# Pathology of Neuromuscular Disease
# Part 1: muscle

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# MUSCLE BIOPSY
# DESCRIPTION OF SPECIMENS, PROCEDURES & STAINS

- 2 blocks of skeletal muscle, **frozen** in isopentane cooled in liquid nitrogen. 12 µm thick sections are cut using a cryostat.
- The following *routine* stains are done :
- Basic histopathological stains: H & E and Gomori trichrome
- Special Stains:, oil red O, PAS, Congo red.
- Enzyme Histochemistry: NADH, SDH, COX, and ATPase, at pH 9.4, 4.6, 4.2. (Myophosphorylase, MAD, acid phosphatase if needed)
- Immune staining: carried out if needed
  - CD3, CD4, CD8, CD20 and CD68 cell markers, MAC
  - dystrophin (dys 1, 2, 3), sarcoglycans (α, β, γ, δ), dystroglycans (α, β), dysferlin, caveolin 3, laminin alpha 2 (merosin), utrophin, spectrin , collagen VI
  - specific antibodies for protein aggregates

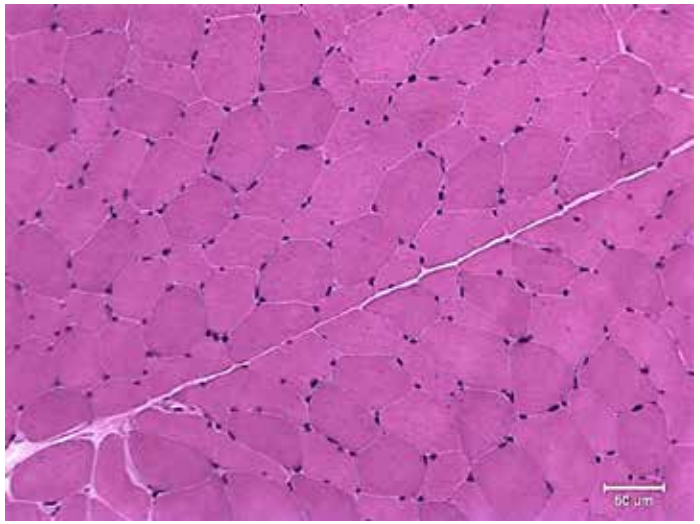
- EM piece placed in glutaraldehyde for further processing
- A separate piece of muscle frozen for biochemical/genetic studies

# H&E and Gomori Trichrome

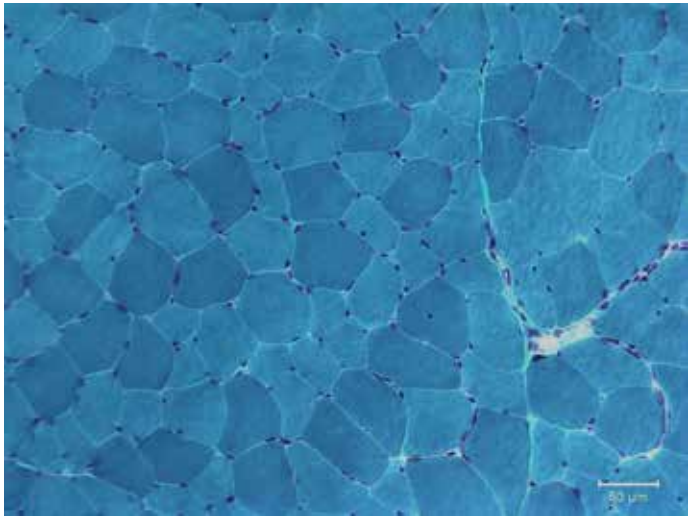
Give wide range of information:

- ✓ Necrosis
- ✓ Regeneration
- ✓ Fiber size – atrophy/hypertrophy
- ✓ Inflammation
- ✓ Fibrosis
- ✓ Structural changes
- ✓ Organelle changes

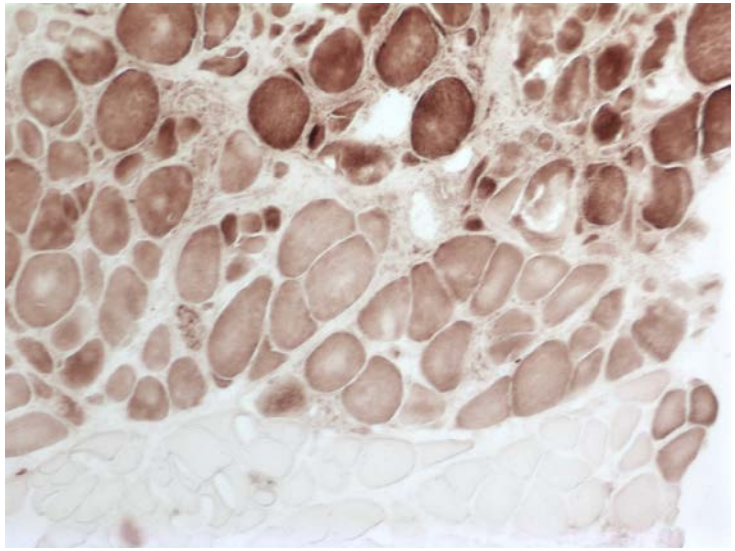
Hematoxylin & Eosin (Gill's)

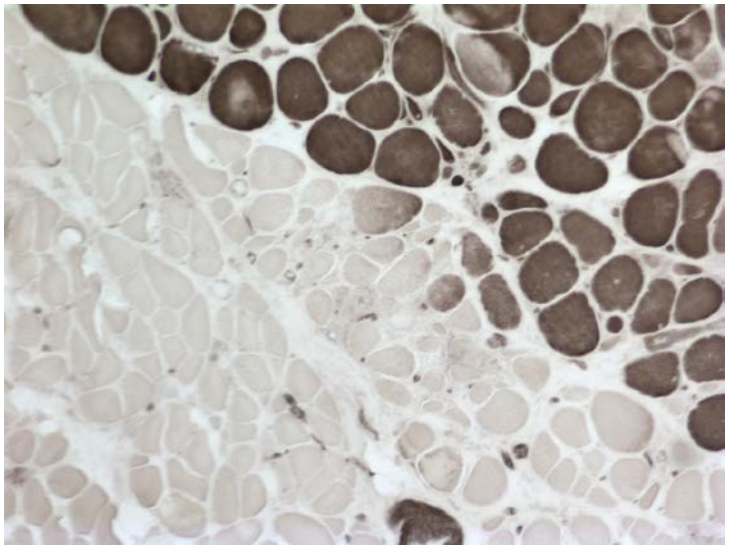


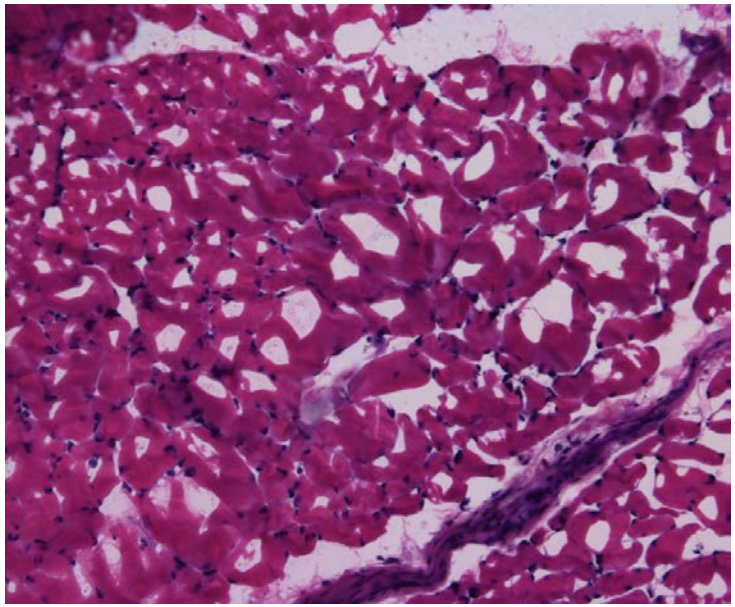
Modified Gomori Trichrome

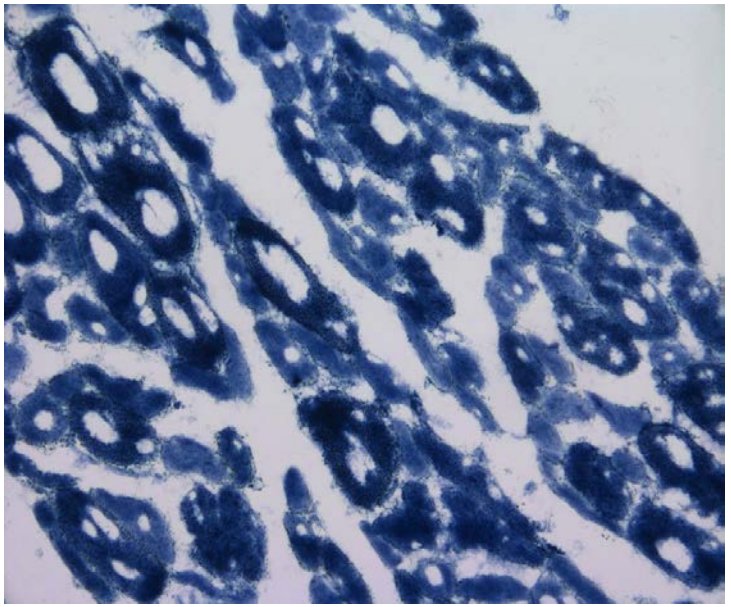


Examples of tissue handling artifacts





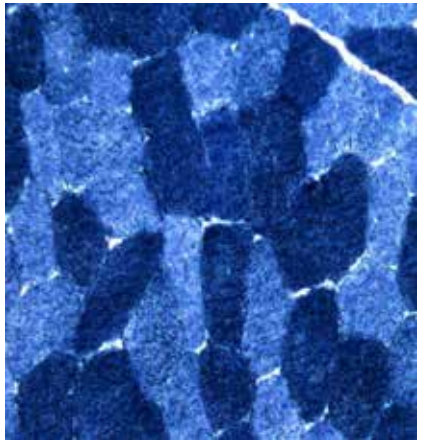




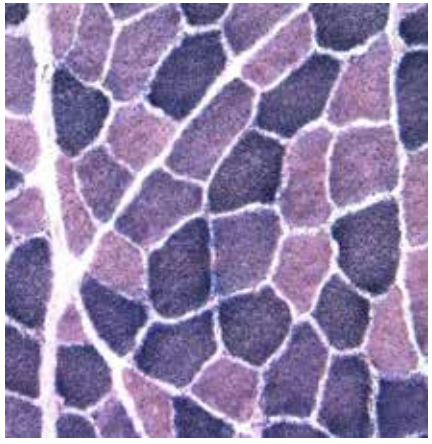
# Useful Histochemical Reactions of Skeletal Muscle Cells

| Reaction | Cellular localization | Source of Reaction | Specificity |
|---|---|---|---|
| **NADH-tetrazolium** | Intermyofibrillar* | Enzyme in mitochondria, | |
| **reductase** | perinuclear, Subsarcolemmal | SR, T-tubules | poor |
| **Succinic dehydrogenase** | Intermyofibrillar* | Enzyme in mitochondria | excellent |
| **Cytochrome oxidase** | Intermyofibrillar* | Enzyme in mitochondria | excellent |
| **Myofibrillar ATPase** | Intermyofibrillar | Myosin or actomyosin | good |

NADH



SDH



COX

ATPase, 9.4

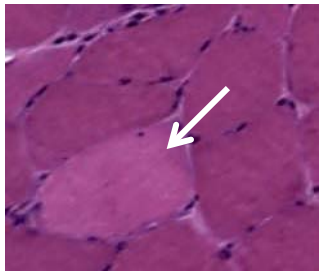
# Necrosis

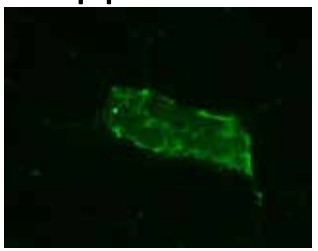
## Factors triggering necrosis in muscle cells:

- Lengthening contractions
  - dystrophic muscle particularly vulnerable
- Ischemia
  - dermatomyositis
- Energy deprivation
  - Glycolytic defects
- Toxic agents
  - Cardiotoxin, neutoxin, statins

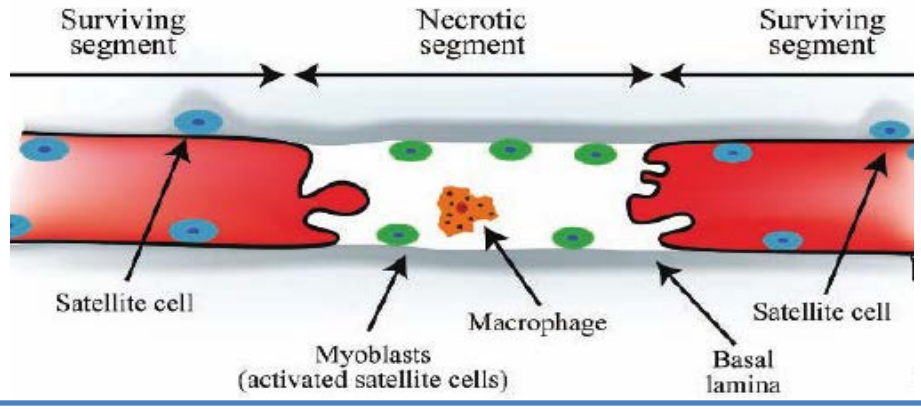
## In the course of necrosis:

- Plasma membrane becomes permeable
  -- $Ca^{++}$ entry, activation of phospholipases, proteases (calpains)
- Some DAG complex- lost early; by 24 hrs dys lost
- Activation of compliment cascade, diffuse cytoplasmic appearance of lytic C5-9 (MAC) within muscle

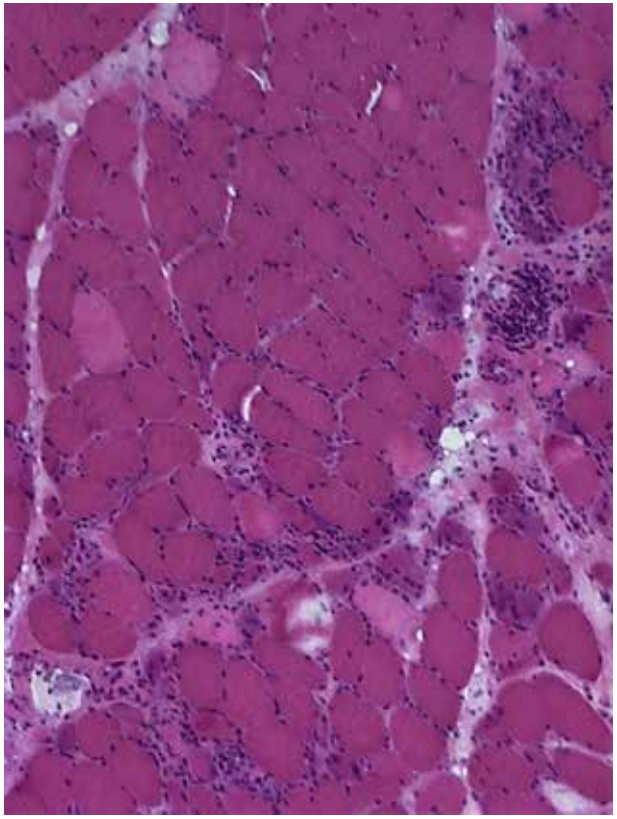


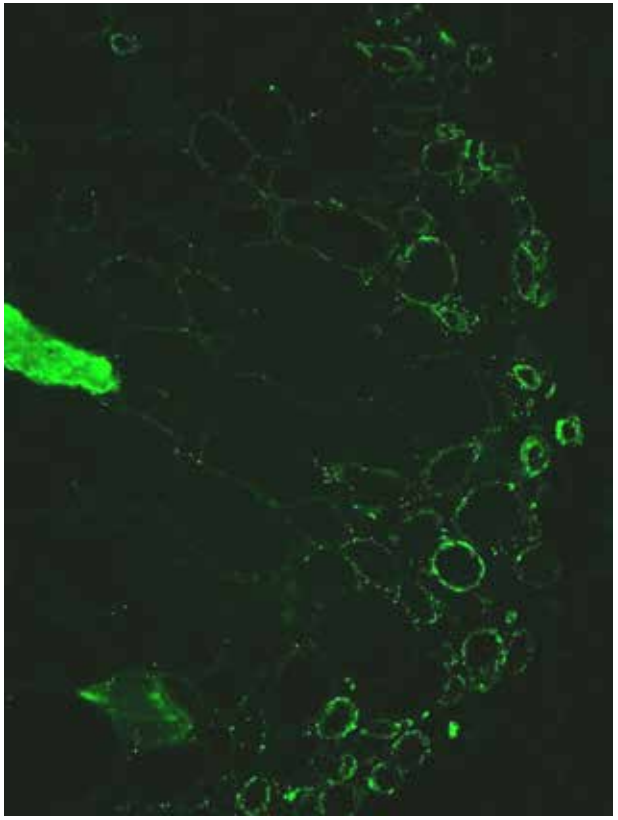


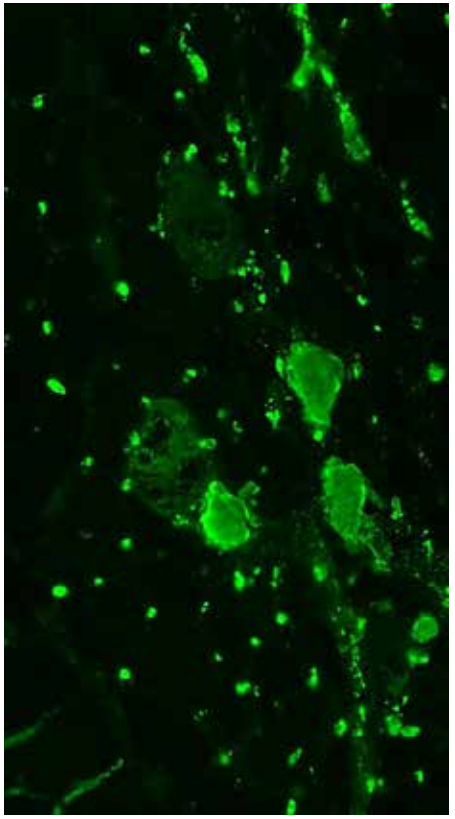
### Segmental Necrosis



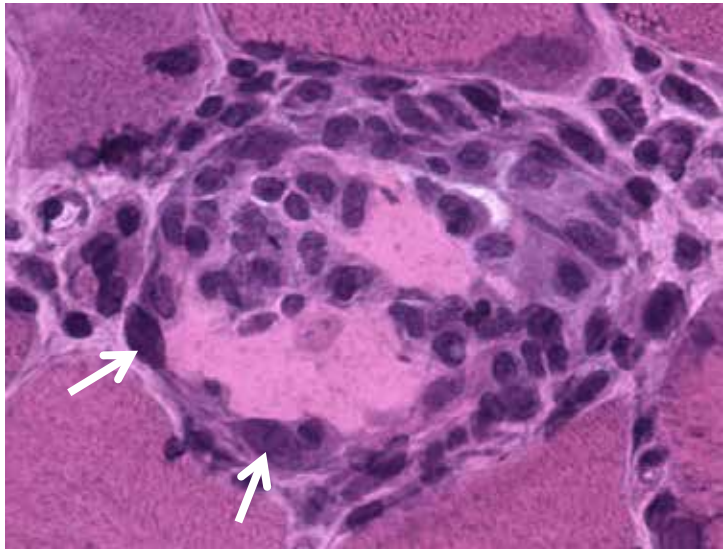
# Dermatomyositis-acute stage (Ischemic necrosis)

