Clinical Introduction to the Dystroglycanopathies

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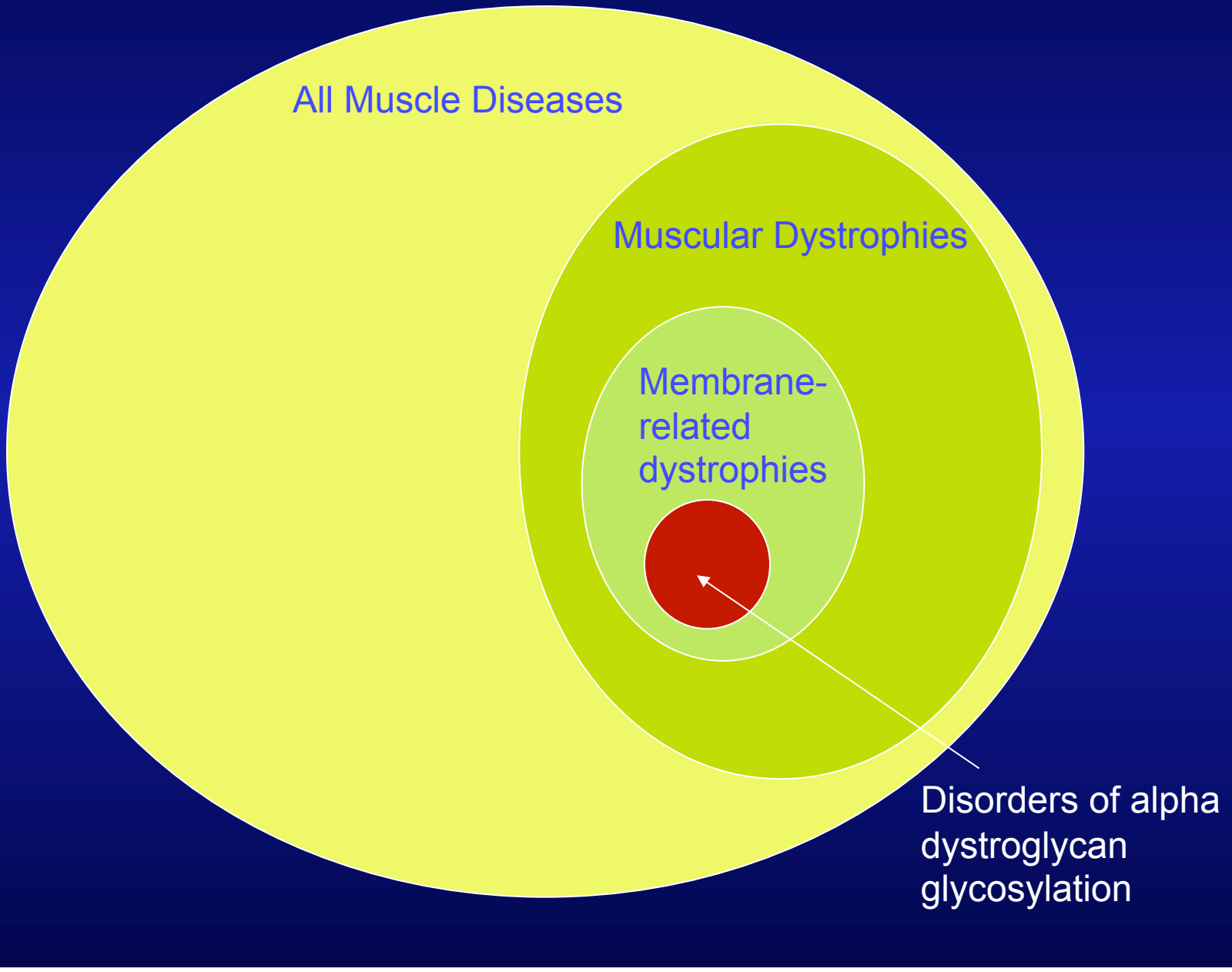
Welcome to Iowa City!

Overview

- Where do the dystroglycanopathies fit in among neuromuscular diseases?
- What is the clinical features of the dystroglycanopathies?
- How are the dystroglycanopathies managed?

Dystroglycanopathies



What are muscular dystrophies?

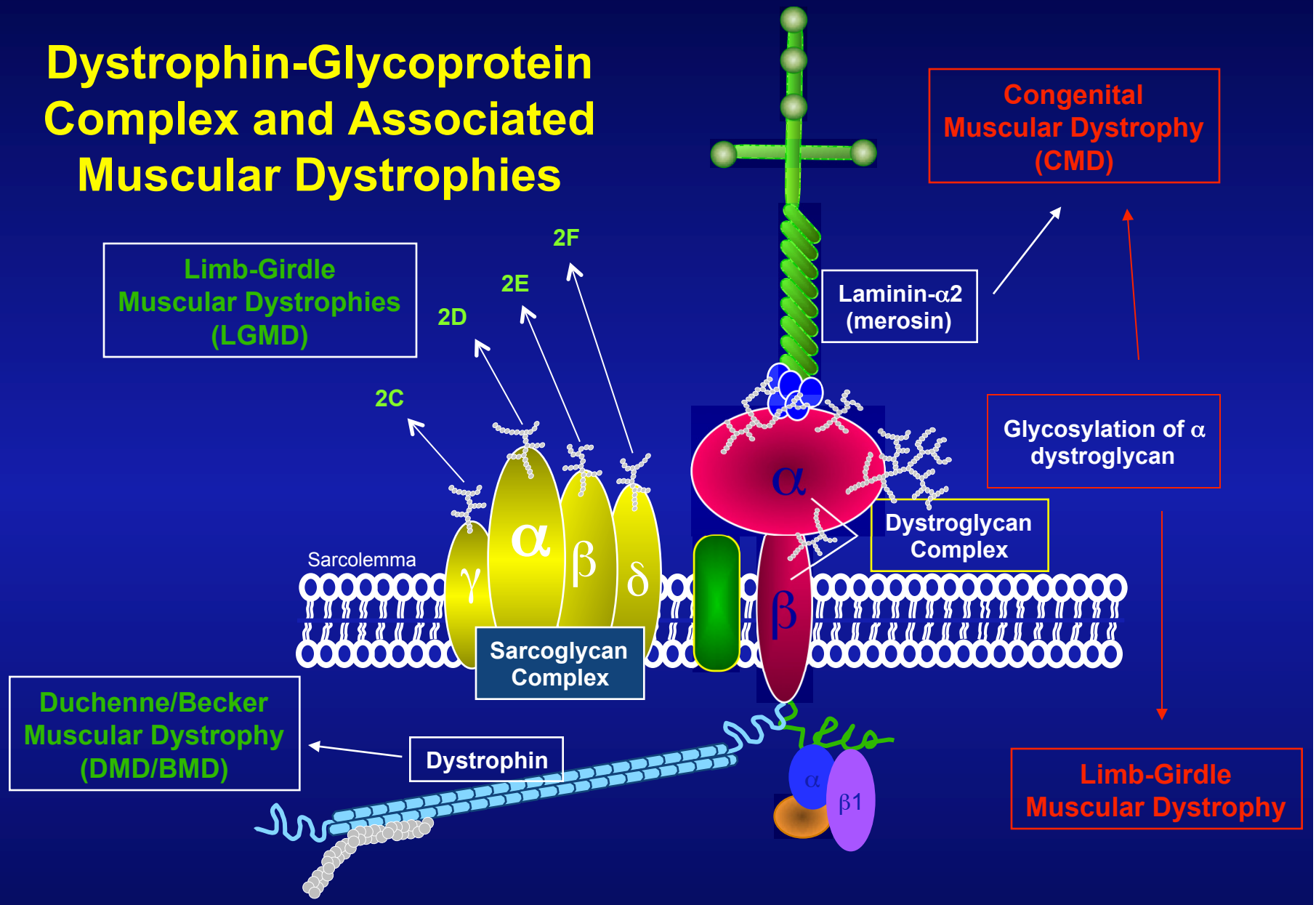
- Inherited diseases of muscle
- Degeneration and regeneration

- Muscular Dystrophies--clinical categories
  - Dystrophinopathies (Duchenne/Becker)
  - Limb girdle Muscular Dystrophies (18 types)
  - Congenital Muscular Dystrophies (14 types)
  - Facioscapulohumeral Muscular Dystrophy
  - Emery-Dreifuss Muscular Dystrophy (XL and AD)
  - Oculopharyngeal Muscular Dystrophy
  - Scapuloperoneal Muscular Dystrophy
  - Distal Muscular Dystrophies

What disorders are included among the dystroglycanopathies?

- NOTE: nomenclature is evolving
- Congenital muscular dystrophies
  - Walker Warburg syndrome
  - Muscle Eye Brain disease
  - Fukuyama Muscular Dystrophy
  - Congenital muscular dystrophy types 1C and 1D
- Limb Girdle muscular dystrophy
  - Types 2I, K, M, N, and O

Dystrophin-Glycoprotein Complex and Associated Muscular Dystrophies

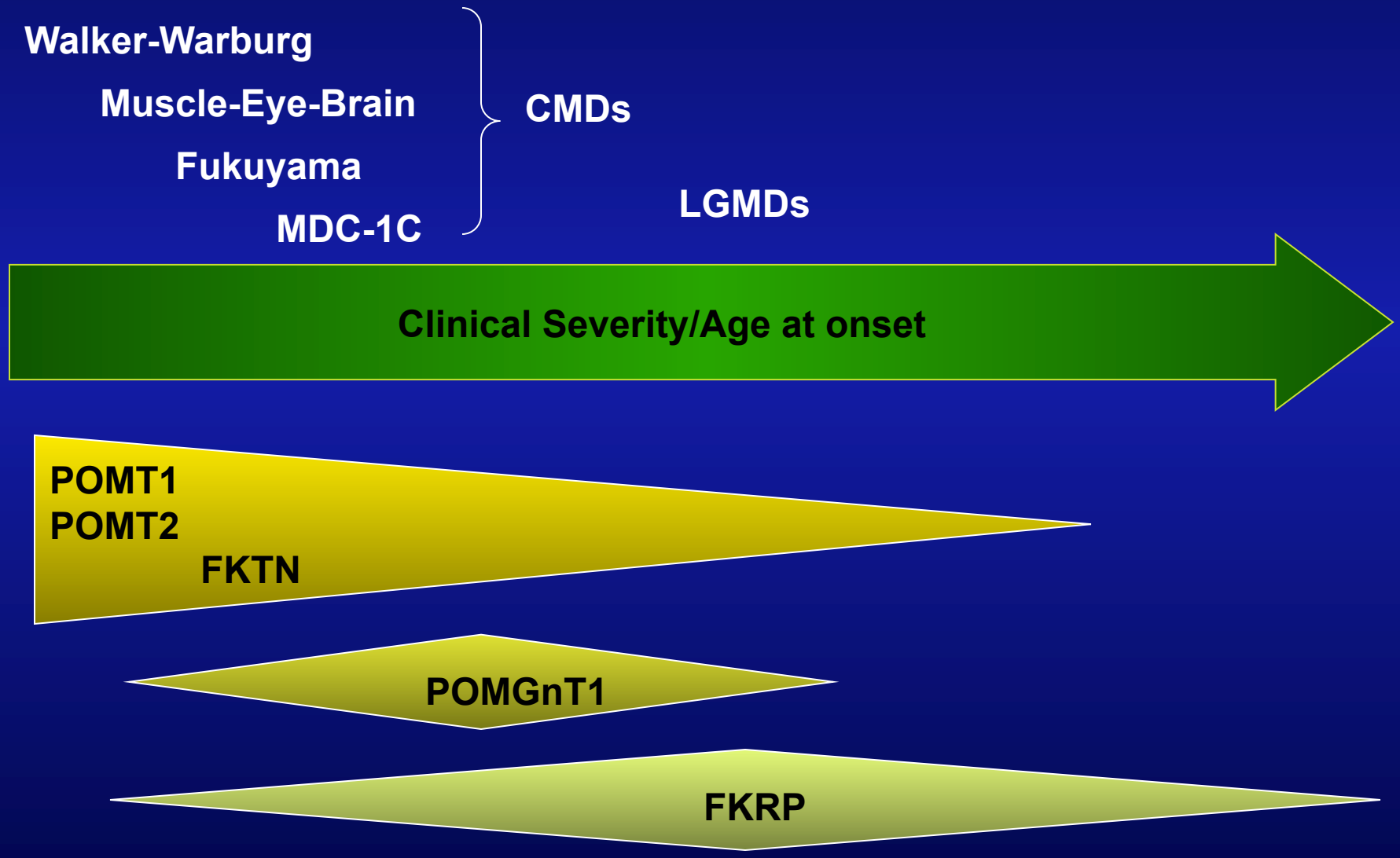


(Courtesy of Kevin Campbell laboratory)

Genes Known to Result in a Dystroglycanopathy

- POMT1 (Protein O-mannosyltransferase 1)
- POMT2 (Protein O-mannosyltransferase 2)
- POMGnT1 (protein O-mannose beta-1,2-N-acetylglucosaminyltransferase)
- Fukutin
- FKRP (Fukutin related protein)
- LARGE
- DAG1 (dystrophin-associated glycoprotein; dystroglycan)

The Dystroglycanopathies--Clinical Spectrum



LGMD 2I

- Caused by mutations in FKRP
- Most common cause of LGMD in northern European population
- Wide range of clinical severity
- Common mutation (c. 826C>A)
  - 2 copies of common mutation= milder disease
  - 1 copy of common mutation + 1 copy of some other mutation = often more severe disease

Iowa FKRP Natural History Study
Age at First Symptom (self-report data)

	Range (years)	Mean age (S.D)
All patients	birth-28	7.4 (7)
Homozygous (826 C>A)	2-28	10.1 (7.7)
Heterozygous (826 C>A + unique)	0.25 -12	4.3 (4)
Heterozygous (2 unique mutations; 1 patient)	Birth	--

Congenital Muscular Dystrophies

- Onset of weakness before age 2
- Range of involvement
  - Muscle weakness present in all
    - Abnormal development of brain and eye
    - Cognitive impairment with minor abnormality of brain formation
    - Cognitive impairment with normal brain structure on MRI
    - Normal intelligence and normal eyes

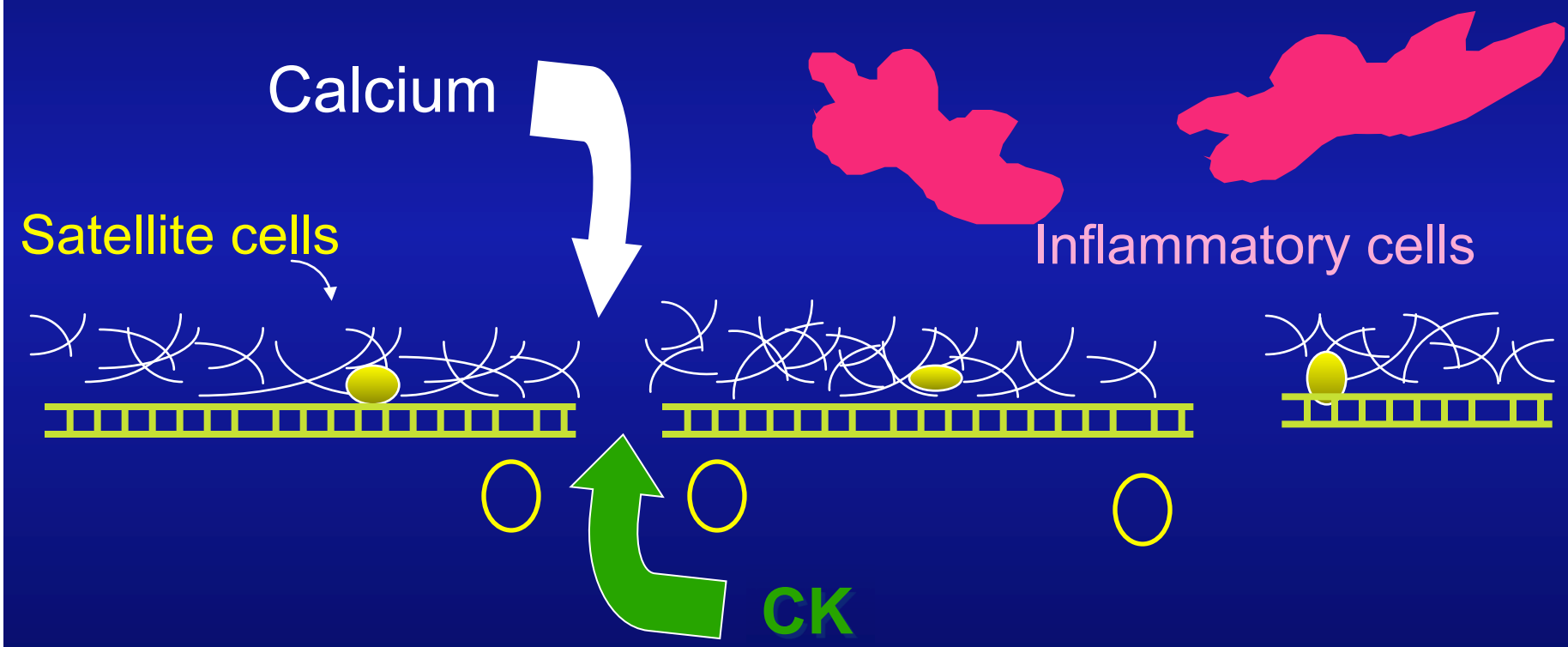
DGs are Autosomal Recessive

- Both parents are carriers
  - Carriers have no symptoms or weakness
- With each pregnancy, 1 in 4 (25%) chance that a child will be affected.
- Non affected sibs
  - 2/3 chance of being a carrier
- Carrier rate in Iowa 1/315

Organ system involvement in Dystroglycanopathies

- Muscles
- Breathing
- Heart
- Bones and joints
- Eyes
- Brain

Mismatch between muscle injury and repair



Muscles get weaker over time

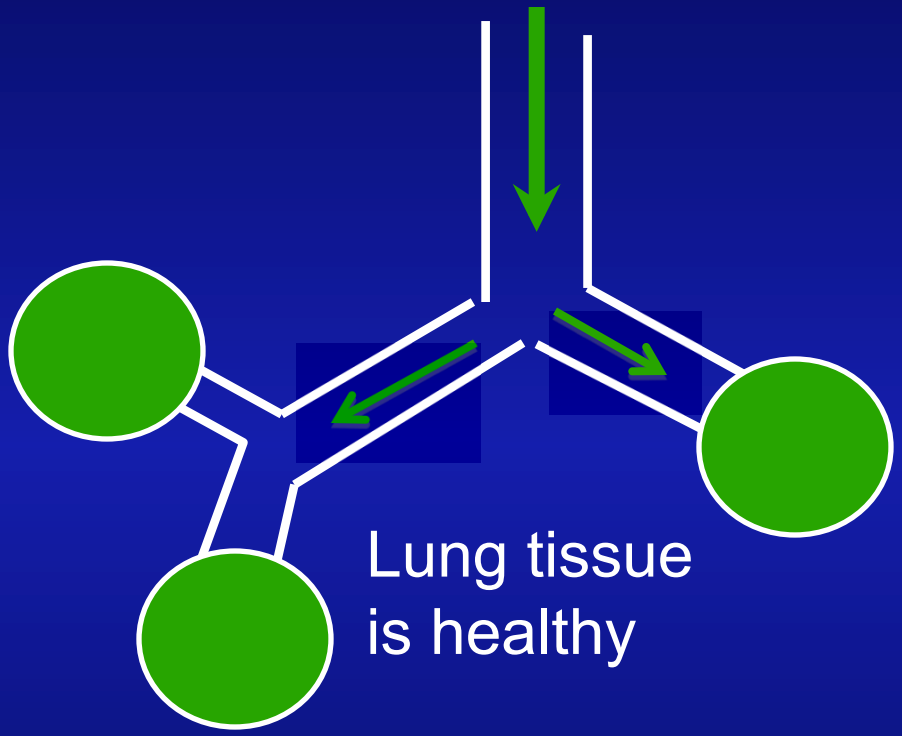
Exercise

- Too little exercise causes muscles to atrophy and become weak
- Normal response to exercise:
  - Membrane breaks
  - CK release
  - Repair of membrane
  - Protein synthesis
  - Fiber hypertrophy

