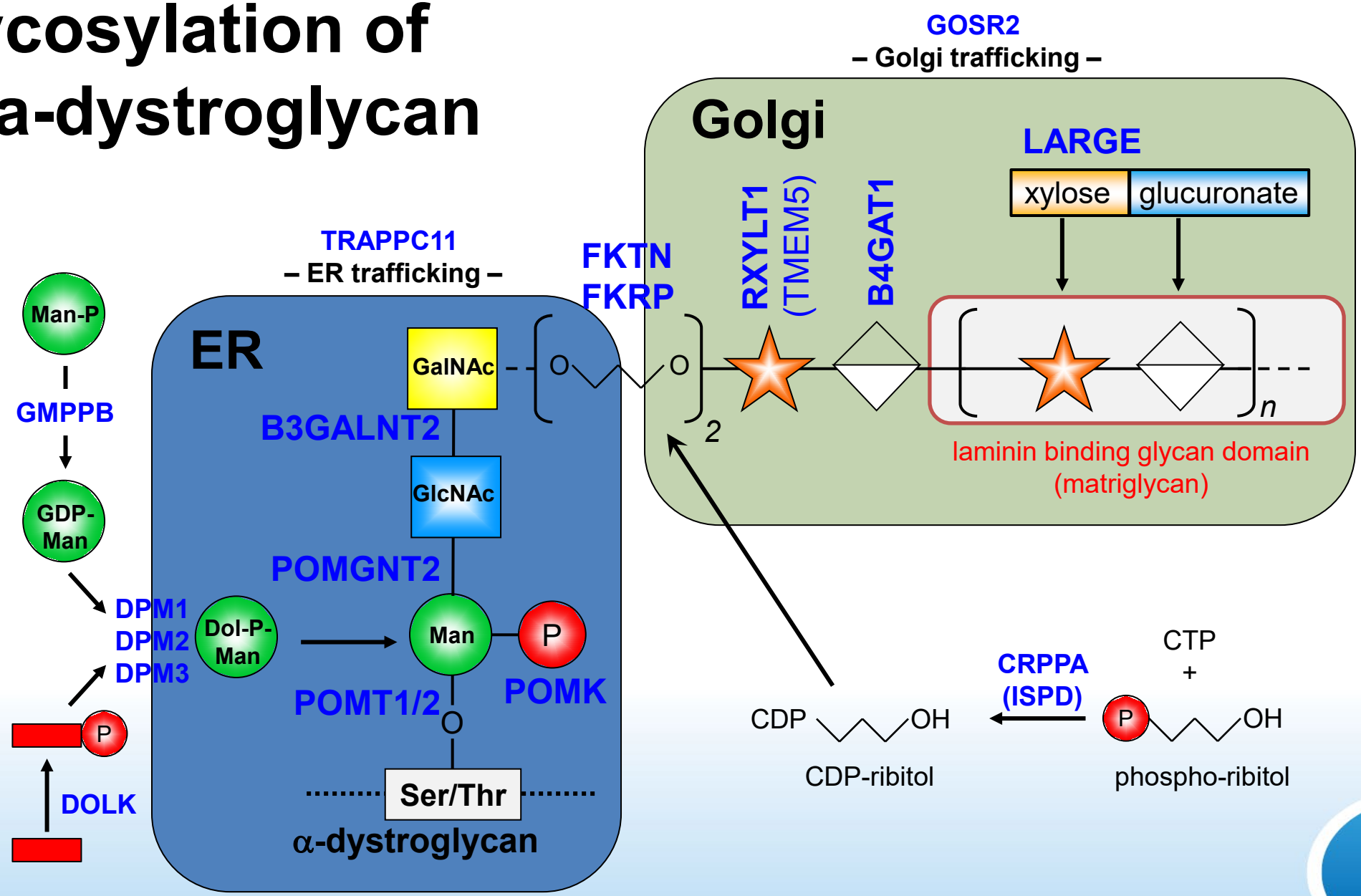
DYSTROGLYCANOPATHIES



Dystrophin-glycoprotein complex

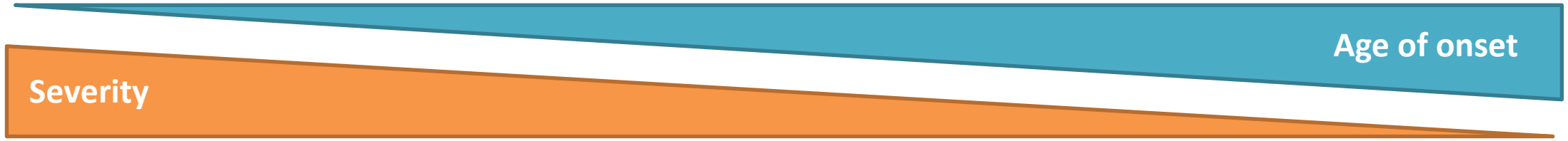
slide courtesy of Steve Moore

glycosylation of alpha-dystroglycan



slide modified from original by Toby Willer

FKRP Phenotypic Variability



Walker-Warburg Syndrome (WWS)

- Met1Val (homozygous)[1]
- Cys318Tyr (homozygous)[2]

Muscle-Eye-Brain Disease (MEB)

- Tyr307Asn (homozygous)[2]

Congenital Muscular Dystrophy (MDC1C)

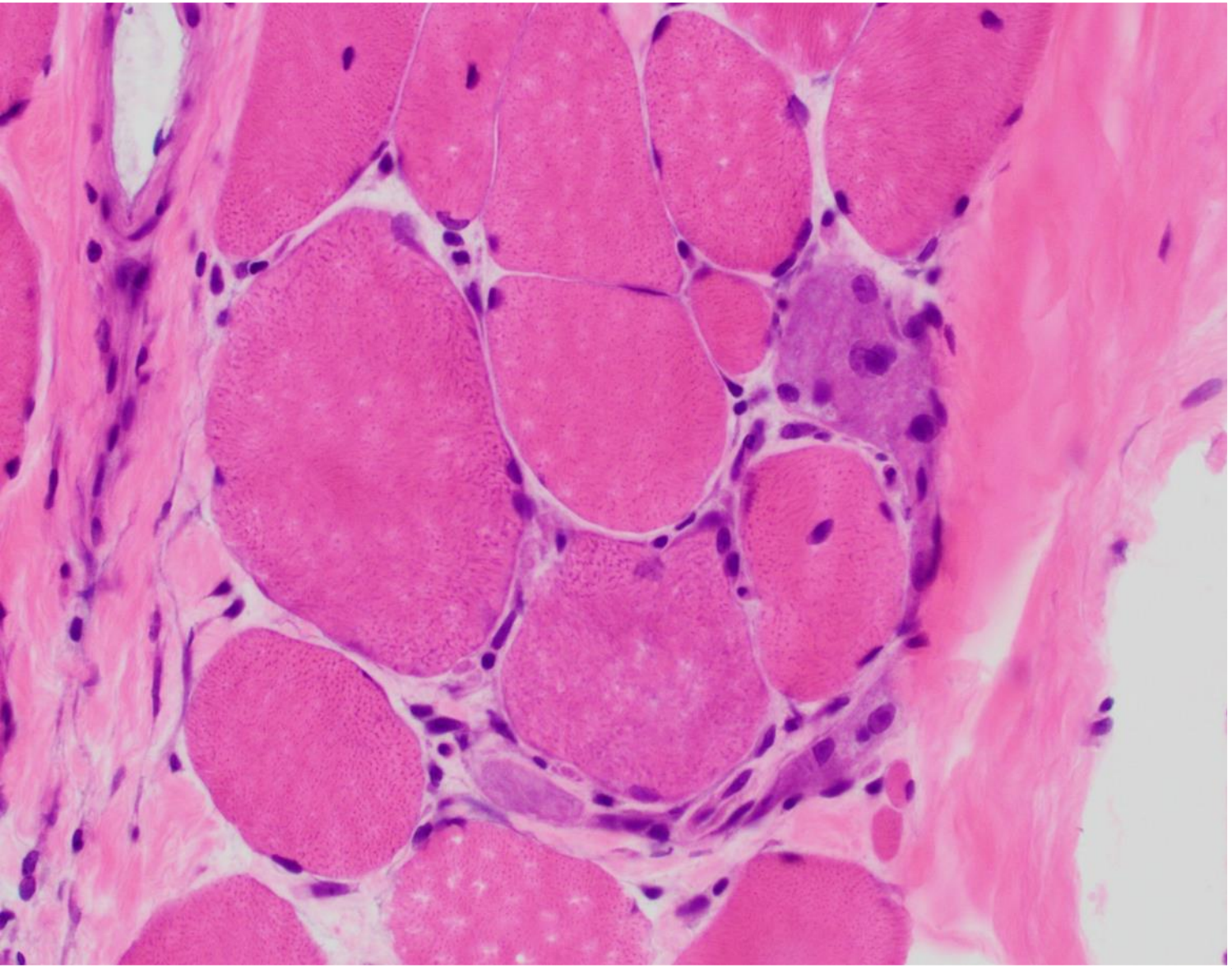
- Ile478Thr (homozygous)[3]
- Pro315Thr (homozygous)[3]
- Ser221Arg (homozygous)[3]
- Asn463Asp (homozygous)[5]

Limb-Girdle Muscular Dystrophy (LGMD2I/R9)

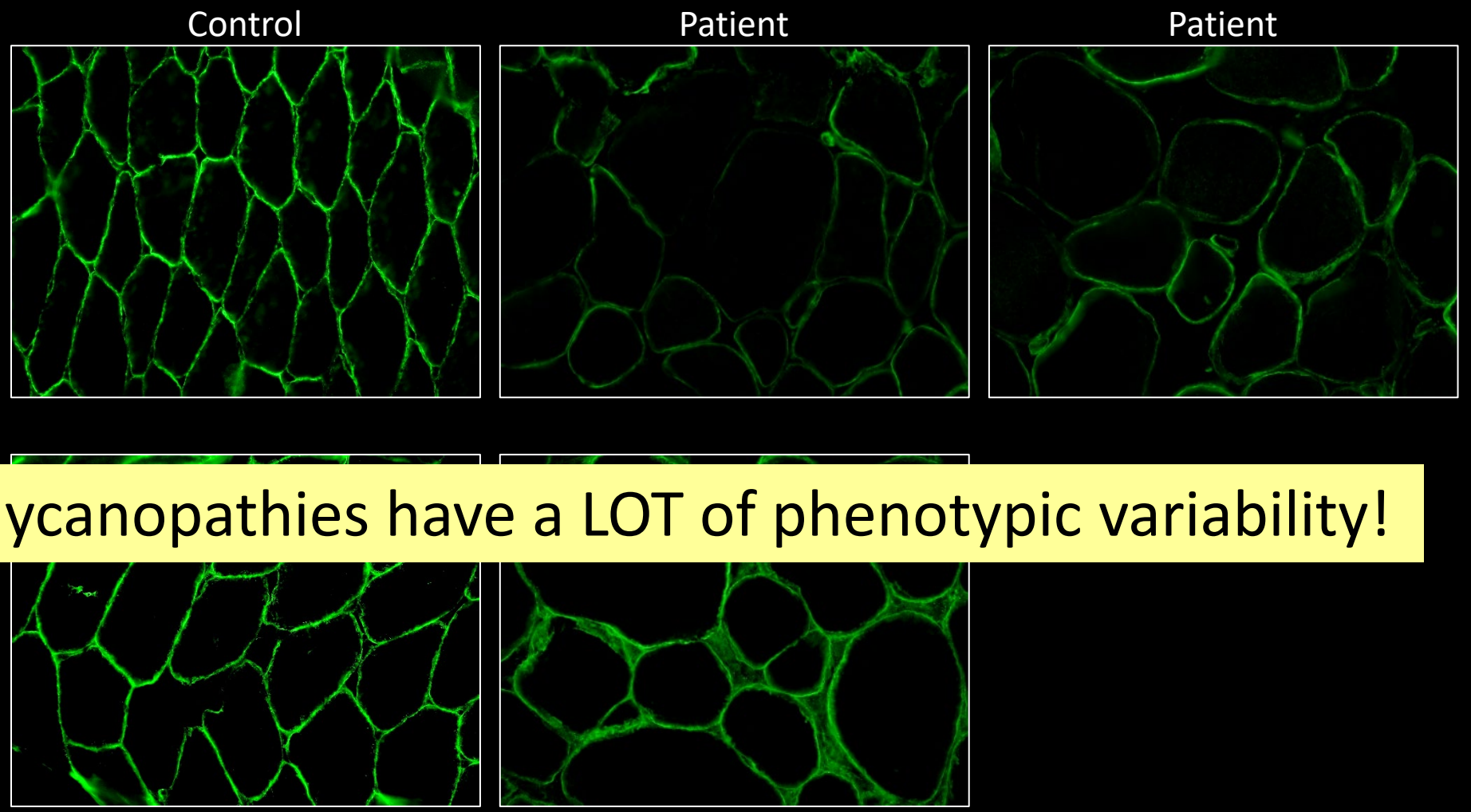
- Leu276Ile (homozygous – common mutation)[4]
- Arg339Leu (homozygous)[4]