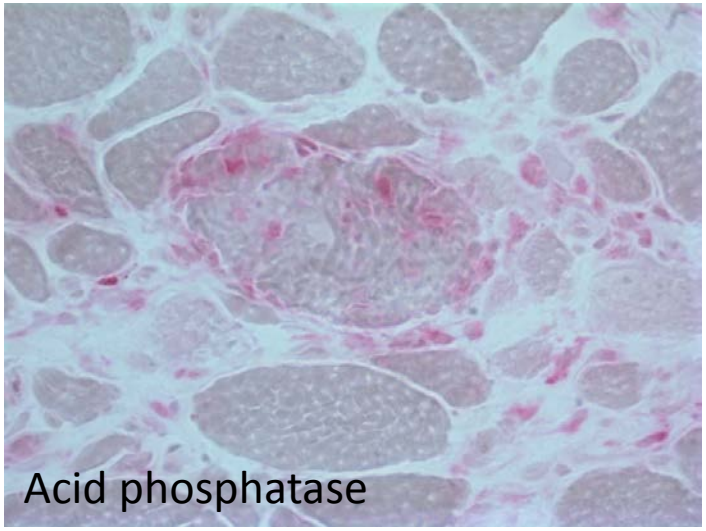






# Phagocytosis



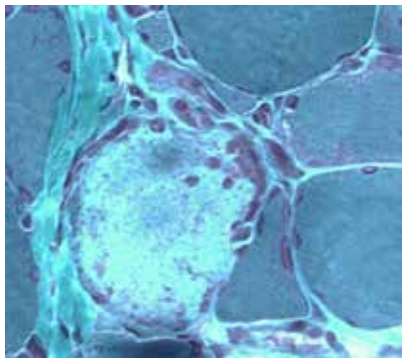


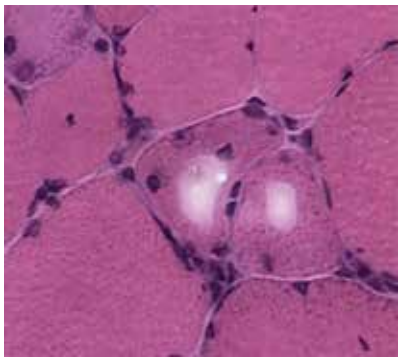

- Starts ~ 6 to 8 hrs after the fiber passed the point of no return

  -- sarcolemmal and myonuclear dissolution

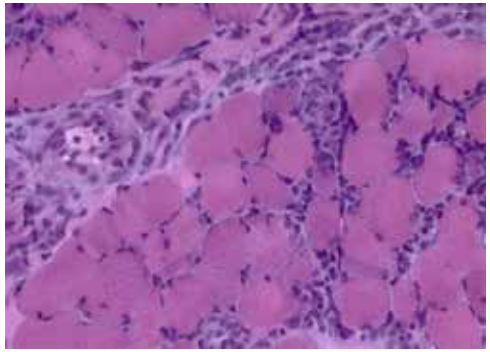
  (earliest change), followed by gradual dissolution of contractile elements

  what is not destroyed: Basal Lamina & Satellite Cells

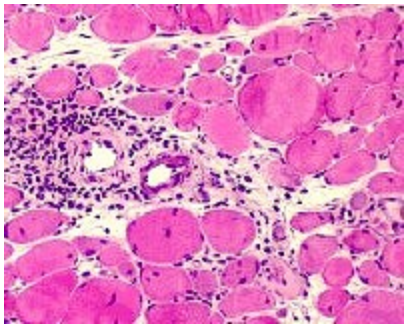




- In surviving stumps- T tubule dilatation
- Abundant MFs within endomysium



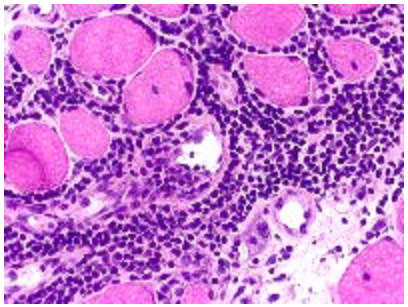
# Patterns of inflammation



H&E

**Perivascular inflammation**

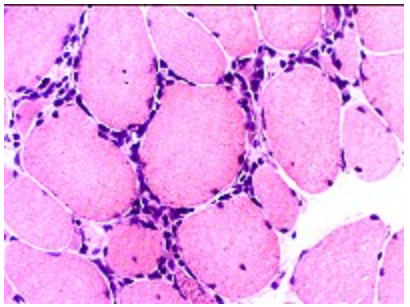
- Variation in muscle fiber size
- Small rounded fibers



H&E

**Perivascular & Perimysial inflammation**

- Mononuclear cell

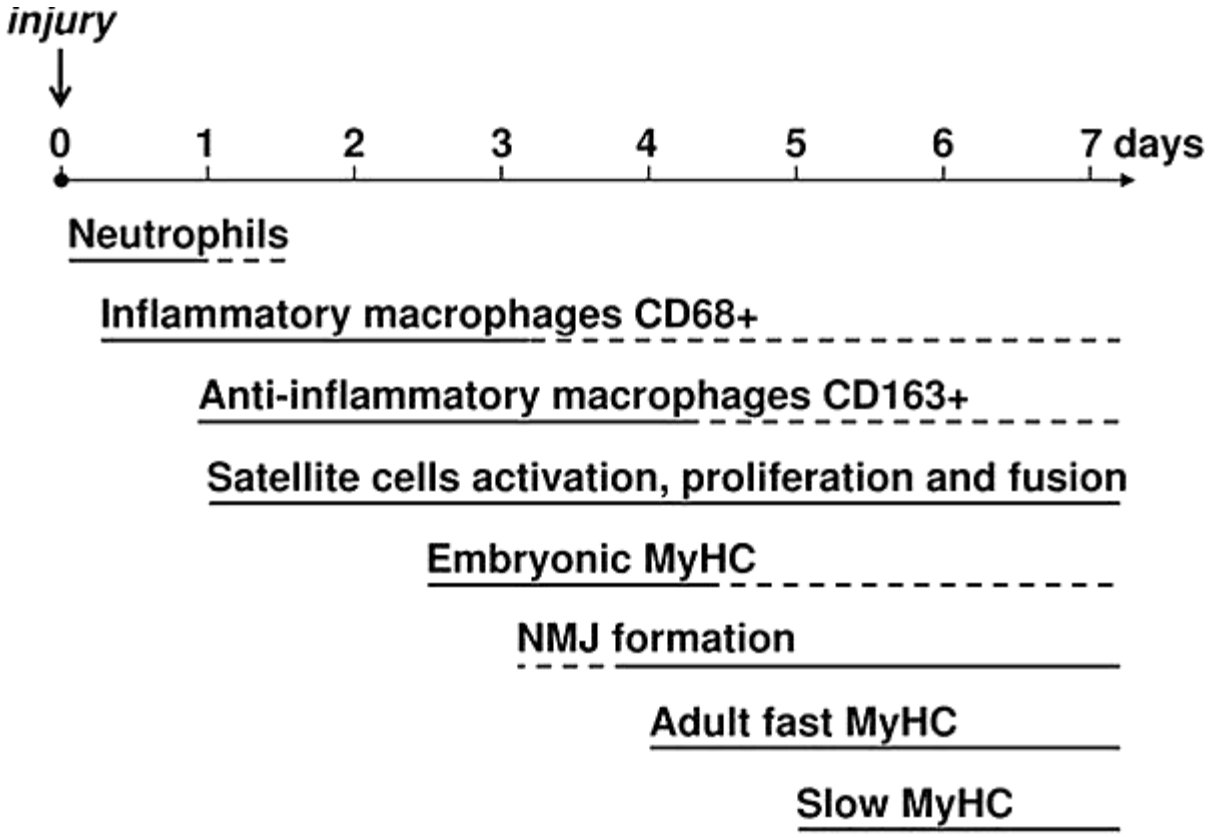


H&E

**Endomysial inflammation**

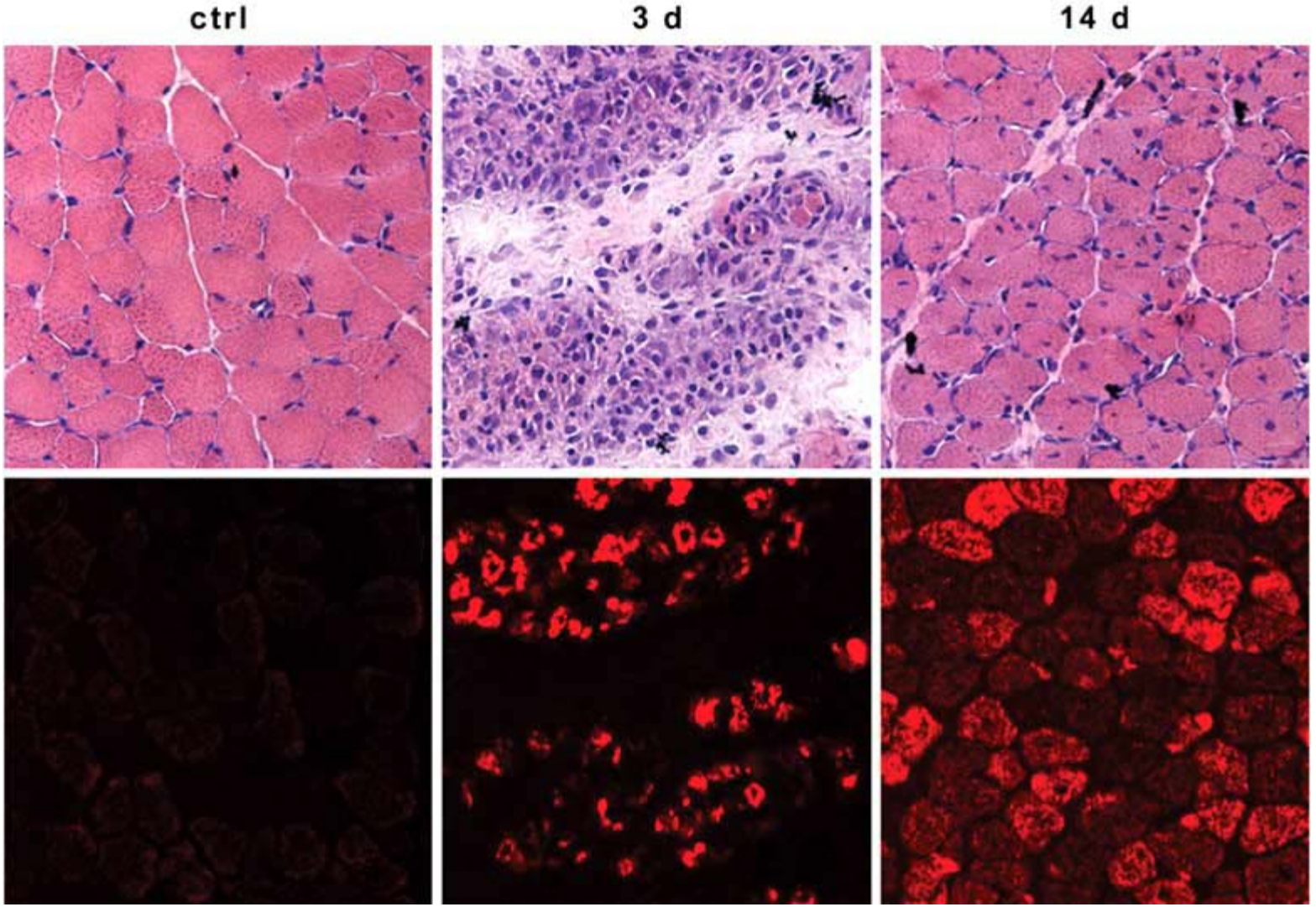
Often associated with focal invasion of muscle fibers

Temporal sequence of inflammatory and regenerative events following muscle injury:




Ciciliot S., Curr. Pharmaceutical Design, 2010

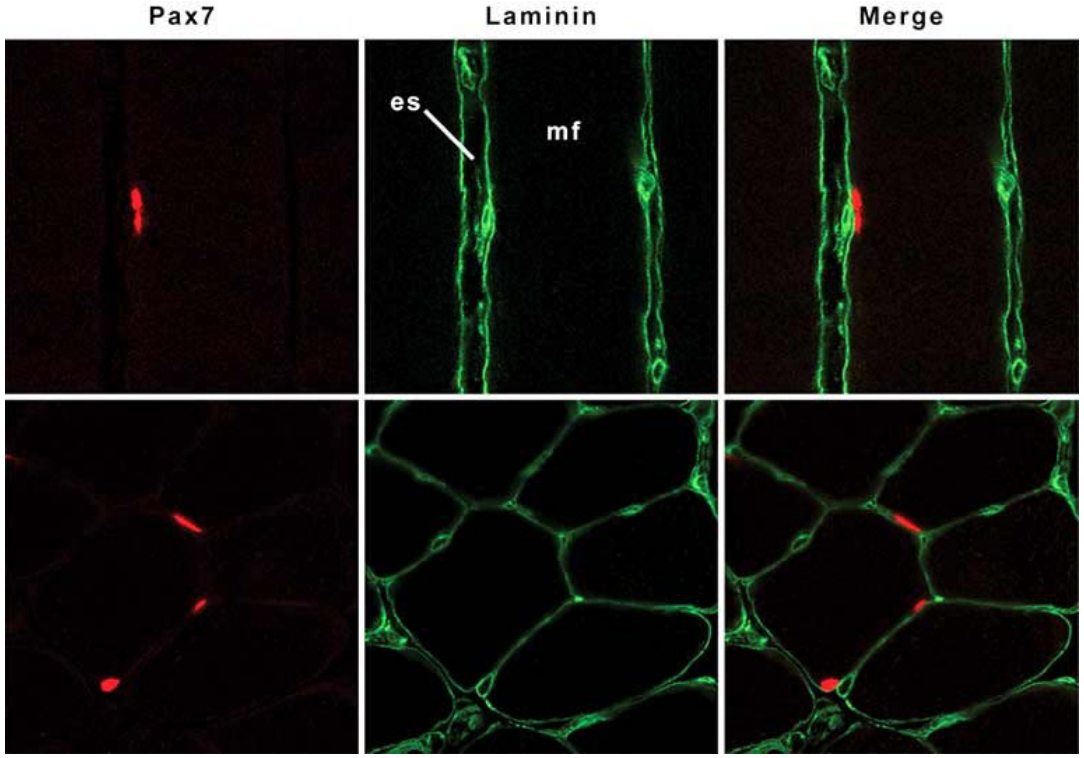
Myofiber growth and embryonic MyHC expression in regenerating skeletal muscle




Ciciliot S., Curr. Pharmaceutical Design, 2010

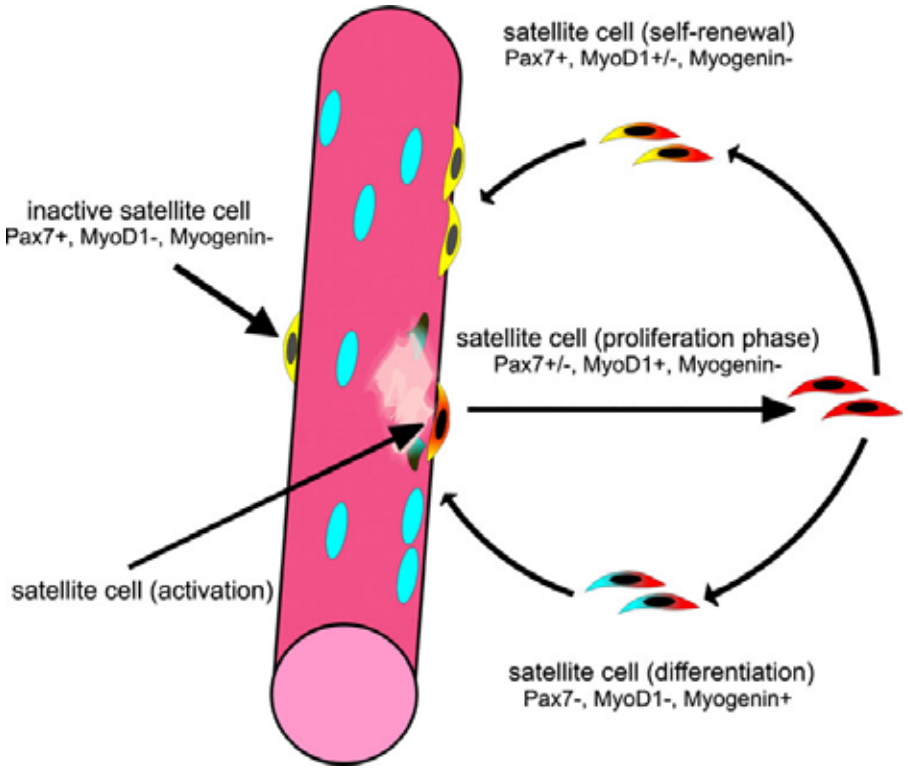
## Satellite Cells

- Muscle specific stem cells located beneath the basal lamina of the myofiber
- Pax7-useful marker for quiescent SCs
- Prevalence = r S/M
- Major role in
  - Natural growth
  - Muscle maintenance, work hypertrophy
  - Regeneration
- Proliferative/differentiating processes lead transformation into myoblast/myotubes in necrotic segments
- Limit of their mitotic cycles?