Exercise
General Recommendations

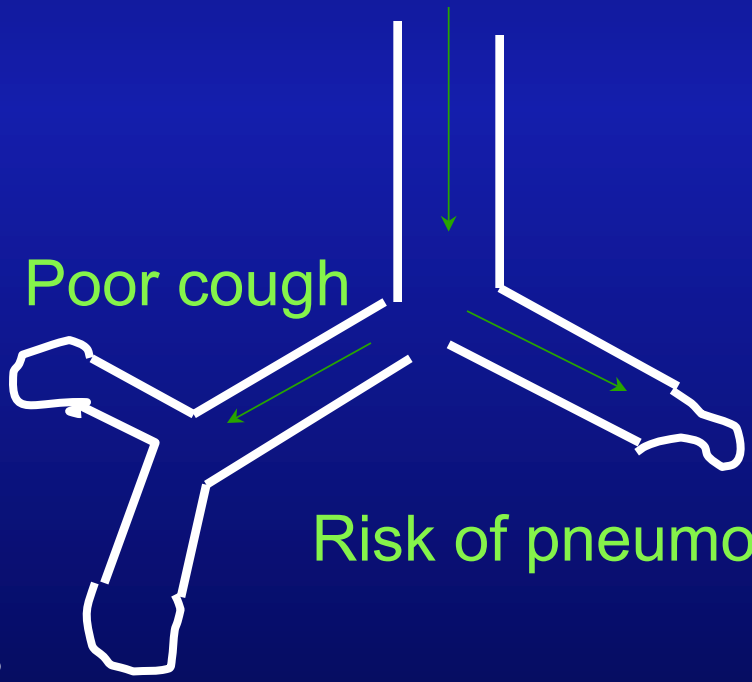
- Maintain active lifestyle, as possible
  - Swimming is an excellent activity
- Pay attention to your body—don't overdo
- Avoid muscle-building exercise, zealous training, overly aggressive PE teachers
- Do regular stretching

Breathing in Dystroglycanopathy



Lung tissue
is healthy

Muscles that move the
air can become weak



Poor cough

Risk of pneumonia

Shallow breaths in sleep

Symptoms:
Morning headache
Excessive fatigue
Daytime sleepiness
Recurrent pneumonia

Breathing in Dystroglycanopathy

- Not a problem early in disease
- Monitor strength of muscles involved in breathing, cough
- Lots of options for management
  - Suction Machine
  - BiPAP (night-time only, fulltime)
  - Cough Assist

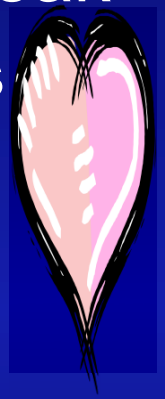
BiPAP results

Patients with progressive neuromuscular weakness

- Prolonged survival (DMD, ALS, SMA)
- Fewer hospitalizations/fewer days in ICU
  - 85% reduction in hosp days compared to the year prior to BiPAP
    - Katz, et al, Arch Dis Child, 2004
- Improved measures of respiratory function by sleep study, ABGs
- Improved quality of life

Cardiomyopathy (Heart Disease) in Dystroglycanopathy

- The heart muscle can also become weak
  - 60% in one series of 23 LGMD2I patients

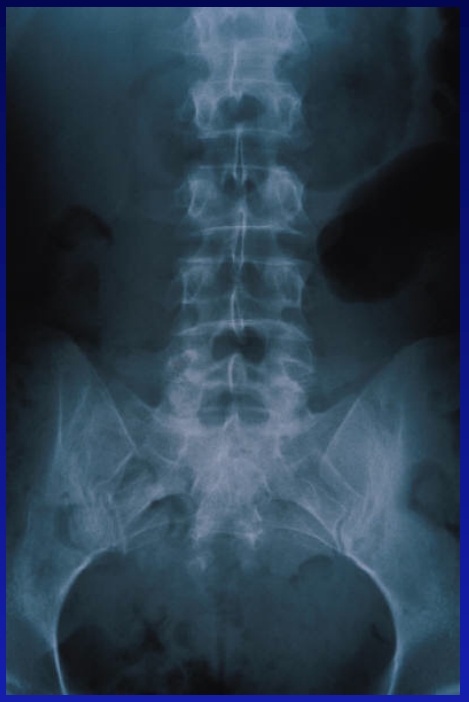


- Limited data is available
  - Highly variable, even within families
  - In patients with FKRP mutations, no apparent relationship between skeletal muscle weakness and cardiomyopathy
    - Cardiomyopathy can occur before weakness
  - Cardiomyopathy generally affects adults

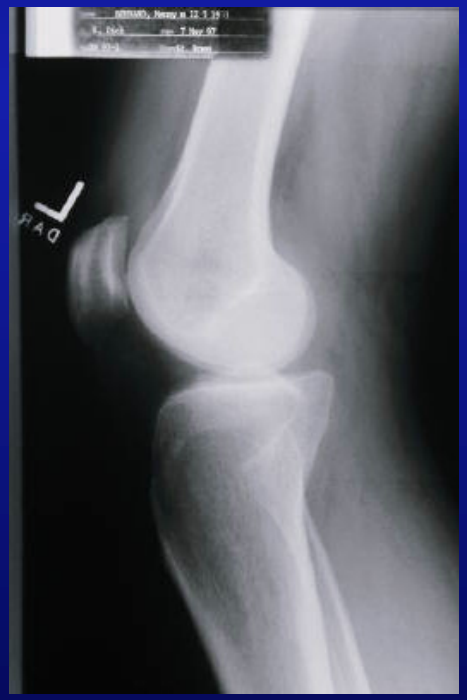
Cardiomyopathy Management

- Monitoring
  - Echocardiogram every 1-2 years and if symptoms
- Consider prevention treatment (Enalapril, Losartan)
  - No data
  - Discuss pros/cons with cardiologist

Osteoporosis

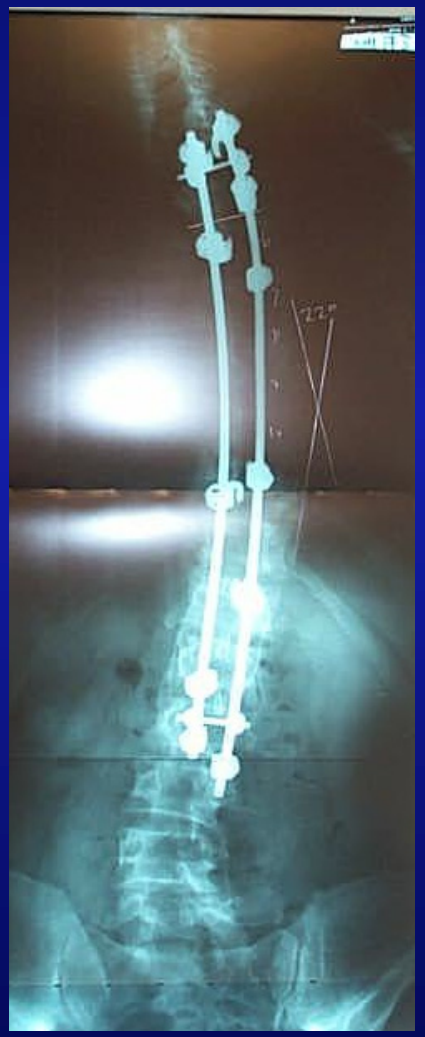


- Risk factors
  - Abnormal forces on bone
  - Non weight bearing
  - Medications?
  - Lack of sun exposure
- Result: Frequent fractures
  - Fracture → ?permanent loss of walking
- Monitoring: DEXAs
- Treatment
  - Calcium, Vitamin D
  - Prolong walking, weight bearing
  - Fosamax or other bisphosphanate



Scoliosis

- ~1-2 years after wheelchair in growing children
- Reasons for surgery
  - Cosmetic
  - Relief of pain
  - Improved respiratory function late in disease
- Against surgery
  - Major operation; pain, complications
  - Trouble eating
  - Trouble fitting into van

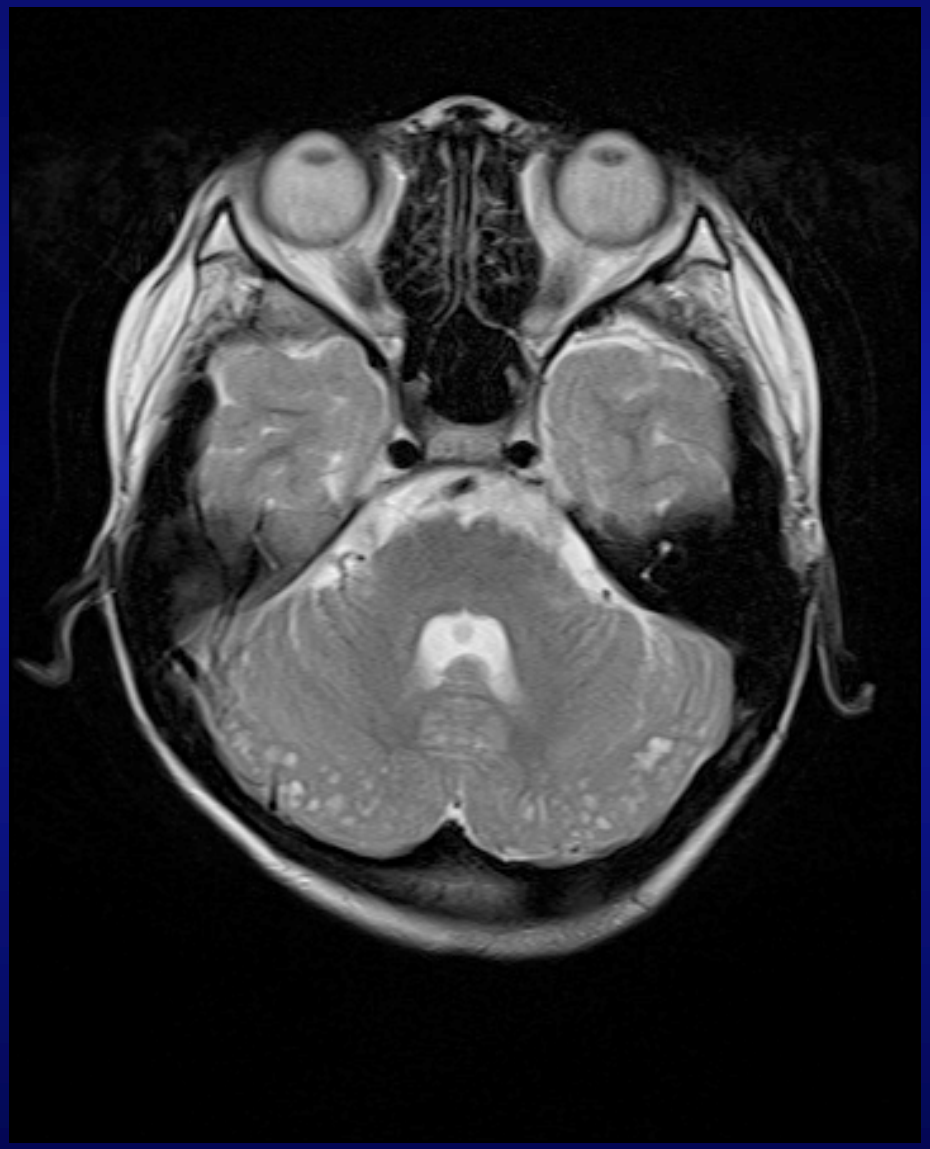


Abnormal Eye Development in DGs

- Reported only in patients with congenital muscular dystrophies
- Wide range of abnormalities
  - Microphthalmia (abnormally small eyes), cataracts, glaucoma, severe myopia, and others
- Everyone with CMD (vs LGMD) should have eye exam

Brain in Dystroglycanopathies

- (Dr. Moore will discuss animal research)
- DG plays a role in brain development
- With some abnormalities of DG, brain structure and/or function are affected



Brain in Dystroglycanopathies

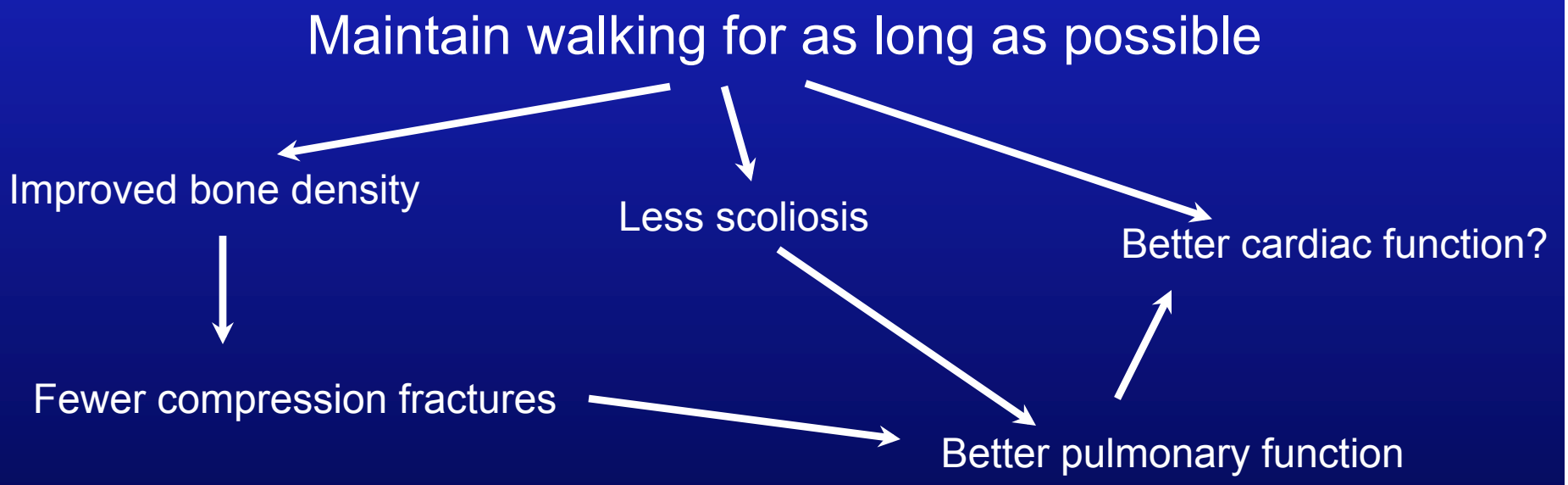
- Cognitive impairment ranging from mild to severe
- Seizures
  - More likely in those with cognitive impairment
- LGMD 2I
  - Normal intelligence
  - Small study suggested mild deficits in planning and organization
    - Needs to be repeated with more patients

Many Other Aspects of Management!

- School modifications
- Emotional adjustments
  - Patient
  - Parents
- Home modifications
- Financial and insurance issues
- Transitions to independent adulthood

Management of Dystroglycanopathy is Multifaceted

- Personalize the management team for each patient
- Optimal treatment of each system can affect outcome in other systems.



Muscle Pathology in the Dystroglycanopathies

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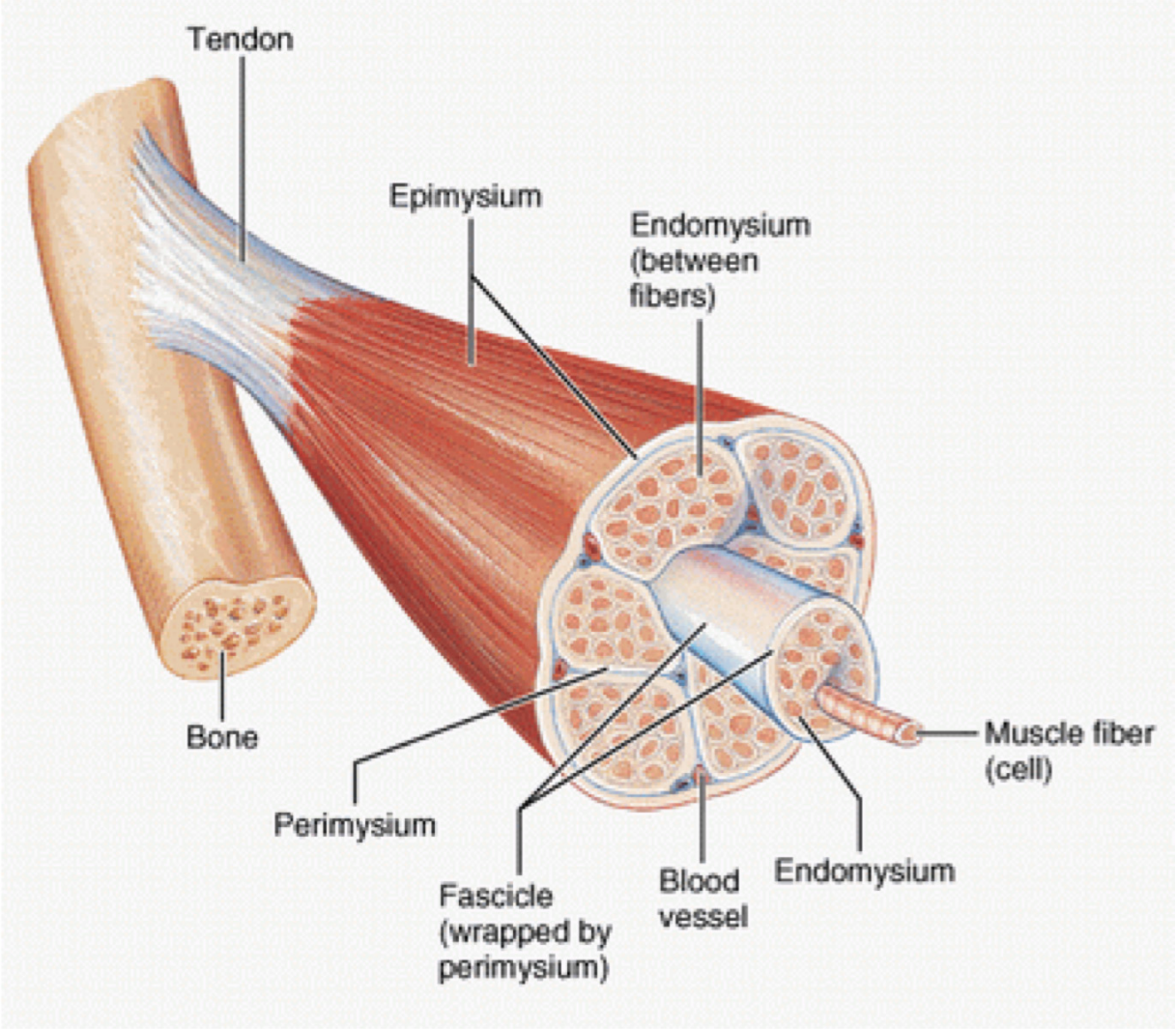
The University of Iowa
Department of Pathology
and
Wellstone Muscular Dystrophy Cooperative
Research Center

Welcome
to Iowa!