(1) Van Reeuwijk et al. Clin Genet. 2010.
(2) Beltran-Valero de Bernabe et al. J Med Genet. 2004.
(3) Mercuri et al. Arch Neurol. 2006.
(4) Brockington et al. Hum Mol Genet. 2001.
(5) Lee et al. Neurol Genet. 2019.

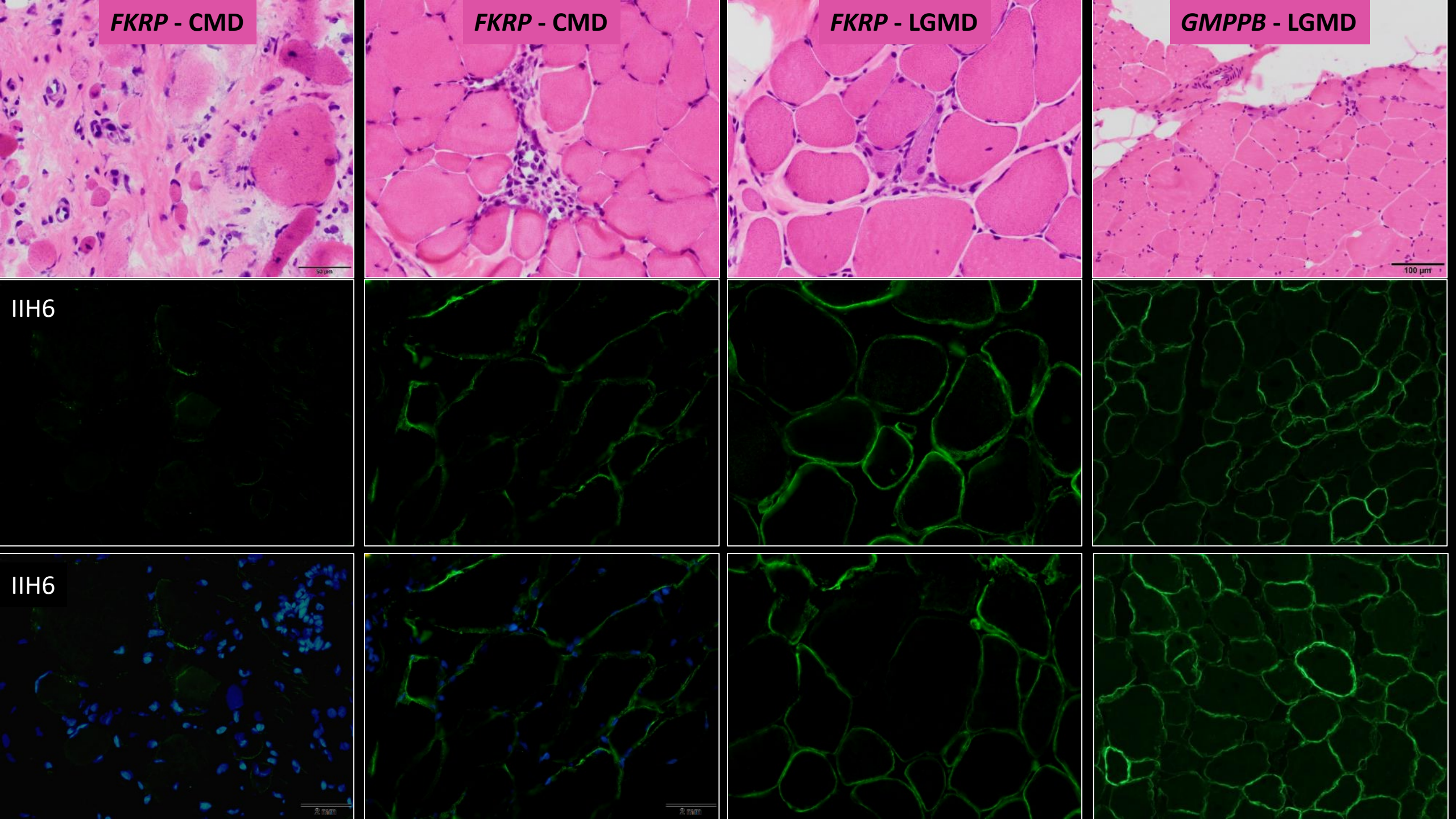
Common dystroglycanopathy pathology



- 26-year-old man with 6 years of progressive proximal muscle weakness
- CK >5000 U/L
- *DMD* deletion/duplication testing and sequencing normal.

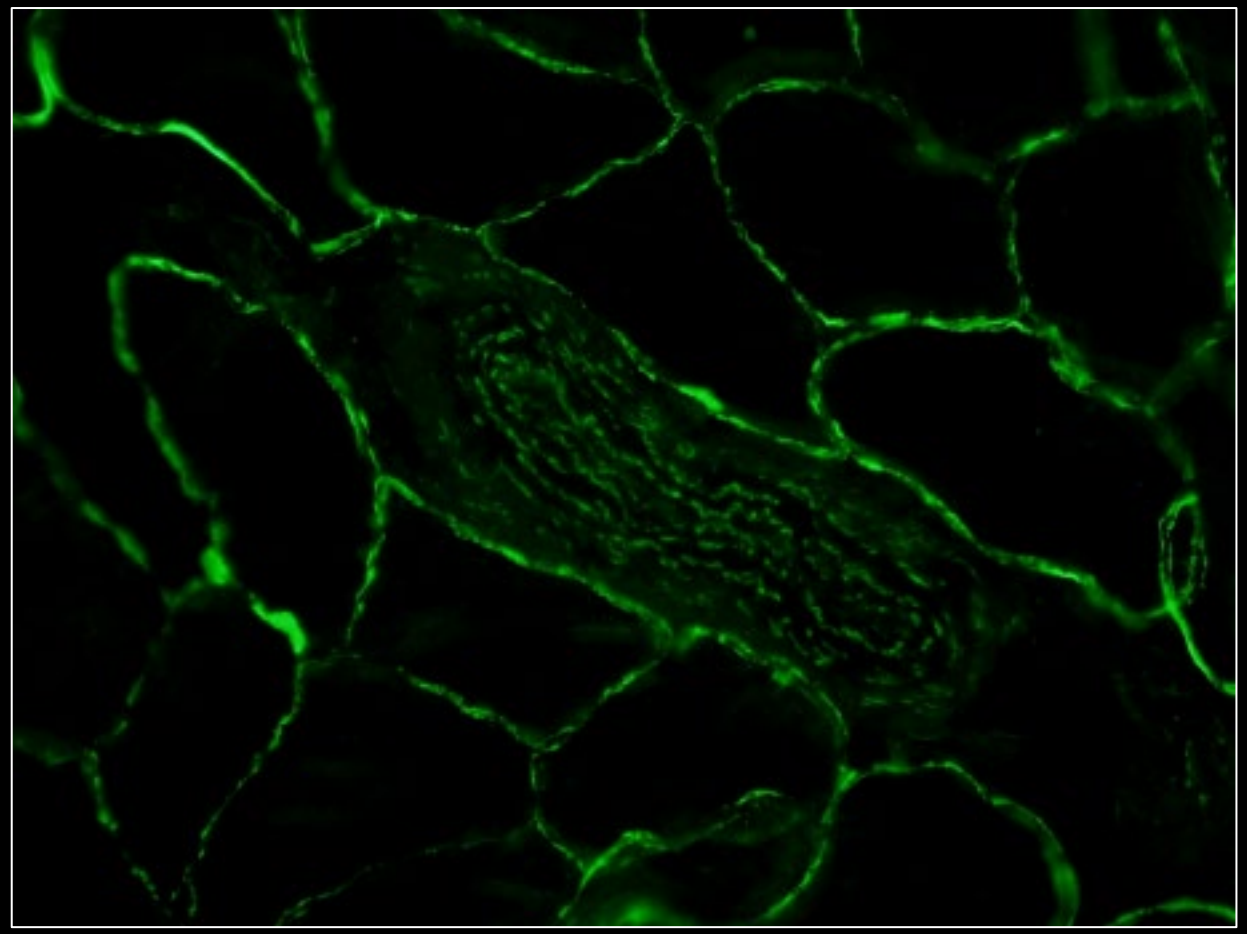


Genetic testing revealed homozygous variants in *FKRP* (c.826C>A) – the “common mutation”