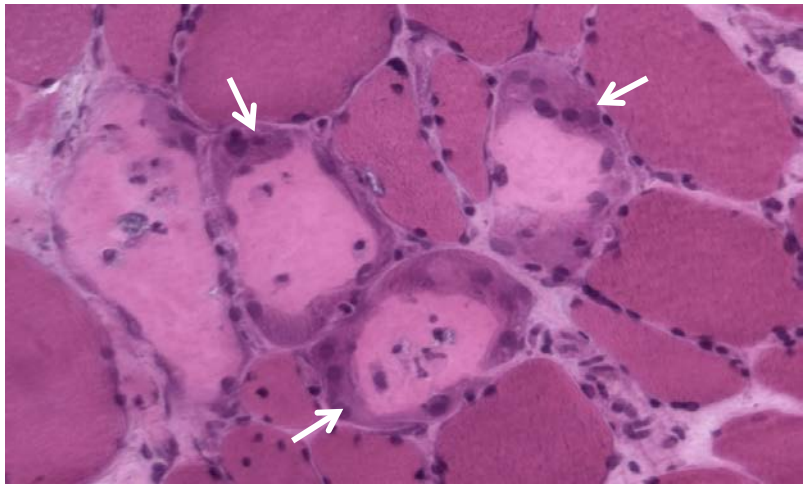


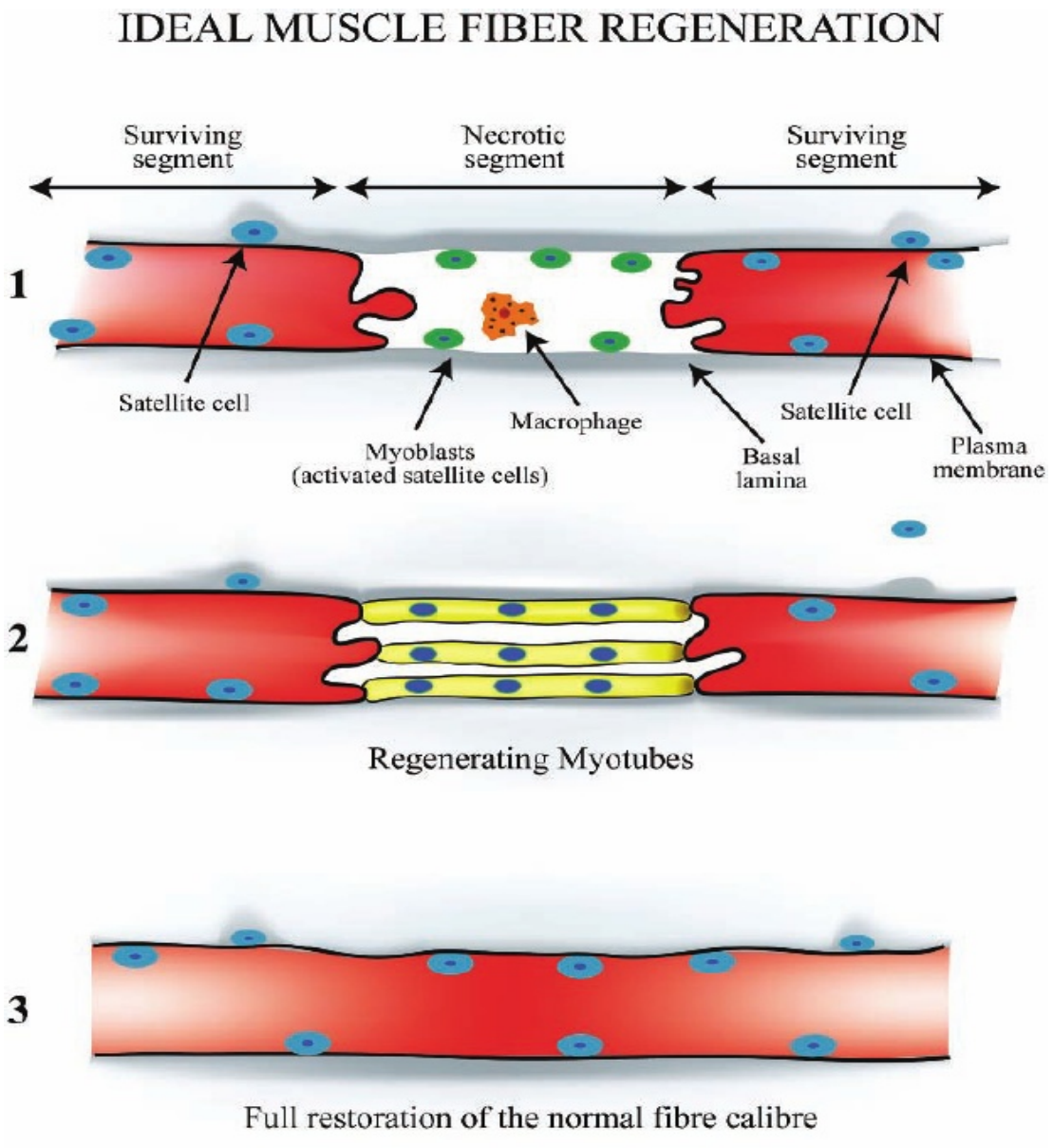

Ciciliot S., Curr. Pharmaceutical Design, 2010

Model for satellite cell self-renewal and differentiation




Activated satellite cells in necrotic fibers

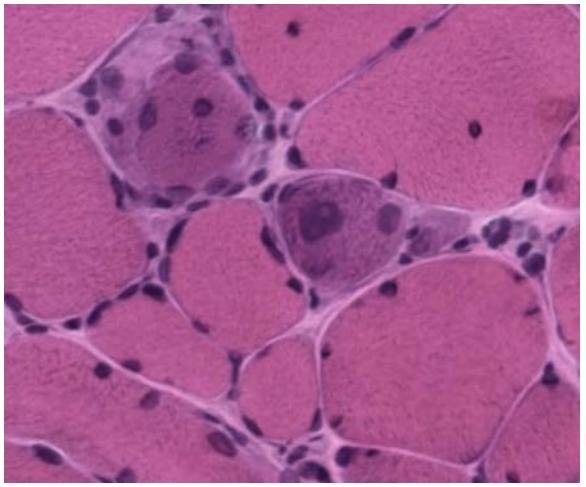




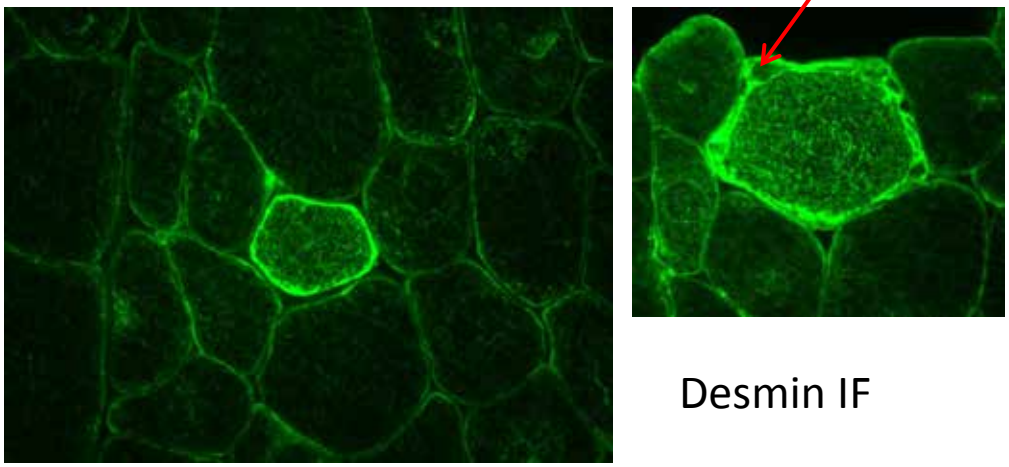

Karpati, G; 2008

# Histological Features of Regenerating Muscle

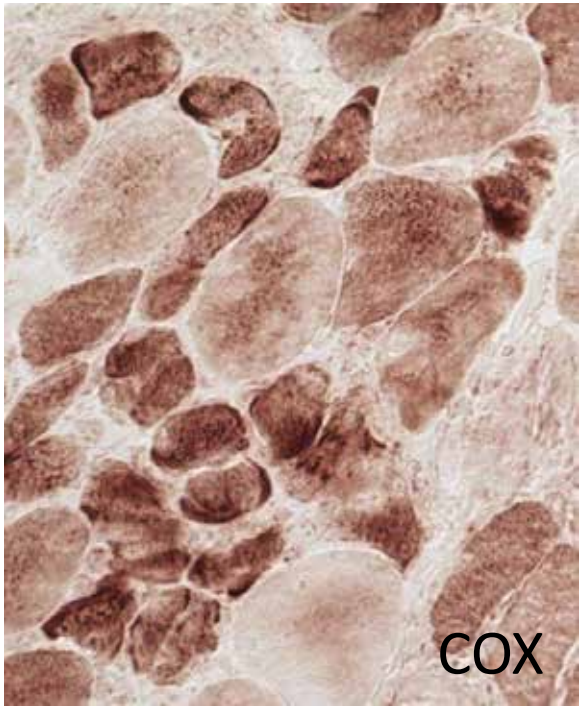
- Eosinophilic cytoplasm, reflecting high content of ribosomes
- Nuclei tend to be pale and large
- Relative excess of glycogen and mitochondria (early)
- Emb & Neo forms of myCH
- Diffuse cytoplasmic desmin stain

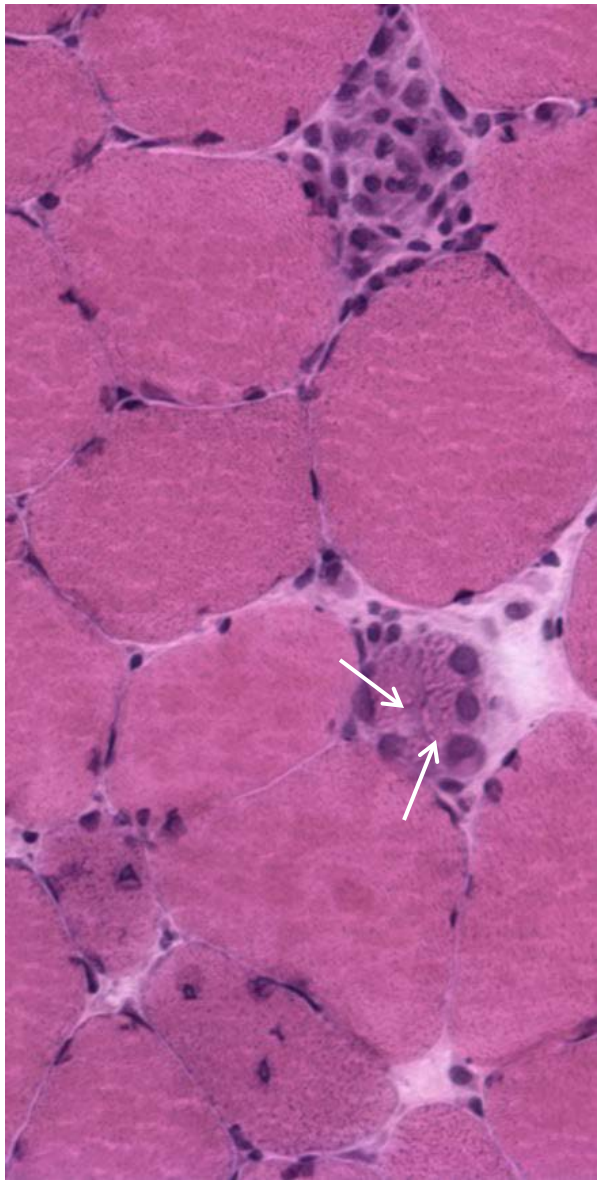


Satellite cell

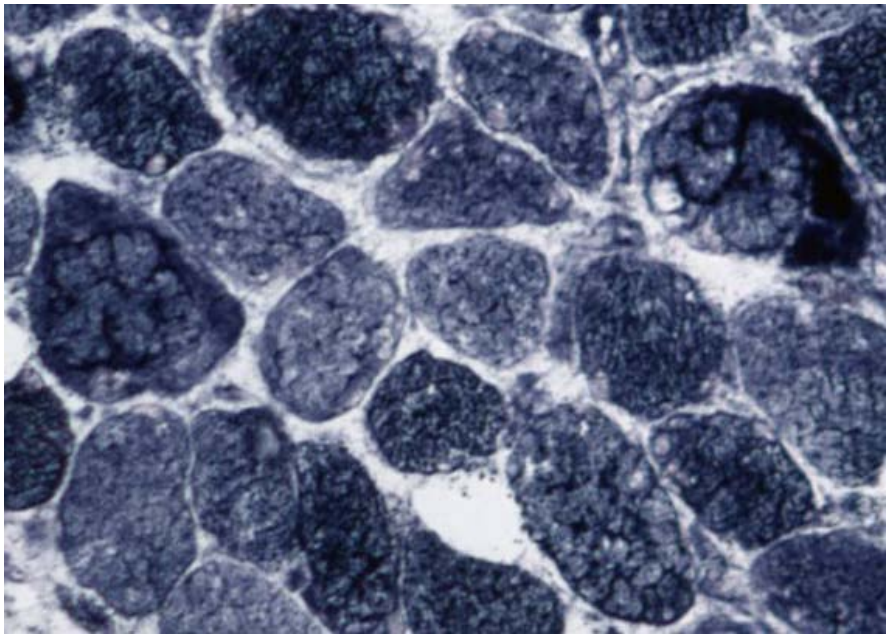


Desmin IF

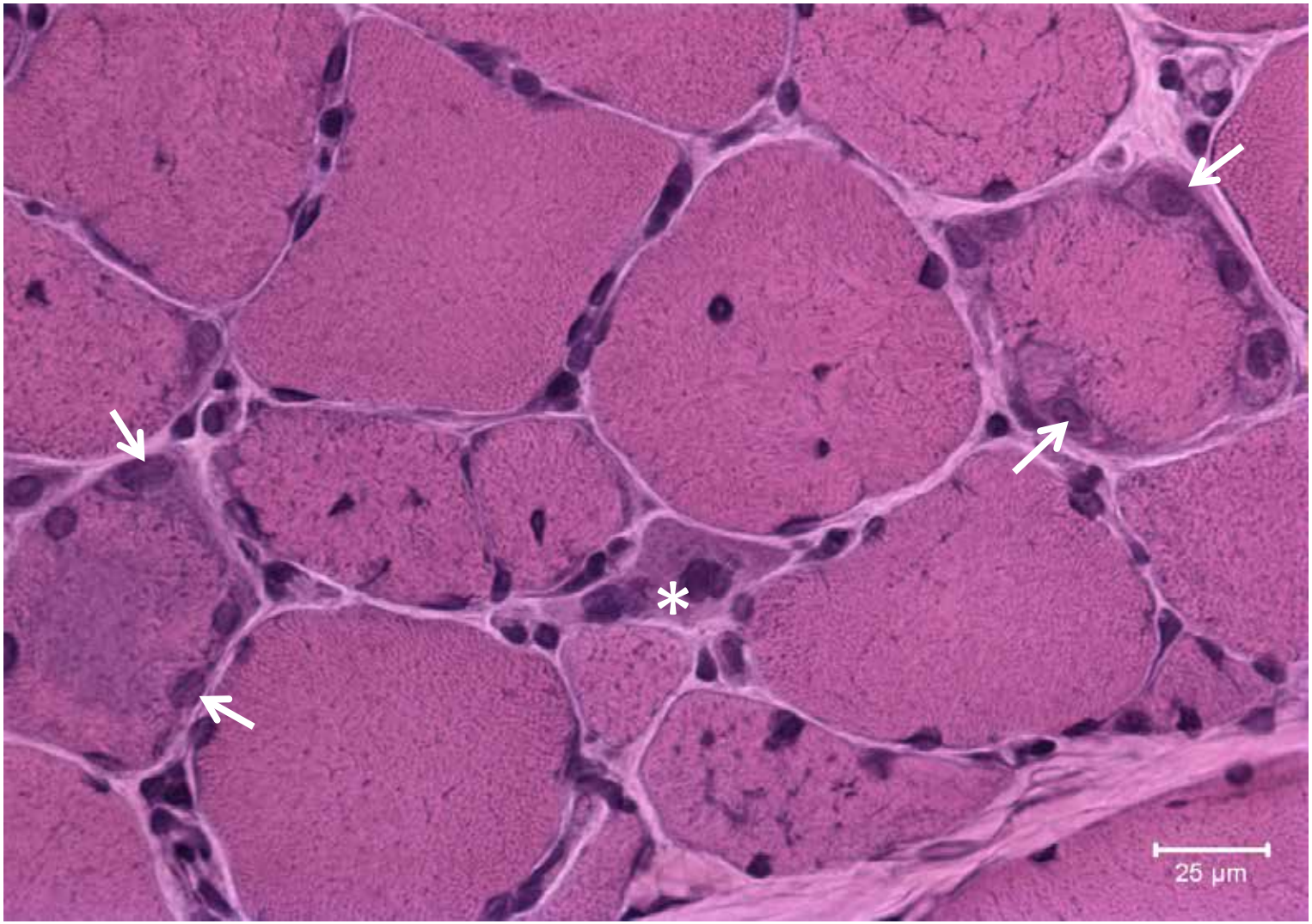




Muscle Fiber Regeneration

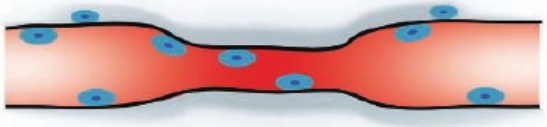


# Muscle Fiber Regeneration



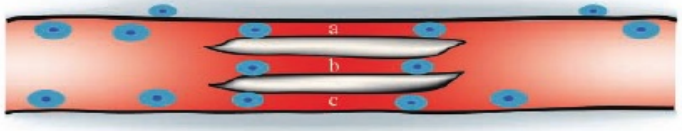
# ABERRATIONS OF MUSCLE FIBER REGENERATION

**1**



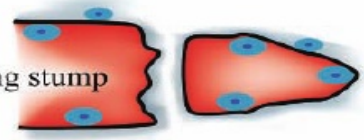
Regenerated segment is of smaller caliber than the rest of the fiber

**2**




Forked fibers due to incomplete lateral fusion of myotubes

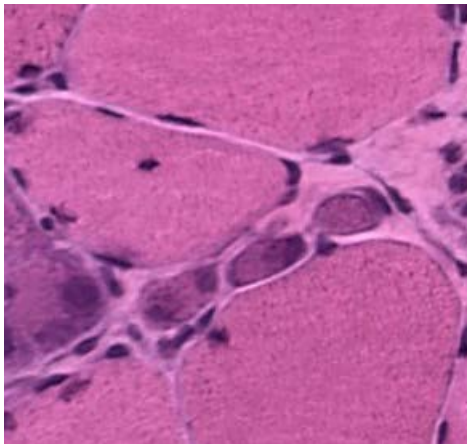
**3**

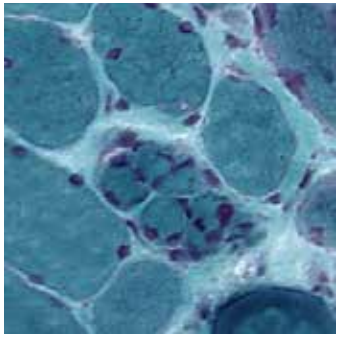



Muliple independent fibers due to lack of fusion of myotubes with the surviving stump

**4**

Empty basement membrane sleeve due to lack of regeneration





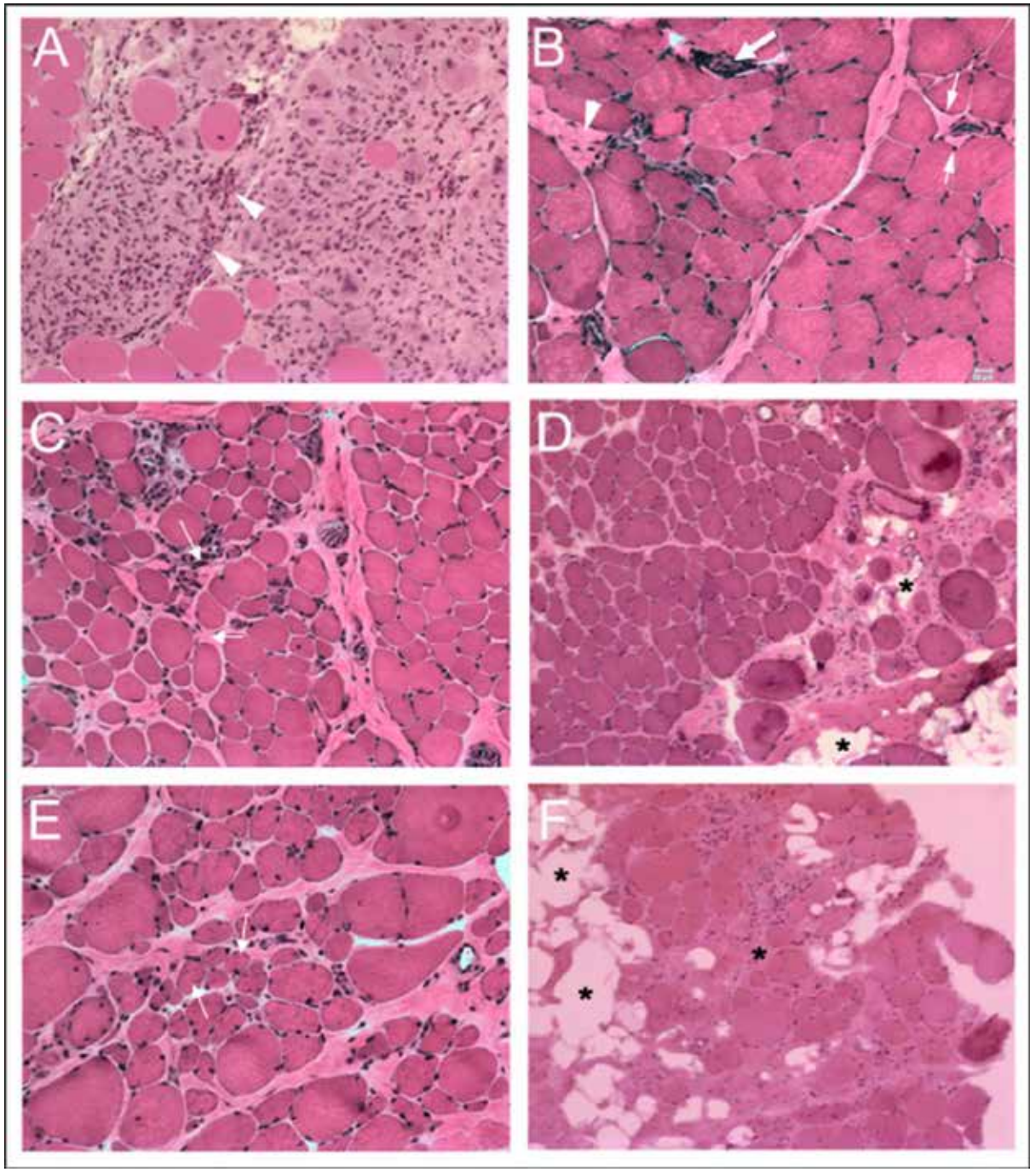
# Dystrophic process and Satellite cells

LGMD2A:

- caused by mutations in the *CAPN3*, encoding $Ca^{2+}$ - activated cysteine protease
- role in sarcomere assembly, turnover and maintenance
- in Calpainopathy there

Is a good correlation between age, duration of symptoms and degree of fibrosis

- microRNA dysregulation leads to inability of Pax7-positive SCs to transit from proliferation to differentiation resulting in impaired regeneration and fibrosis in LGMD 2A



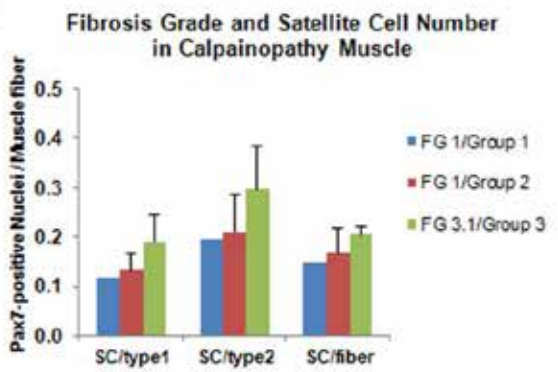
# Satellite Cells in Dystrophic Process (calpainopathy)

