Pathology of congenital myopathies and allied disorders

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Historical perspective and definition

- Shy and Magee; A new congenital non-progressive myopathy.
- Increased application of histochemical techniques in the 1950s and 1960s identified a group of conditions with shared clinical features and characteristic morphological changes in biopsies
- Congenital myopathies are a molecularly, clinically and pathologically heterogenous group of diseases defined by muscle weakness and hypotonia, usually present at birth or early childhood, with a static or slowly progressive clinical course, in association with a characteristic morphological defect
- Historically classified on basis of major morphological findings: rods (nemaline), cores (CCD and MmD), central nuclei, and selective type I hypotrophy (CFTD)

Challenges in classification

- Spectrum of pathological changes with known gene defects has widened eg. RYR1, MYH7, Titin
- New genes responsible for rare structural defects are identified
- Defects in the same gene can cause diverse clinical phenotypes eg. FHL1, RYR1, Titin
- The same pathological phenotype may result from defects in different genes eg. NM, CNM
- The same class of proteins implicated in CM can give rise to arthrogrypotic syndromes (MYH)

Sewry CA 2008. Congenital myopathies with known genetic defects

- **Nemaline rods and nemaline myopathy**
- ACTA1 1q42 AD or AR Skeletal a-actin [10]
- NEB 2q2 AR Nebulin [11]
- TPM3 1q2 AD a-tropomyosin [12]
- TPM2 9p13 AD b-tropomyosin [13]
- TNNT1 19q13 AR Troponin T [14]
- CFL2 14q12 AD Cofilin-2 [15]

- **Other myopathies with abundant rods**
- Rods and cores RYR1 19q13 AD Ryanodine receptor [16]
- Rods and caps TPM2 9q13 AD b-tropomyosin [17]

- **Congenital myopathies with cores**
- Central core disease RYR1 19q13 AD or AR Ryanodine receptor [18,19]
- Multiminicore disease SEPN1 1p36 AR Selenoprotein N1 [20]
- Congenital myopathy and fatal cardiomyopathy TTN 2q31 AR Titin [21]

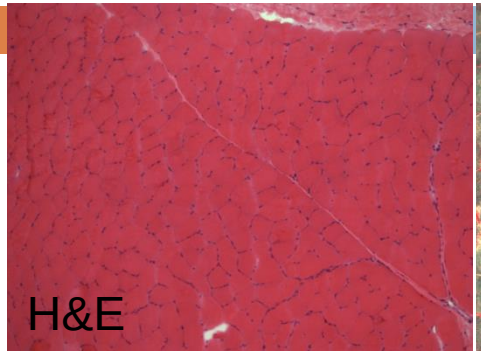
- **Central nuclei and centronuclear myopathies**
- Myotubular myopathy MTM1 Xq28 XLR Myotubularin [22]
- Centronuclear myopathy DNM2 19p13 AD Dynamin 2 [23]
- BIN1 2q14 AR Amphiphysin [24]
- RYR1 19q13 AD Ryanodine receptor [25]
- Congenital myopathy and fatal cardiomyopathy TTN 2q31 AR Titin [21]

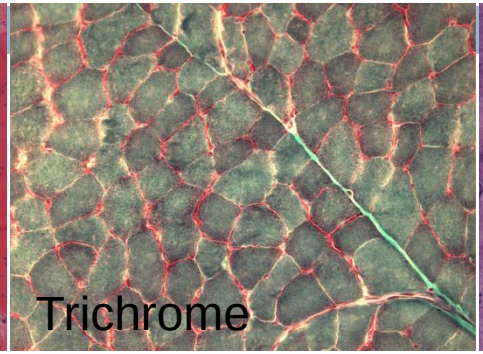
- **Surplus protein congenital myopathies**
- Actin aggregation myopathy ACTA1 1q42 AD Skeletal a-actin [10]
- Hyaline body myopathy MYH7 14q11 AD Slow/b-cardiac myosin heavy chain [26]
- Cap disease TPM2 9q13 AD b-tropomyosin [17,27]
- Reducing body myopathy FHL1 Xq26 XLD/R Four and a half lim domain-1 protein [5]
- Spheroid body myopathy MYOT 5q23 AD Myotilin [4]

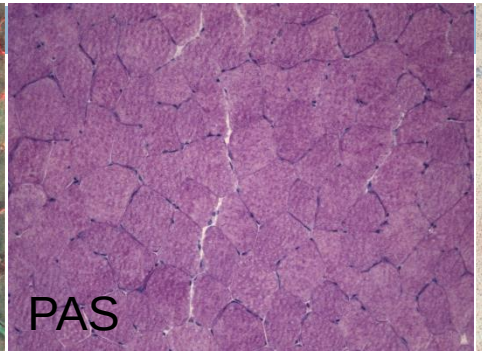
- **Congenital fibre-type disproportion**
- ACTA1 1q42 AD Skeletal a-actin [28]
- SEPN1 1p36 AR Selenoprotein N1 [29]
- TPM3 1q2 AD a-tropomyosin [30]