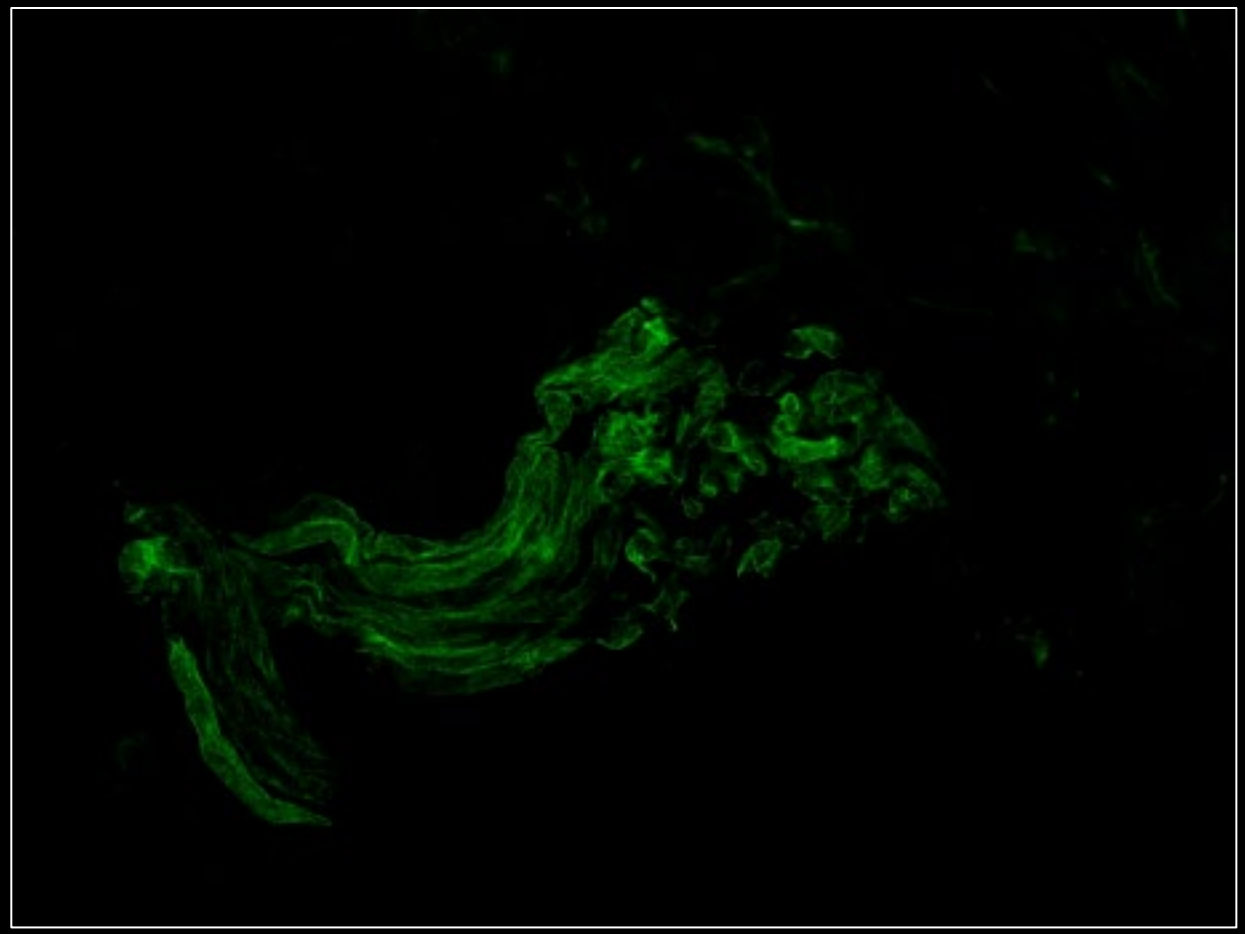

FKRP - CMD
FKRP - CMD
FKRP - LGMD
GMPPB - LGMD
50 µm
100 µm
IIH6
IIH6

Glycosylated alpha-dystroglycan (IIH6) in peripheral nerve

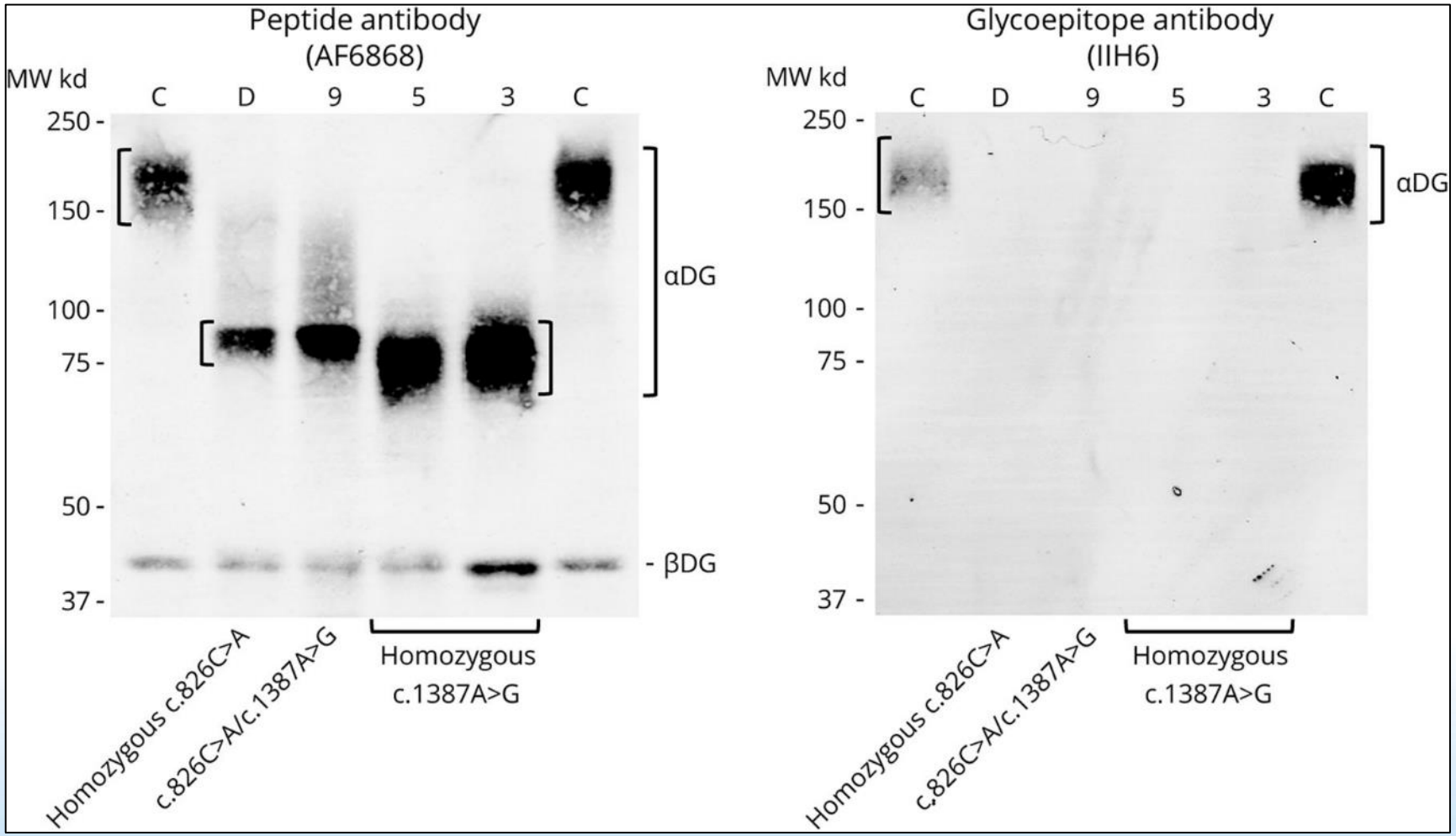
Control



Patient



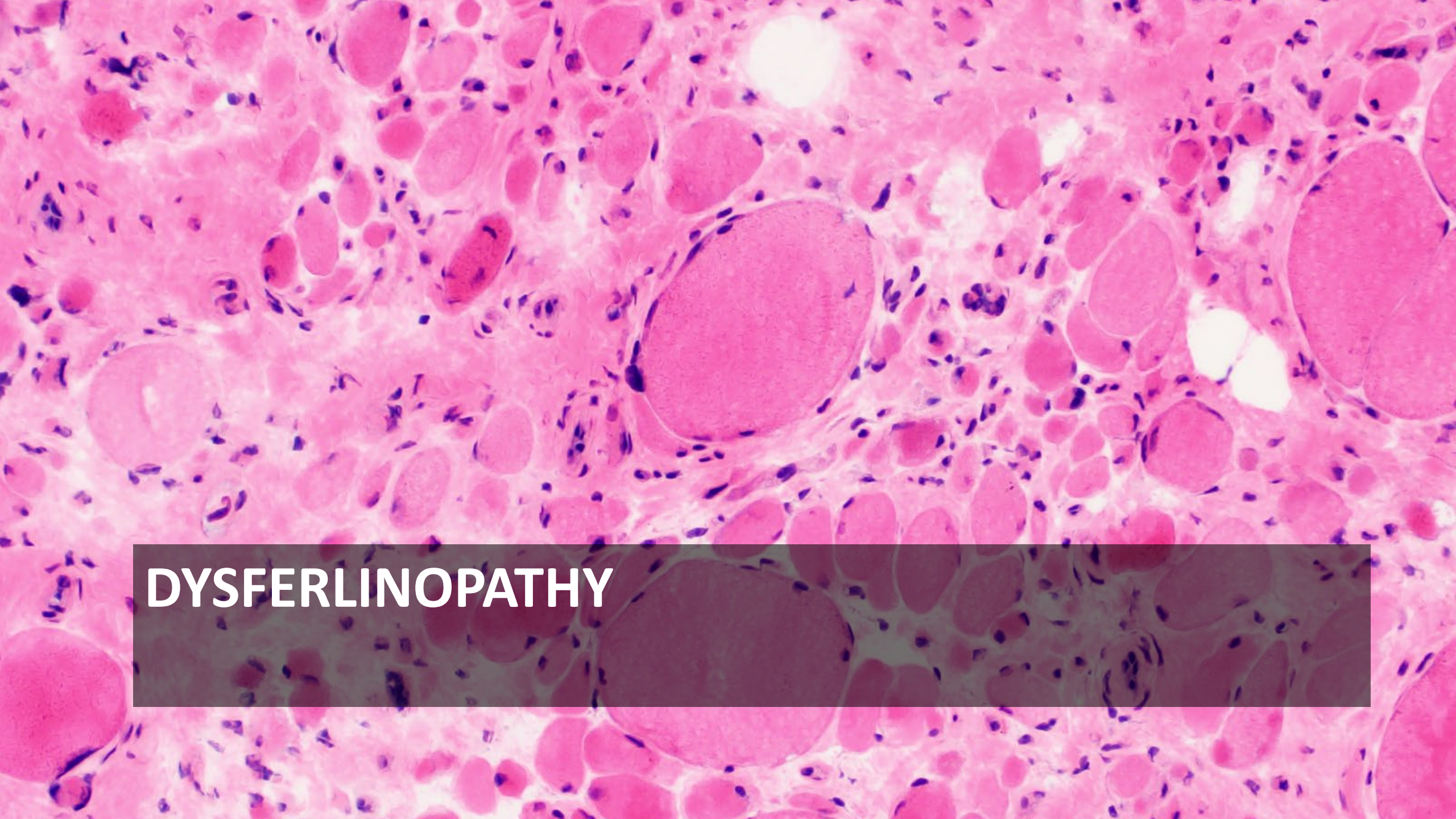
Western blot



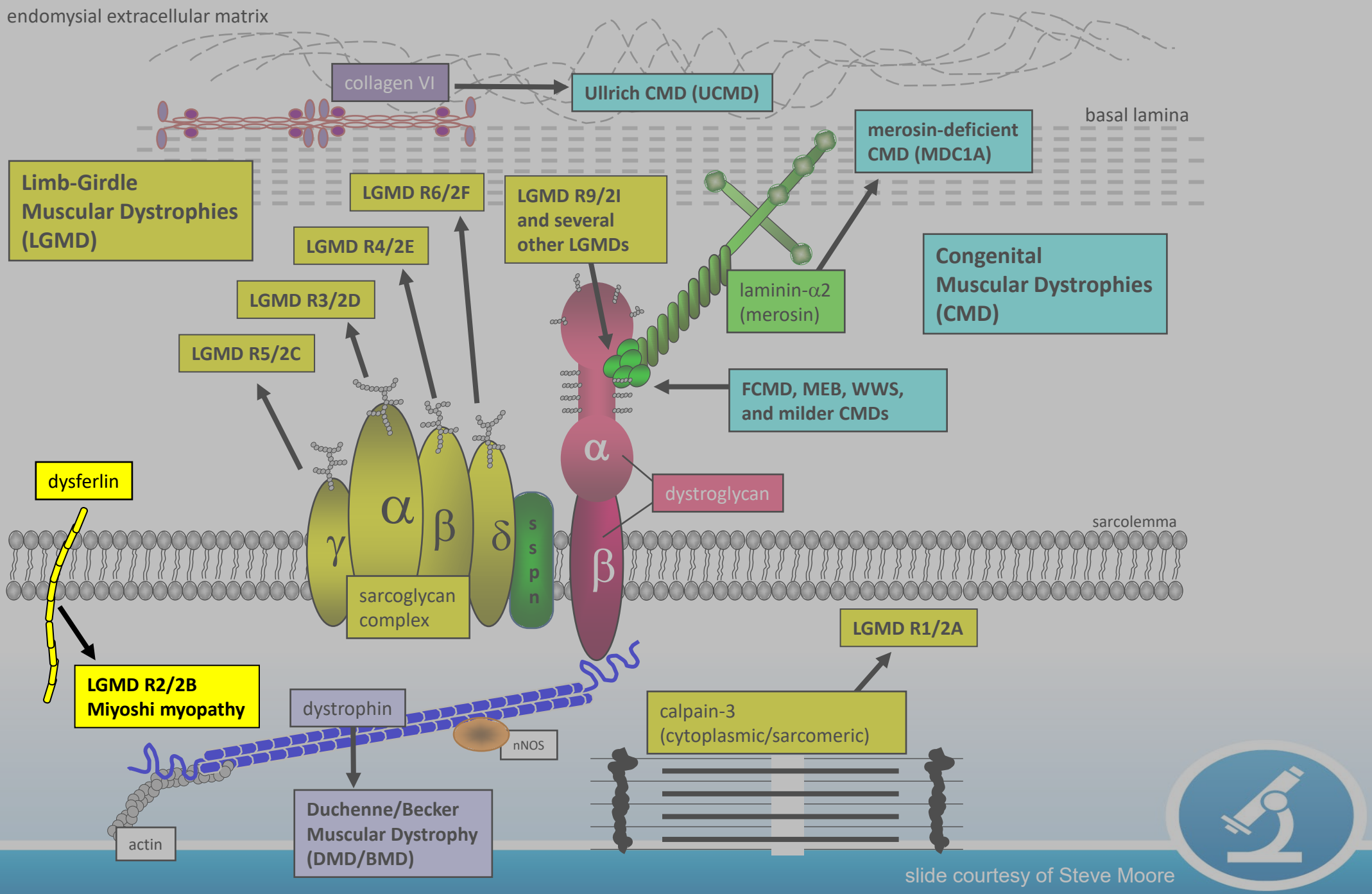
Lee et al. Neurol Genet. 2019. PMID: 31041397

Dystroglycanopathies take-home points

- Widely variable clinical and pathologic findings ranging from mild to severe
  - Even within the same gene!
- Pathophysiology involves abnormal glycosylation of alpha-dystroglycan
  - To diagnose you need to look at a glycosylation specific antibody
  - Pitfall - both alpha-DG protein and beta-DG protein staining will be mostly, if not entirely, normal
- Western blotting can help in cases with difficult to interpret immunostaining

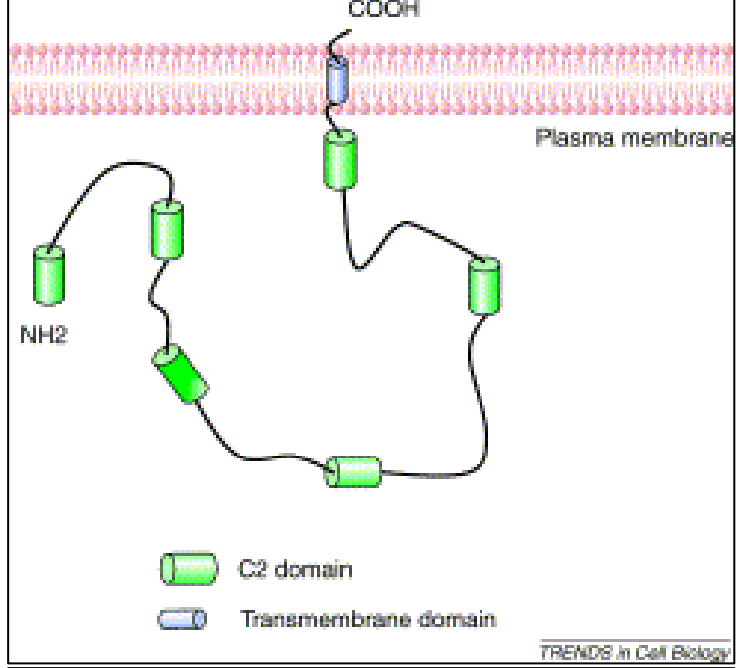