Dystroglycanopathy Muscle Pathology – the basics

- Skeletal muscle structure
- Muscle biopsy evaluation
- What looks different in muscular dystrophy
- How to distinguish dystroglycanopathy from similar muscular dystrophies

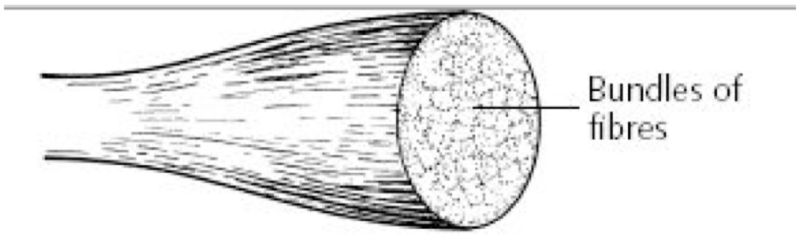
muscle structure



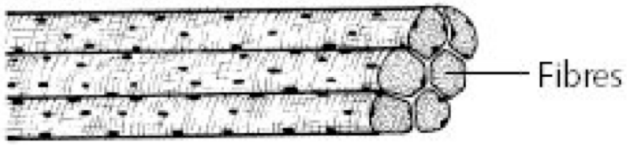
http://cyhsanatomy1.wikispaces.com/file/detail/Skeletal_Muscles-1.gif

muscle structure

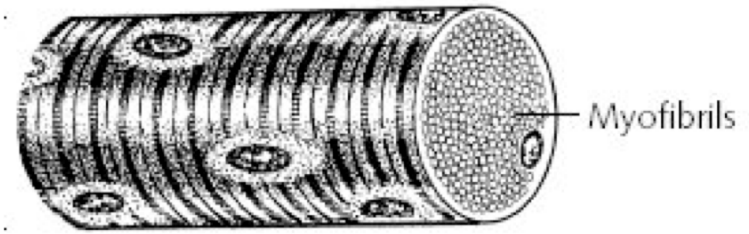
a muscle



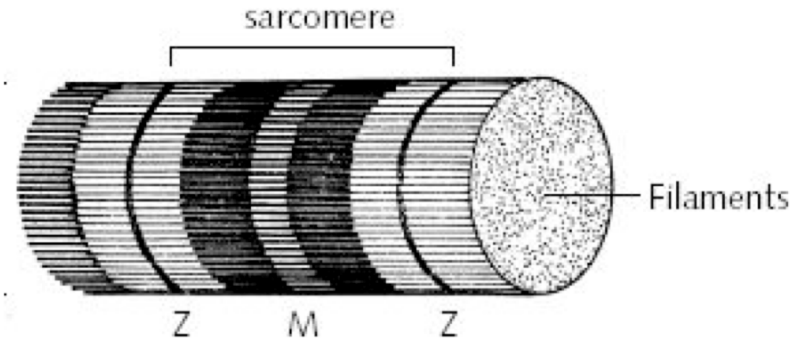
a group of cells



a single cell



contractile proteins inside a single cell



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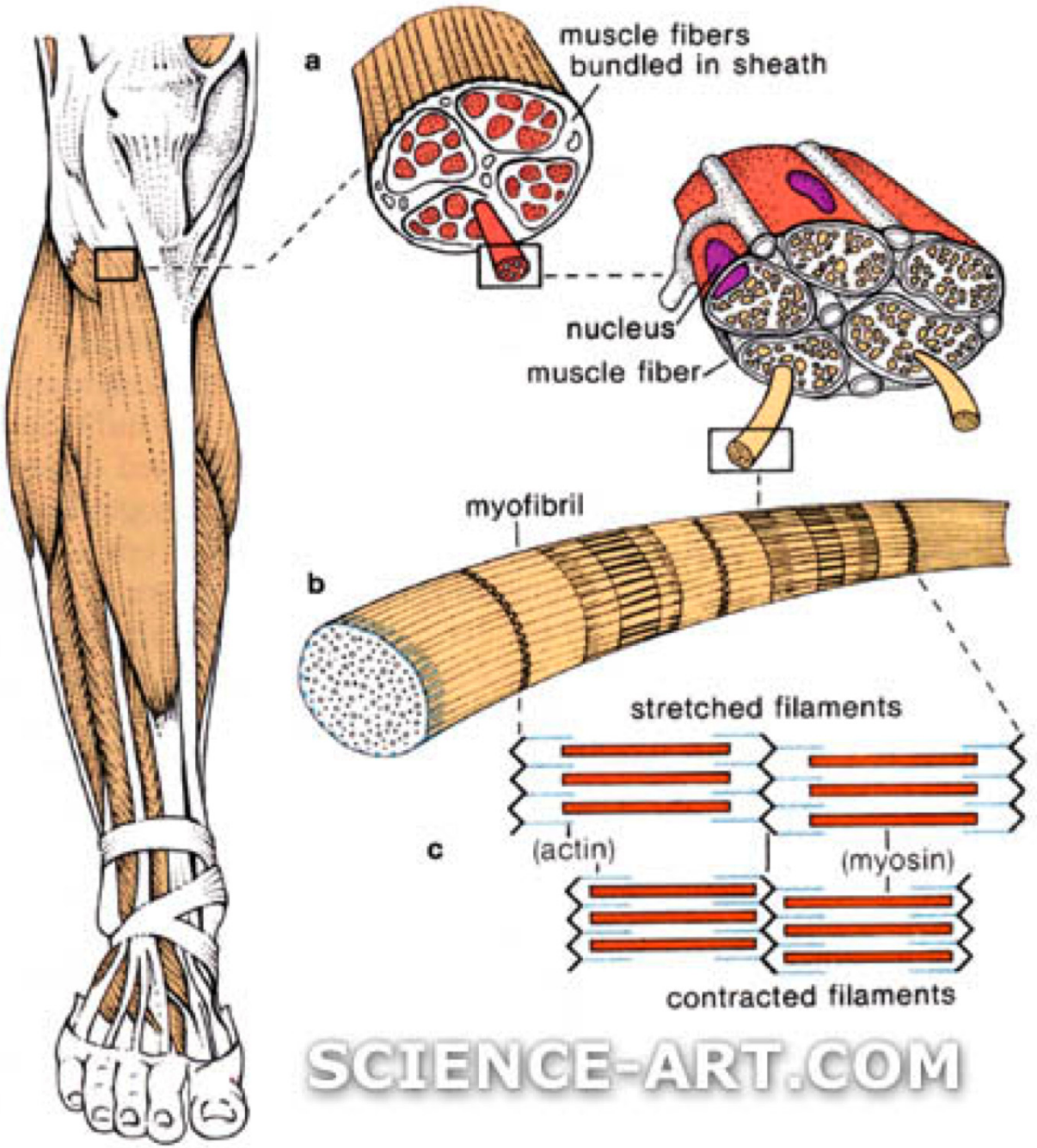
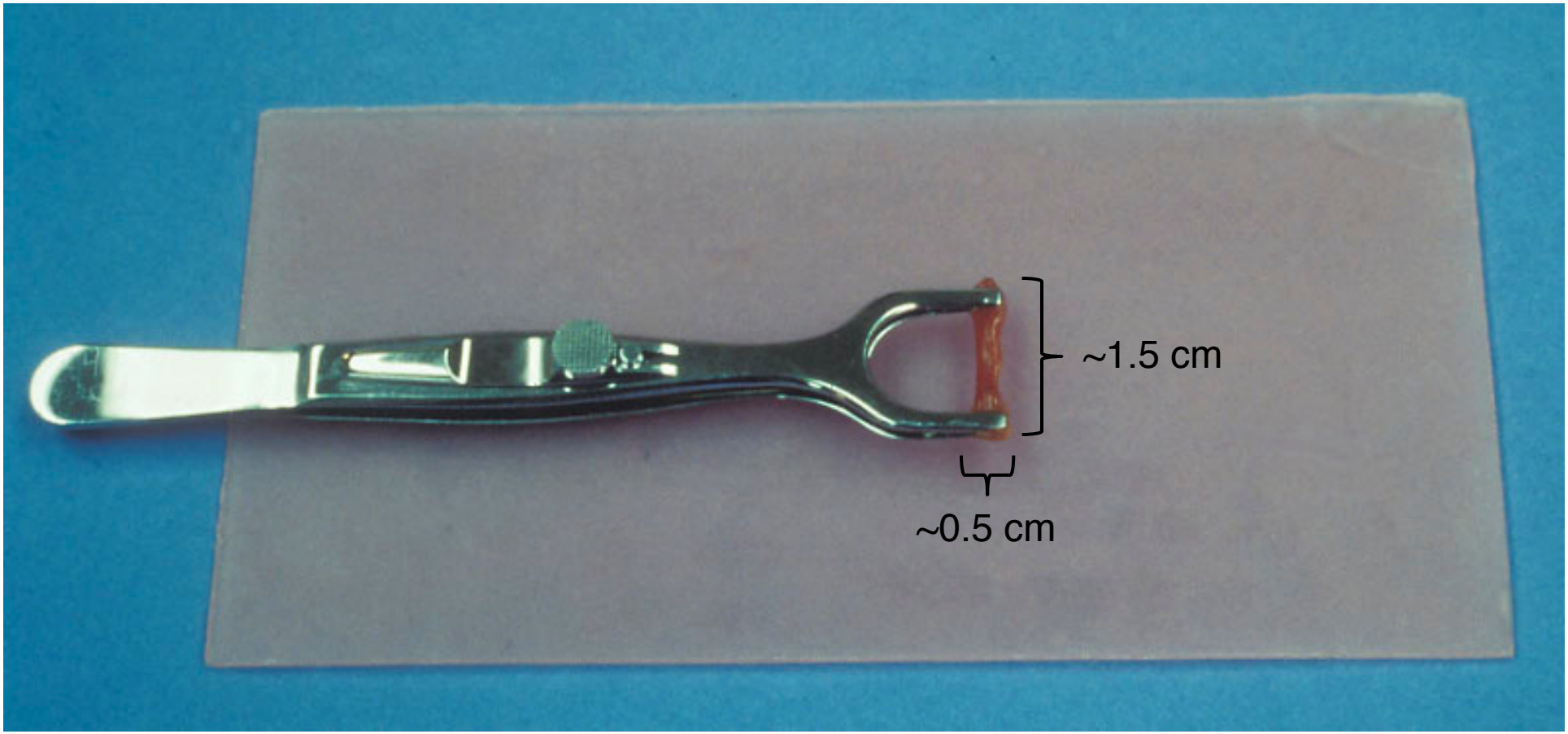
muscle structure

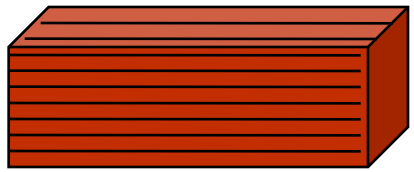


muscle biopsy in a clamp

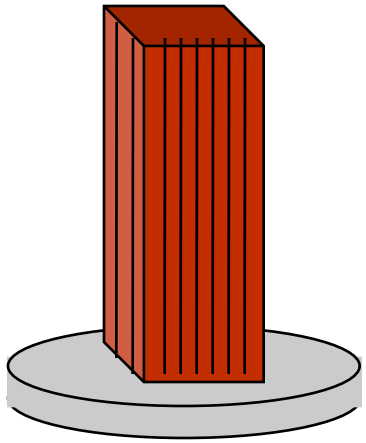


mount muscle on cork for cross sections

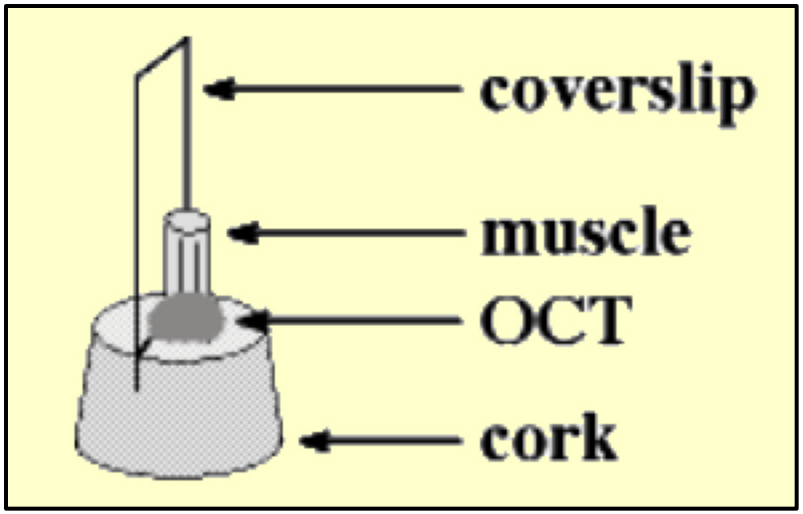
1) cut biopsy from clamp



2) mount on cork
3) freeze in isopentane

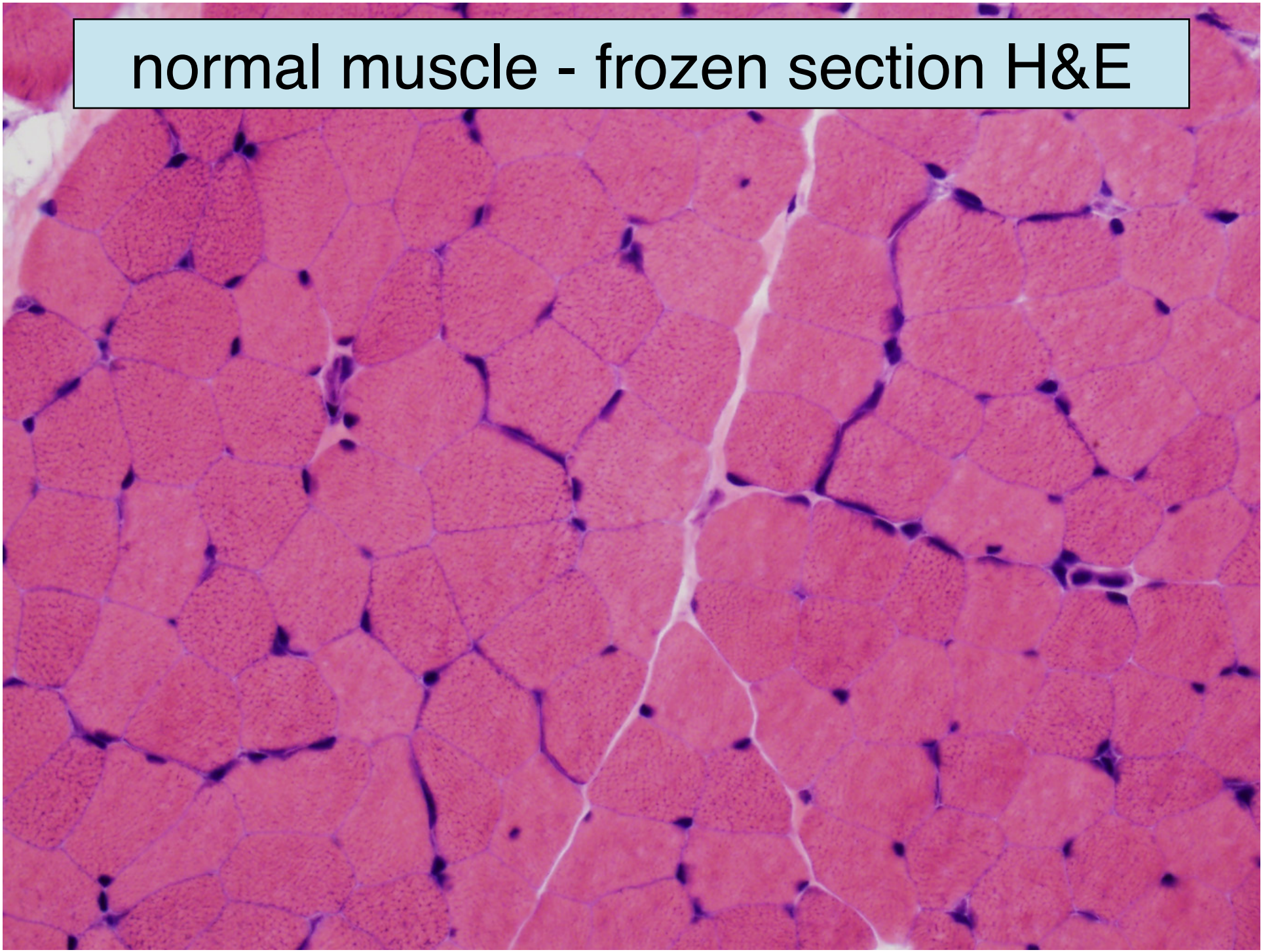


~1.0 cm



freeze muscle in **very** cold isopentane

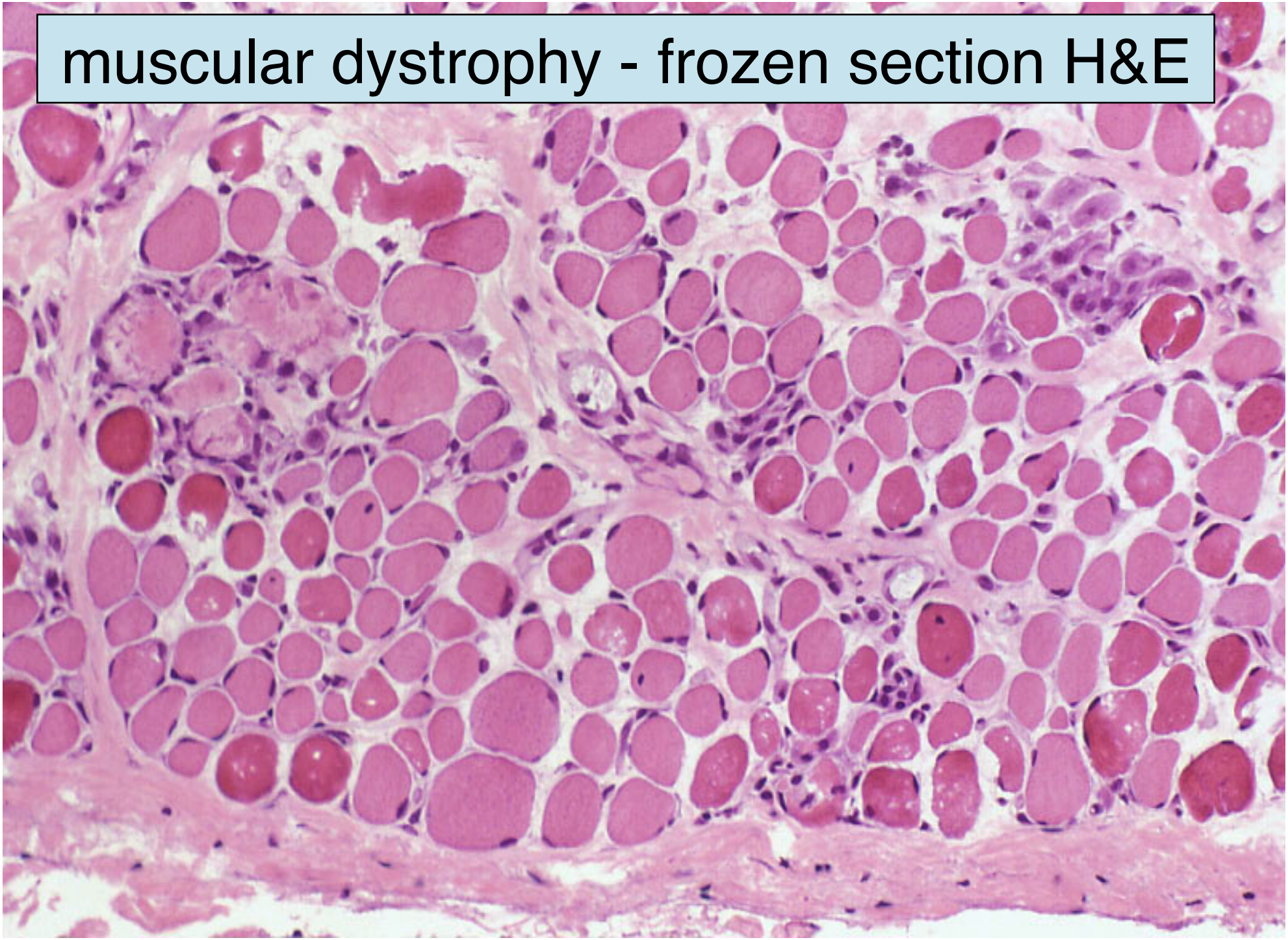
Cool isopentane inside a metal cup by suspending the cup in liquid nitrogen. The optimum freezing temperature is about -160^{o}C.