A

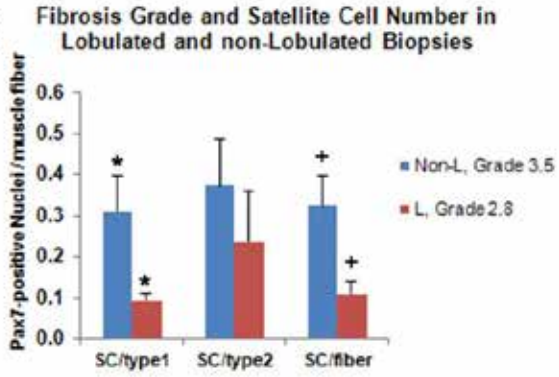
**Fiber type specific distribution of satellite cells in calpainopathy biopsies**

| Biopsies | | n | Age | DD | FG | SC /type1 | SC/type2 | SC/fiber |
|---|---|---|---|---|---|---|---|---|
| Group 1 | | 1 | 10 | 1 | 1 | 0.117 | 0.196 | 0.147 |
| Group 2 | | 3 | 19.7 ± 2.7 | 7.7 ± 3.3 | 1 ± 0.0 | 0.134 ± 0.032 | 0.210 ± 0.076 | 0.168 ± 0.051 |
| Group 3 | | 9 | 37.8 ± 4.8 | 19.7 ± 3.6 | 3.1 ± 0.2 | 0.189 ± 0.054 | 0.298 ± 0.087 | 0.205 ± 0.052 |
| Group 3 | LF | 5 | 36.4 ± 2.8 | 19.4 ± 3.6 | 2.8 ± 3.6 | 0.093 ± 0.018* | 0.236 ± 0.124 | 0.109 ± 0.029+ |
| Group 3 | no LF | 4 | 39.5 ± 11.0 | 20.0 ± 7.6 | 3.5 ± 0.5 | 0.310 ± 0.092* | 0.374 ± 0.130 | 0.325 ± 0.080+ |
| Control | | 3 | 45.7 ± 5.0 | | | 0.081 ± 0.001 | 0.056 ± 0.010 | 0.065 ± 0.006 |

B




C

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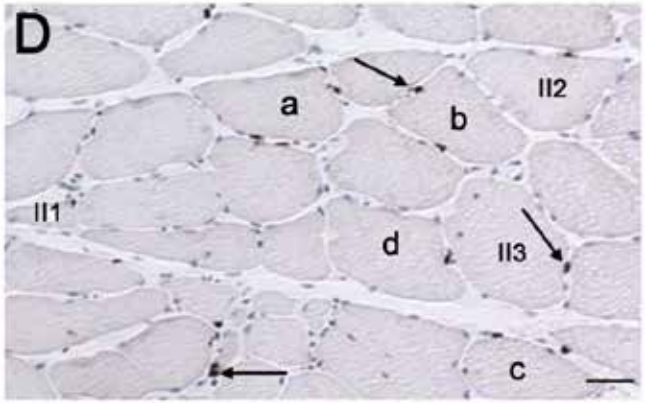
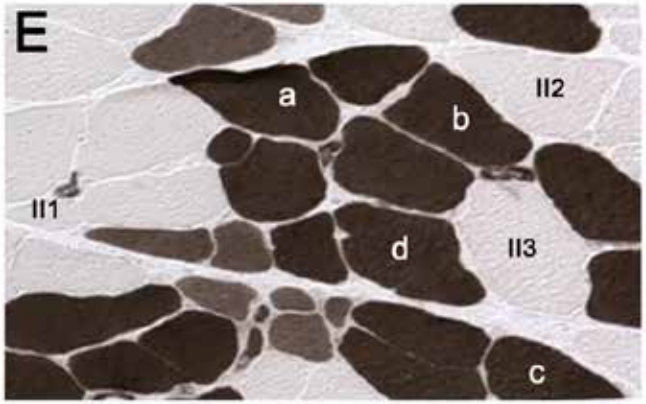
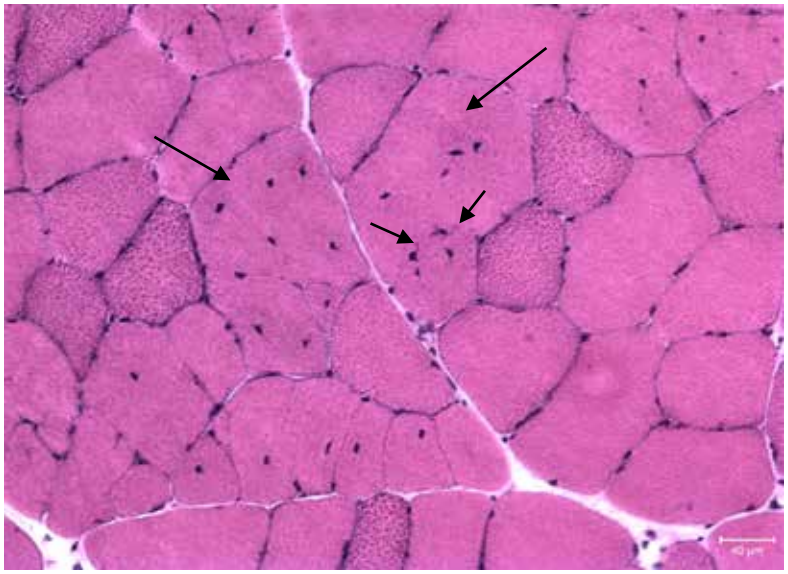
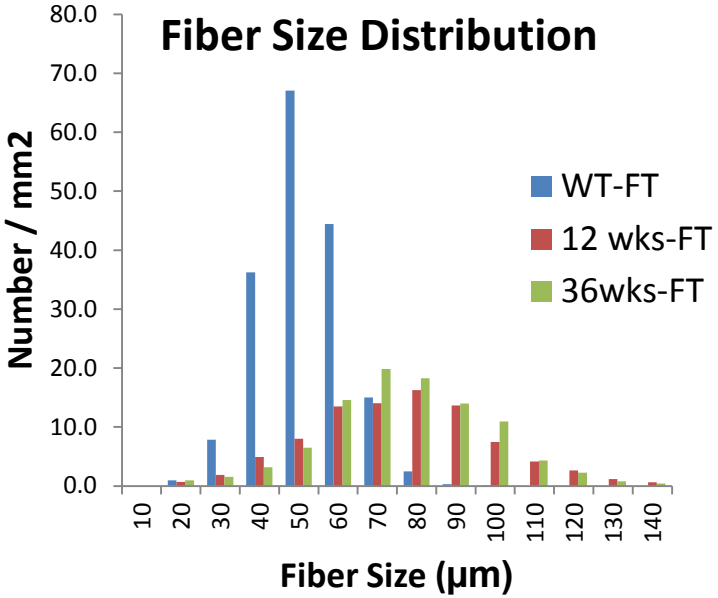



E



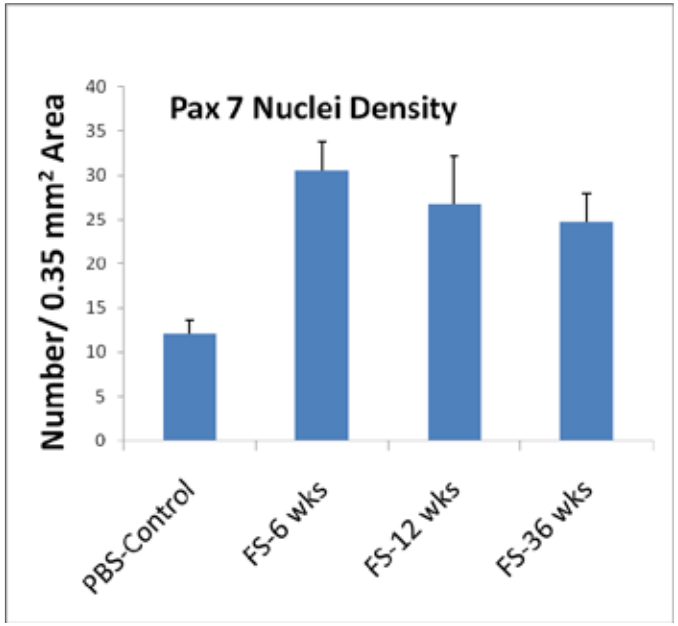
# Fiber Hypertrophy and Satellite Cells

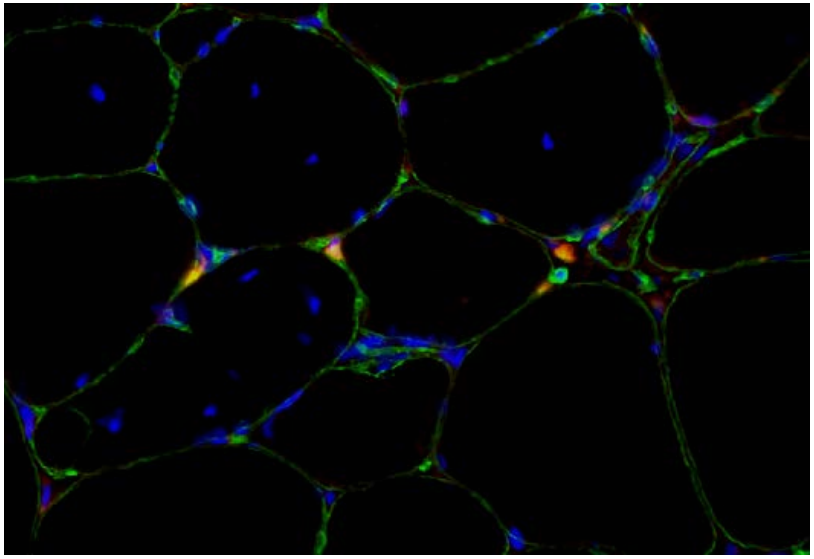


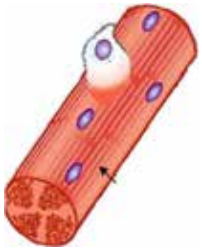



Multinucleated hypertrophic cells following AAV1.CMV.follistatin gene therapy

# Satellite Cells in Muscle Hypertrophy



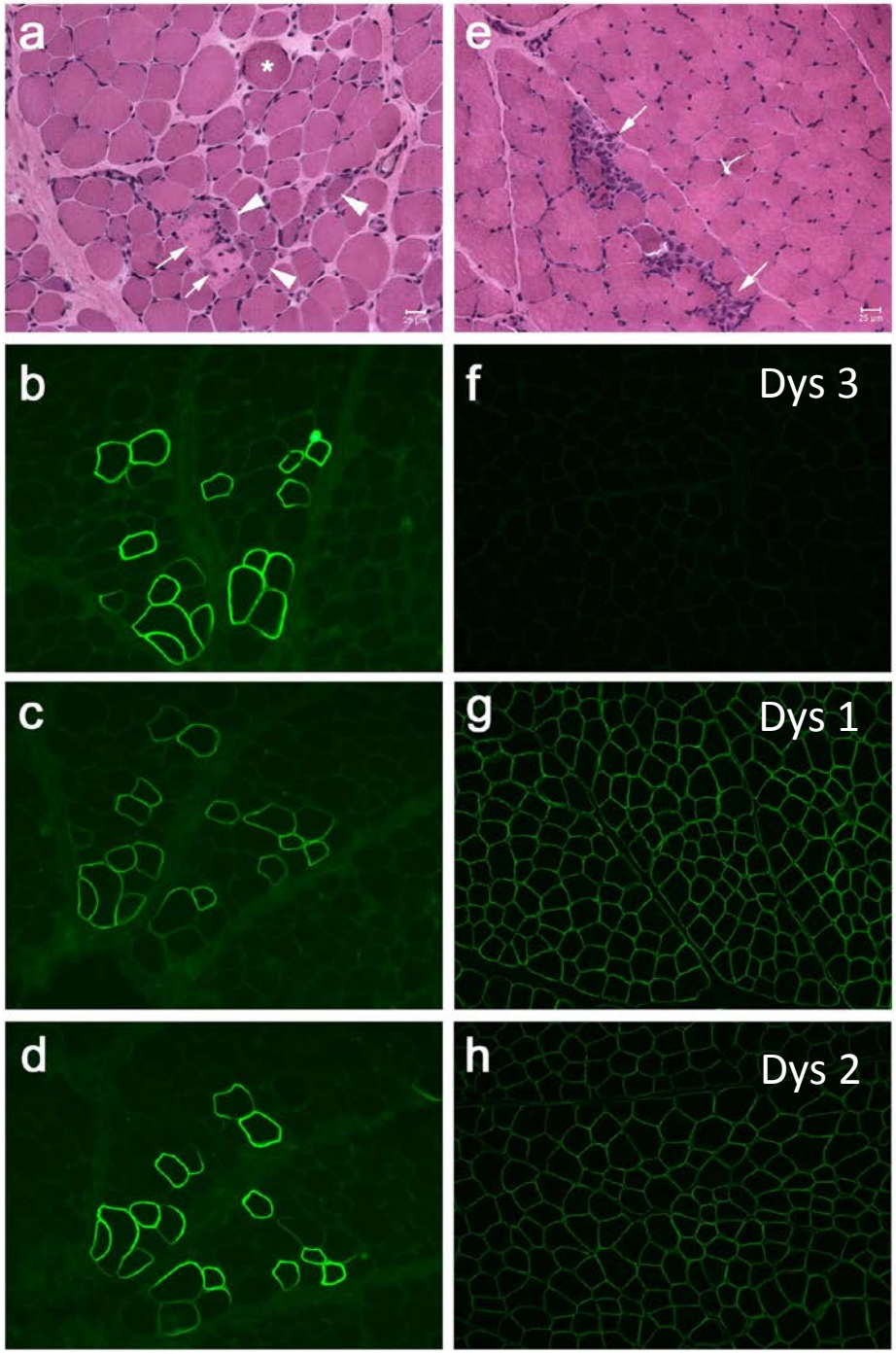


Follistatin induces muscle hypertrophy through:

- SC proliferation, Mstn and Act inhibition
- Overexpression in muscle lead to increased DNA & muscle protein content and increased fiber size

- <u>The nuclei are contributed to by satellite cells that the muscle fiber incorporates as it grows in size.</u>