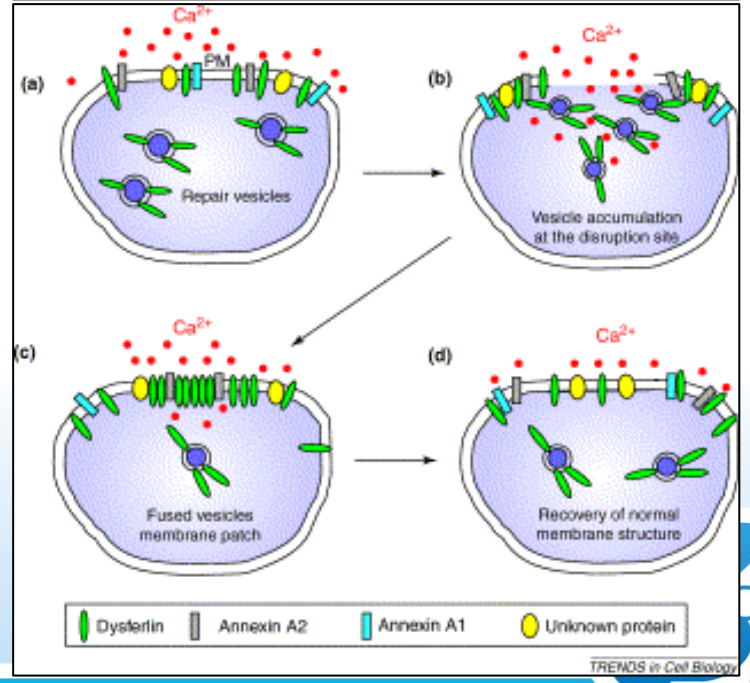
DYSFERLINOPATHY


endomysial extracellular matrix
collagen VI
Ullrich CMD (UCMD)
basal lamina
merosin-deficient CMD (MDC1A)
Limb-Girdle Muscular Dystrophies (LGMD)
LGMD R6/2F
LGMD R9/2I and several other LGMDs
LGMD R4/2E
LGMD R3/2D
LGMD R5/2C
laminin-α2 (merosin)
Congenital Muscular Dystrophies (CMD)
FCMD, MEB, WWS, and milder CMDs
α
γ
α
β
δ
s s p n
β
dystroglycan
dysferlin
sarcolemma
sarcoglycan complex
LGMD R1/2A
LGMD R2/2B Miyoshi myopathy
dystrophin
nNOS
calpain-3 (cytoplasmic/sarcomeric)
actin
Duchenne/Becker Muscular Dystrophy (DMD/BMD)
slide courtesy of Steve Moore

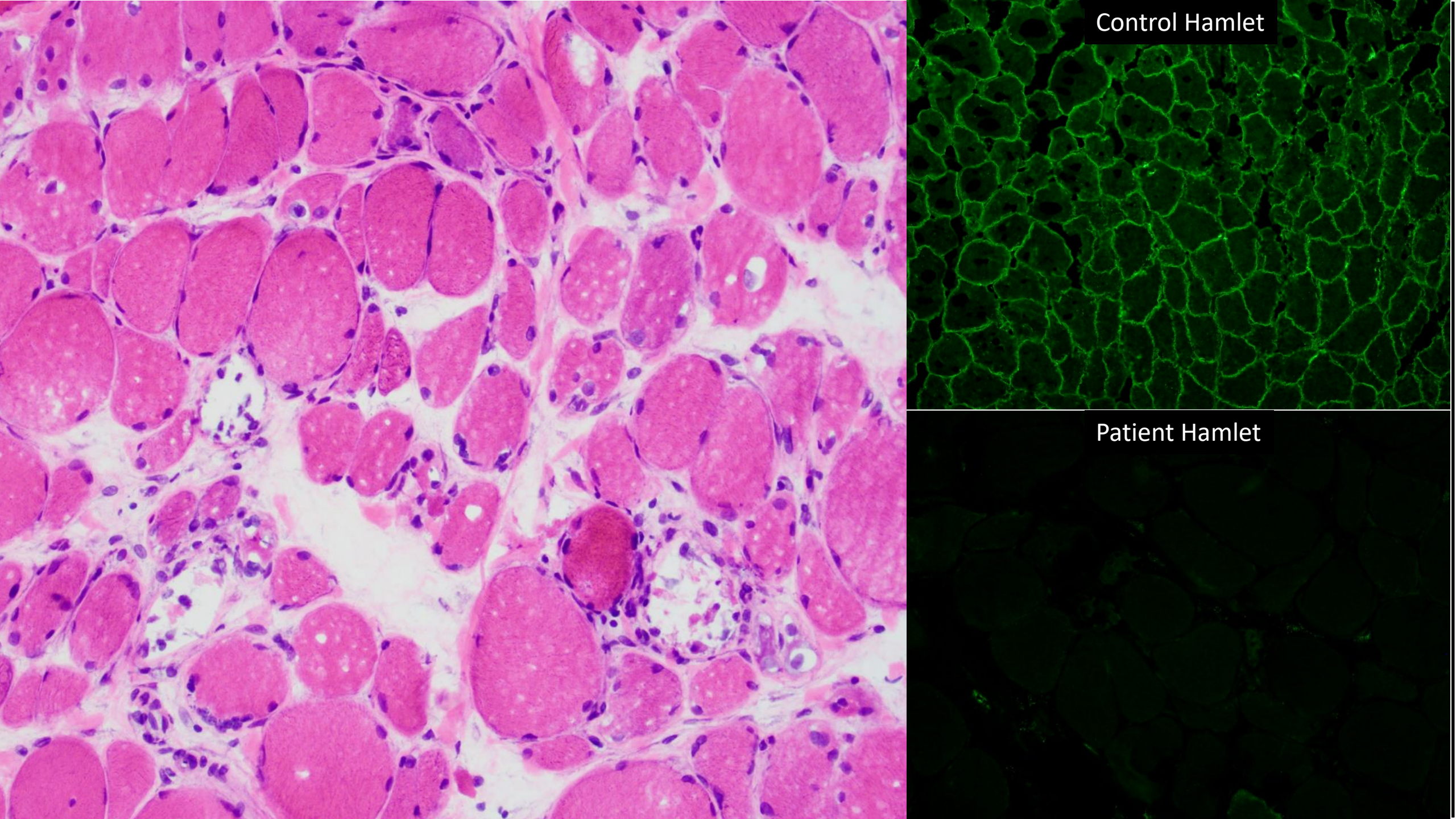
Dysferlinopathy – *DYSF* gene – autosomal recessive

- Protein localized to the sarcolemma
  - Important for membrane repair
- Clinical phenotype variable
  - LGMD 2B/R2
  - Miyoshi myopathy (distal)
- Unique histopathologic findings for a muscular dystrophy