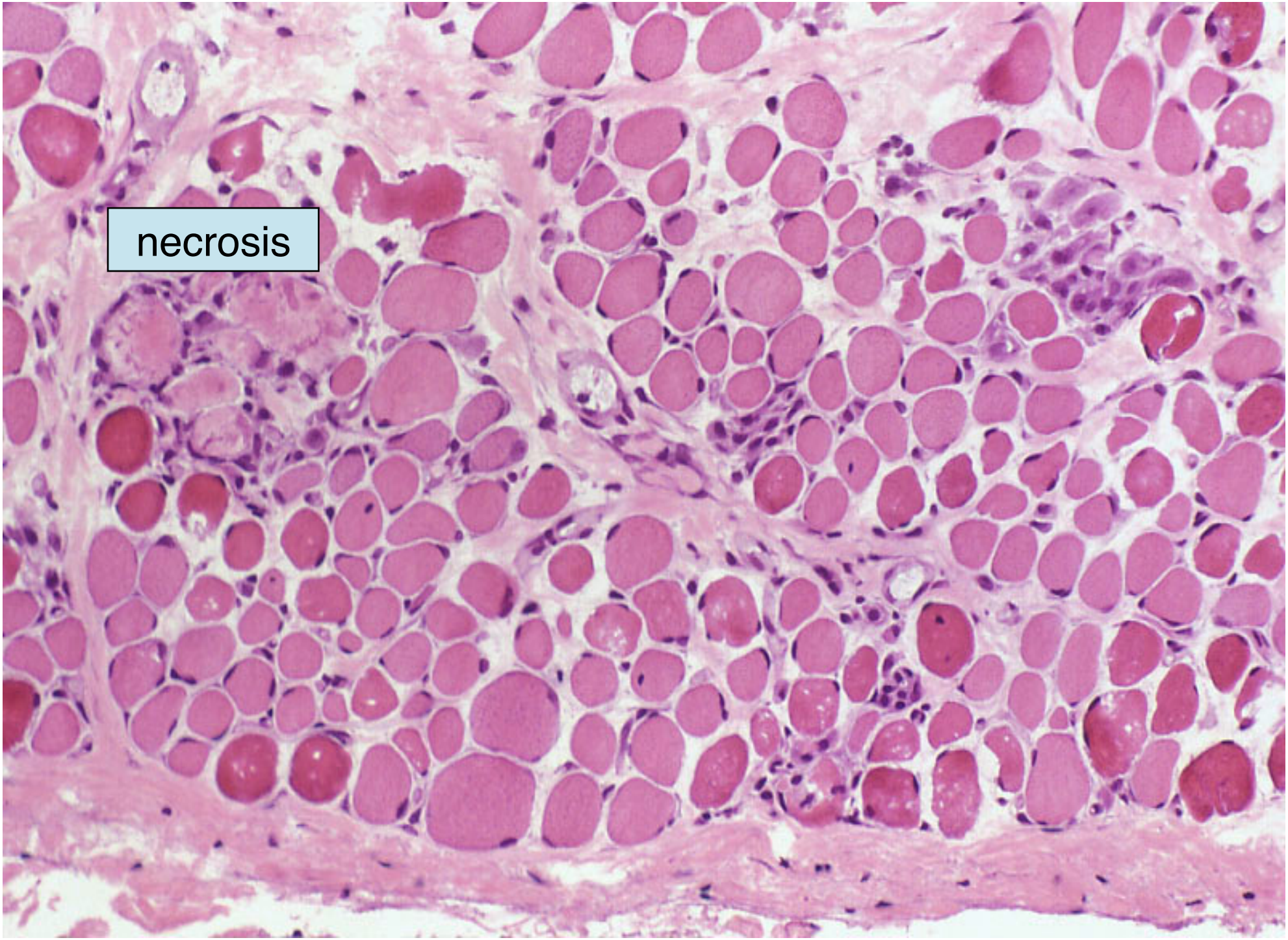

normal muscle - frozen section H&E

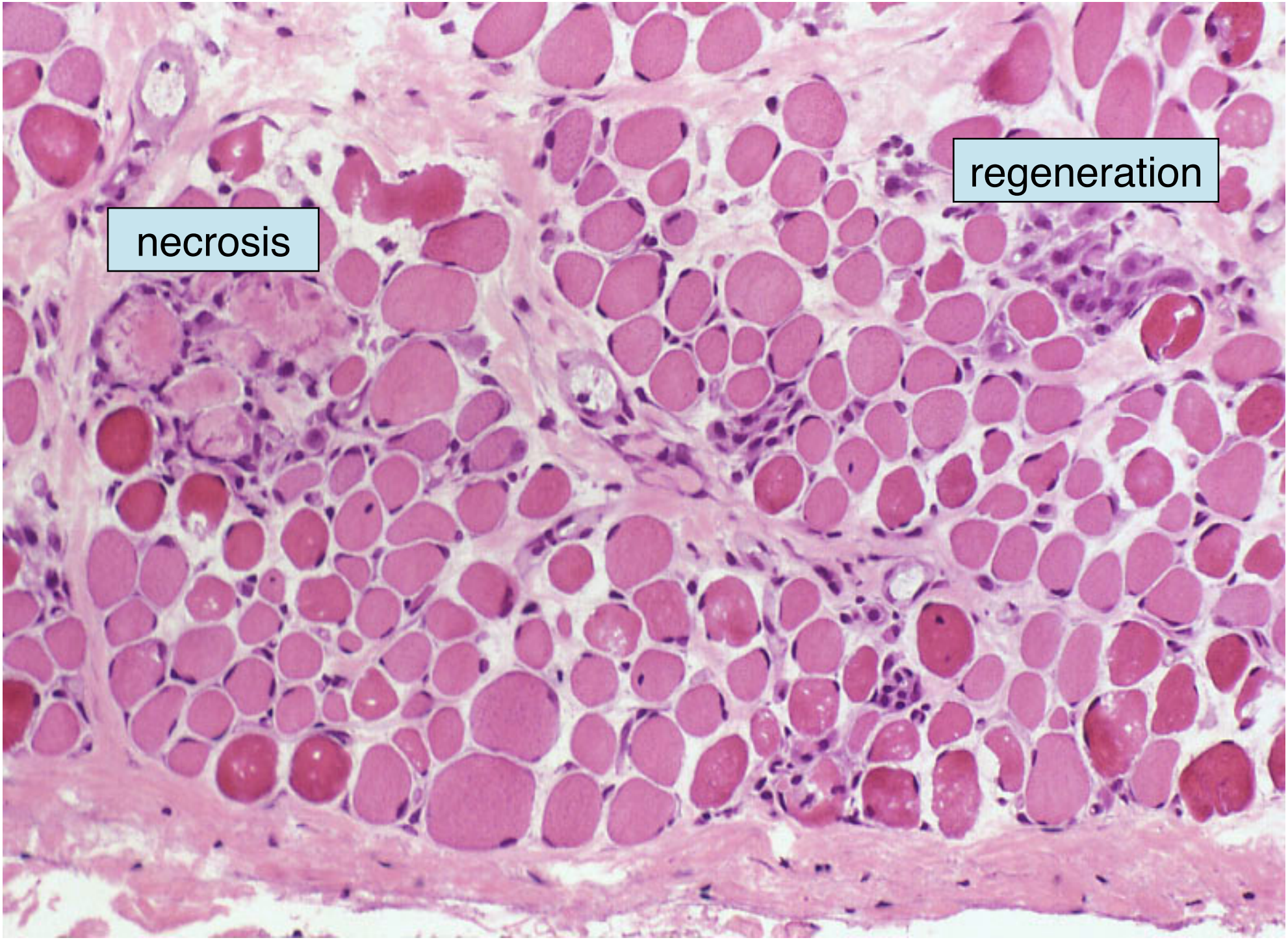
What is muscular dystrophy?

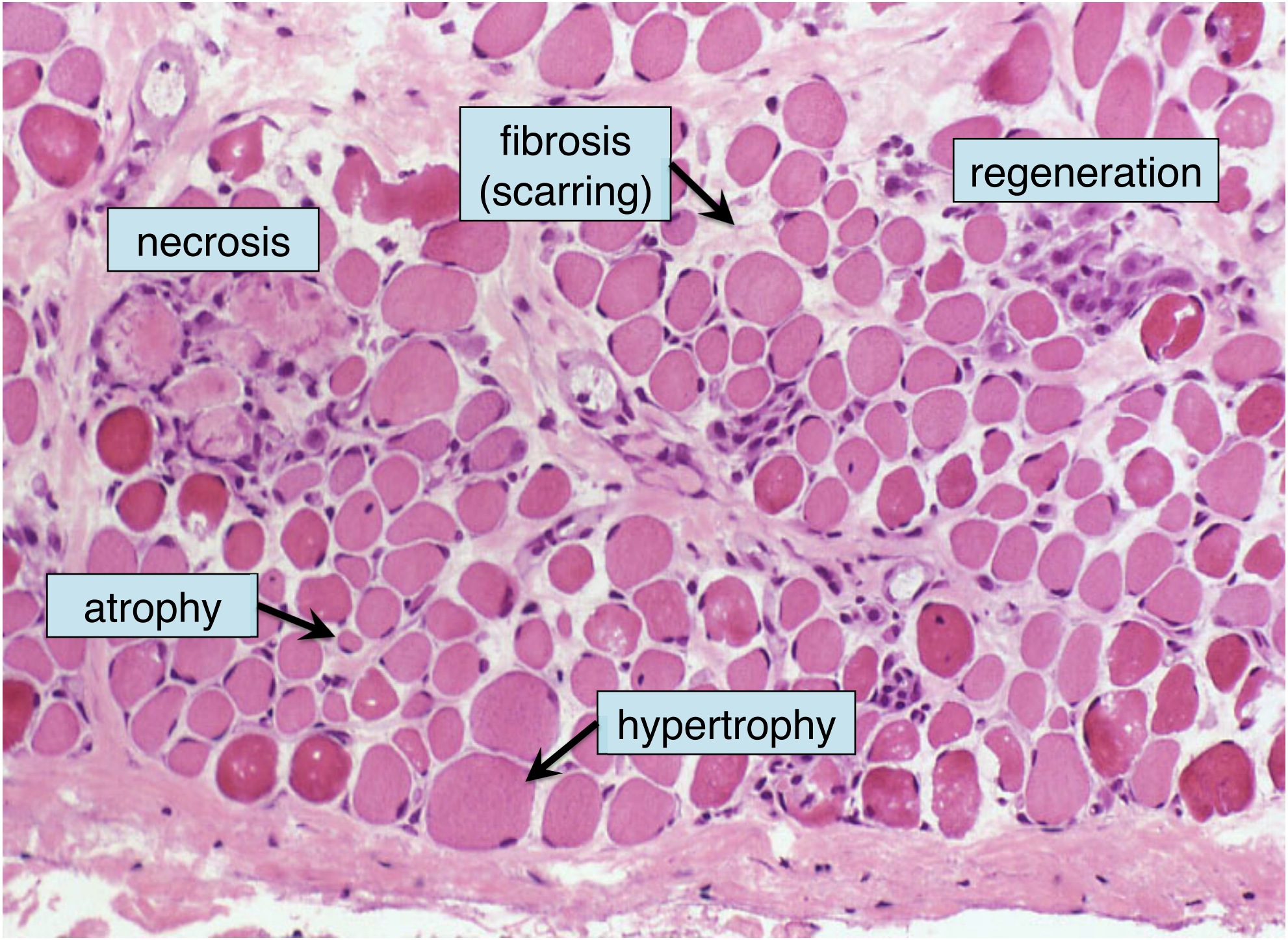
- inherited muscle disorder characterized by repeated cycles of muscle degeneration (necrosis) and regeneration
- patients present with weakness and a wide variety of other signs and symptoms
- classified by patterns of inheritance, distribution of muscle involvement, age of onset, the abnormal gene (protein)