Immune stains:
Dystrophinopathies

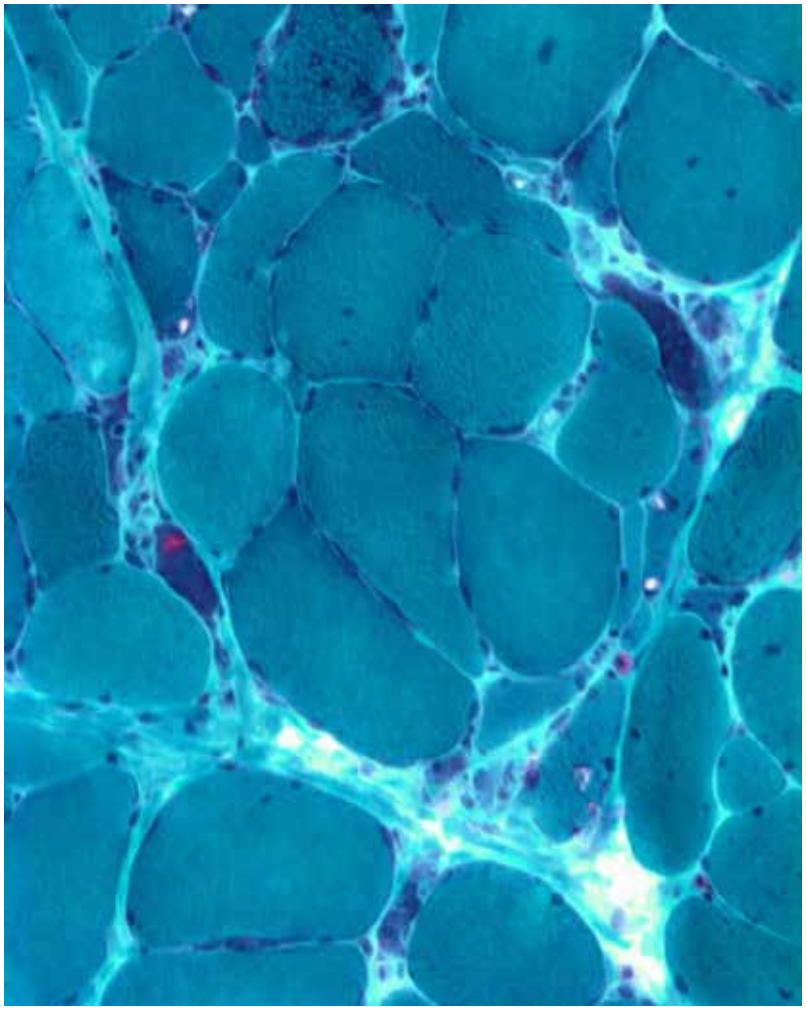


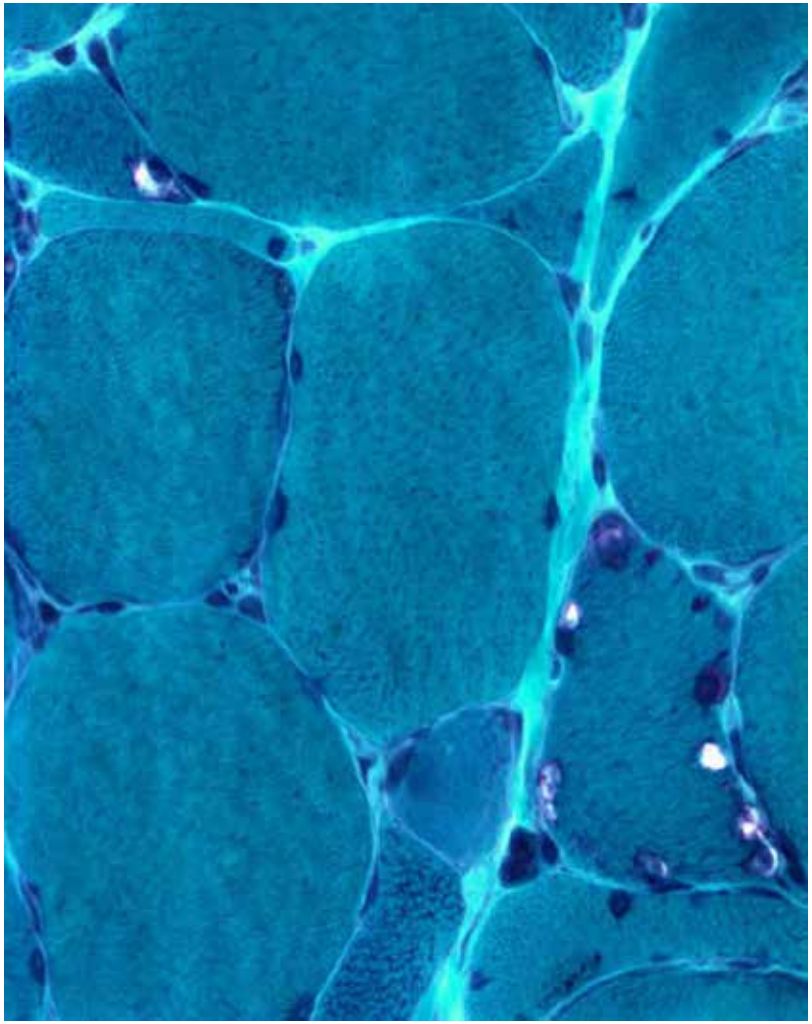

DMD
Exon 55-63 duplication

BMD
Exon 19-29 duplication

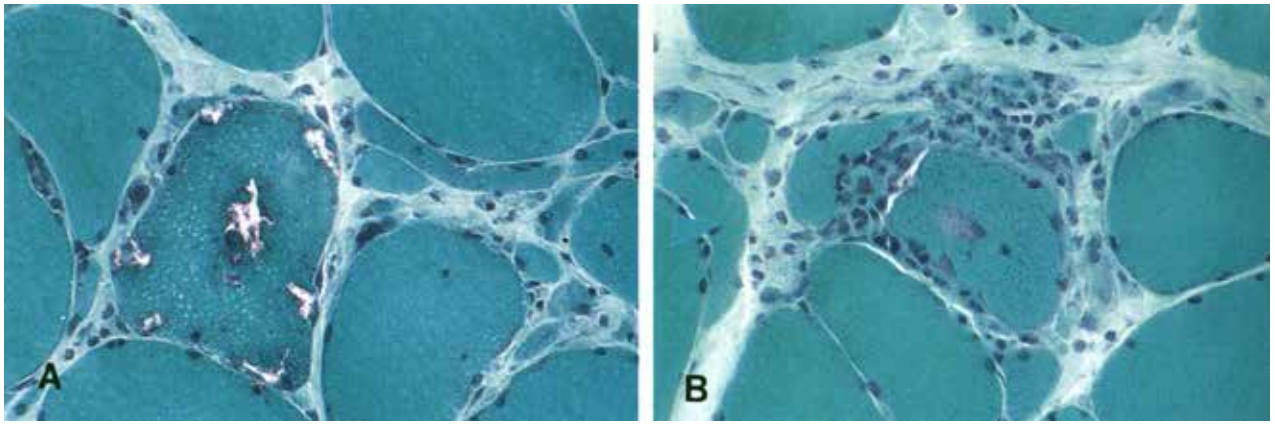
# Structural abnormalities: vacuoles

C09-103 Inclusion body myositis

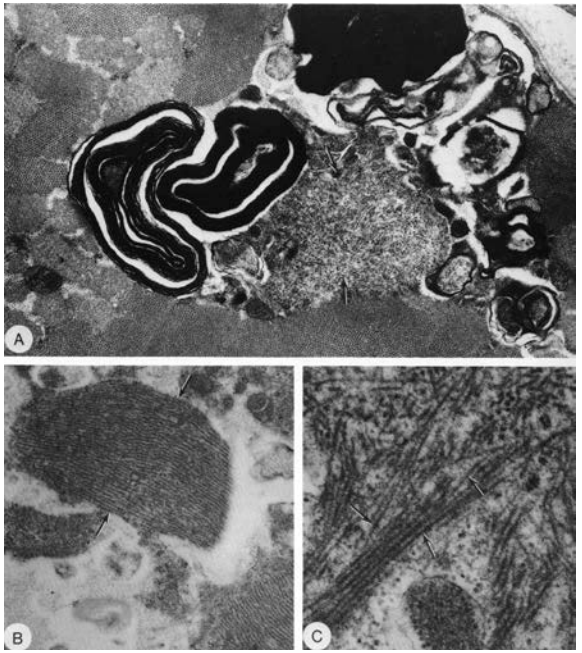




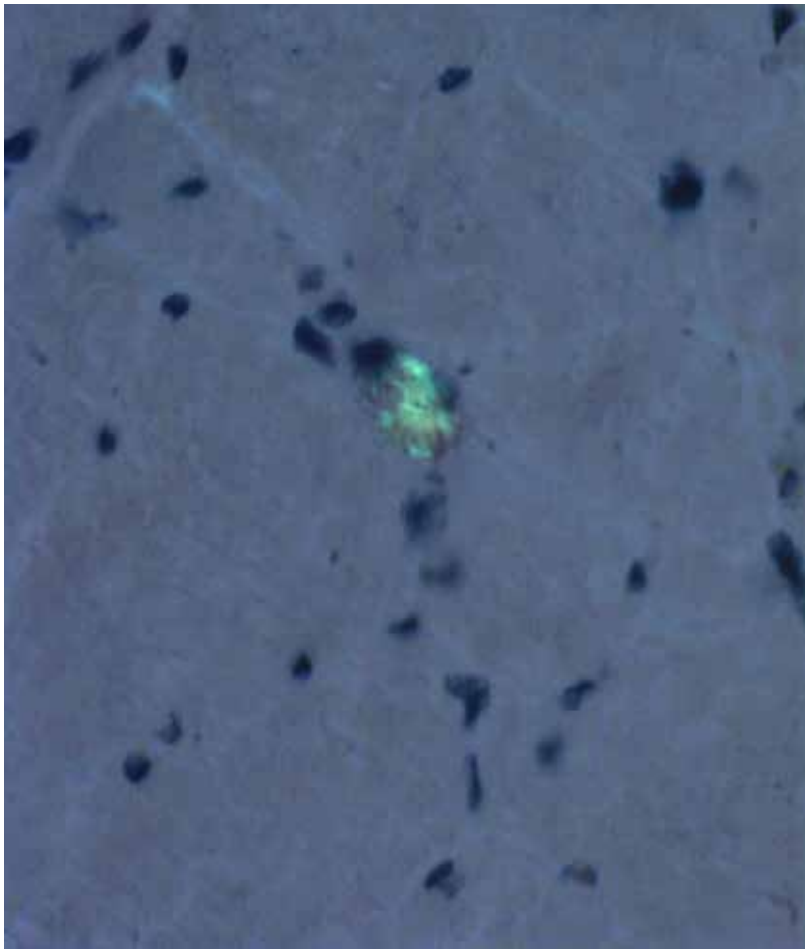
# Inclusion Body Myositis (IBM)

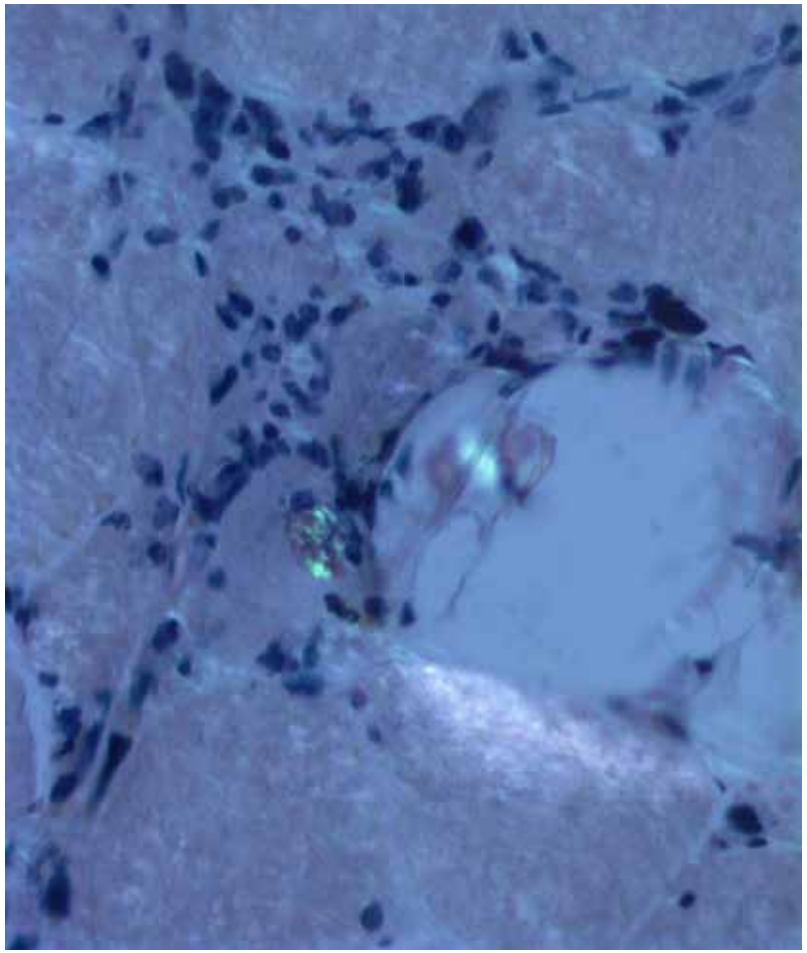



- The term IBM coined in 1971 by Yunis & Samaha
- Histopathologic differentiation from PM by:
  - vacuolated fibers
  - Nuclear and cytoplasmic fibrillary inclusions, which are congophilic



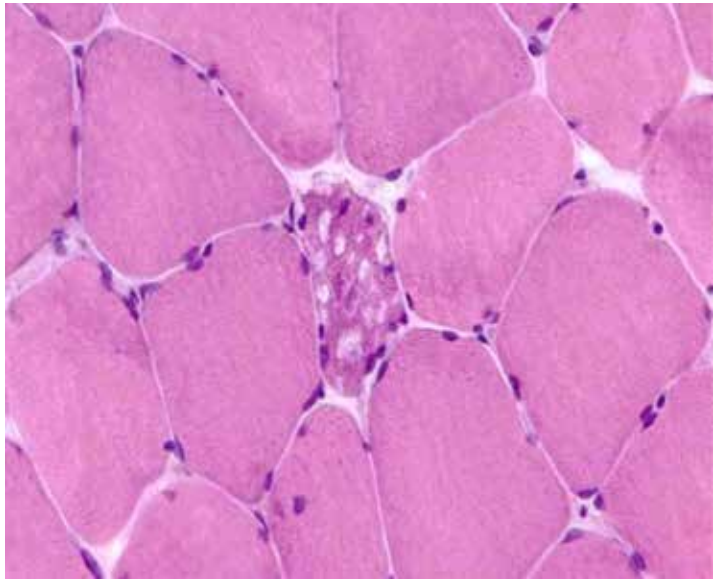
# Structural abnormalities: vacuoles

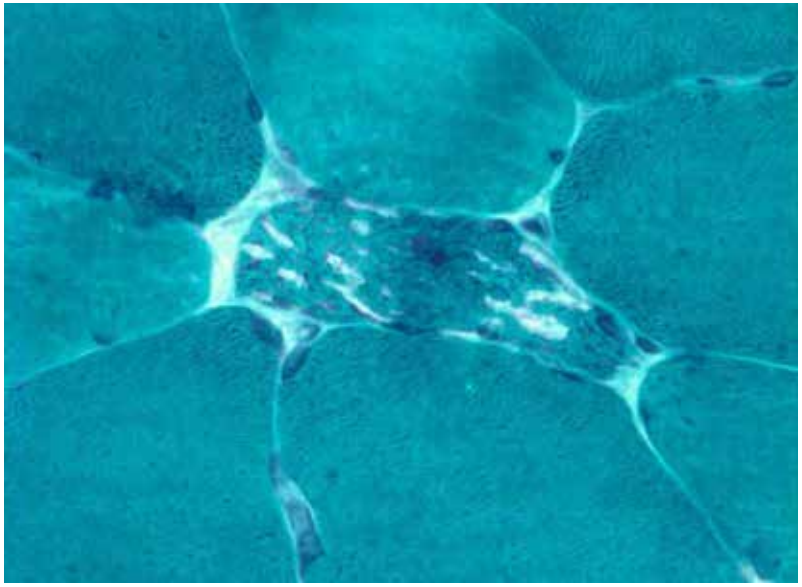




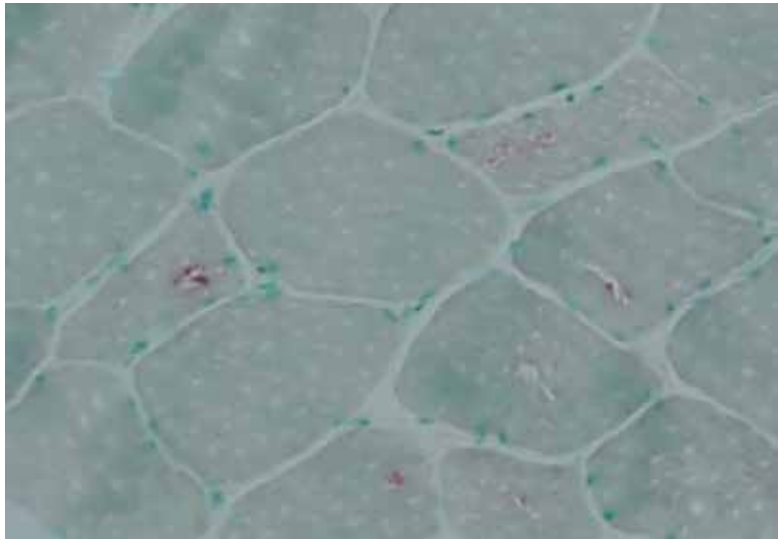
C09-103, IBM, Congo red stain

# Structural abnormalities: vacuoles



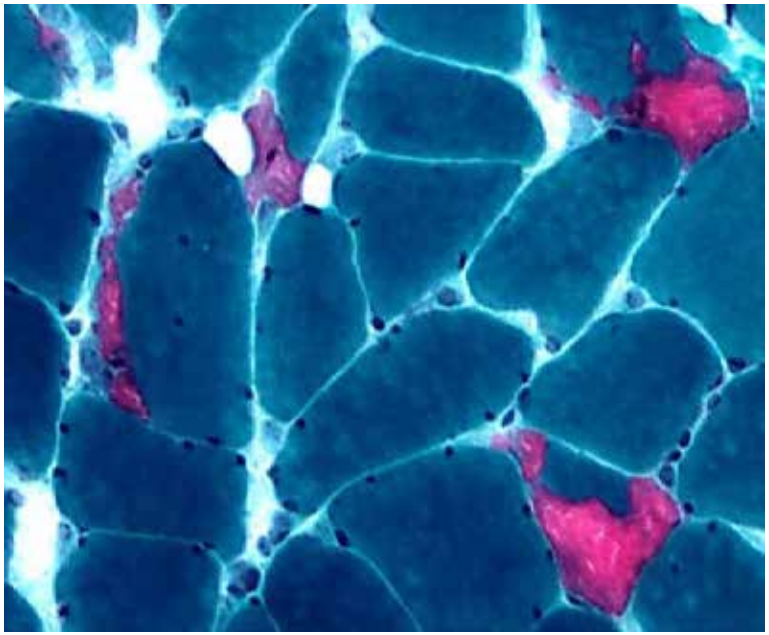


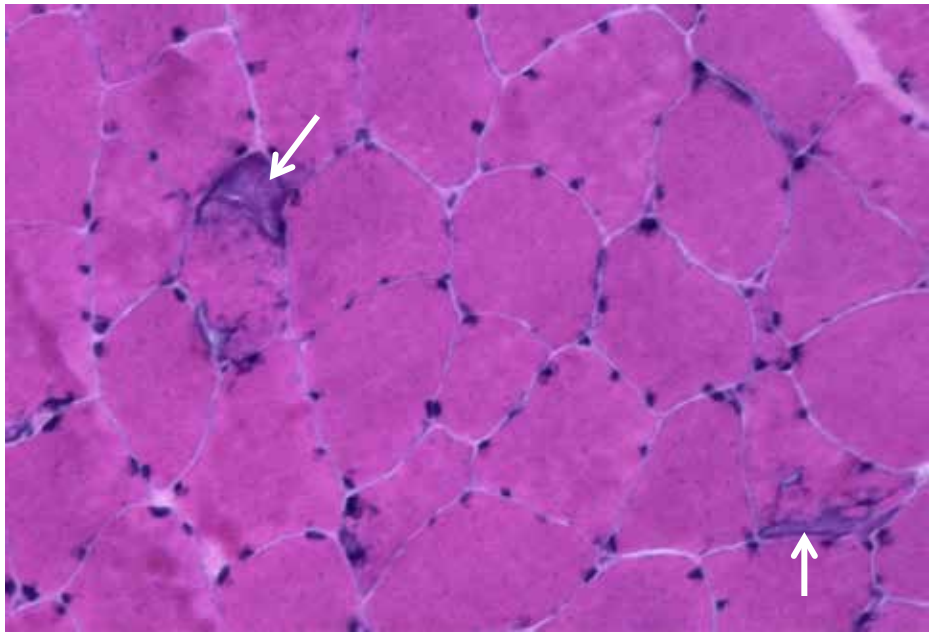
C09-115
Adult onset
acid maltase
deficiency

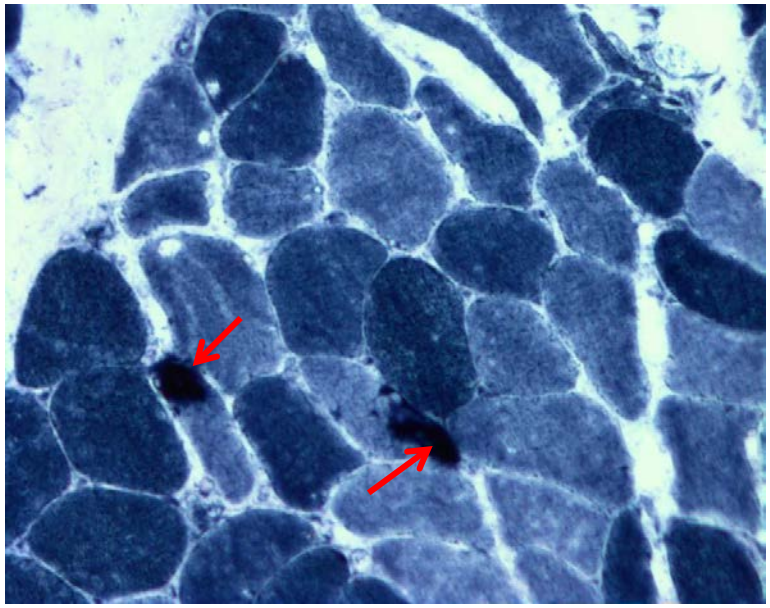


Acid phosphatase

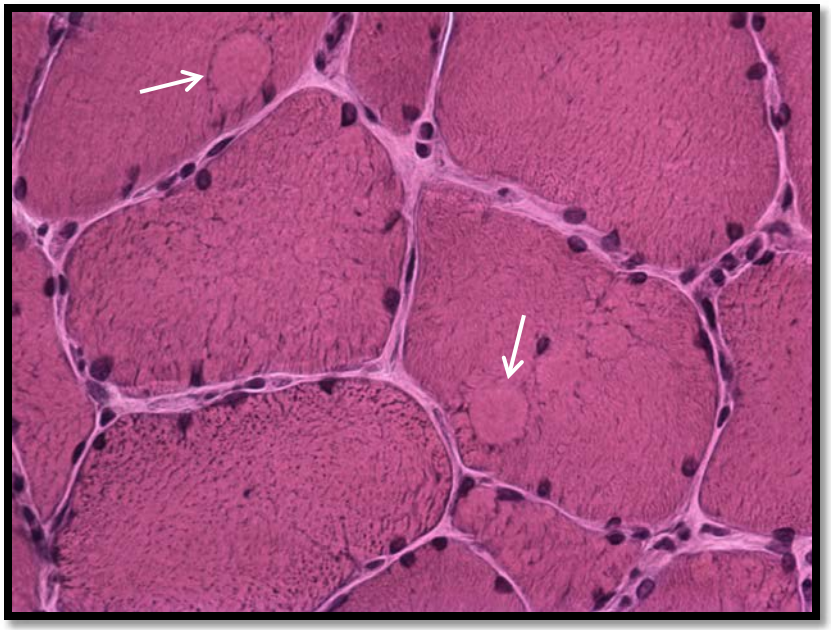
# Structural Abnormalities: Tubular Aggregates

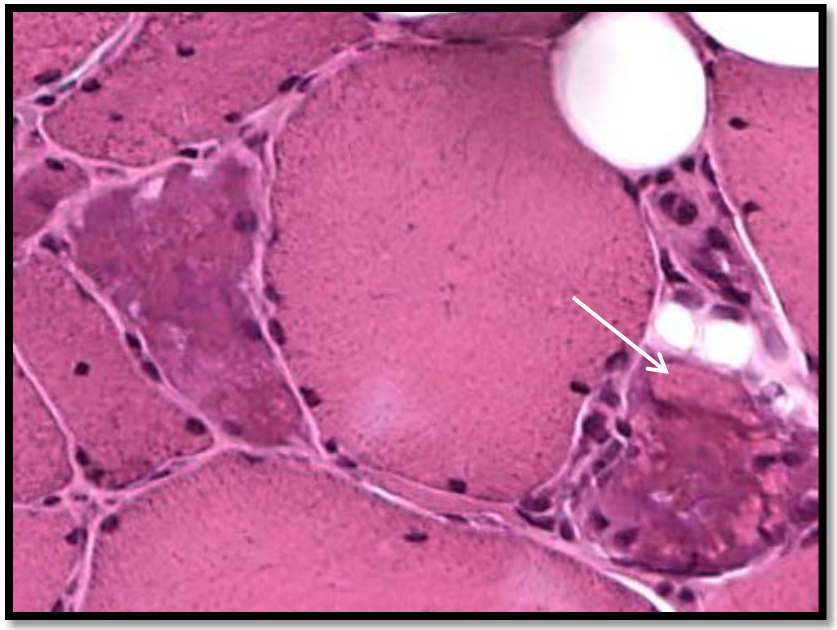






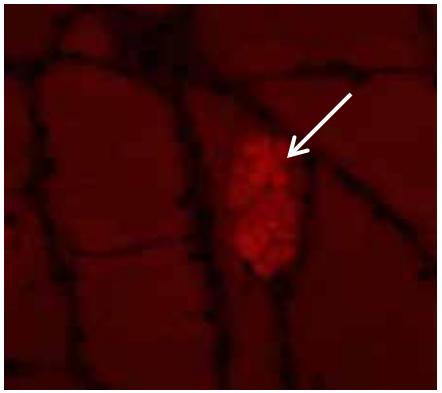
# Structural Abnormalities: Protein Aggregate Myopathy (PAM)