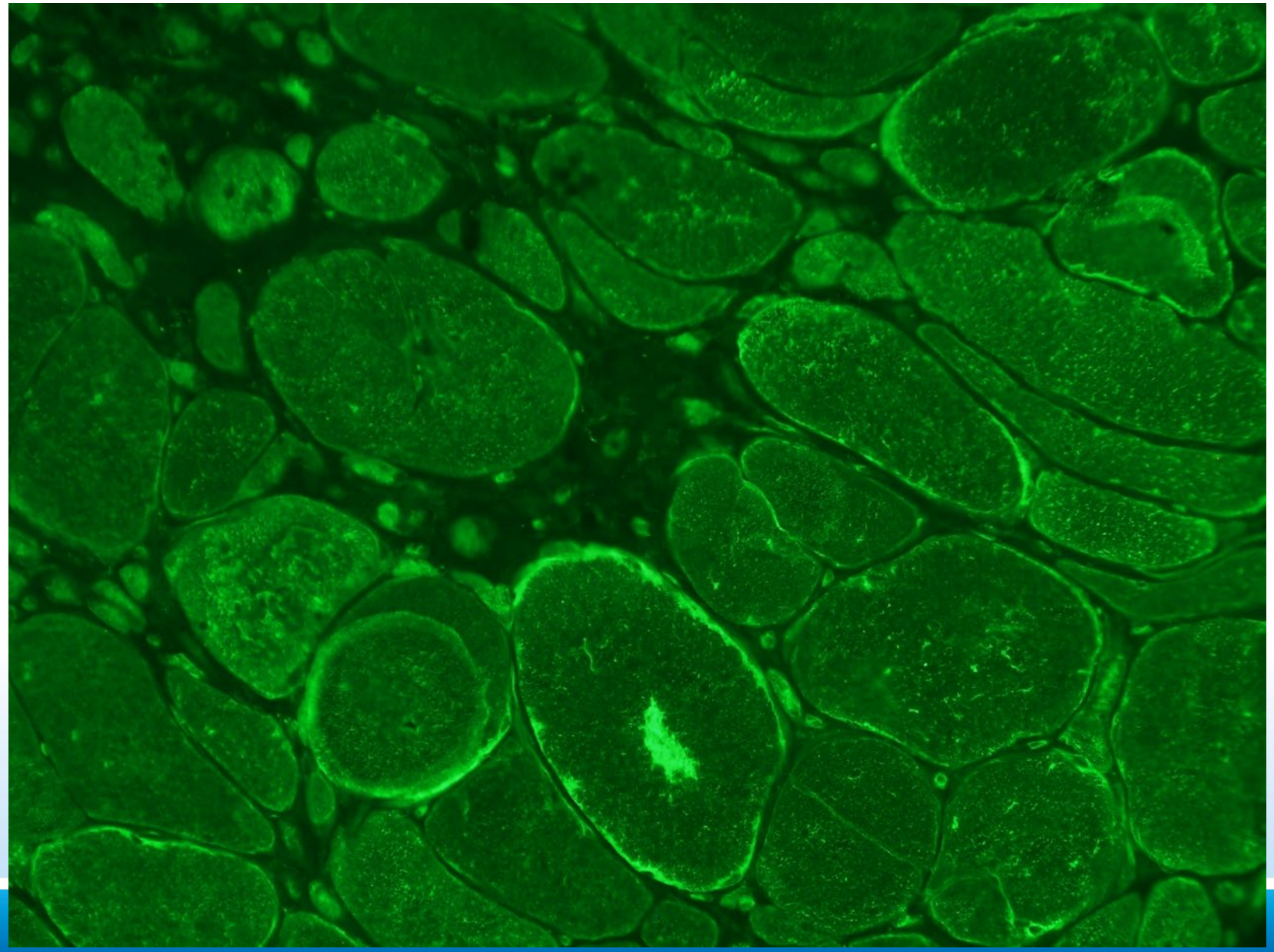




Bansal and Campbell. Trends in Cell Biology. 2004. PMID: 15066638


Control Hamlet
Patient Hamlet

Pitfall – nonspecific sarcoplasmic staining



Dysferlin (Hamlet) and calpain-3 (12A2) Western blot

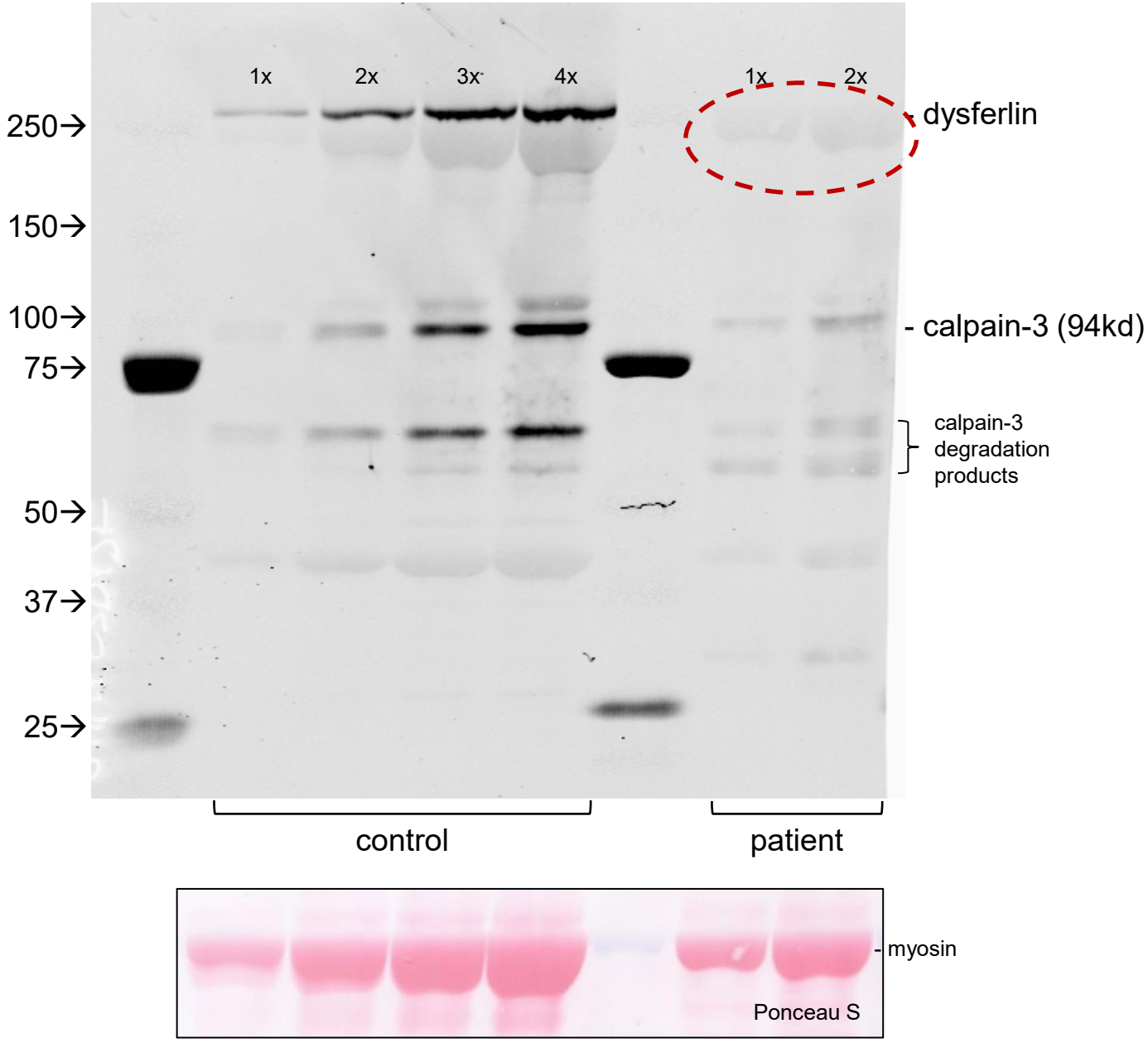
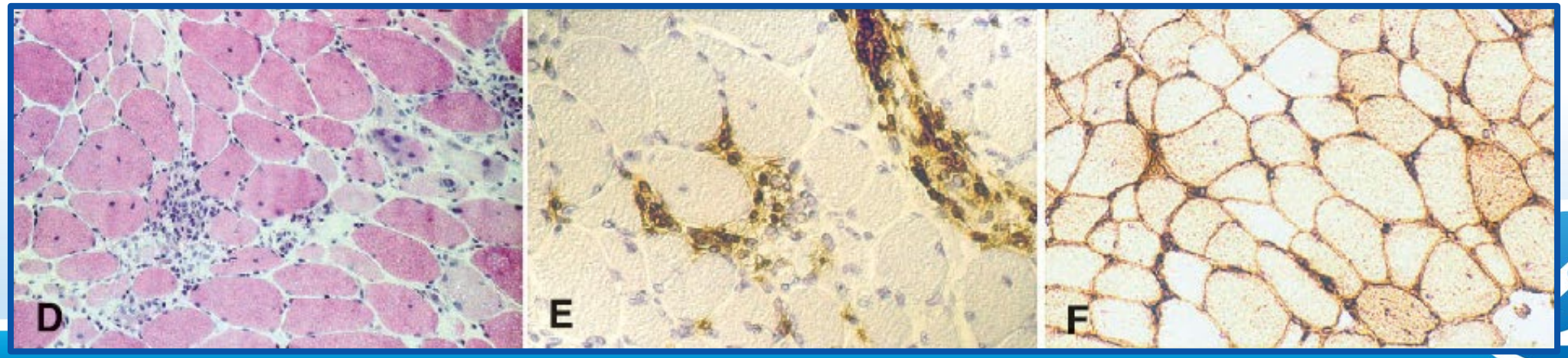


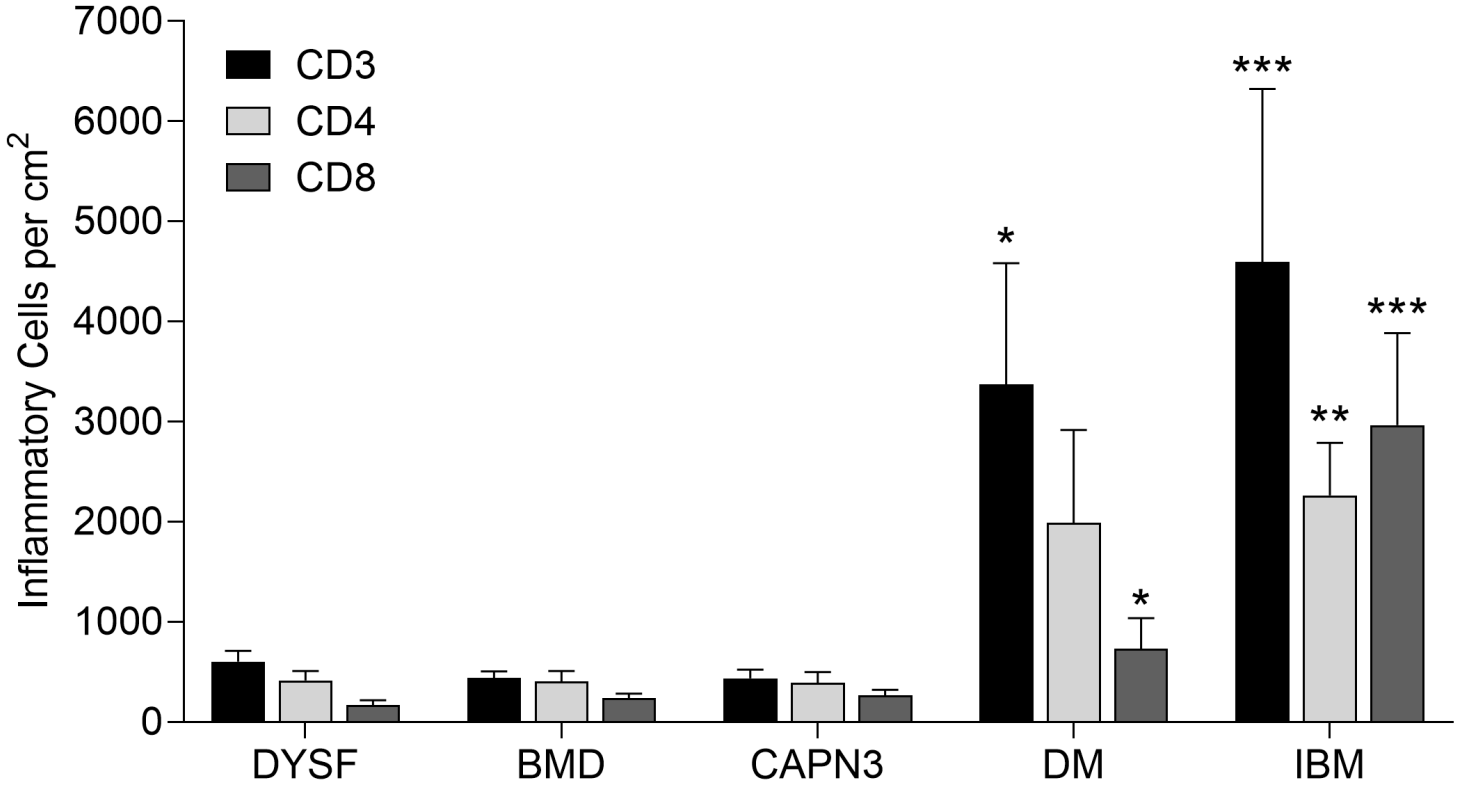
Image and WB by Steve Moore and Mary Cox