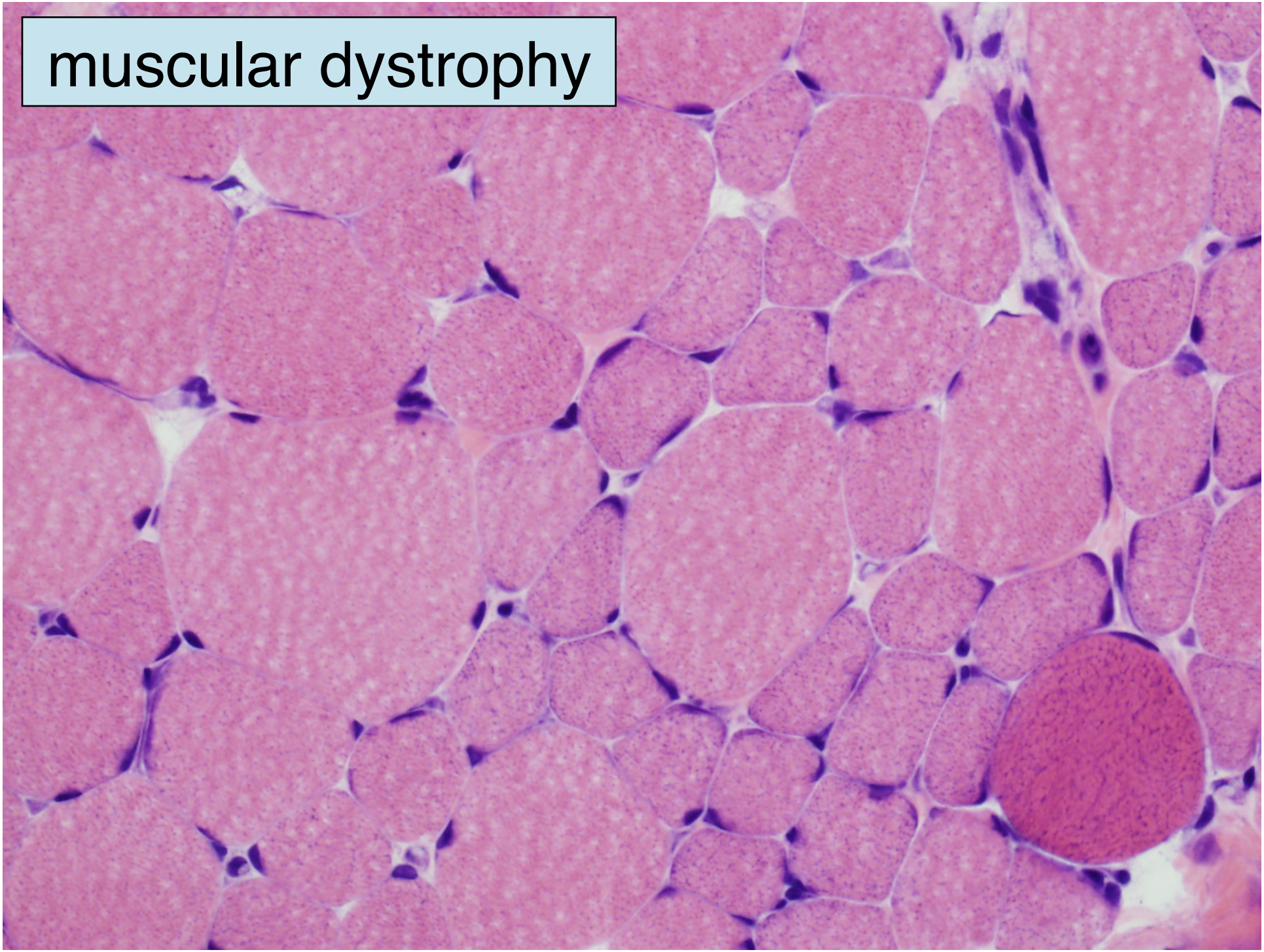
muscular dystrophy - frozen section H&E

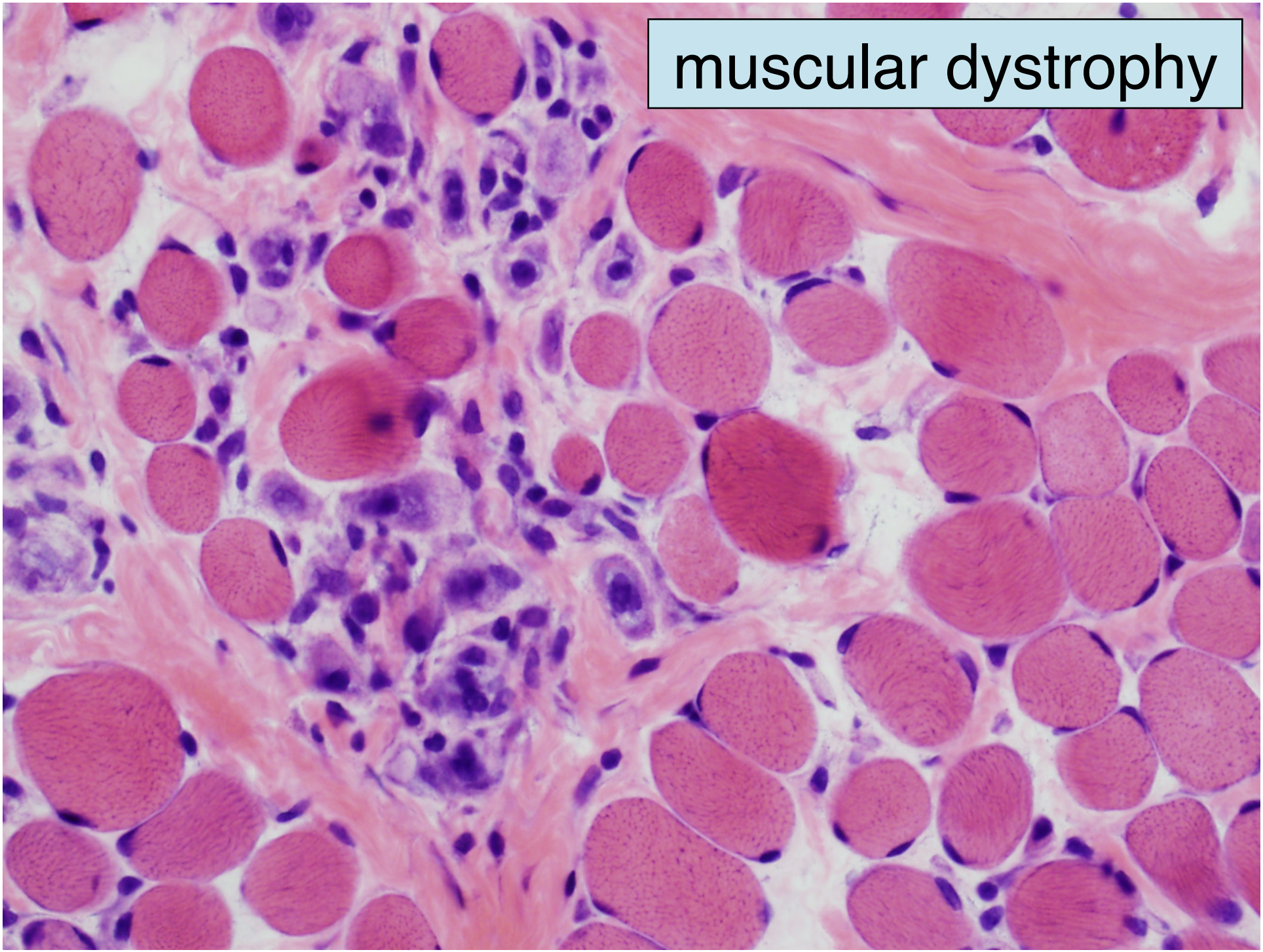


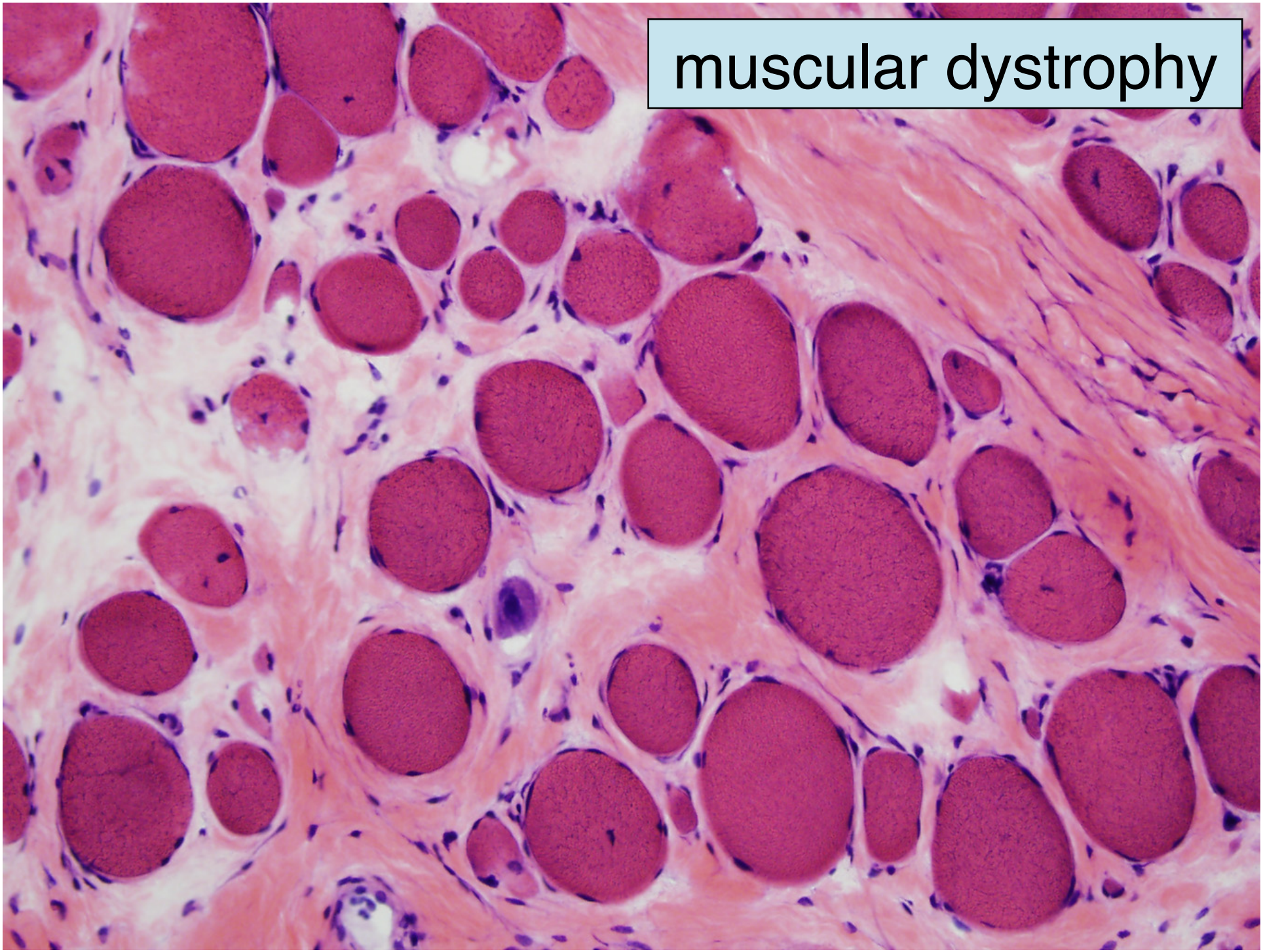

necrosis

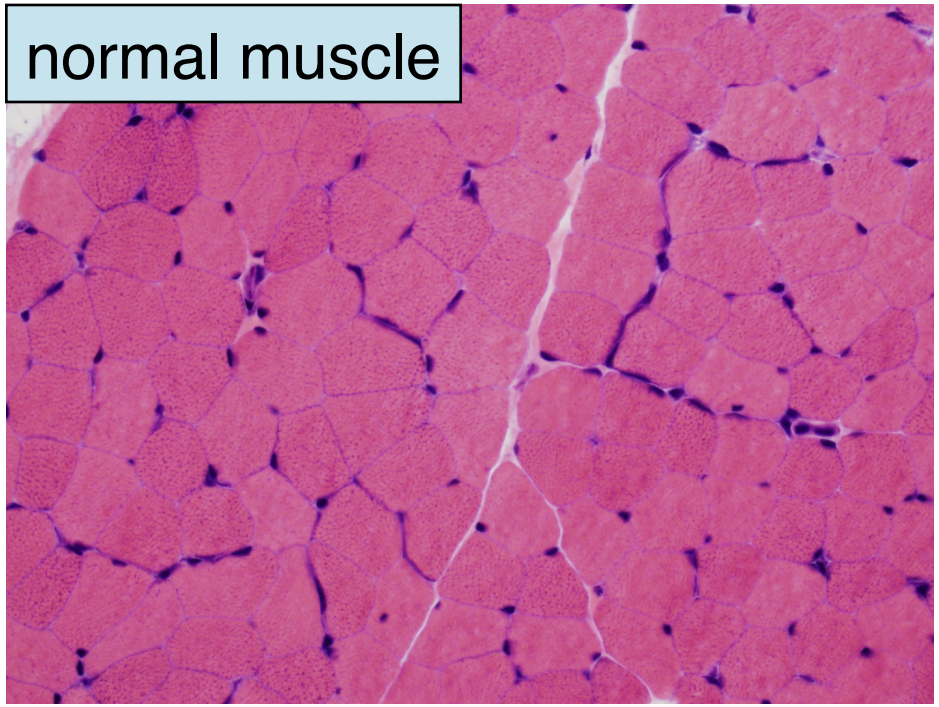

necrosis
regeneration

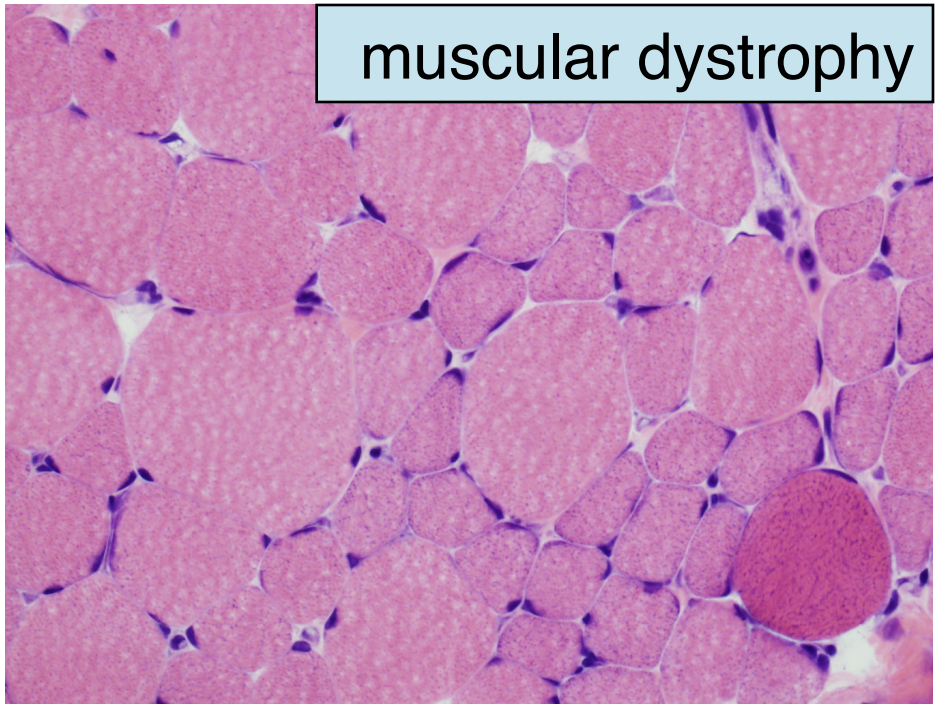

fibrosis
(scarring)
regeneration
necrosis
atrophy
hypertrophy

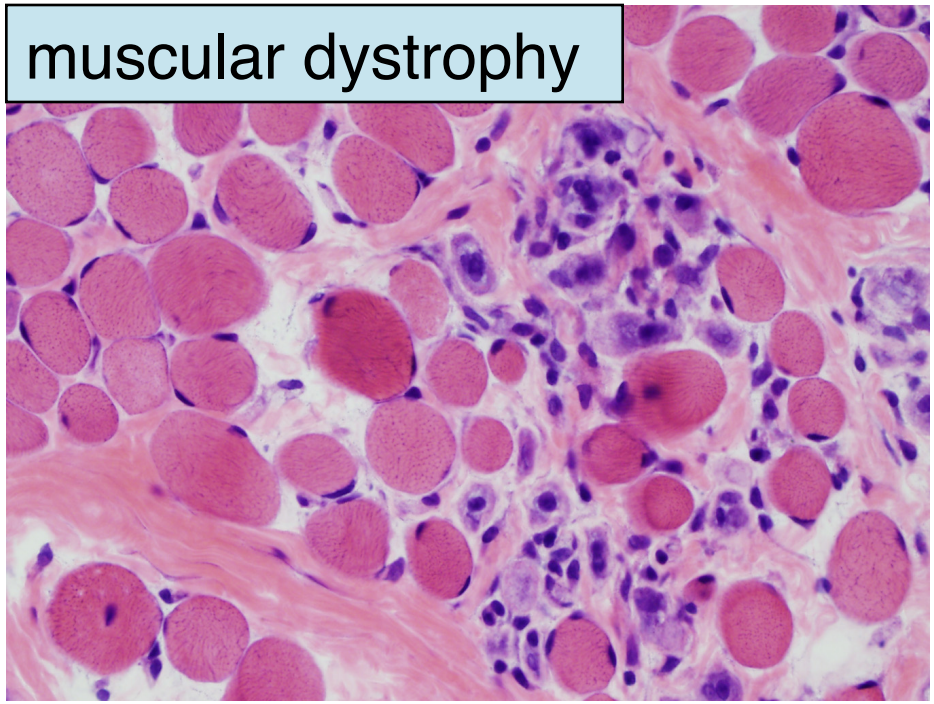

muscular dystrophy

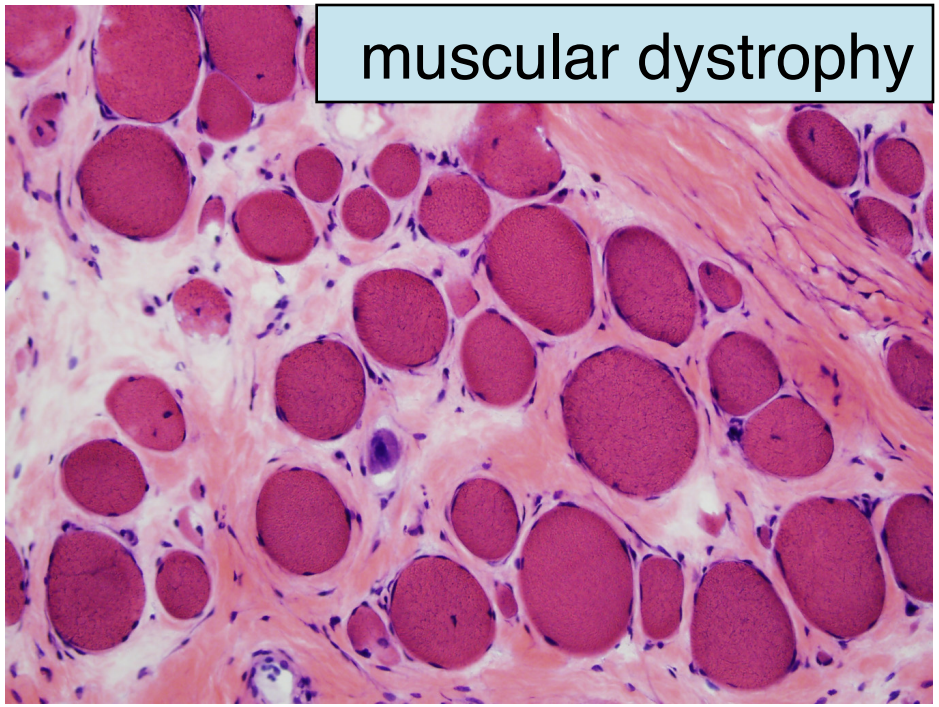

muscular dystrophy

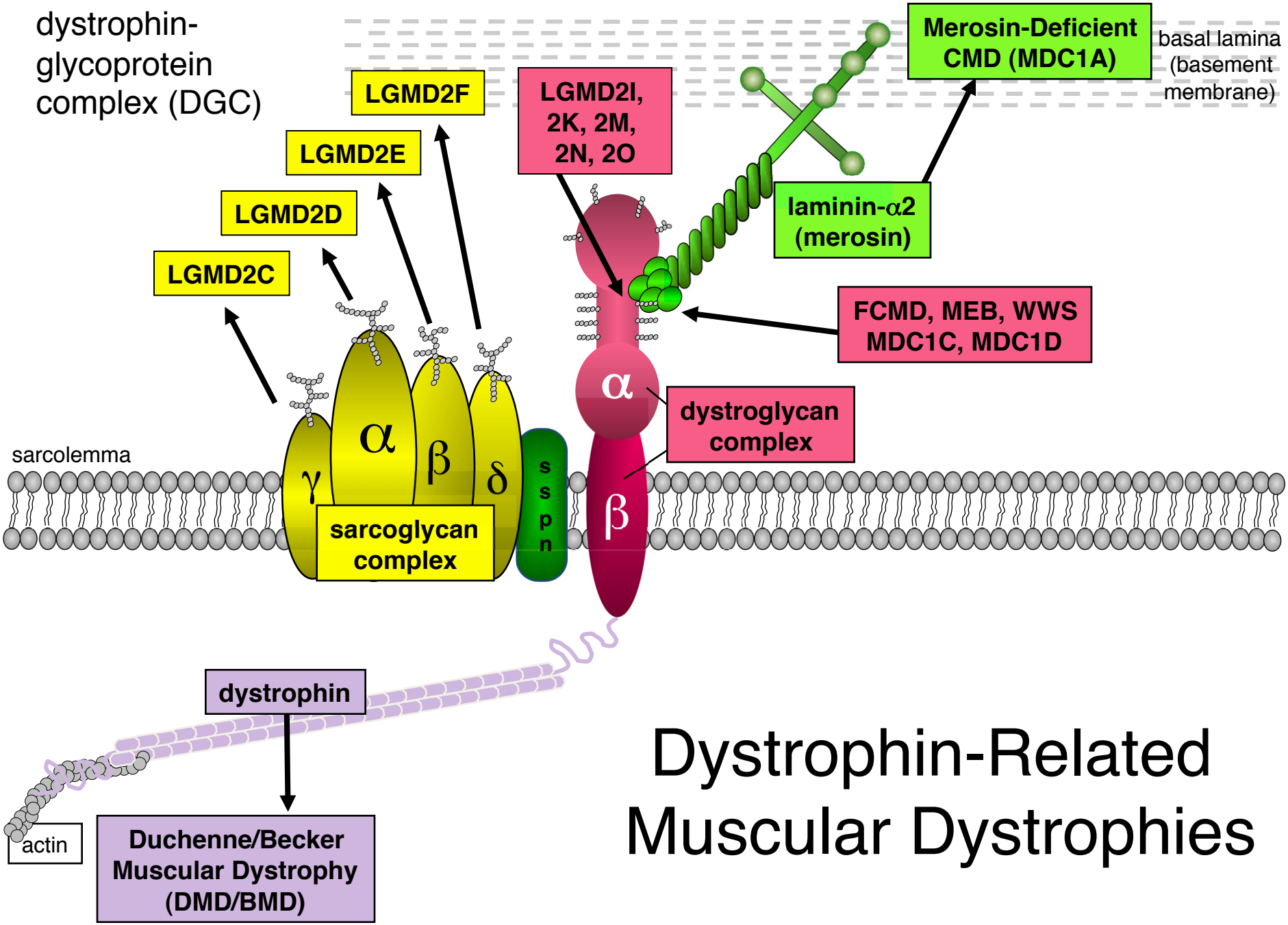

muscular dystrophy


normal muscle


muscular dystrophy


muscular dystrophy


muscular dystrophy



Dystrophin-Related Muscular Dystrophies

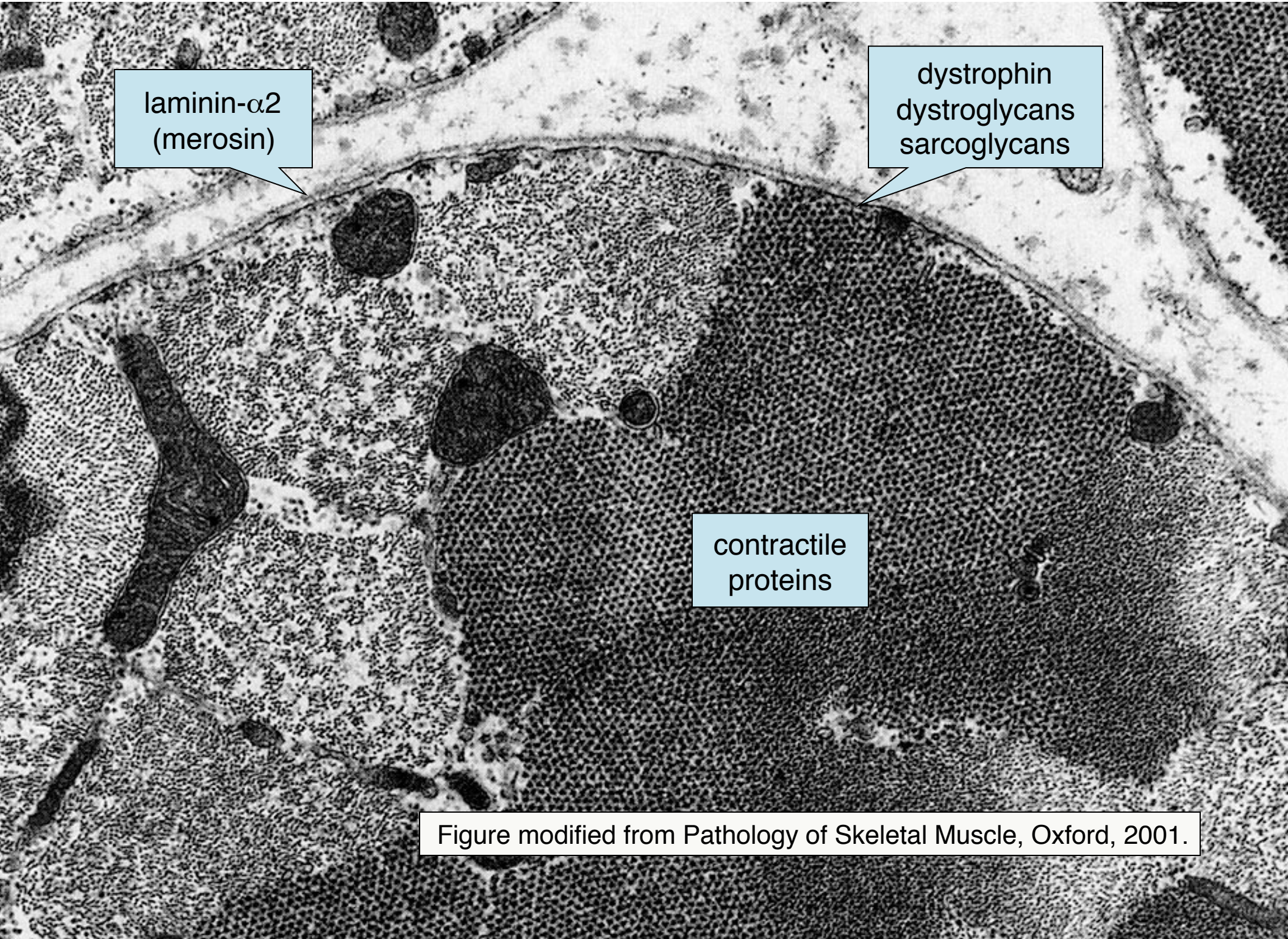
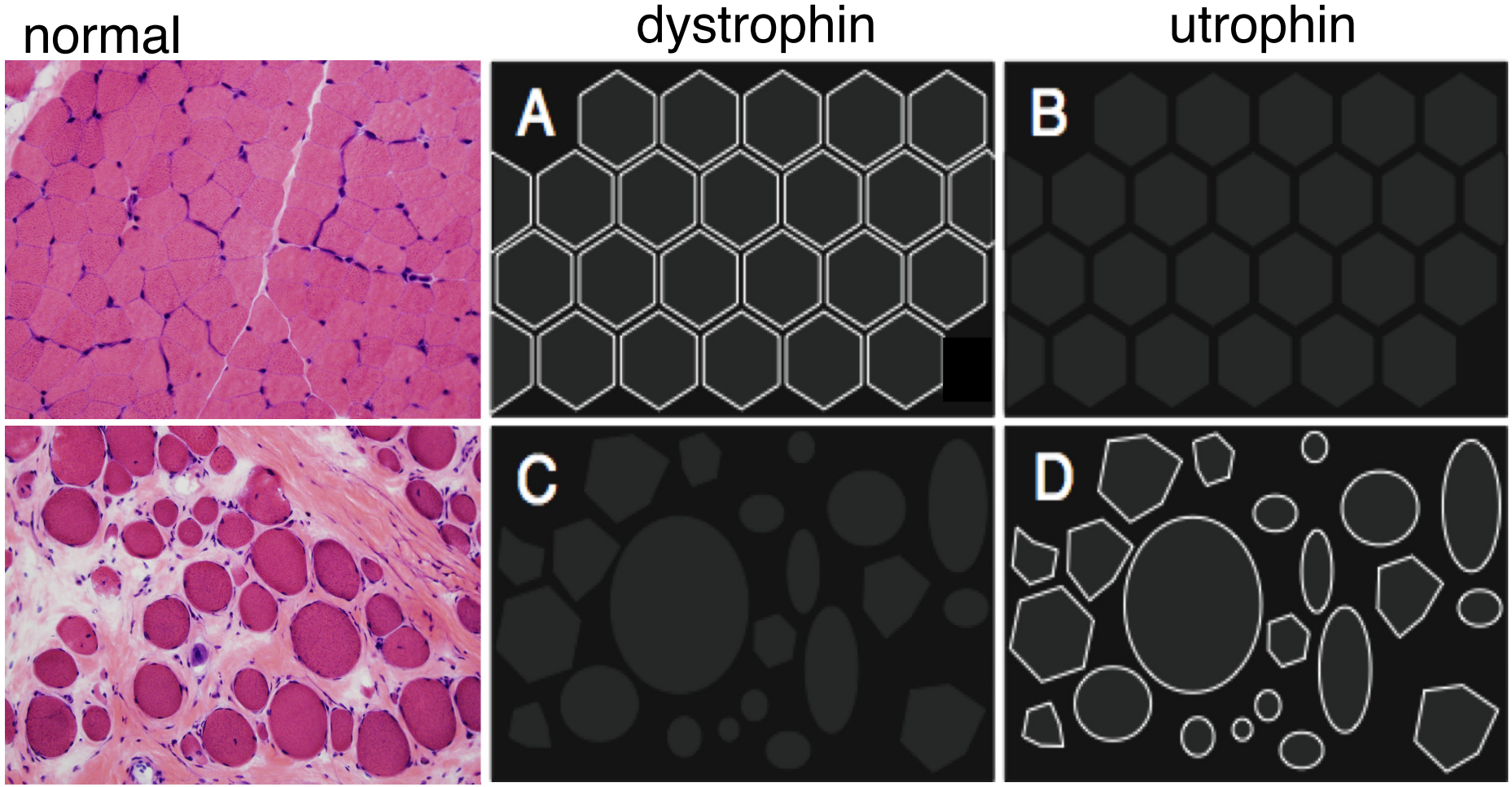
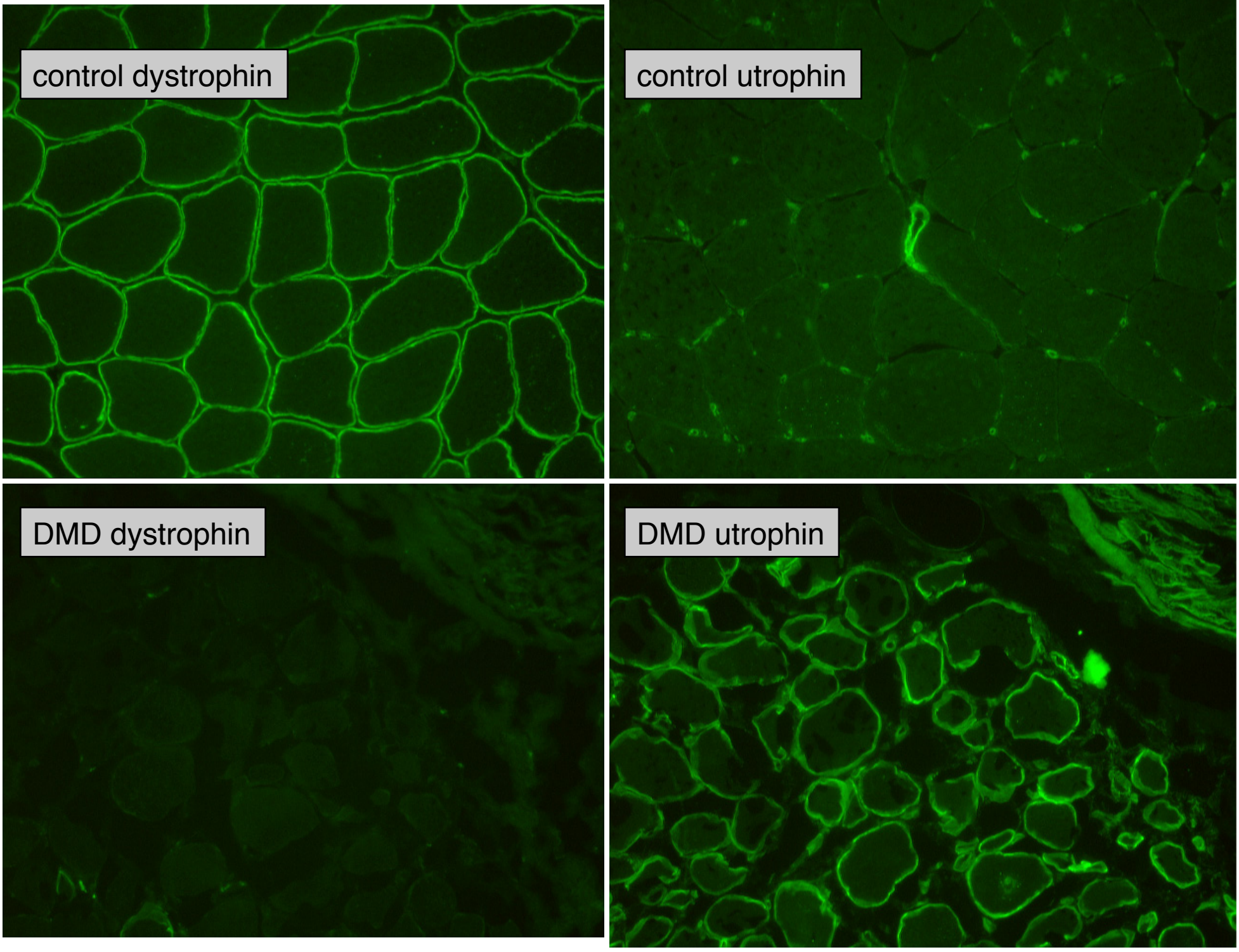