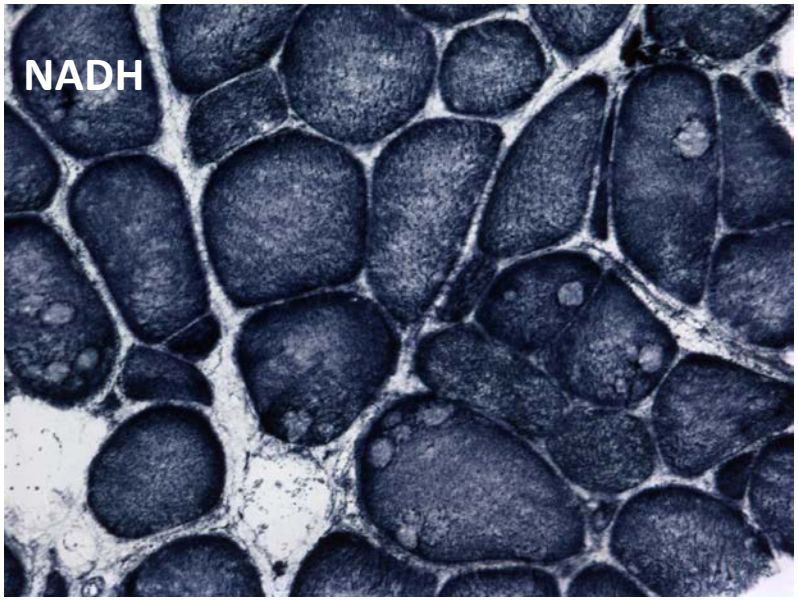


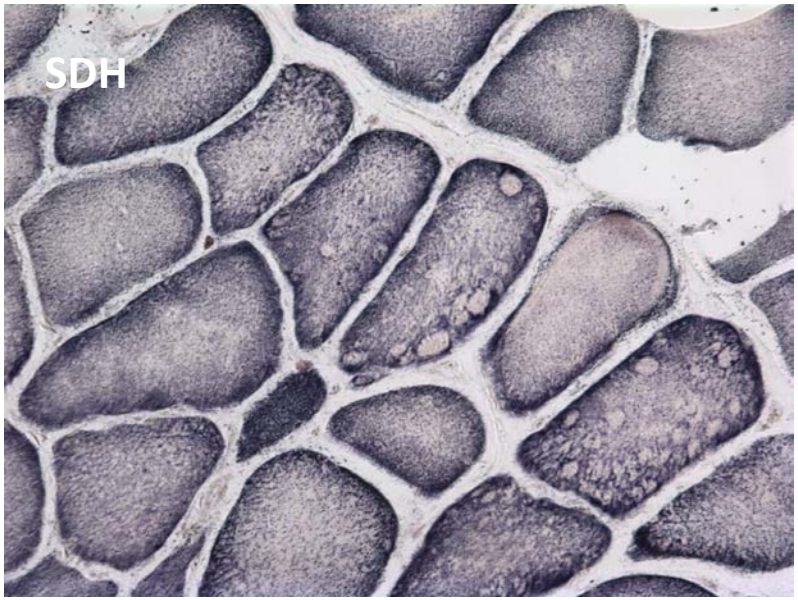
**Myofibrillar Myopathies**

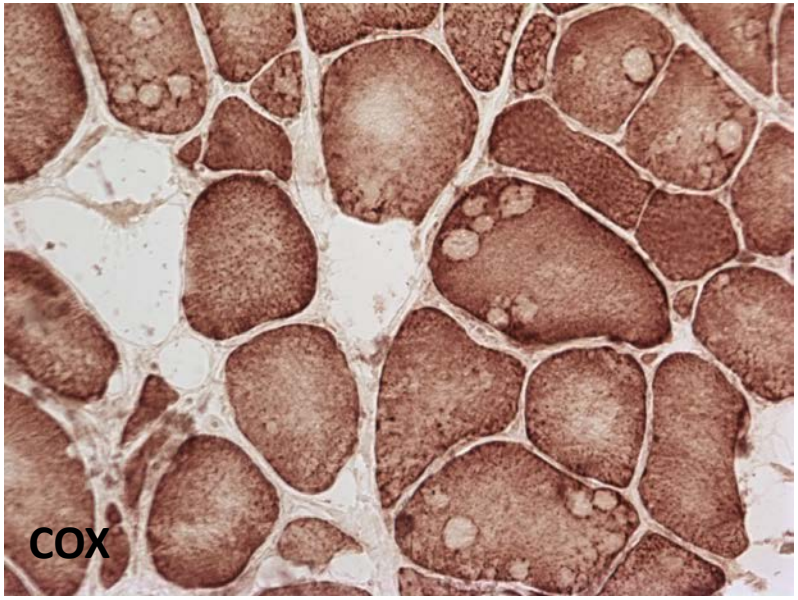
- Desmin
- αB-crystallin (HSP20)
- Myotilin
- ZASP (Z-band alternatively spliced PDZ)
- Filamin (filamin C)

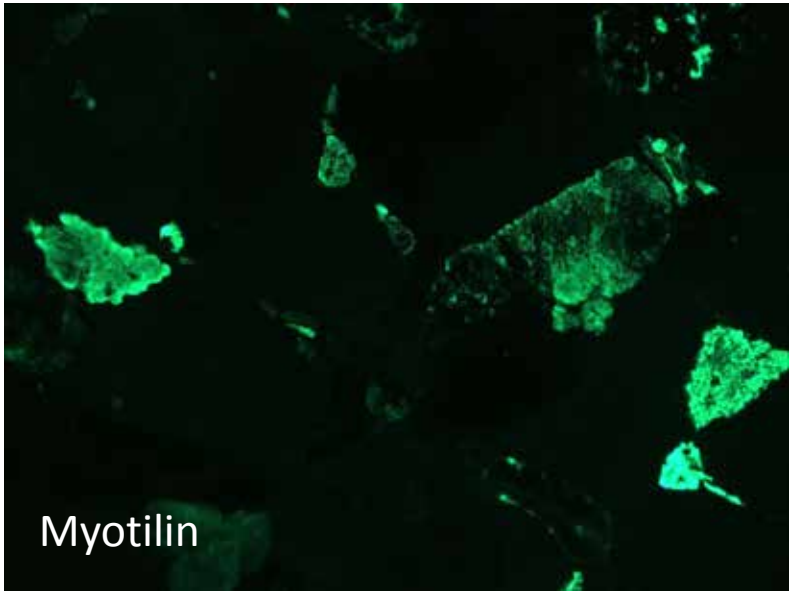


# Structural Abnormalities: Protein Aggregate Myopathy (PAM)

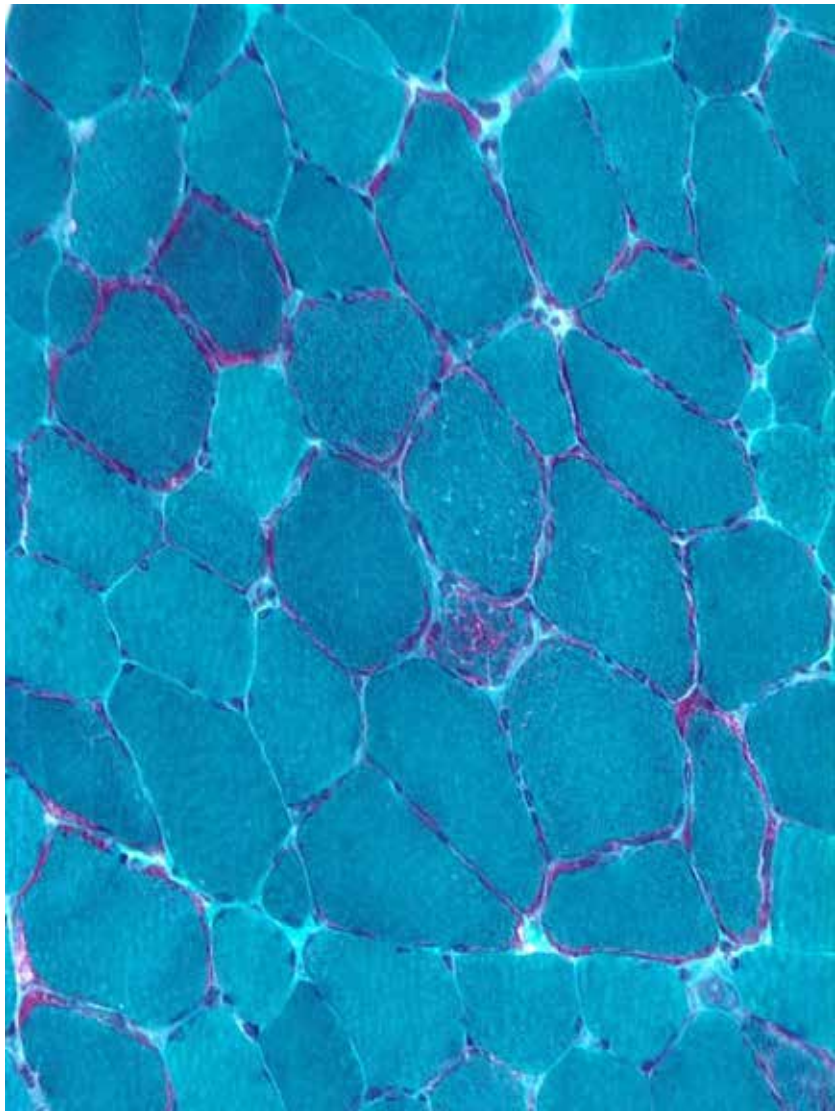


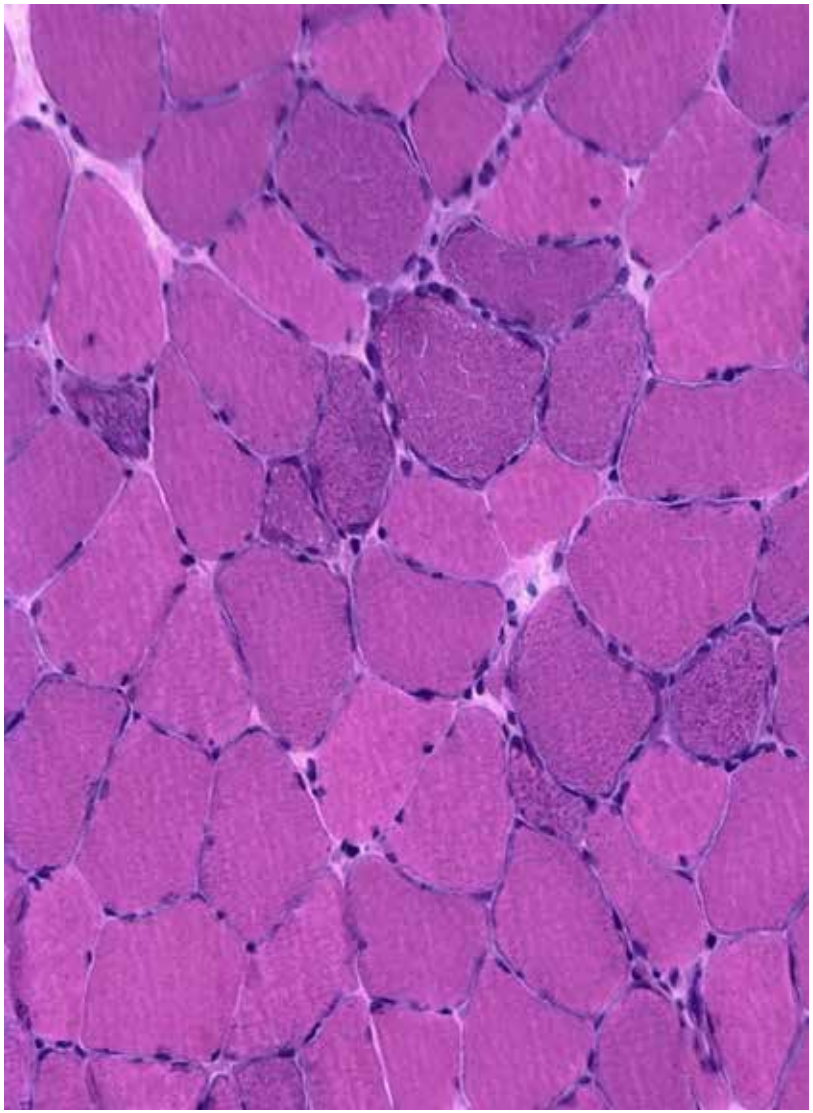



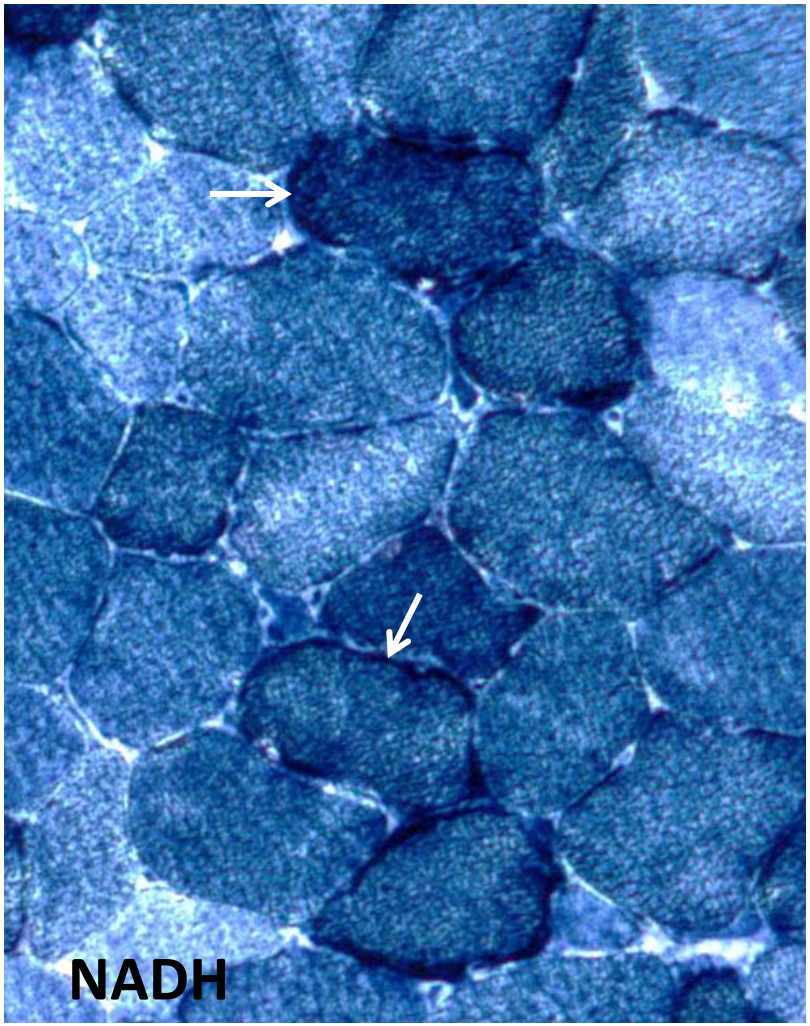
# Organelle change: Mitochondria content and distribution

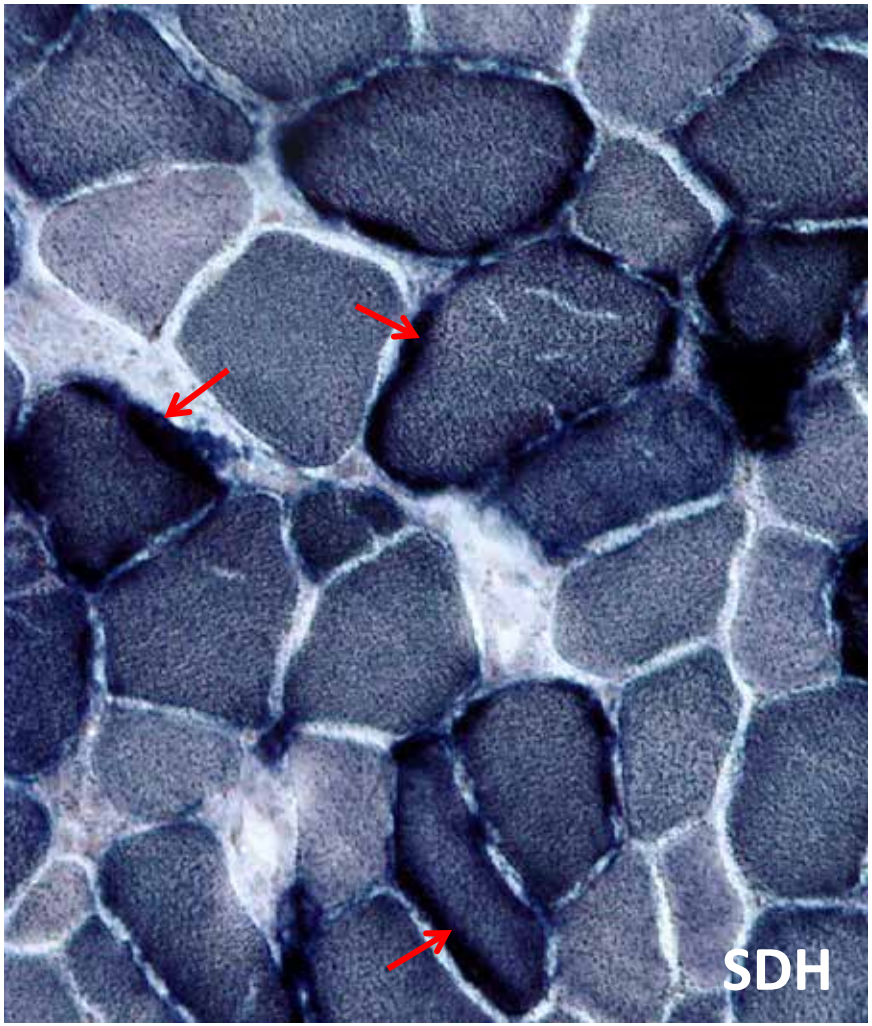




C10-33 Mitochondrial myopathy

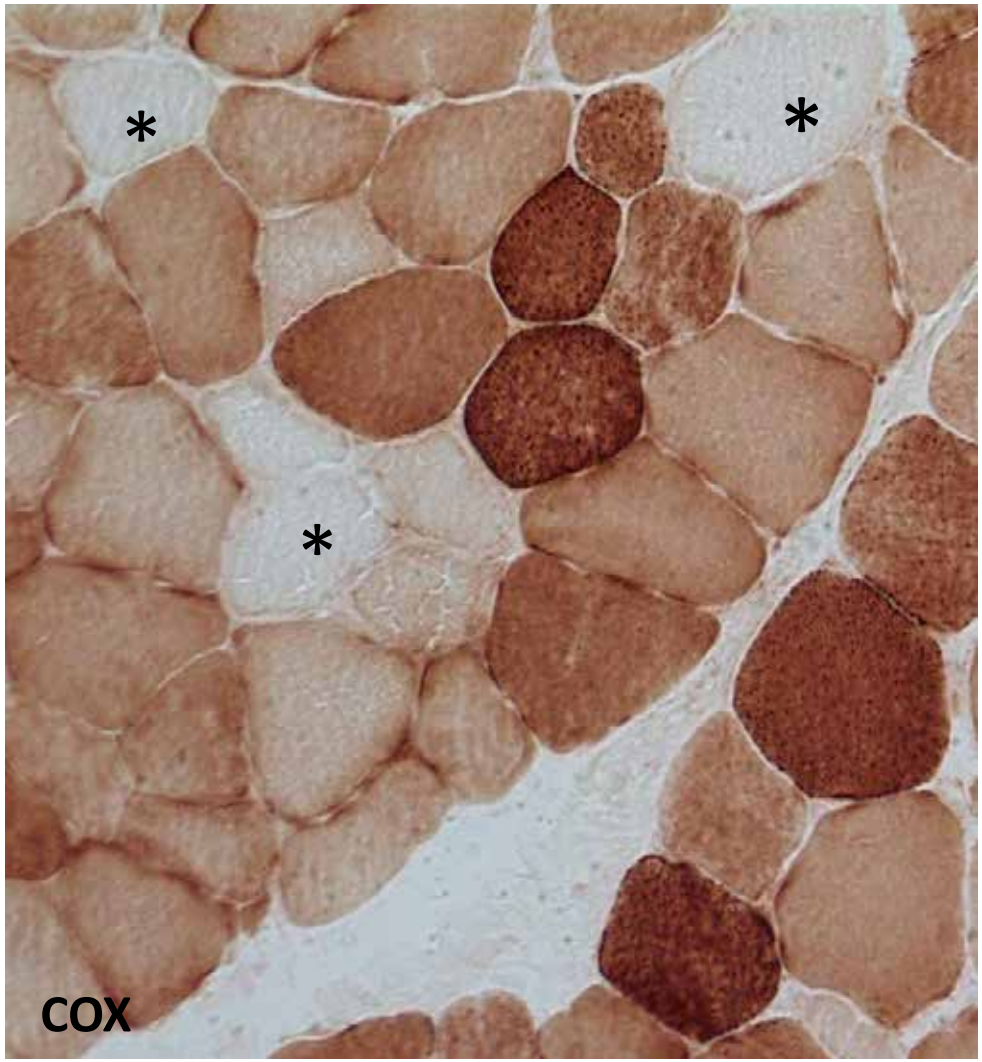
# Organelle change: Mitochondria content and distribution