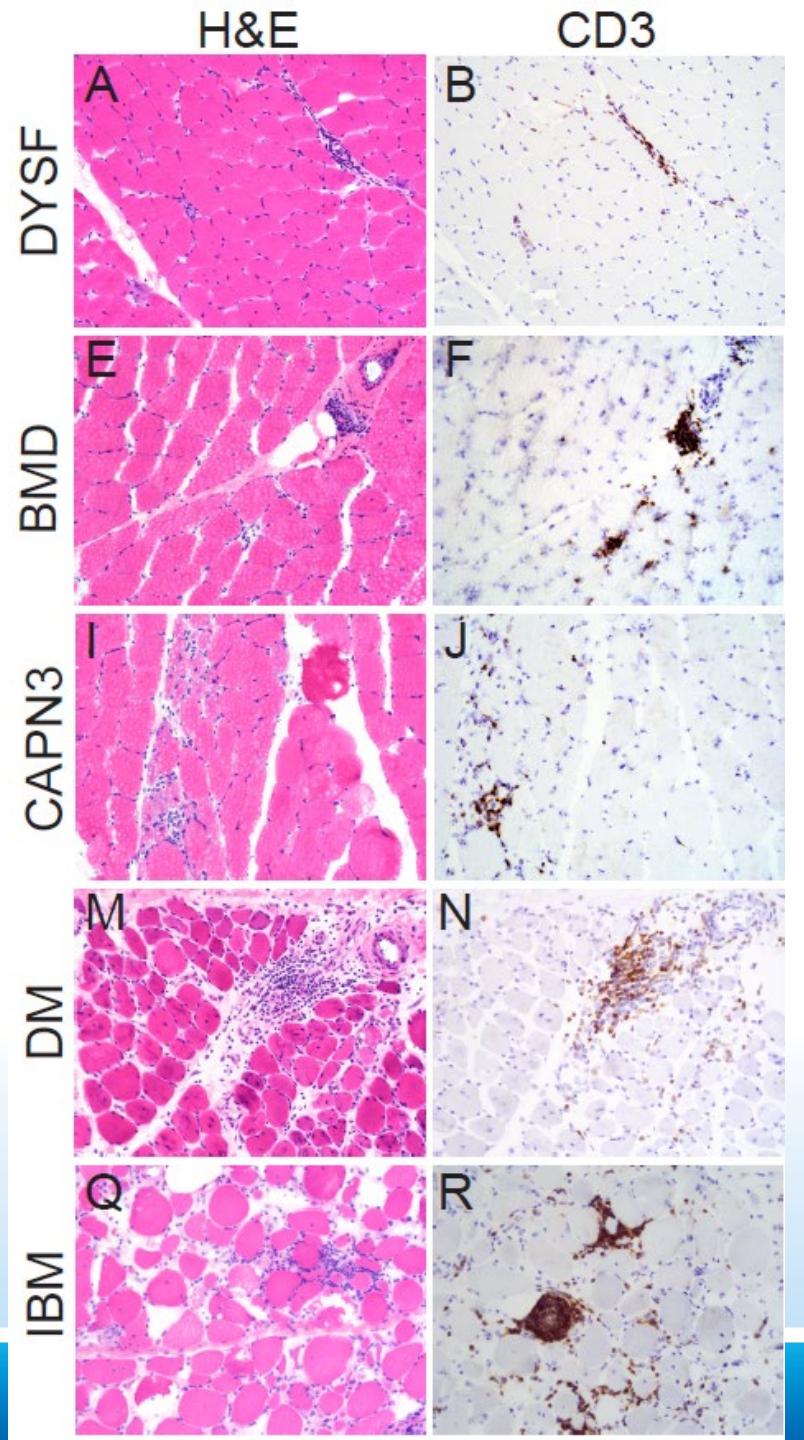
Dysferlinopathy and inflammation

- Multiple papers have reported perivascular or endomysial inflammation as being a recurrent finding in dyferlinopathy biopsies
  - Fanin and Angelini, Neuropathy and Applied Neurobiology 28:461-470, 2002
  - Confalonieri, et al., Journal of Neuroimmunology. 2003. 142: 130-136
  - Brunn et al., Acta Neuropathologica 112:325–332, 2006
  - Choi et al, J Korean Med Sci 24:1015-1023, 2009
  - Krahn et al., Neuromuscular Disorders 21:503–512, 2011

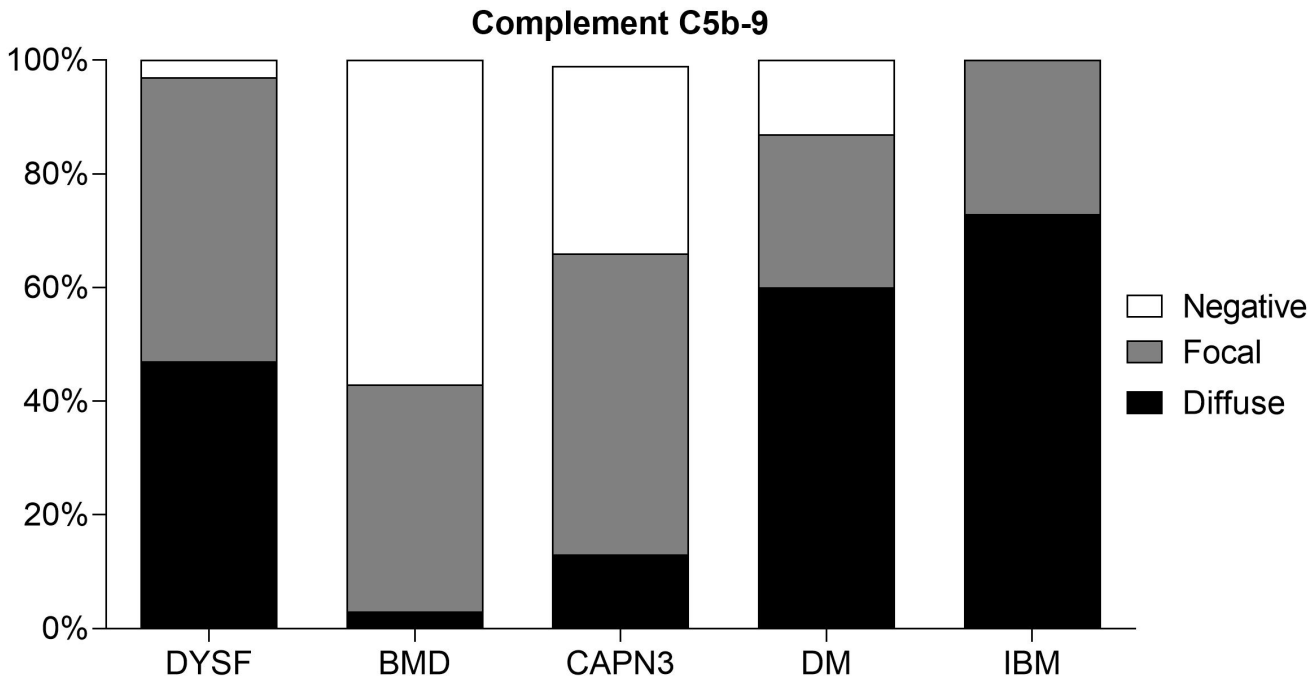


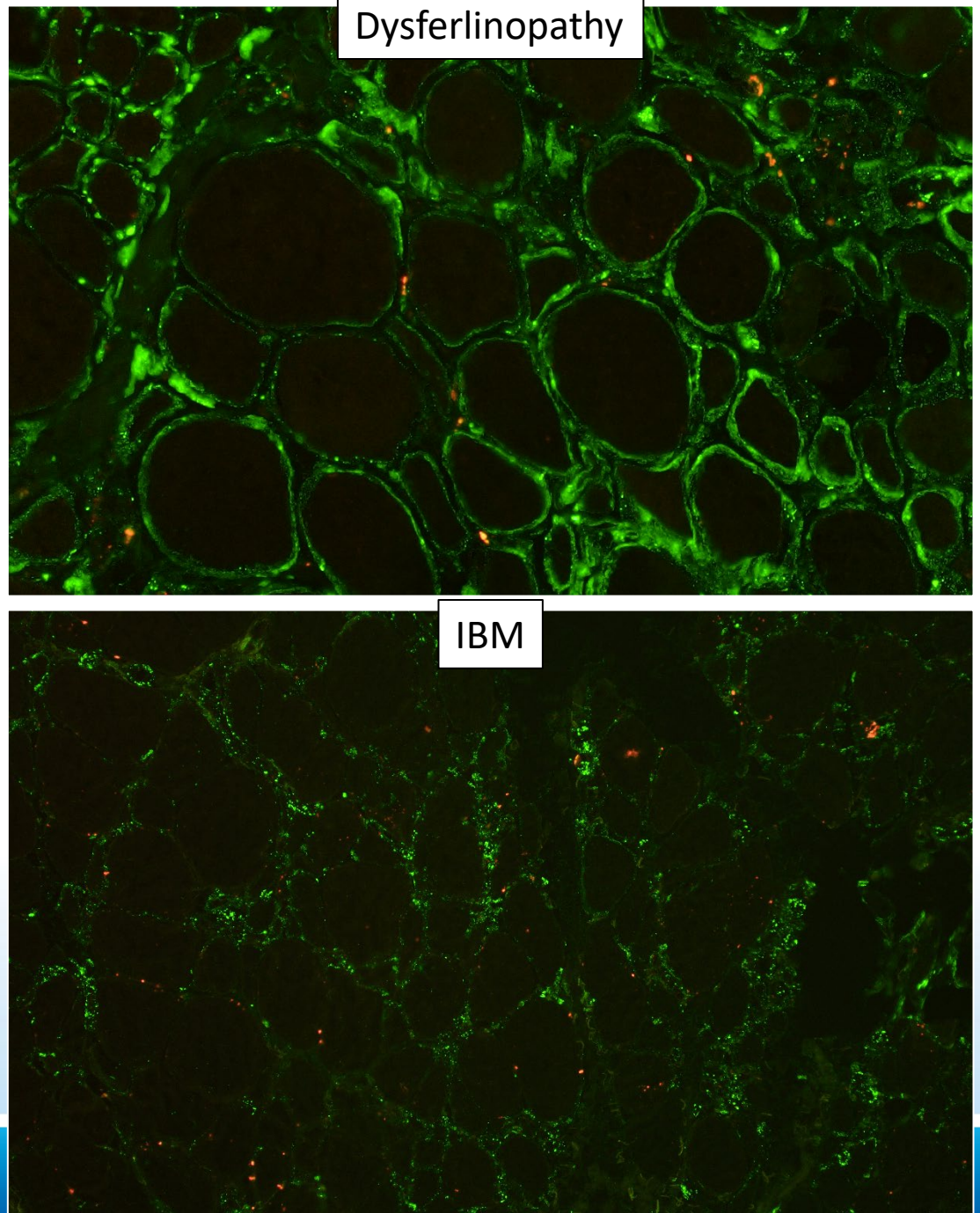
Inflammation OK, but how much?