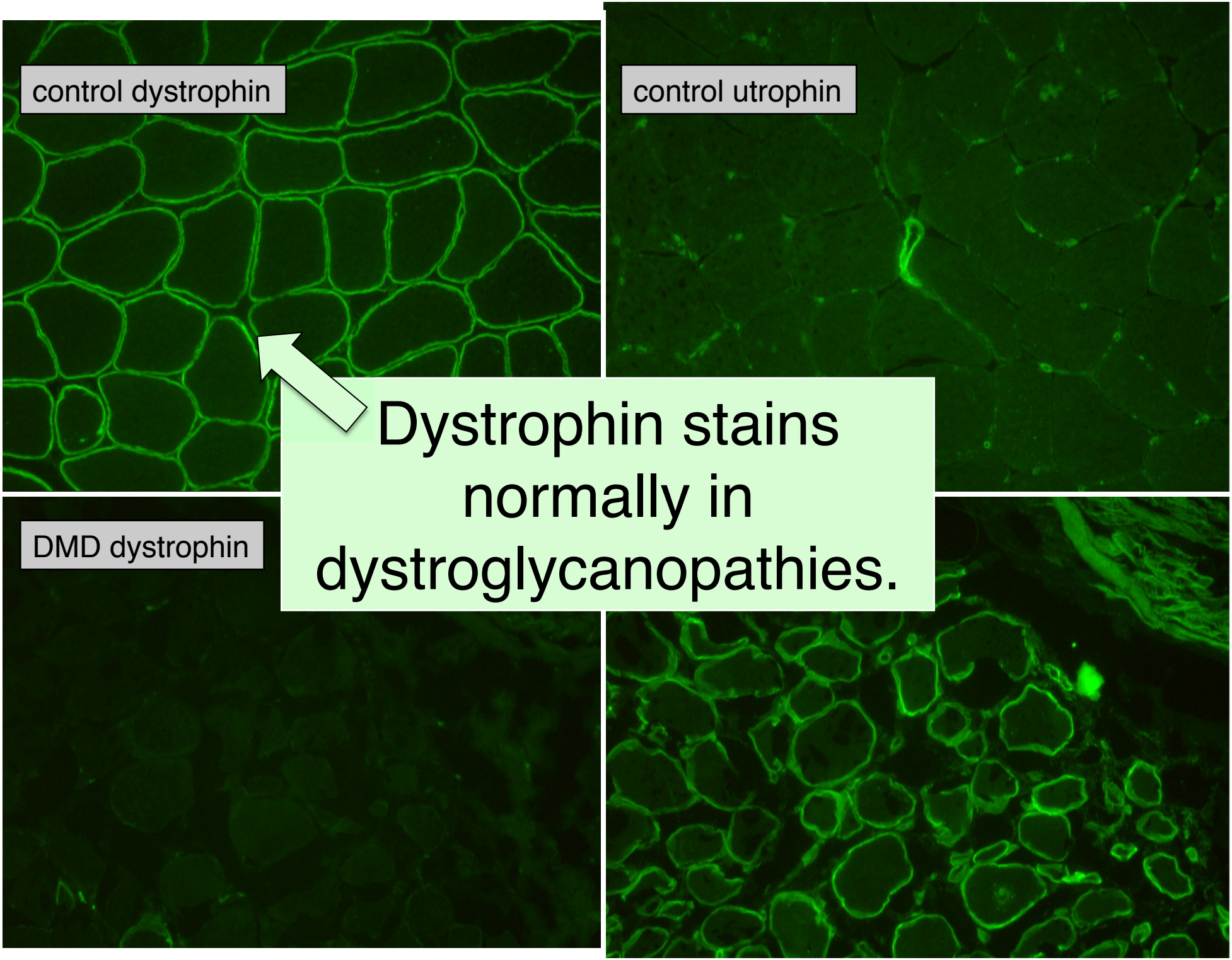
Figure modified from Pathology of Skeletal Muscle, Oxford, 2001.

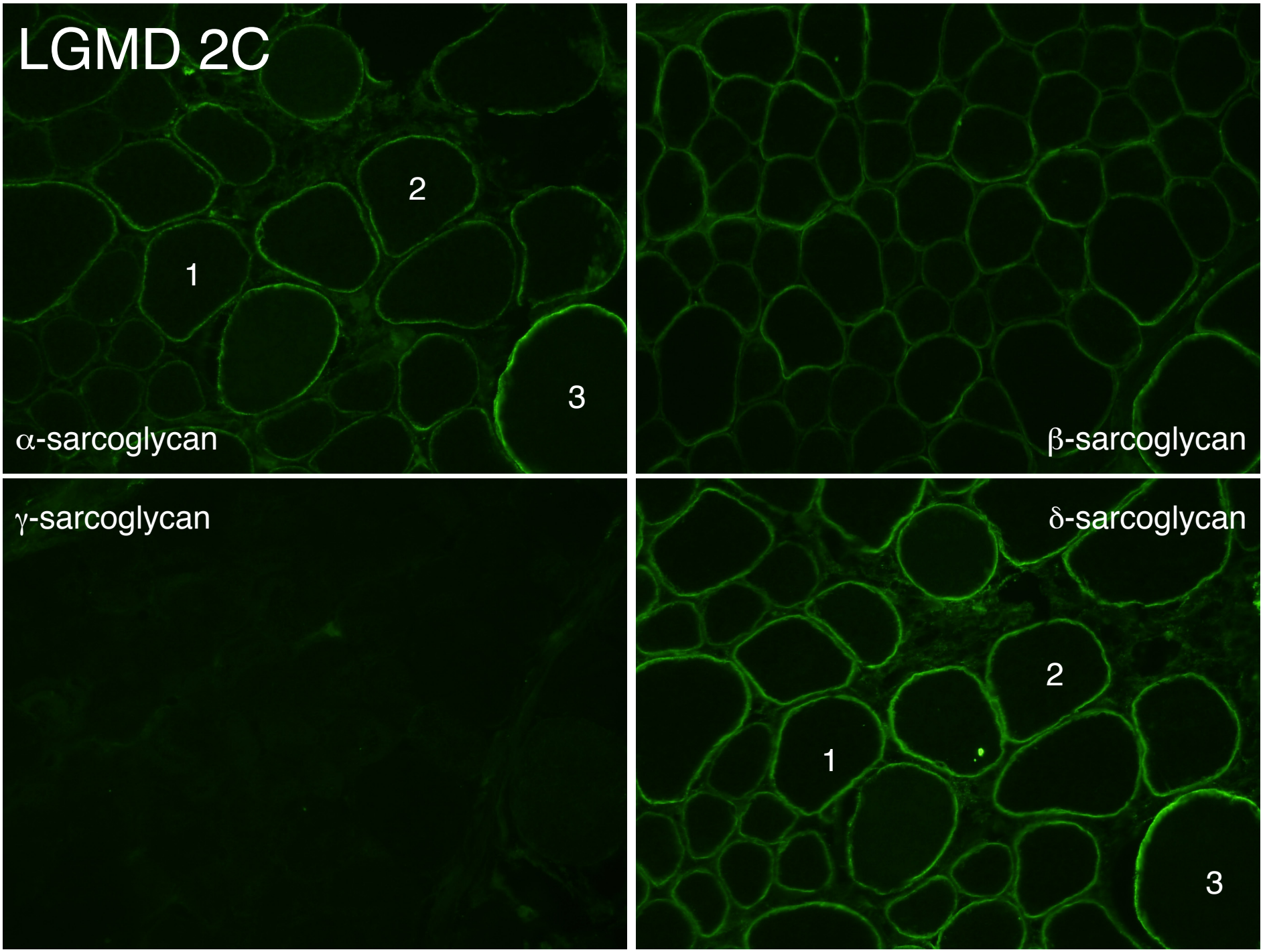
immunostaining for the diagnosis of muscular dystrophy

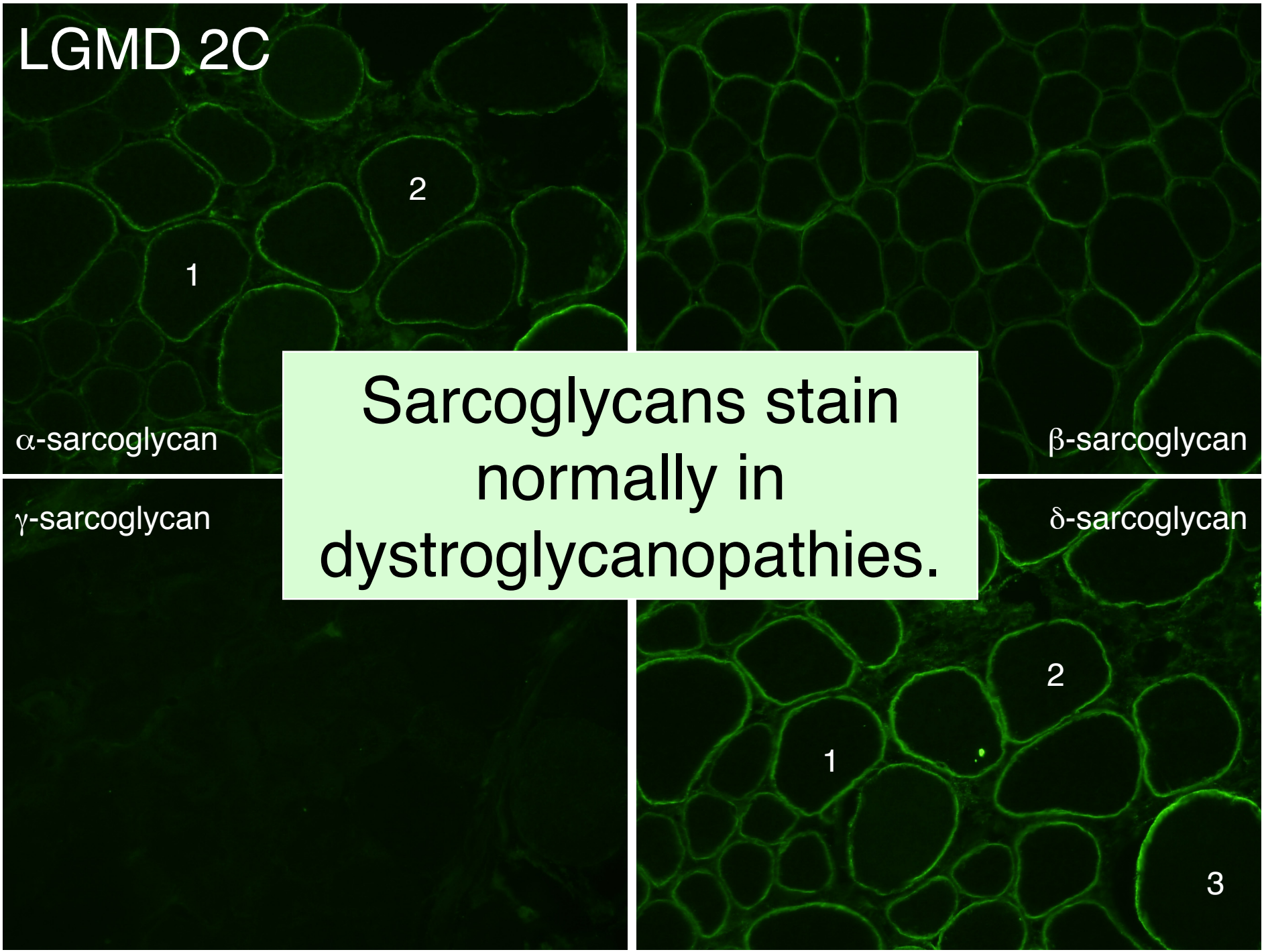


Duchenne muscular dystrophy (DMD)


control dystrophin
control utrophin
DMD dystrophin
DMD utrophin

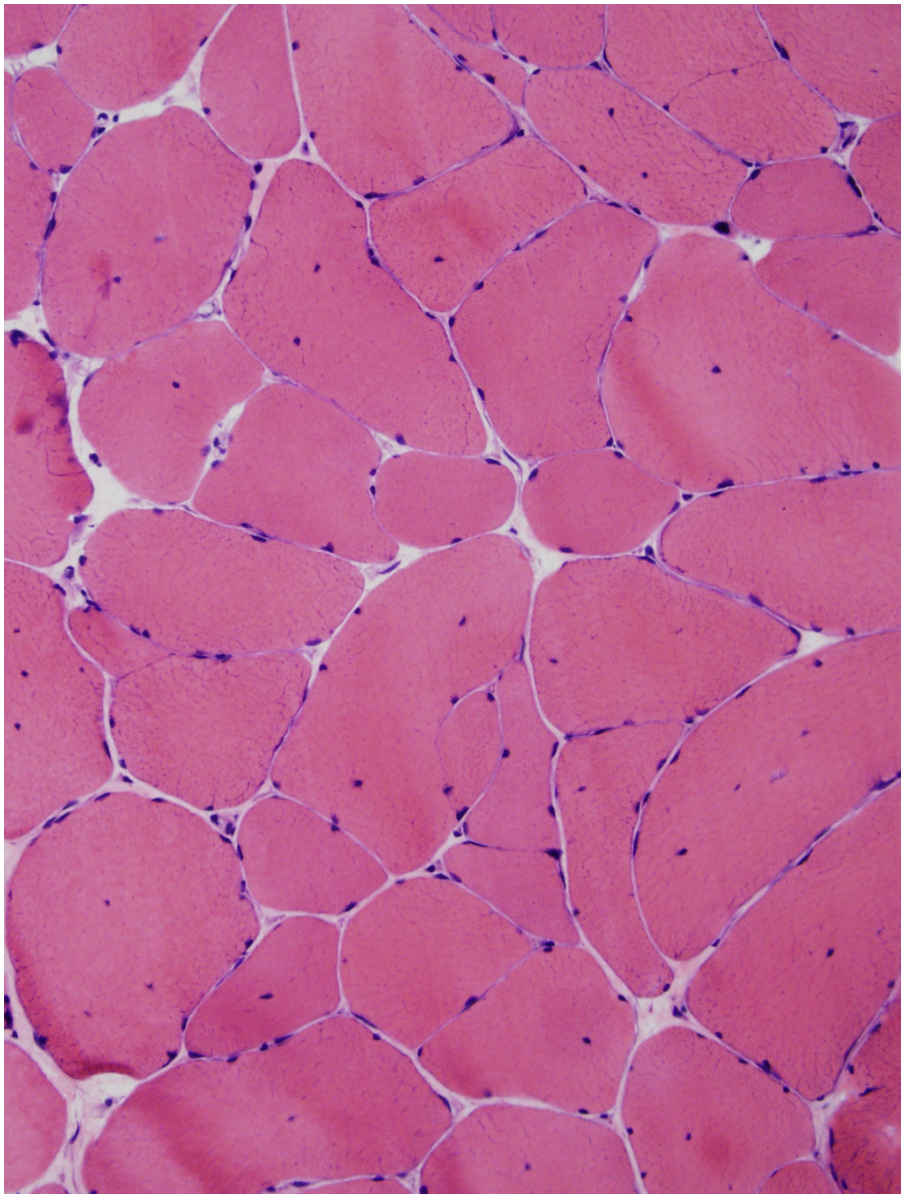

control dystrophin
control utrophin
Dystrophin stains normally in dystroglycanopathies.
DMD dystrophin