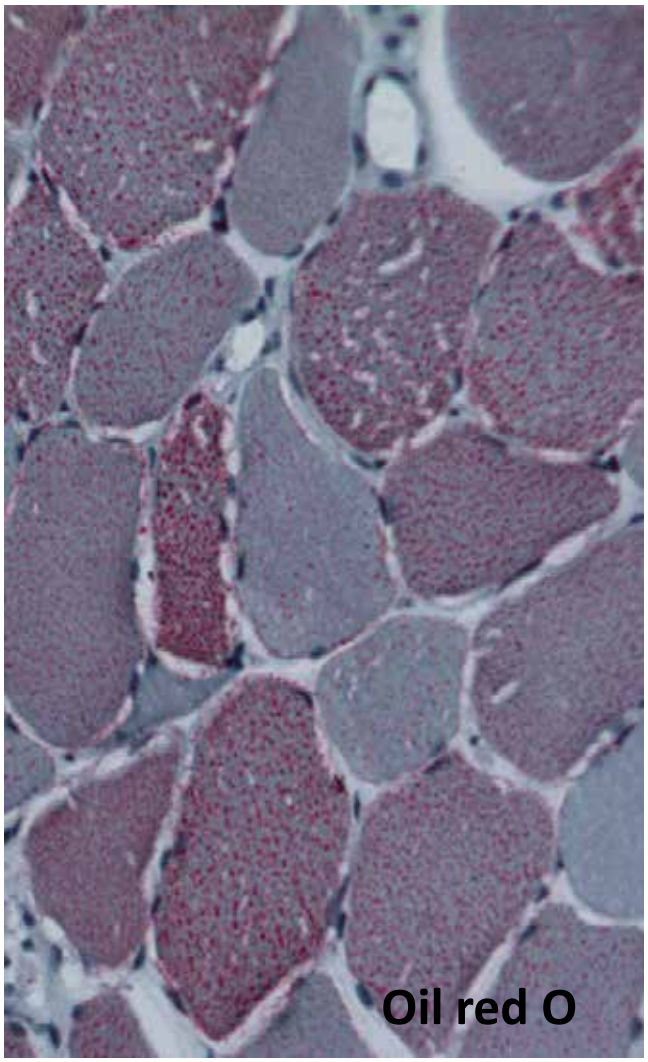



C10-33 Mitochondrial myopathy

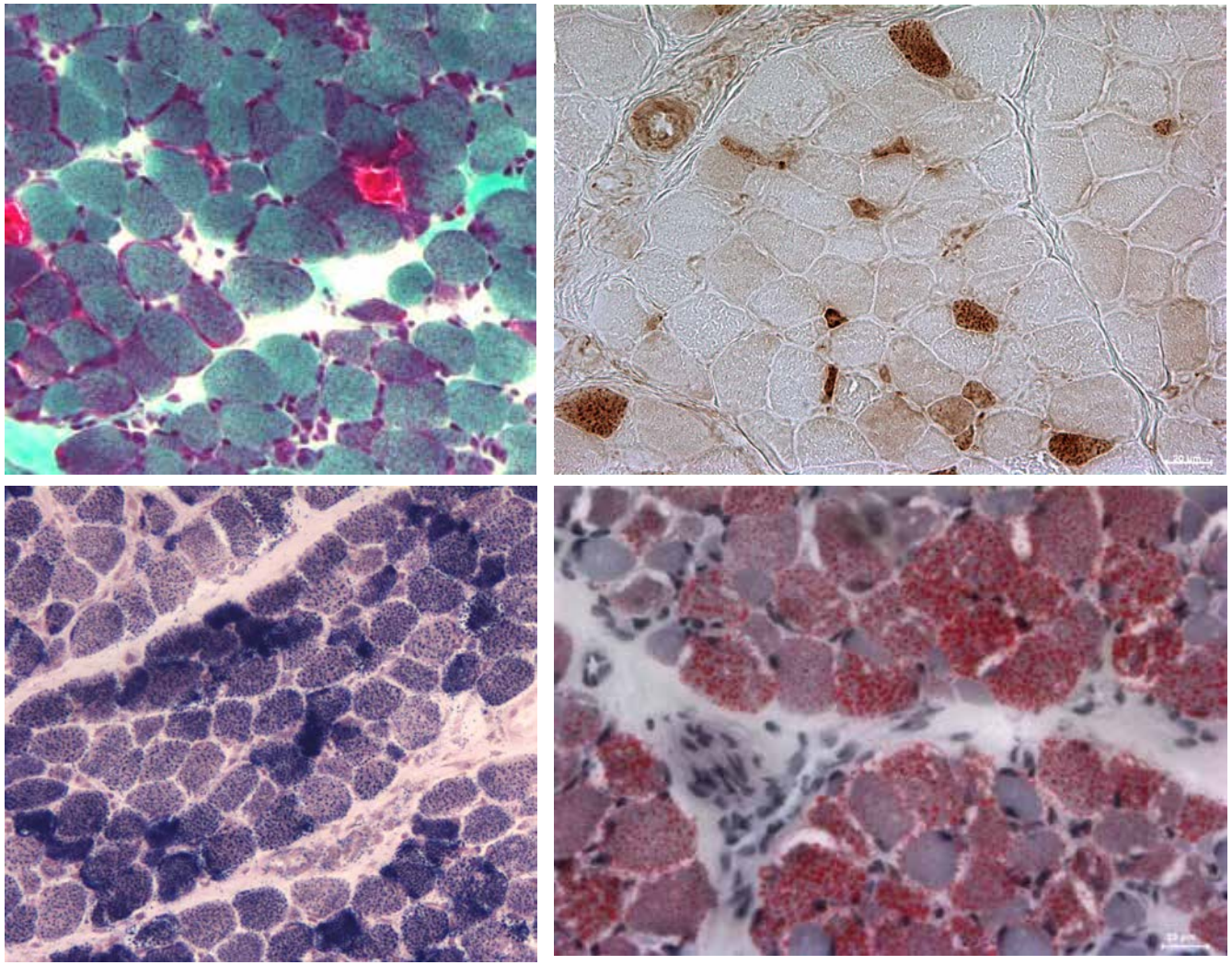
# Organelle change: Mitochondria content and distribution



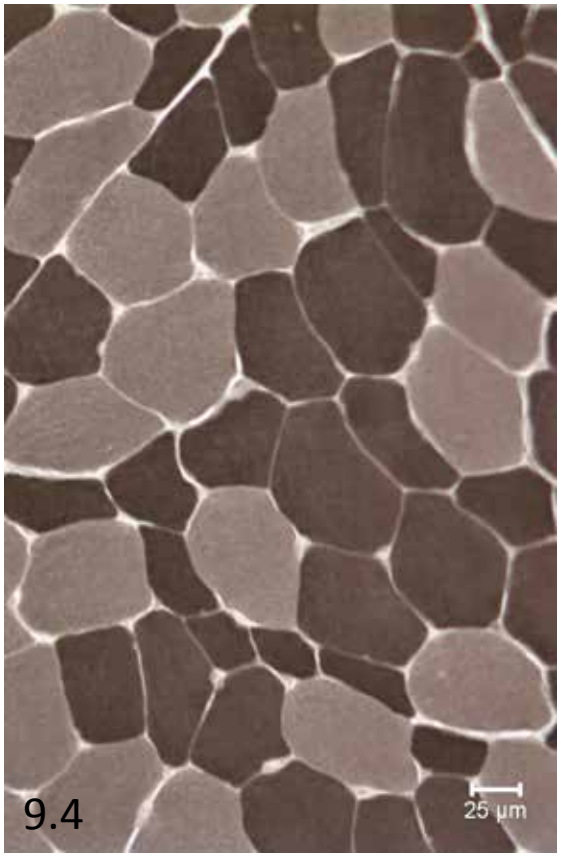

C10-33 Mitochondrial myopathy

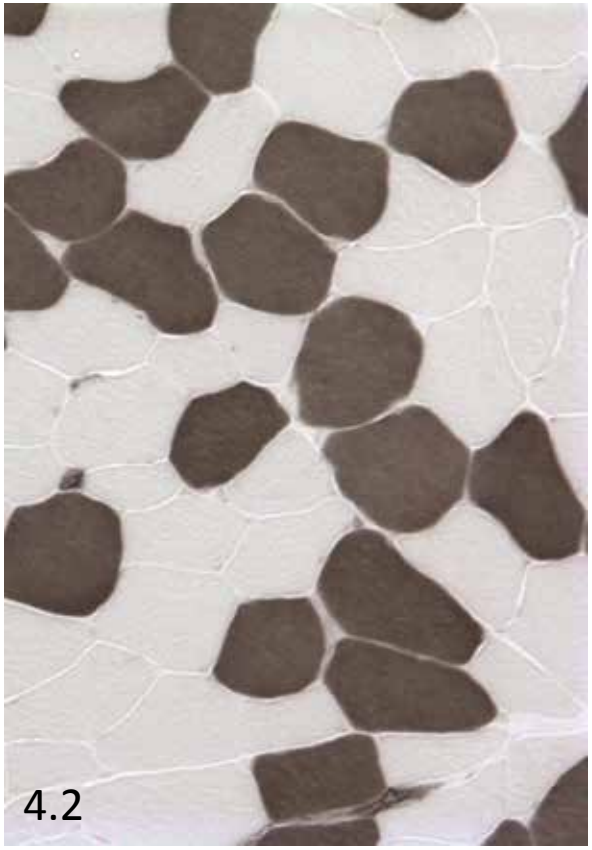
# Organelle Change : Mitochondria content and distribution

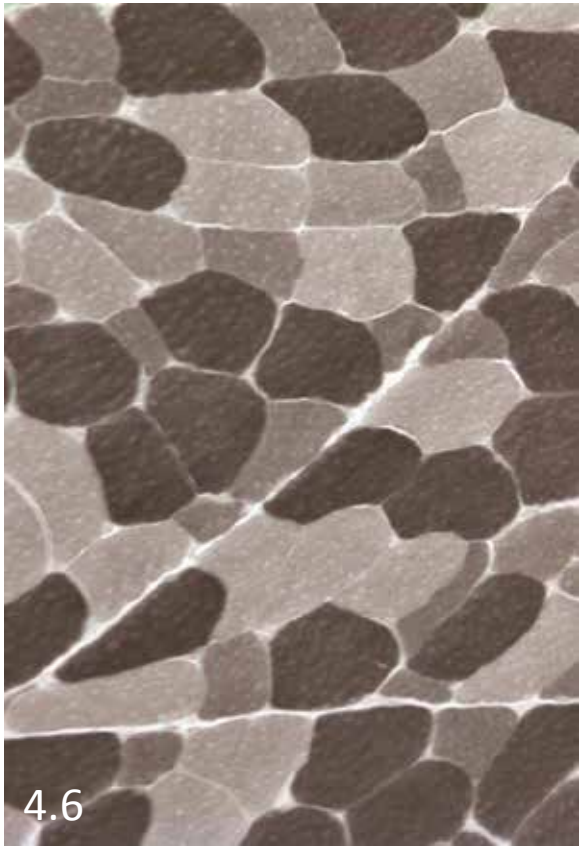


C05-97 Thymidine Kinase 2 deficiency

## Muscle Fiber Types

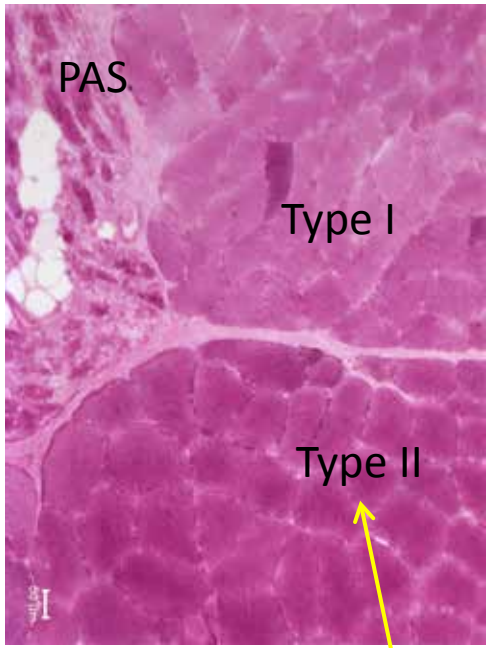


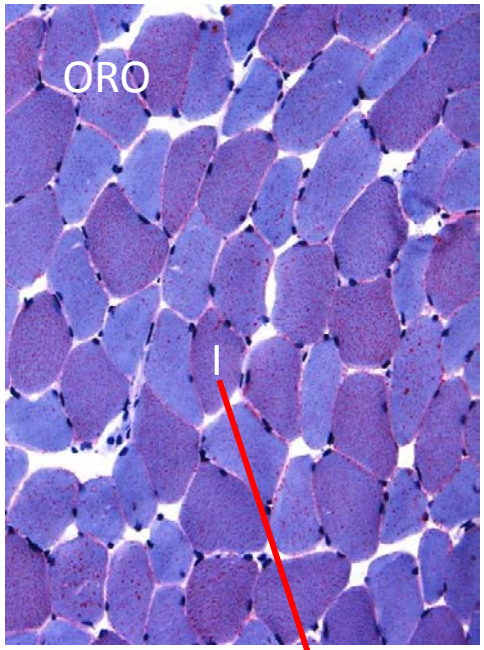



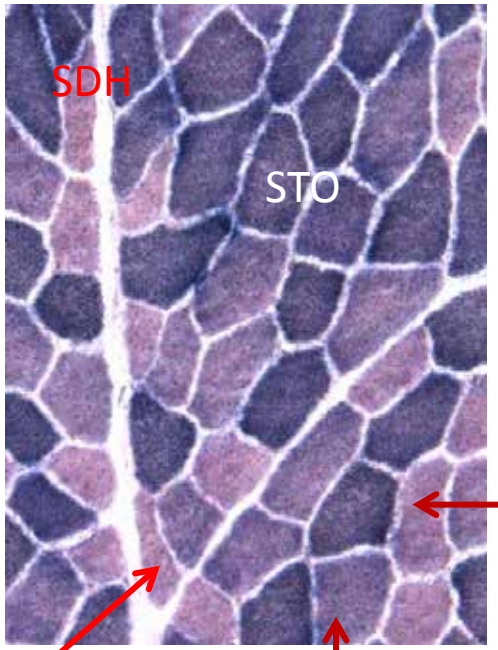




Myofibrillar ATPase

# Muscle Fiber Types

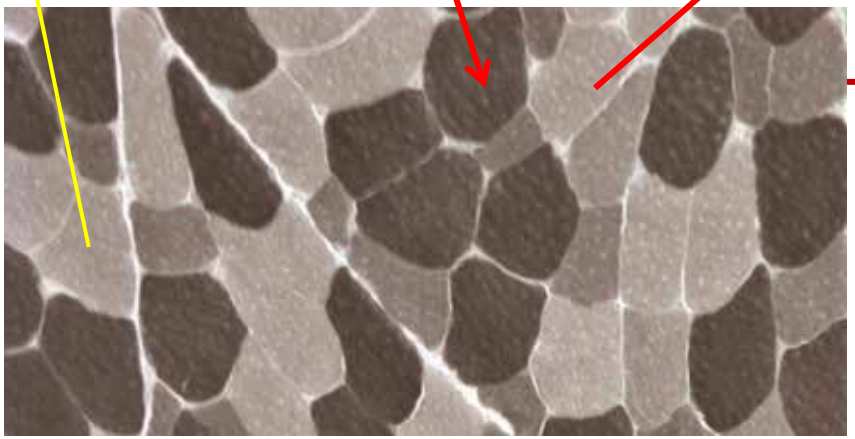



STO/type I (high lipid, mito.)

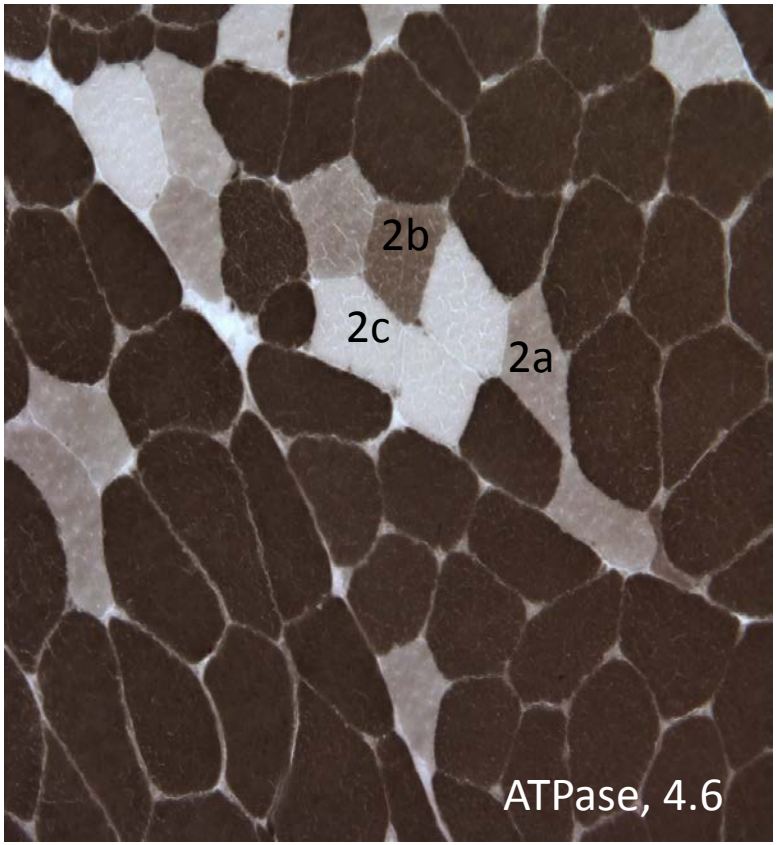
FTG/type IIA
FTO/type IIB
(high glycogen)