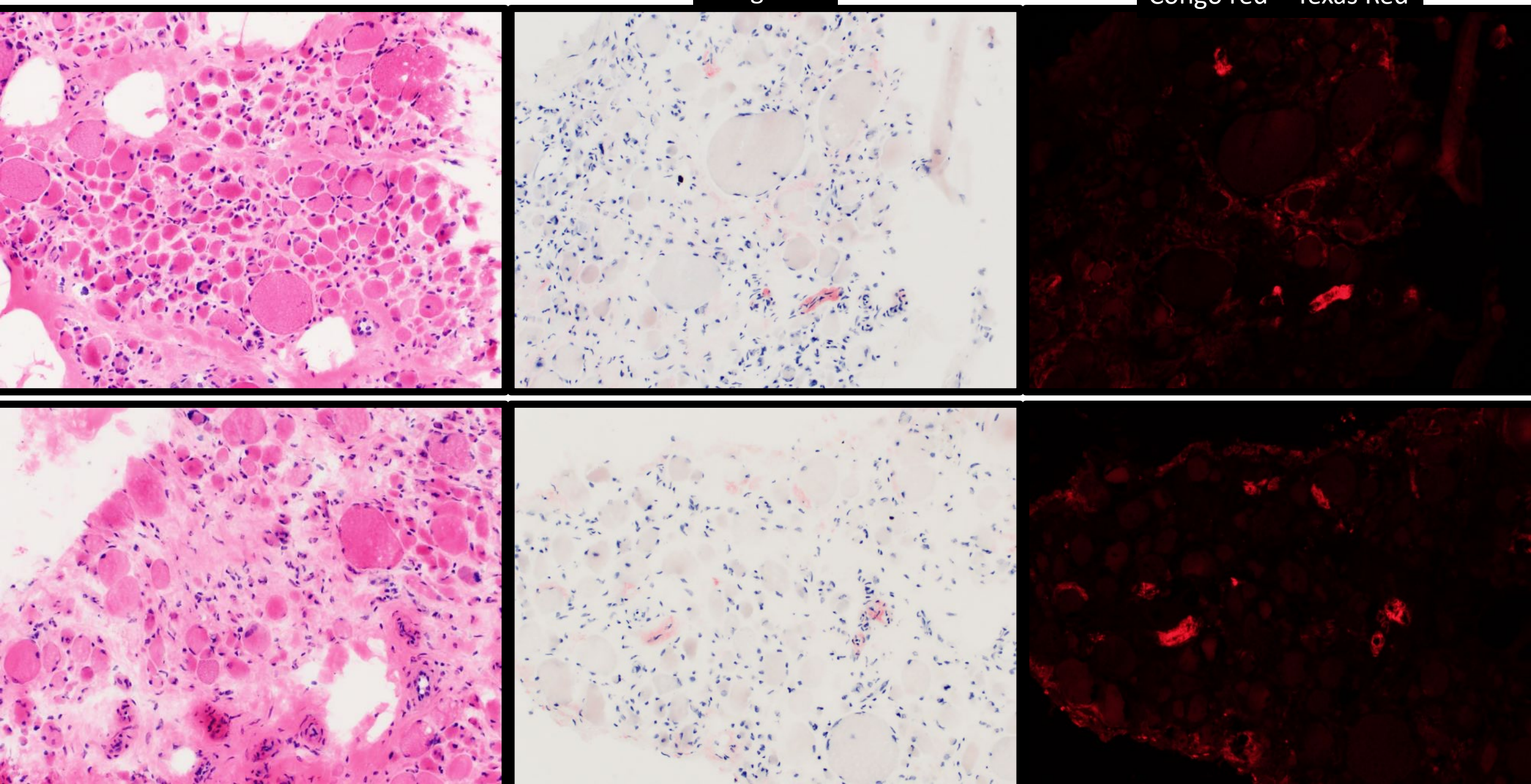
Dysferlinopathy and complement





Becker N, Moore SA, Jones KA. 2021. In preparation.

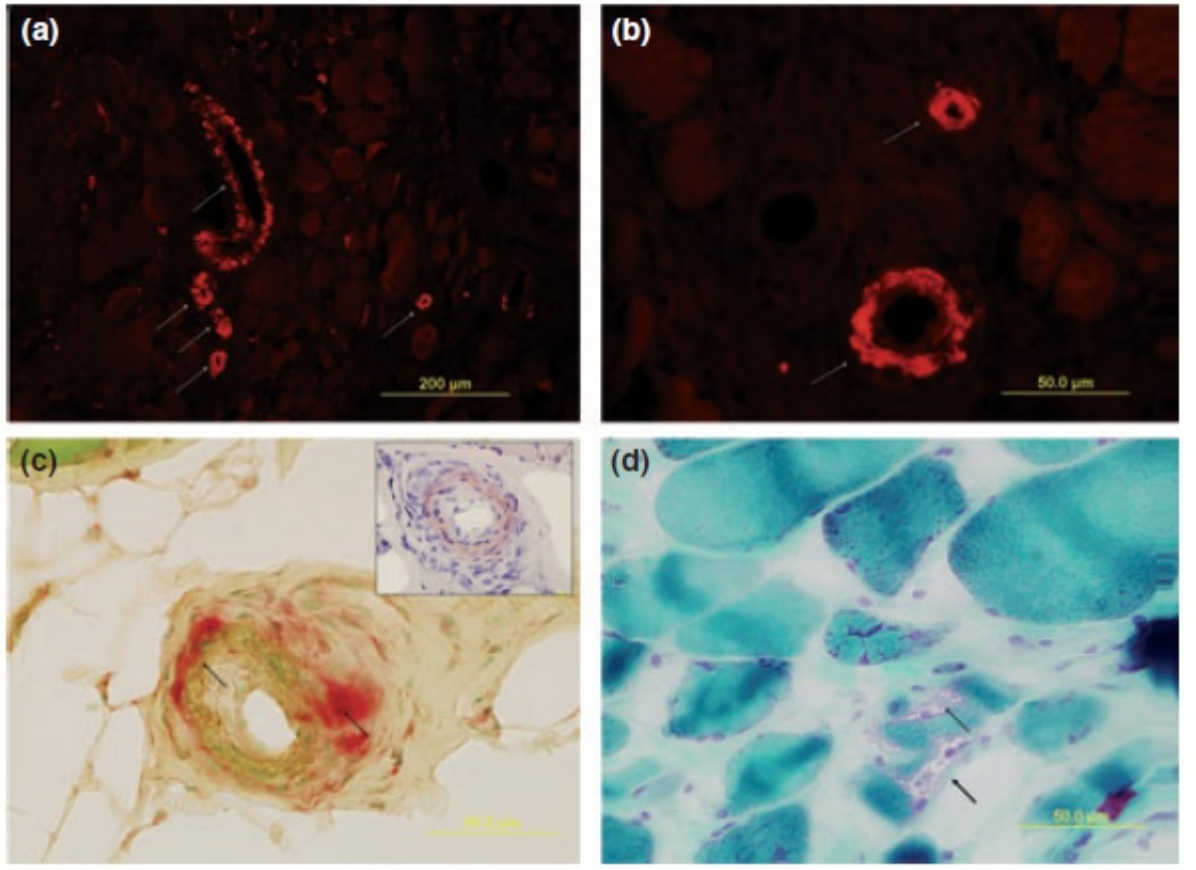
Dysferlinopathy and amyloid



Anoctaminopathy (*ANO5*) and laminopathy (*LMNA*) share features with dysferlinopathy

- ANO5 and LMNA disease can also show complement C5b-9 deposition along myofibers (Iowa experience)
- ANO5 has also been reported to show amyloid deposition

Amyloid deposition in ANO5



Liewluck et al. Eur J Neurol. 2013. PMID: 23663589

Dysferlinopathy take-home points

- Proximal or distal phenotypes
- Total loss of dysferlin expression is diagnostic of disease
  - Loss of sarcolemmal positivity with increased sarcoplasmic positivity is NONSPECIFIC and can be a pitfall
- While inflammation can be seen in dysferlinopathies, it is seen at a level comparable to other MDs and less than expected in myositis
- Complement C5b-9 myofiber deposition is very common in dysferlinopathies and uncommon in other MDs
  - Also a shared feature with ANO5 and LMNA disease
- Amyloid deposition is a unique feature of dysferlinopathies
  - Also shared with ANO5 diseease