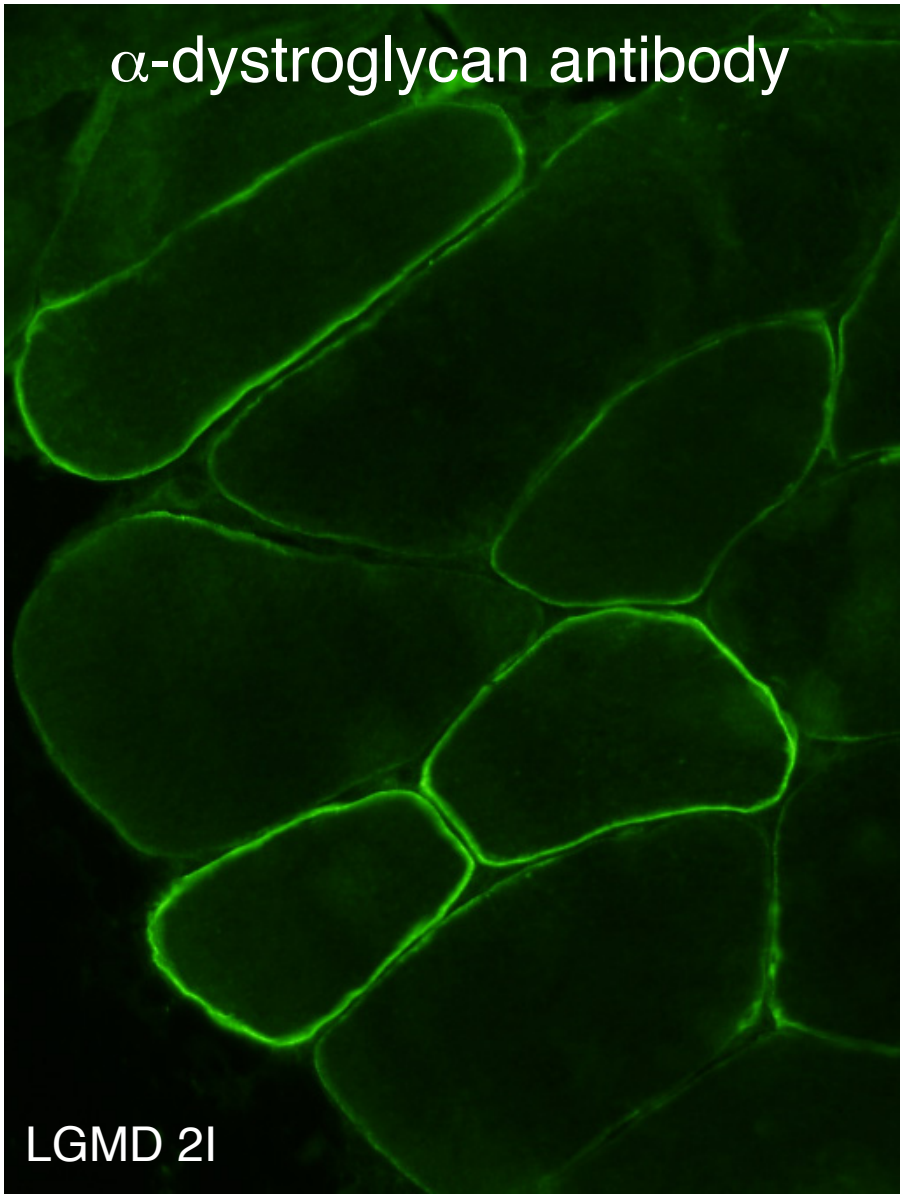

LGMD 2C
1
2
3
α-sarcoglycan
β-sarcoglycan
γ-sarcoglycan
δ-sarcoglycan
1
2
3



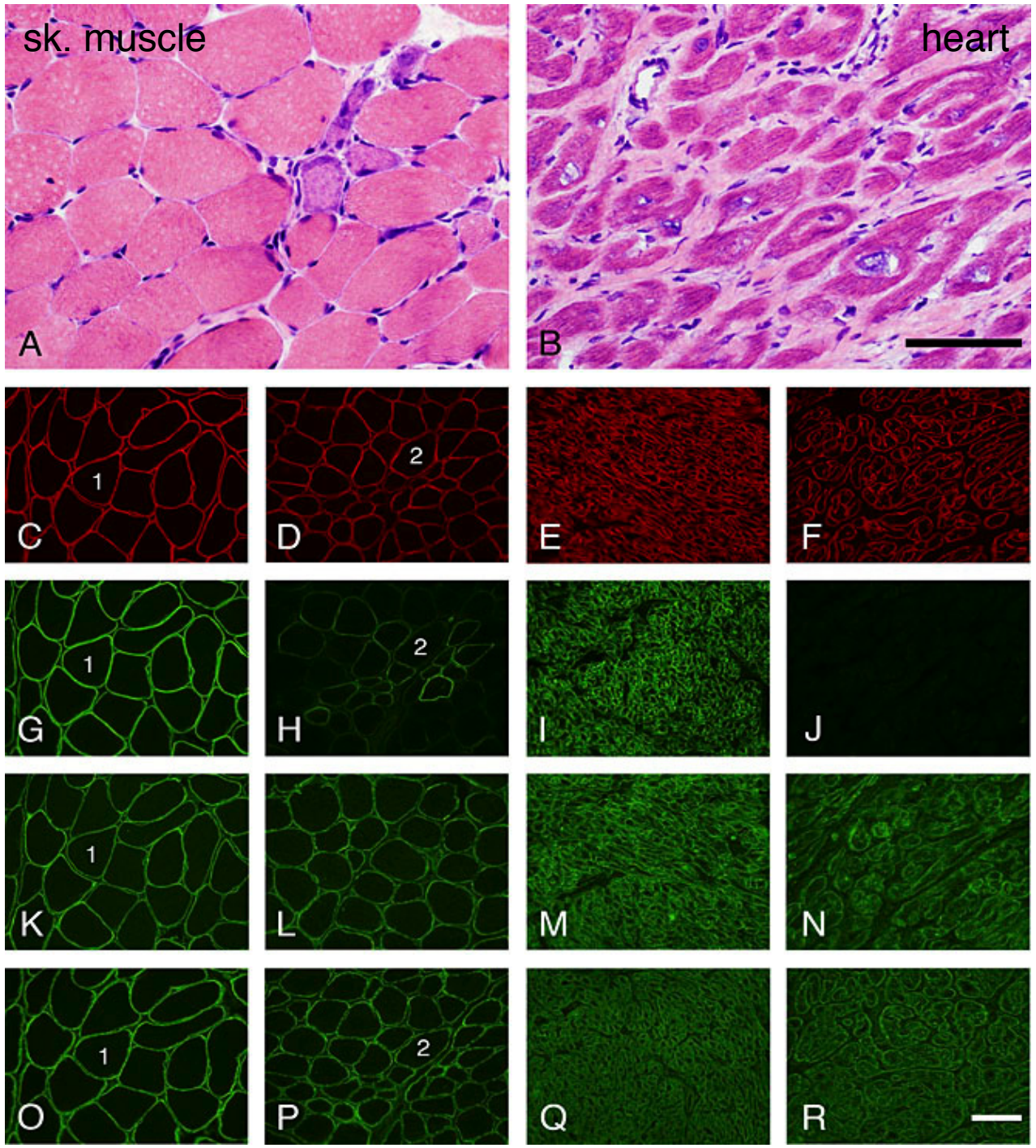
Sarcoglycans stain normally in dystroglycanopathies.

adult onset LGMD – dystroglycanopathy
patchy, reduced staining for alpha-dystroglycan

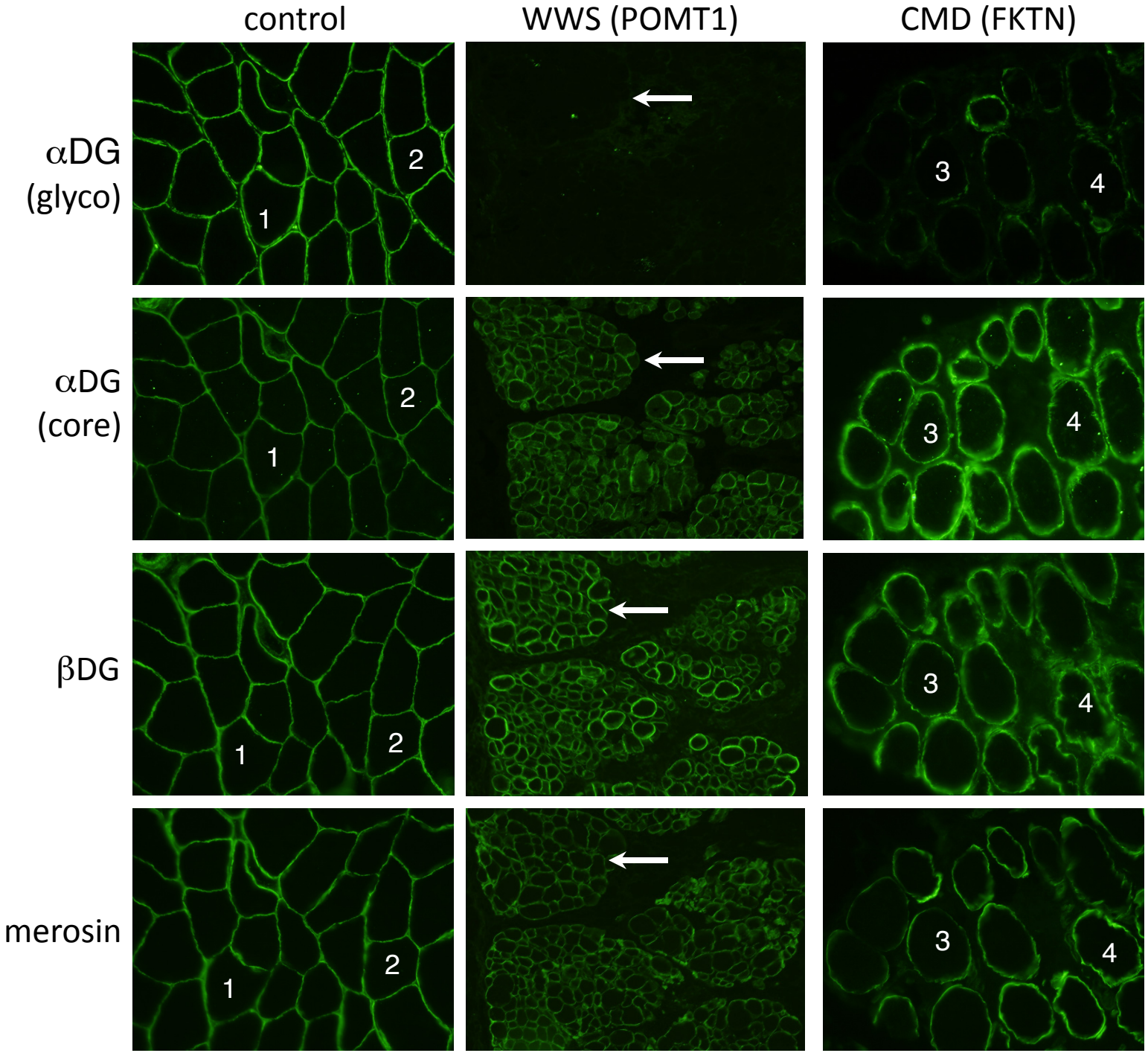




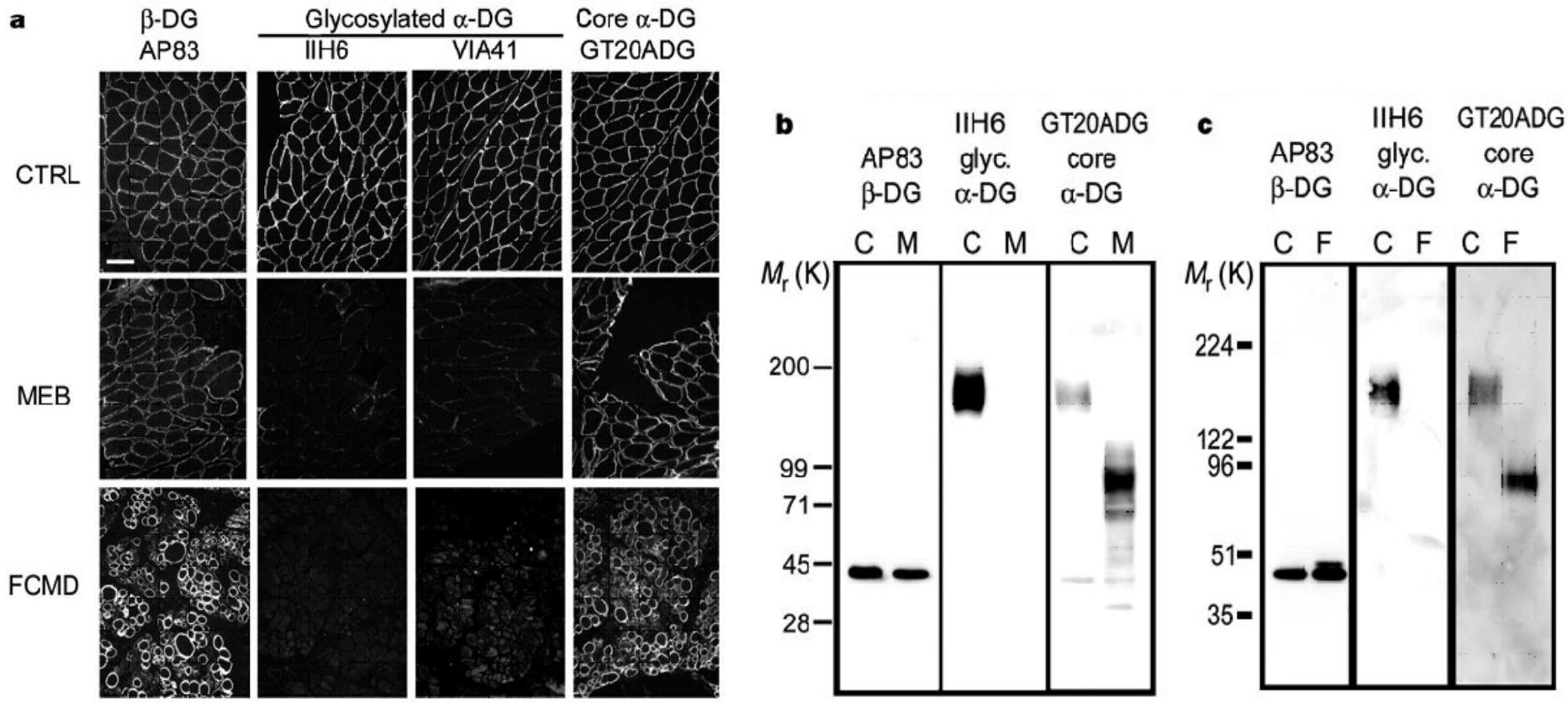
LGMD 2I patient with cardiomyopathy



Margeta et al., Muscle & Nerve 40:883-889, 2009


control
WWS (POMT1)
CMD (FKTN)
αDG (glyco)
αDG (core)
βDG
merosin
1
2
3
4

α-dystroglycan is missing "laminin binding glycosylation" in the dystroglycanopathies



Michele et al., Nature 418:417-422, 2002.

Glycosylation

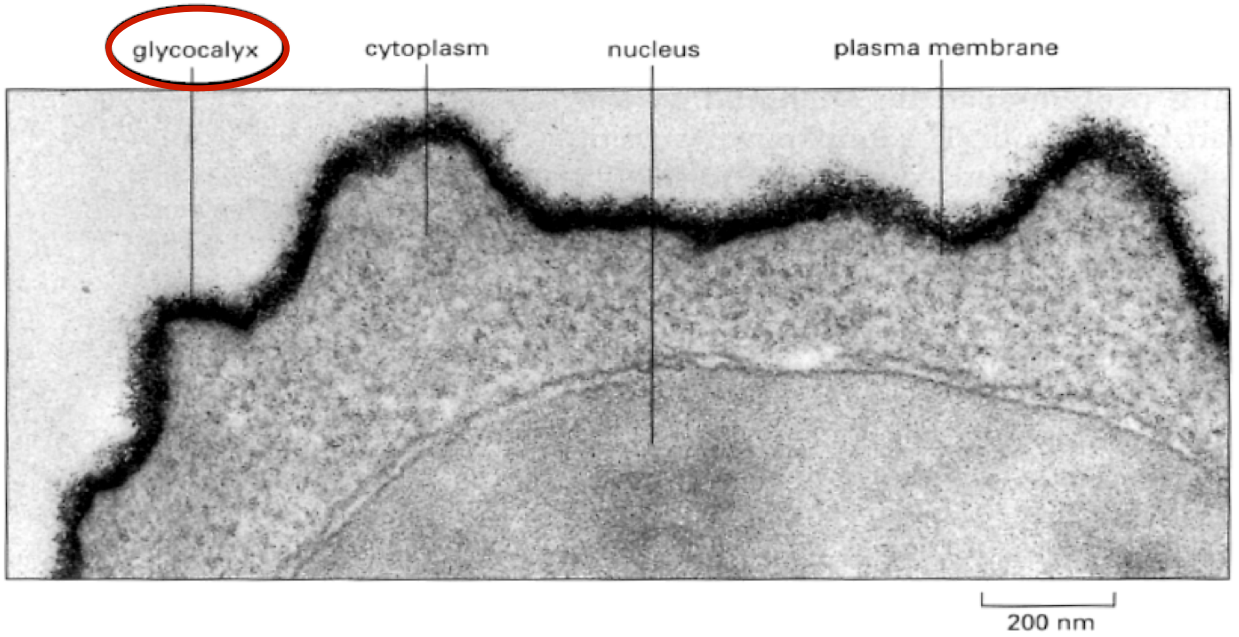
Glyco –
A Greek word that means sweet

Glycobiology-
The branch of science that studies the role of carbohydrates (sugar molecules) and their implication on health and disease.

Tobias Willer, Ph.D.

Assistant Research Scientist in Dr. Kevin Campbell's lab
Department of Molecular Physiology and Biophysics
University of Iowa College of Medicine

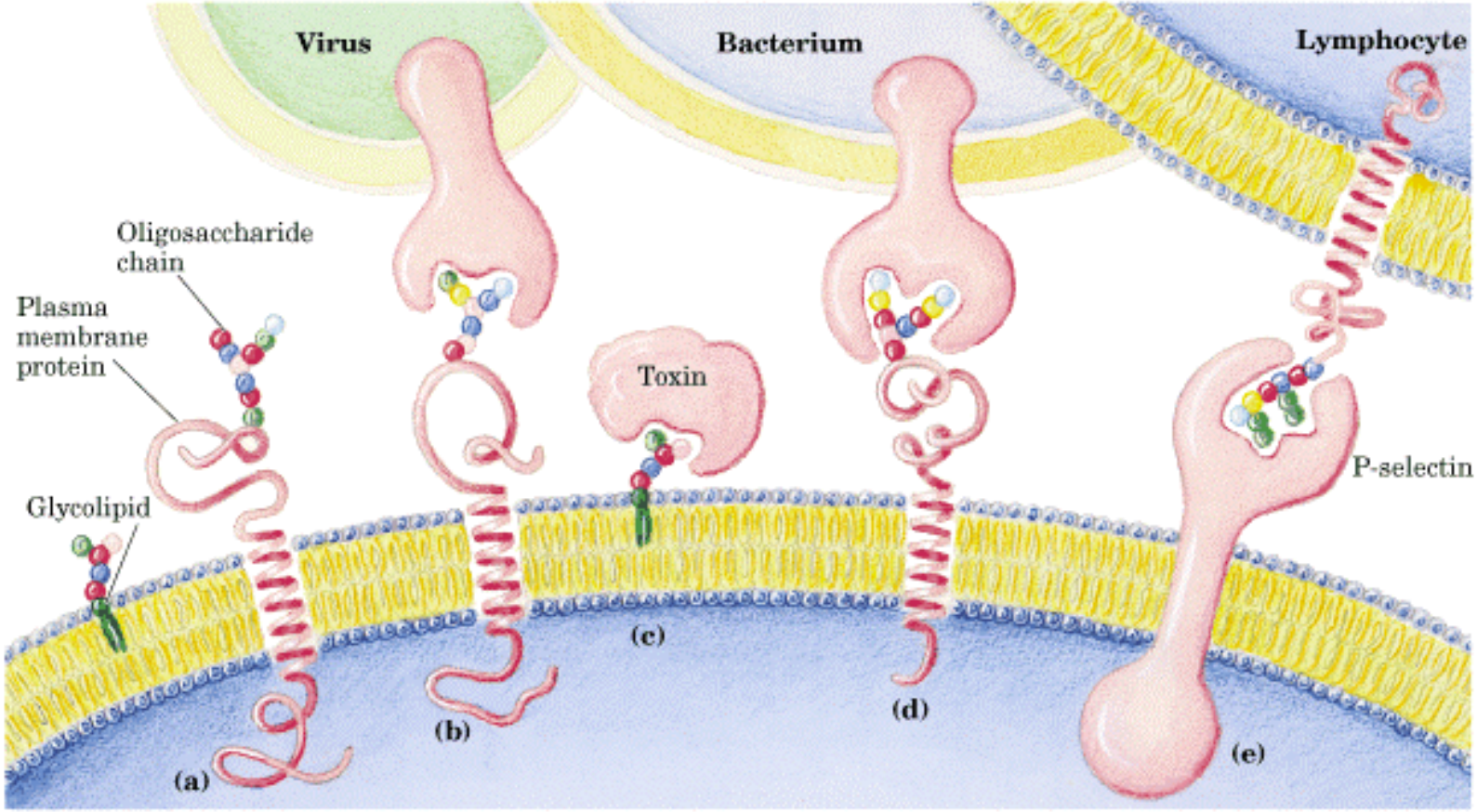
All cells are coated with “glycans”



Electron micrograph of a human lymphocyte (Ruthenium Red staining)

Glycoproteins are found on the outer surface of plasma membrane, in the extracellular matrix, in the blood, and in specific organelles, Golgi complexes, lysosomes, and secretory granules.