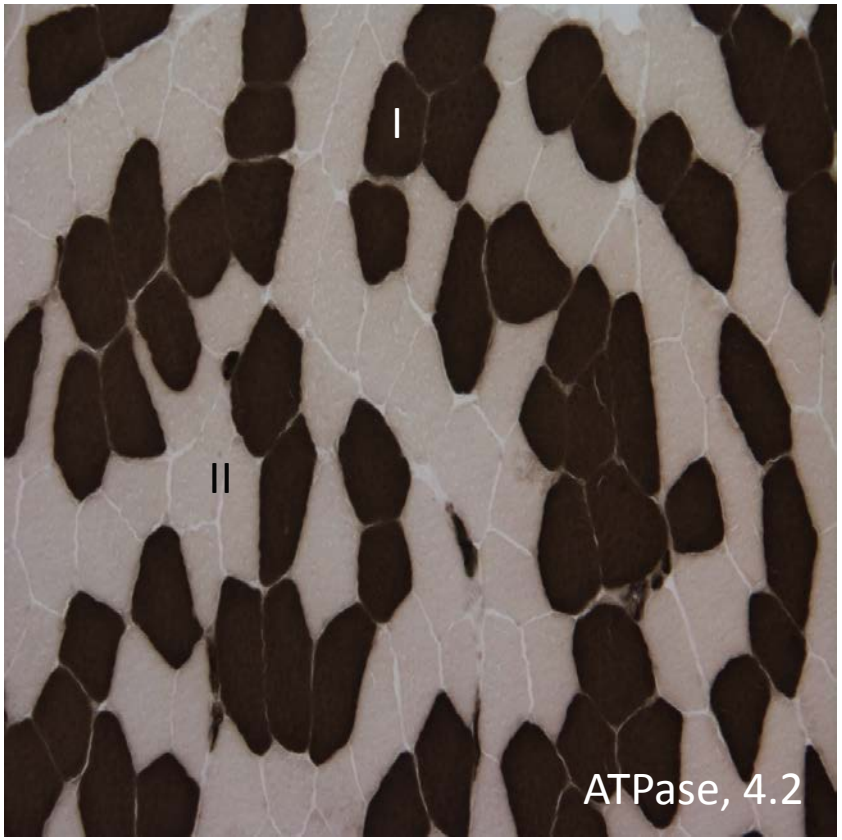
ATPase 4.6

# Fiber Types and Performance

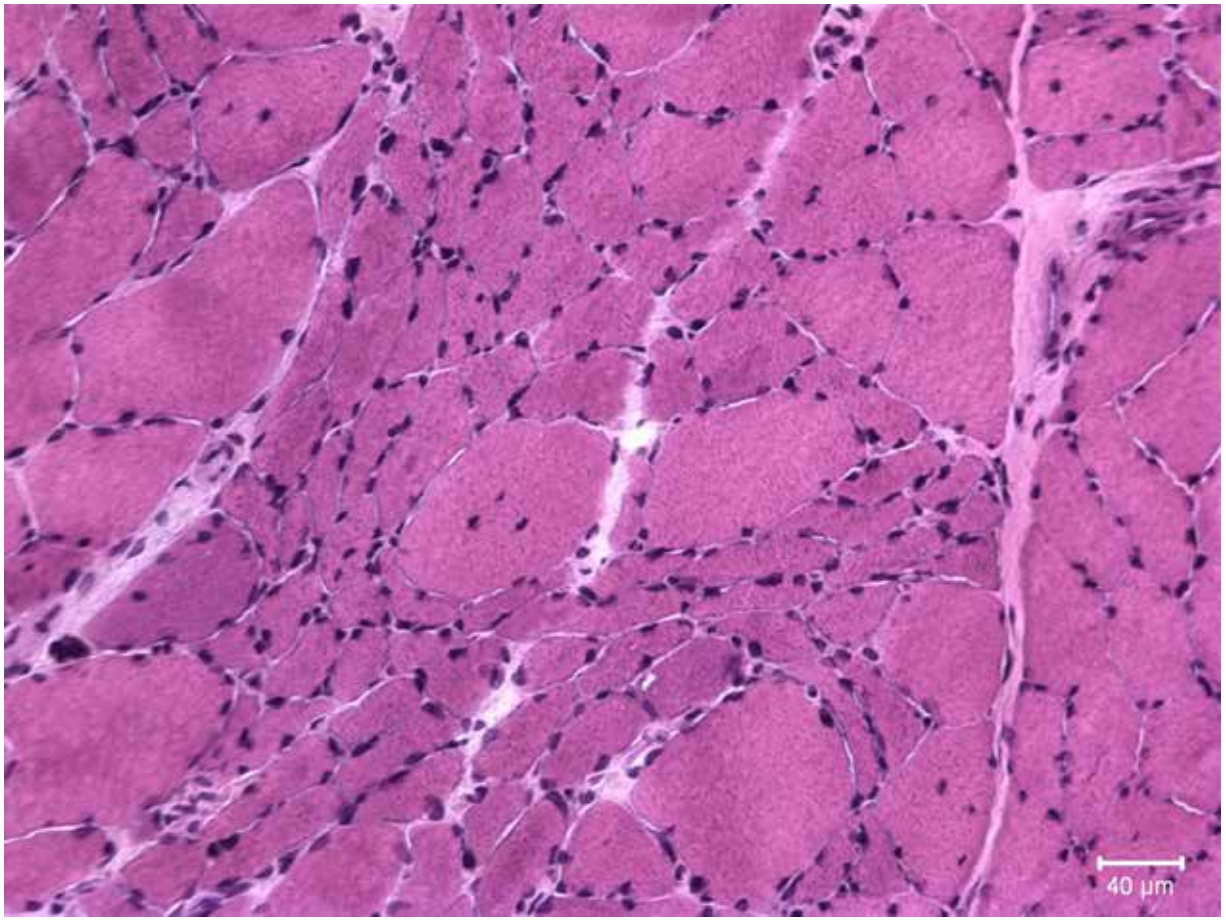
Endurance Athletes



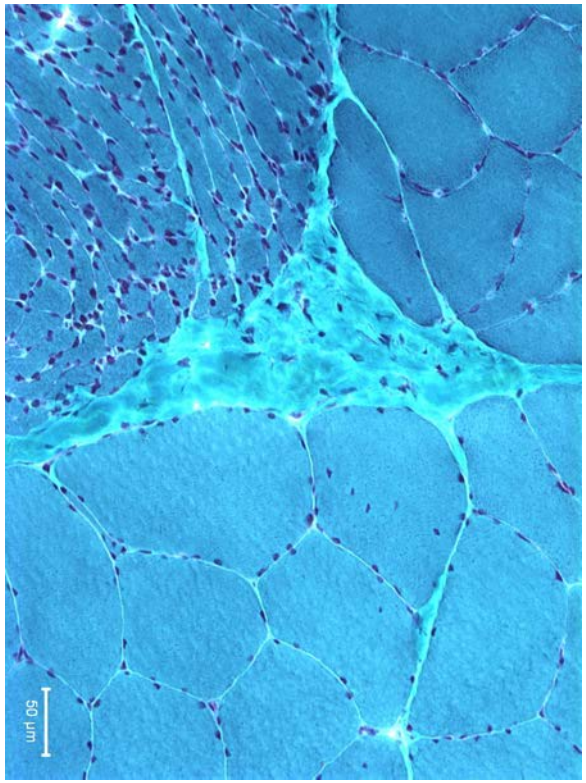

Weight lifters

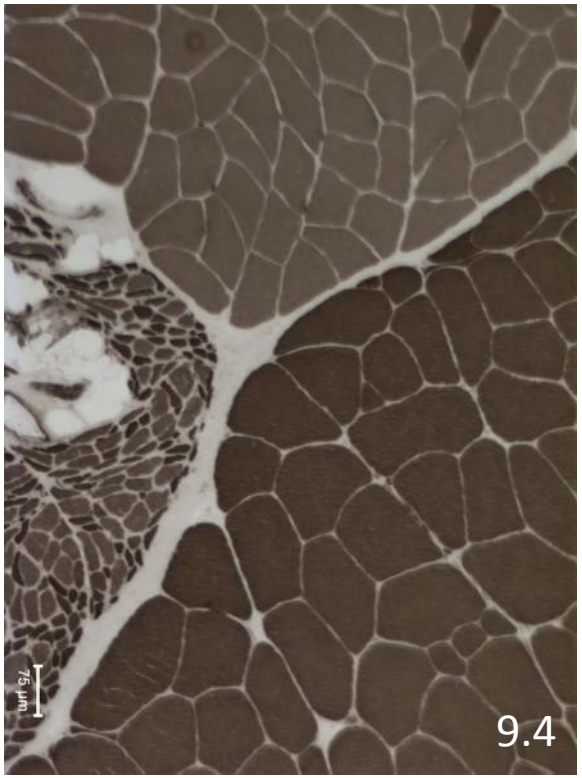


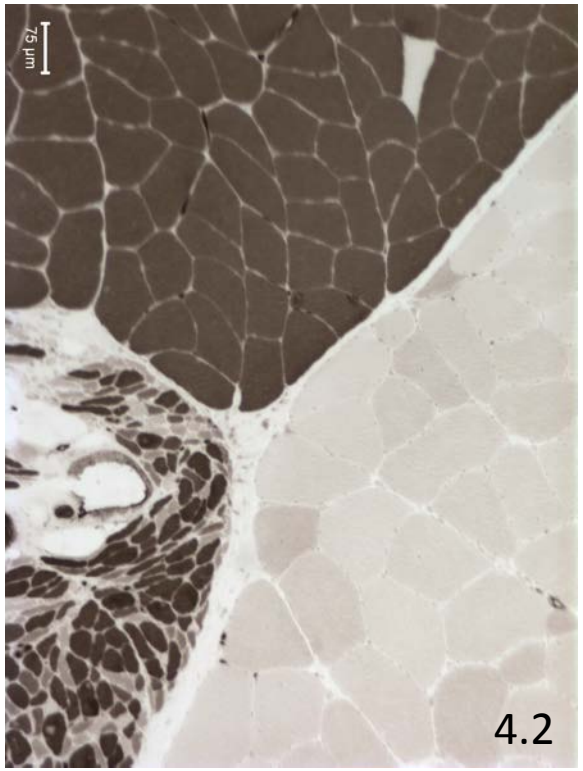
# Neurogenic Changes



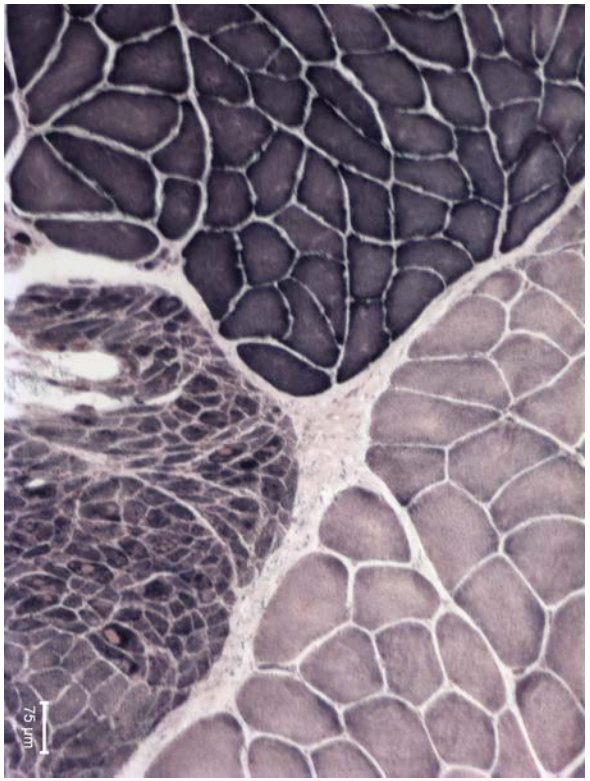
Group atrophy and muscle fiber type grouping

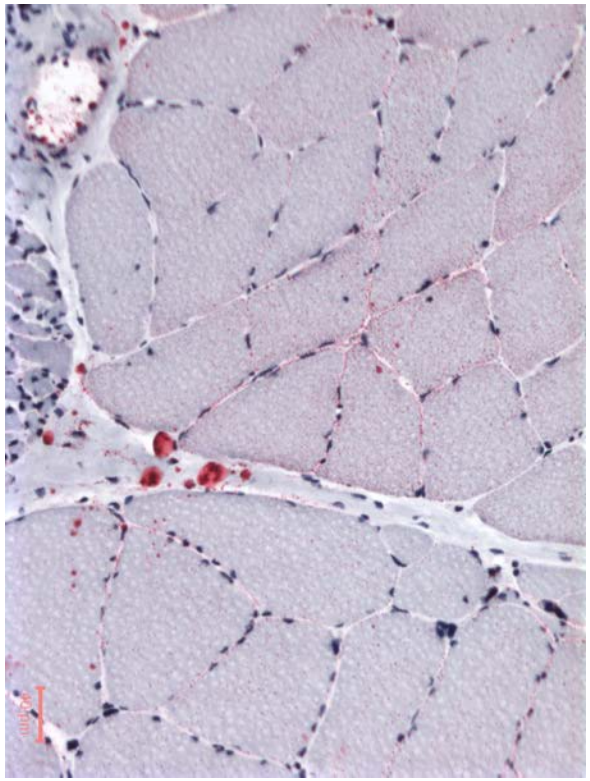


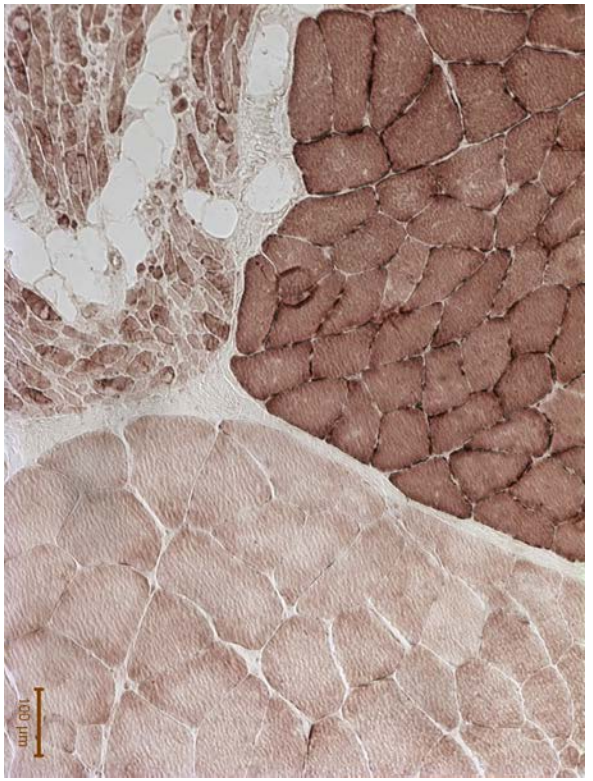



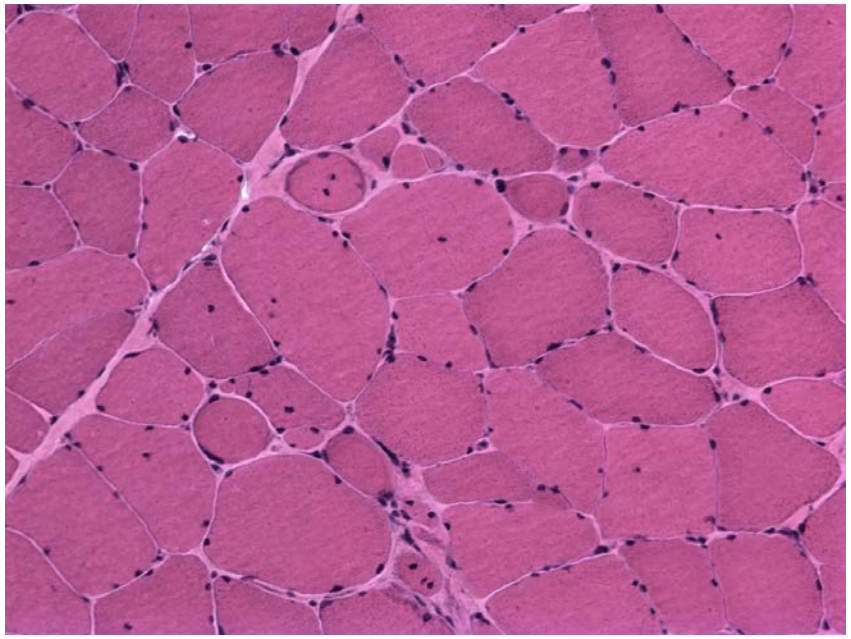
Group atrophy and muscle fiber type groupings



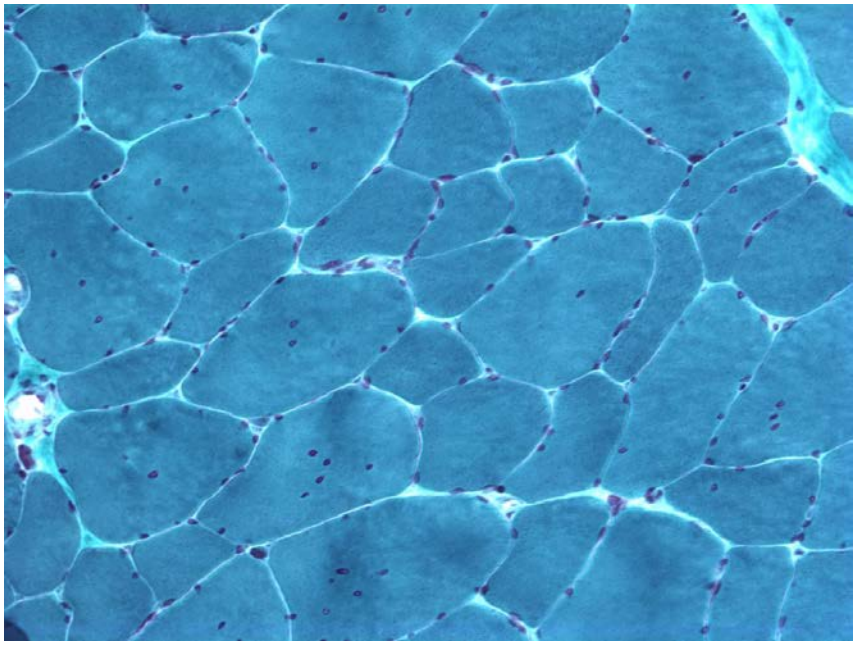


DM1



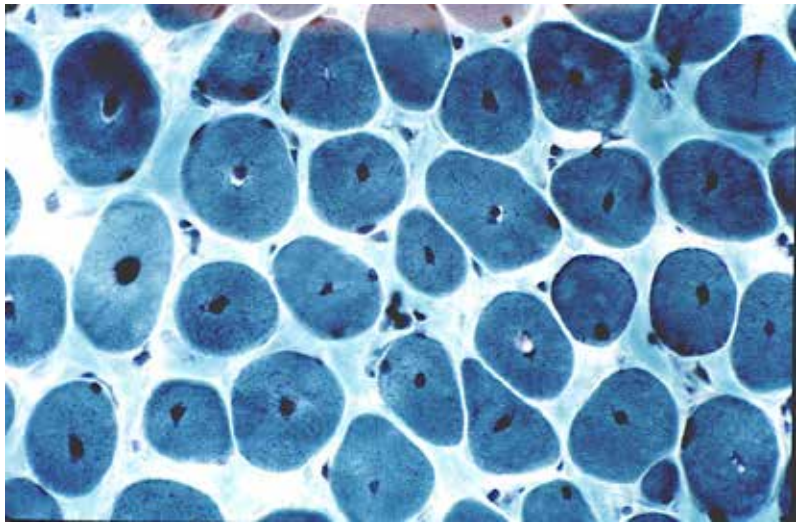
> CTG $_{4\text{-}37}$ repeats in the terminal exon of DMPK gene

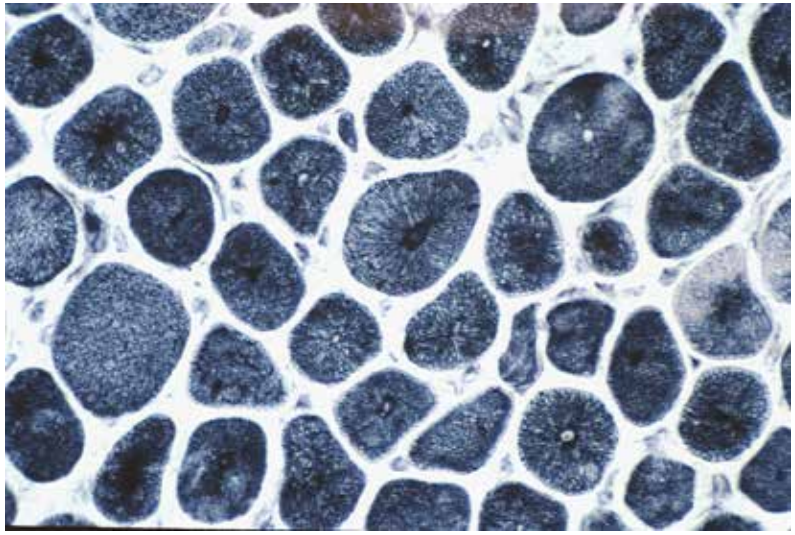
DM2 (PROMM)



> 104 to 176 bp CCTG repeats in intron 1 of exon of ZNF9 gene

# Congenital Myopathies





## Centronuclear Myopathy: X-linked

- Onset, infancy with severe hypotonia
- Mutations in MTM1
- Protein expressed in sarcolemma, I band, T-tubule triads, associated with endosomes
- Role in muscle fiber maturation

## Centronuclear Myopathy: Autosomal recessive

- Onset, infancy, childhood, adult

## Centronuclear Myopathy: Autosomal Dominant

- Onset, adolescence and adult
- Mutations in DNM2
- Protein associated with MTs, binds to BIN1, implicated in endocytosis and cell motility