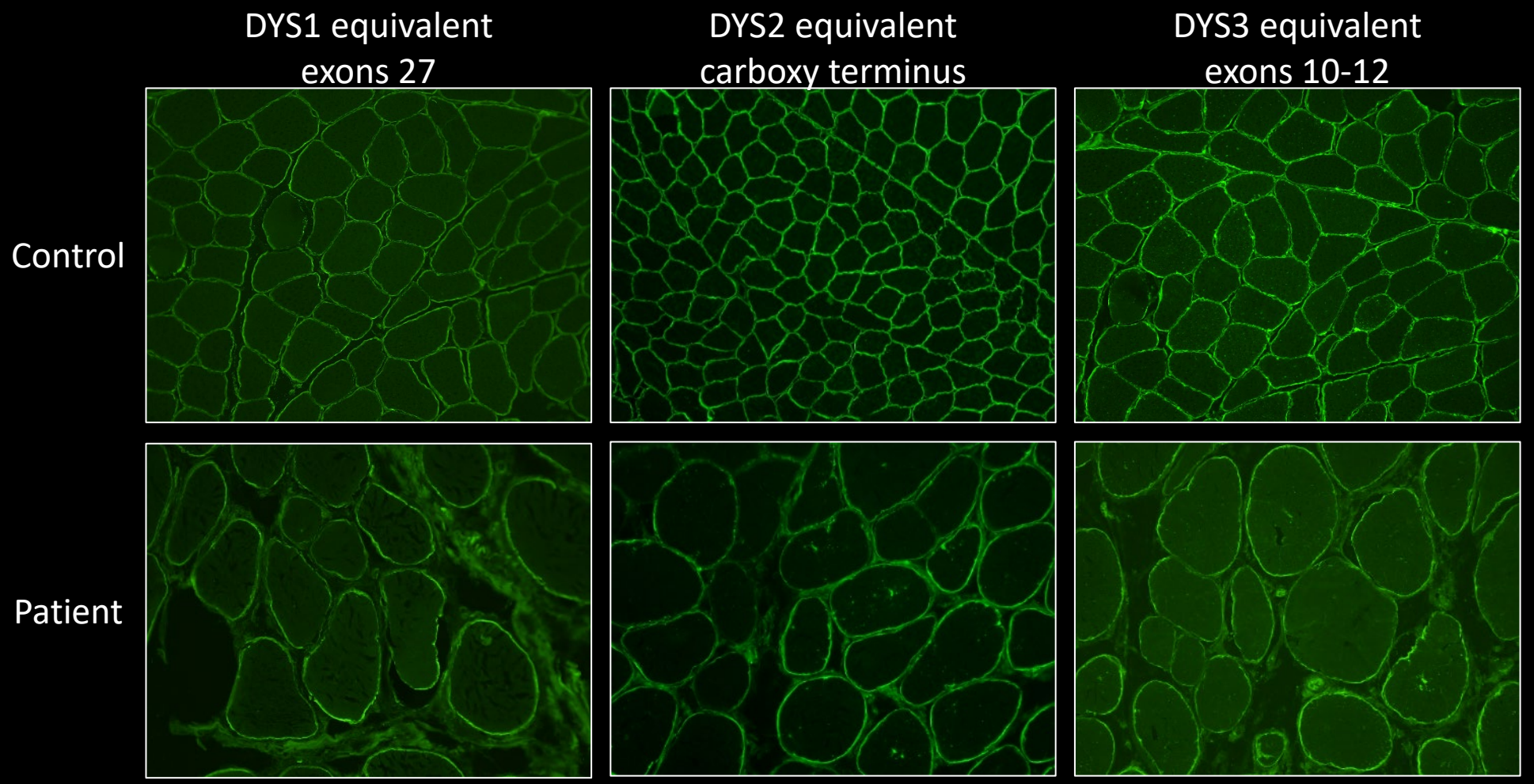
PathPresenter

https://pathpresenter.net/#/public/presentation/display?token=f465172f

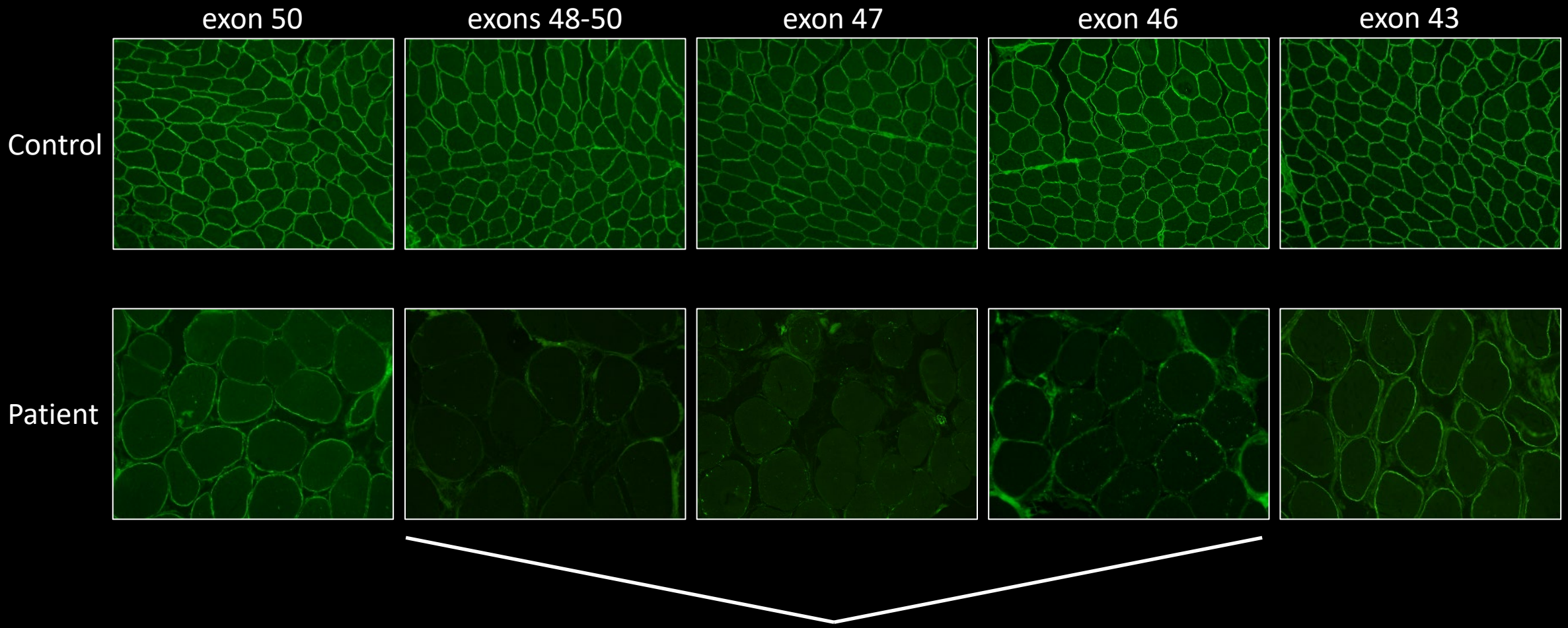
Unknown case:

- 72-year-old man
- Multi-year history of gradually progressive proximal muscle weakness
- CK elevated while on statin, taken off statin, and CK remained elevated at >10,000 U/L
- Clinical concern for an immune-mediated necrotizing myopathy or myositis

Unknown case – dystrophin immunostaining

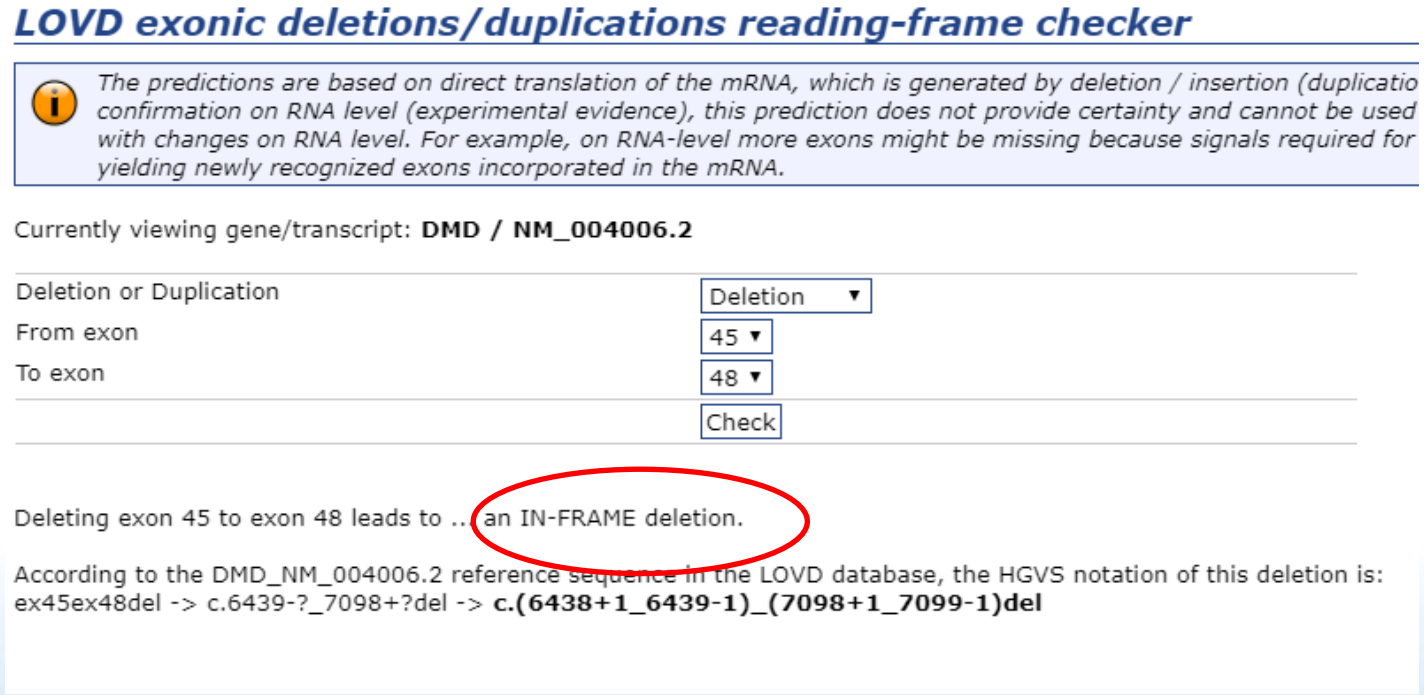


Unknown case – dystrophin immunostaining



Case solved – strong implications for the patient and his family

- Genetic testing revealed deletion of exons 45-48



LOVD exonic deletions/duplications reading-frame checker

The predictions are based on direct translation of the mRNA, which is generated by deletion / insertion (duplicatio confirmation on RNA level (experimental evidence), this prediction does not provide certainty and cannot be used with changes on RNA level. For example, on RNA-level more exons might be missing because signals required for yielding newly recognized exons incorporated in the mRNA.

Currently viewing gene/transcript: **DMD / NM_004006.2**

Deletion or Duplication	Deletion
From exon	45
To exon	48
	Check

Deleting exon 45 to exon 48 leads to .. an IN-FRAME deletion.

According to the DMD_NM_004006.2 reference sequence in the LOVD database, the HGVS notation of this deletion is:
ex45ex48del -> c.6439-?_7098+?del -> **c.(6438+1_6439-1)_(7098+1_7099-1)del**

https://databases.lovd.nl/shared/scripts/readingFrameChecker.php?gene=DMD&transcript=NM_004006.2


Questions?

Useful references

1. Bönnemann CG, Wang CH, Quijano-Roy S, Deconinck N, Bertini E, Ferreiro A, et al. Diagnostic approach to the congenital muscular dystrophies. Neuromuscul Disord. 2014 Apr;24(4):289-311. PMID: 24581957; PMCID: PMC5258110.
2. Dowling JJ, Weihl CC, Spencer MJ. Molecular and cellular basis of genetically inherited skeletal muscle disorders. Nat Rev Mol Cell Biol. 2021 Jul 13. PMID: 34257452.
3. Gieron-Korthals M, Fernandez R. New Developments in Diagnosis, Treatment, and Management of Duchenne Muscular Dystrophy. Adv Pediatr. 2020 Aug;67:183-196. PMID: 32591061.
4. Liewluck T, Milone M. Untangling the complexity of limb-girdle muscular dystrophies. Muscle Nerve. 2018 Aug;58(2):167-177. PMID: 29350766.
5. Moore SA, Shilling CJ, Westra S, Wall C, Wicklund MP, Stolle C, et al. Limb-girdle muscular dystrophy in the United States. J Neuropathol Exp Neurol. 2006 Oct;65(10):995-1003. PMID: 17021404.
6. Nix JS, Moore SA. What Every Neuropathologist Needs to Know: The Muscle Biopsy. J Neuropathol Exp Neurol. 2020 Jul 1;79(7):719-733. PMID: 32529201; PMCID: PMC7304986.
7. Tuffery-Giraud S, Béroud C, Leturcq F, Yaou RB, Hamroun D, Michel-Calemard L, et al. Genotype-phenotype analysis in 2,405 patients with a dystrophinopathy using the UMD-DMD database: a model of nationwide knowledgebase. Hum Mutat. 2009 Jun;30(6):934-45. PMID: 19367636.