Roles of oligosaccharides in recognition and adhesion at the cell surface



Surface carbohydrates on cells serve as points of attachment for other cells, infectious bacteria, viruses, toxins, hormones and many other molecules

Why glycoproteins? – The biological advantages of modifying proteins with sugars

"… while the function of DNA and proteins are generally known … it is much less clear what carbohydrates do …"

Ciba Foundation Symposium 1988

- important for function

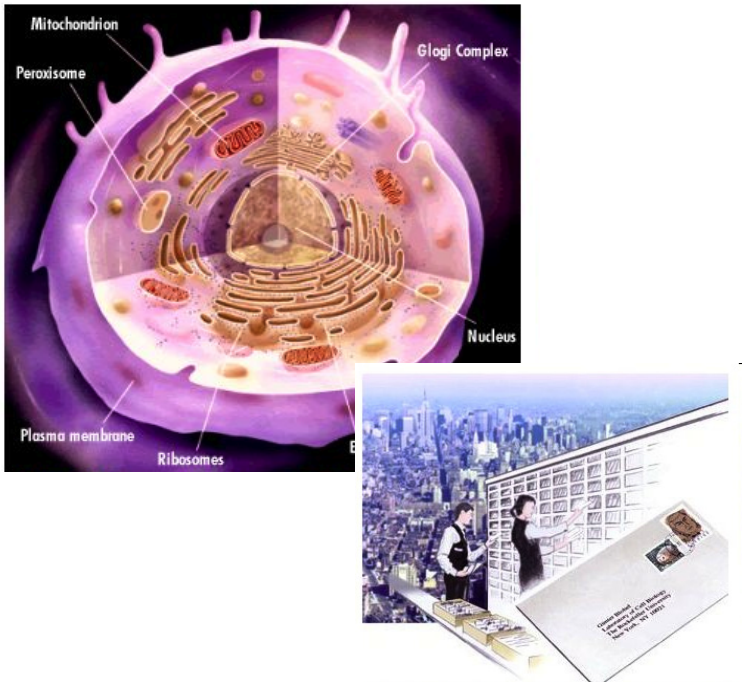
sugars can be important for receptor function

- important for folding

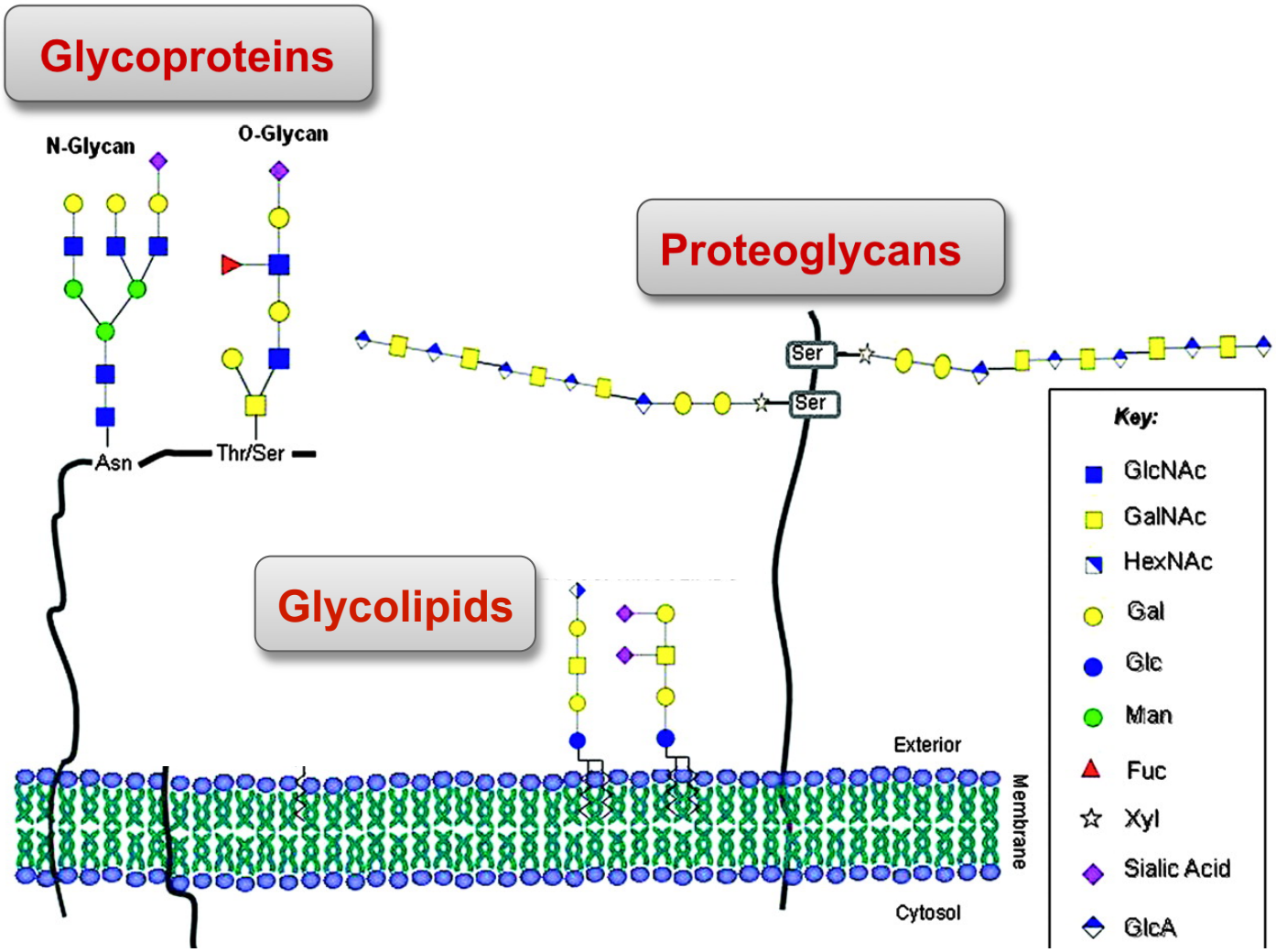
enhances stability of proteins

- important for targeting

sugars can act as a ZIP code to direct proteins to a specific cellular compartment



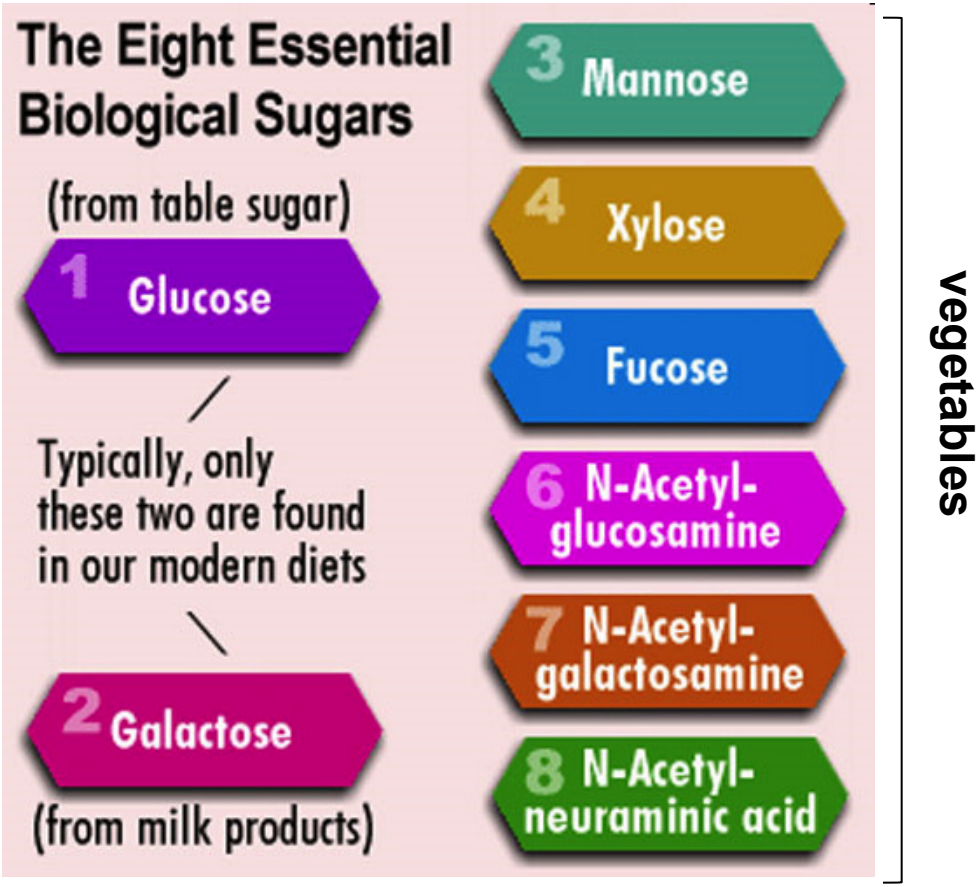
Schematic representation of common classes of glycoconjugates expressed in human cells



Reis C A et al. J Clin Pathol 2010;63:322-329

Glyconutrients

With growing interest in Glycobiology, these **essential sugars** and their complex carbohydrates are gaining increased recognition for their physiological importance in every day life.



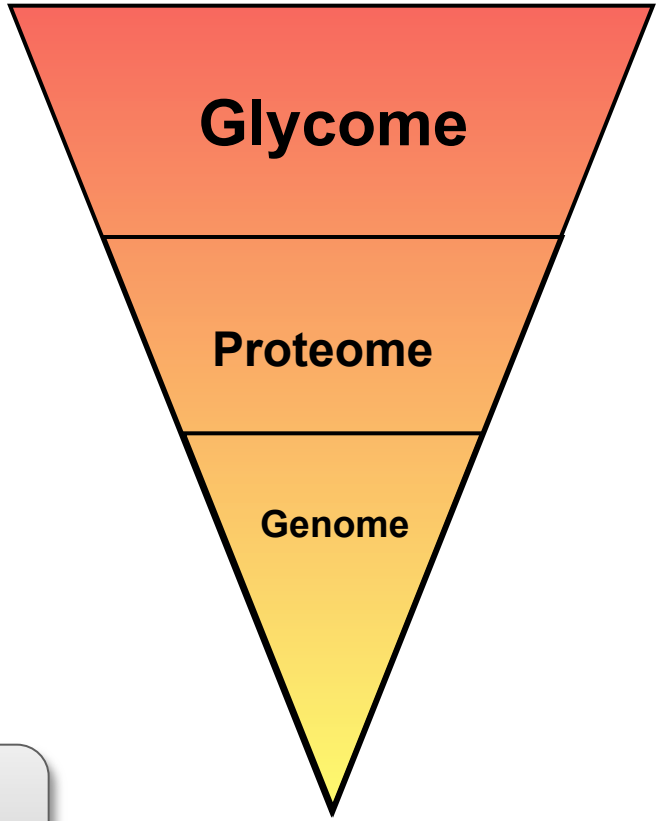
Next challenge: Decoding the glycome

Two different hexoses can combine in many different ways!
What a vast number of different structures for recognition purposes.

> 10^{15} hexa-oligosaccharides
with 20 different monosaccharide.

> 10^{7} (= 20^{6}) hexapeptides
with 20 amino acids.

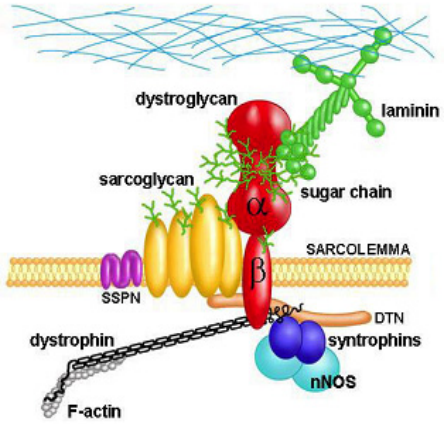
~4000 (= 4^{6}) hexanucleotides
with 4 nucleotide subunits



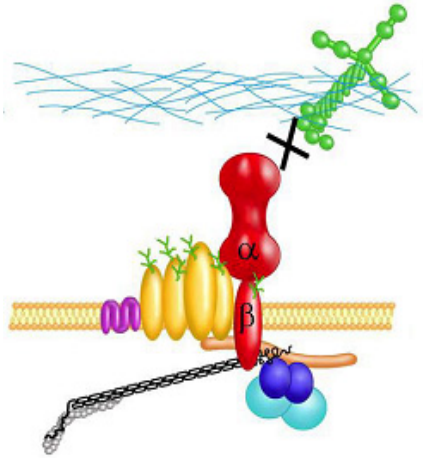
By comparison “Glyco-Legos” can build more complex structures than amino acids and nucleotides combined

Glycosylation and disease

- **Congenital disorders of glycosylation** (CDG)
  defect in N-glycan synthesis, metabolic disease that affects the brain and many other organs.
- **Cancer**
  glycans are used as marker for progressive tumors
- **Autoimmune disease**
- **Dystroglycanopathies**
  α-dystroglycan glycosylation defect => loss of receptor function
  => loss of laminin binding

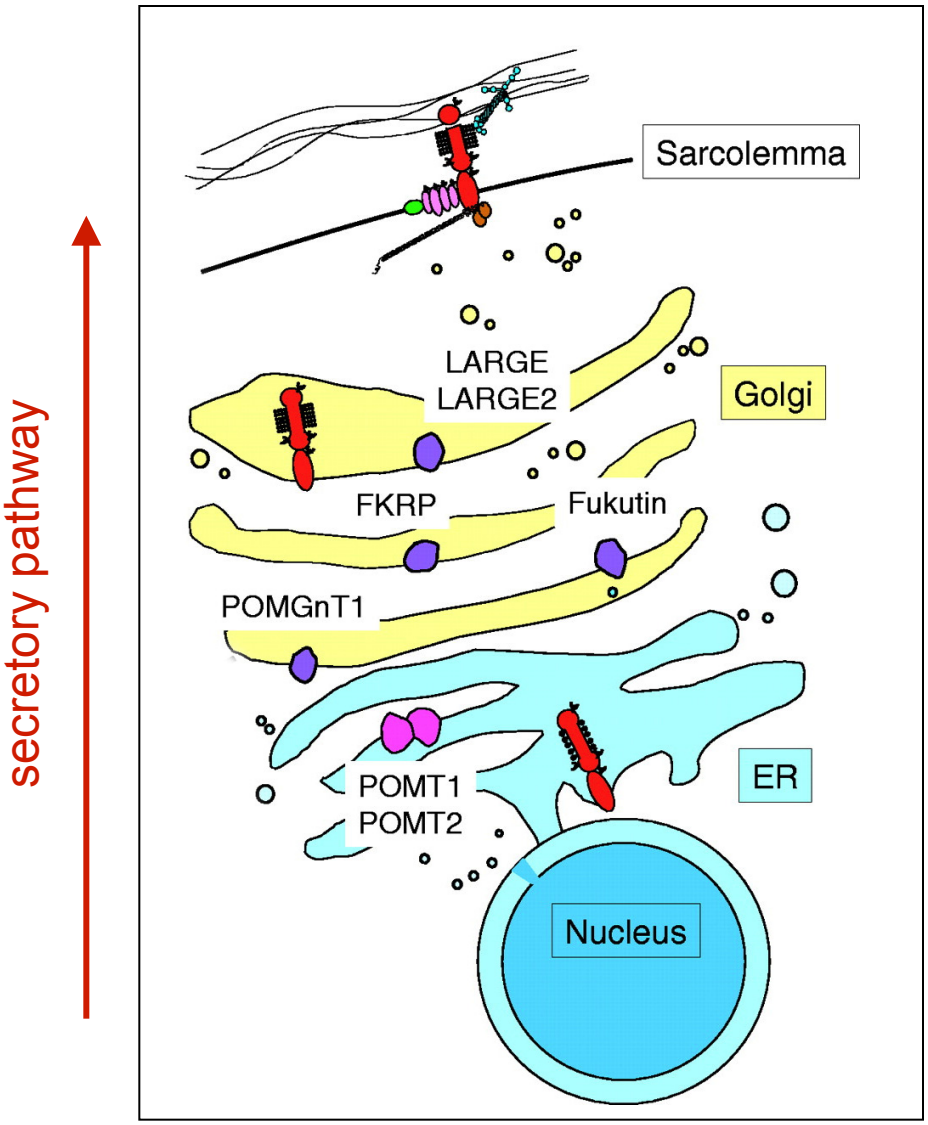


normal



dystroglycanopathy

Glycosylation of α-dystroglycan



Barresi, R. et al. J Cell Sci 2006;119:199-207

6 genes know to be involved in α-dystroglycan glycosylation

Endoplasmic reticulum
- POMT1
- POMT2

Golgi
- POMGnT1
- FKRP
- Fukutin
- LARGE1

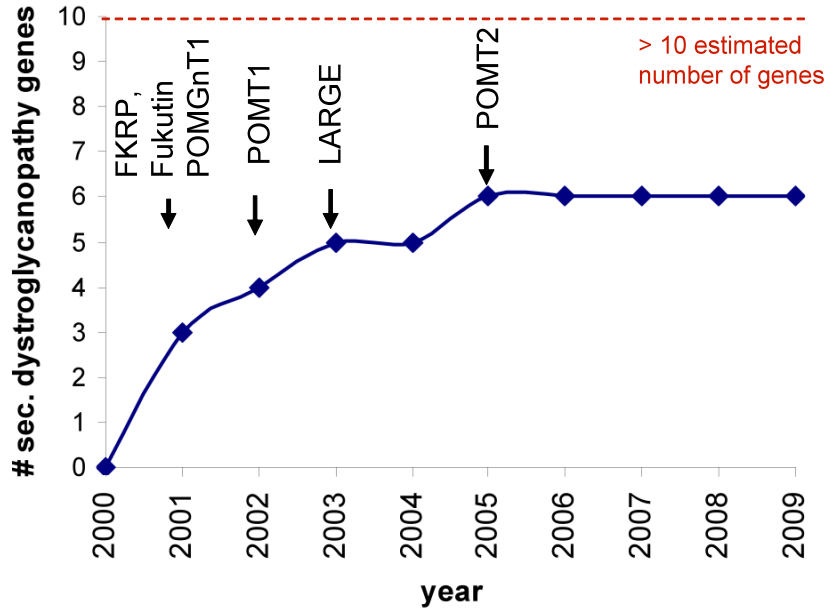
Glycosylation happens during secretion along the secretory pathway

Outlook: α-Dystroglycan glycosylation …. there is still a lot to discover

primary dystroglycanopathy: dystroglycan (DAG1) defect, 1 patient identified

secondary dystroglycanopathy: 6 known/putative genes causing

- ***POMT1*** (9q34.1)
- ***POMT2*** (14q24.3)
- ***POMGnT1*** (1p34.1)
- ***FKRP*** (19q13.32)
- ***Fukutin*** (9q31)
- ***LARGE*** (22q12.3)



Currently only 50% of dystroglycanopathy patients can be explained with known genes and can be provided with genetic diagnosis.
Preliminary linkage data suggest ~ 5 additional candidate genes that still remain unidentified.