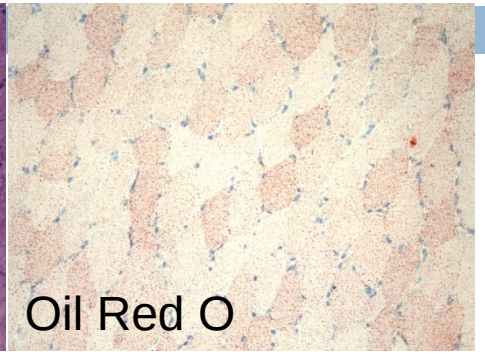
- **Congenital myopathies characterized by distal involvement or distal arthrogryposis or both**
- NEB 2q2 AR Nebulin [31]
- TPM2 9q13 AD b-tropomyosin [32]
- MYH3 17p13 AD Myosin heavy chain 3 [33]
- MYH8 17p13 AD Perinatal myosin [34]
- TNNI2 11p15 AD Troponin I [32]
- TNNT3 11p15 AD Troponin T3 [35]

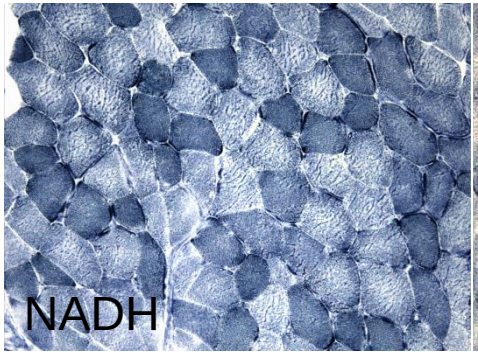
Histology of normal muscle

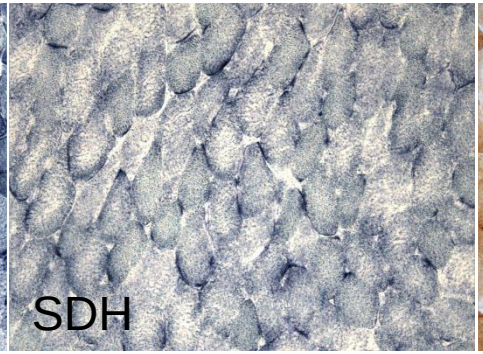


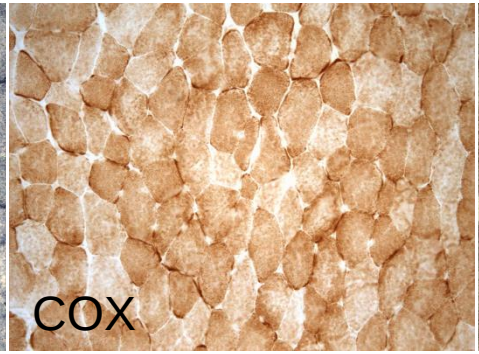


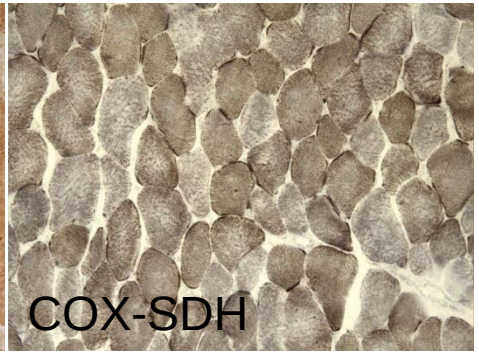


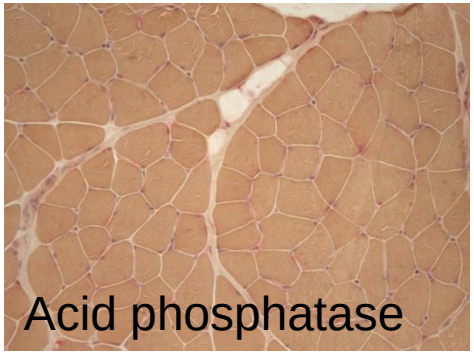


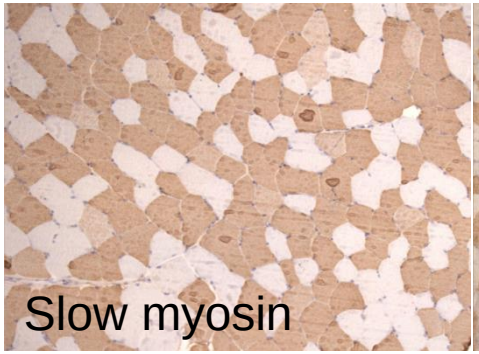


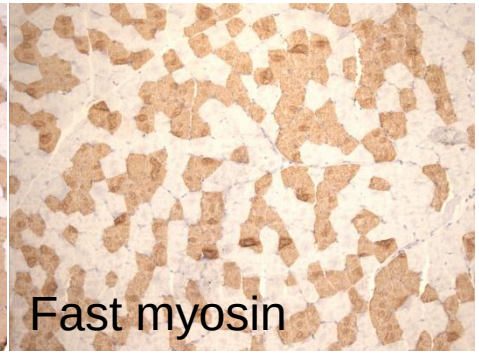






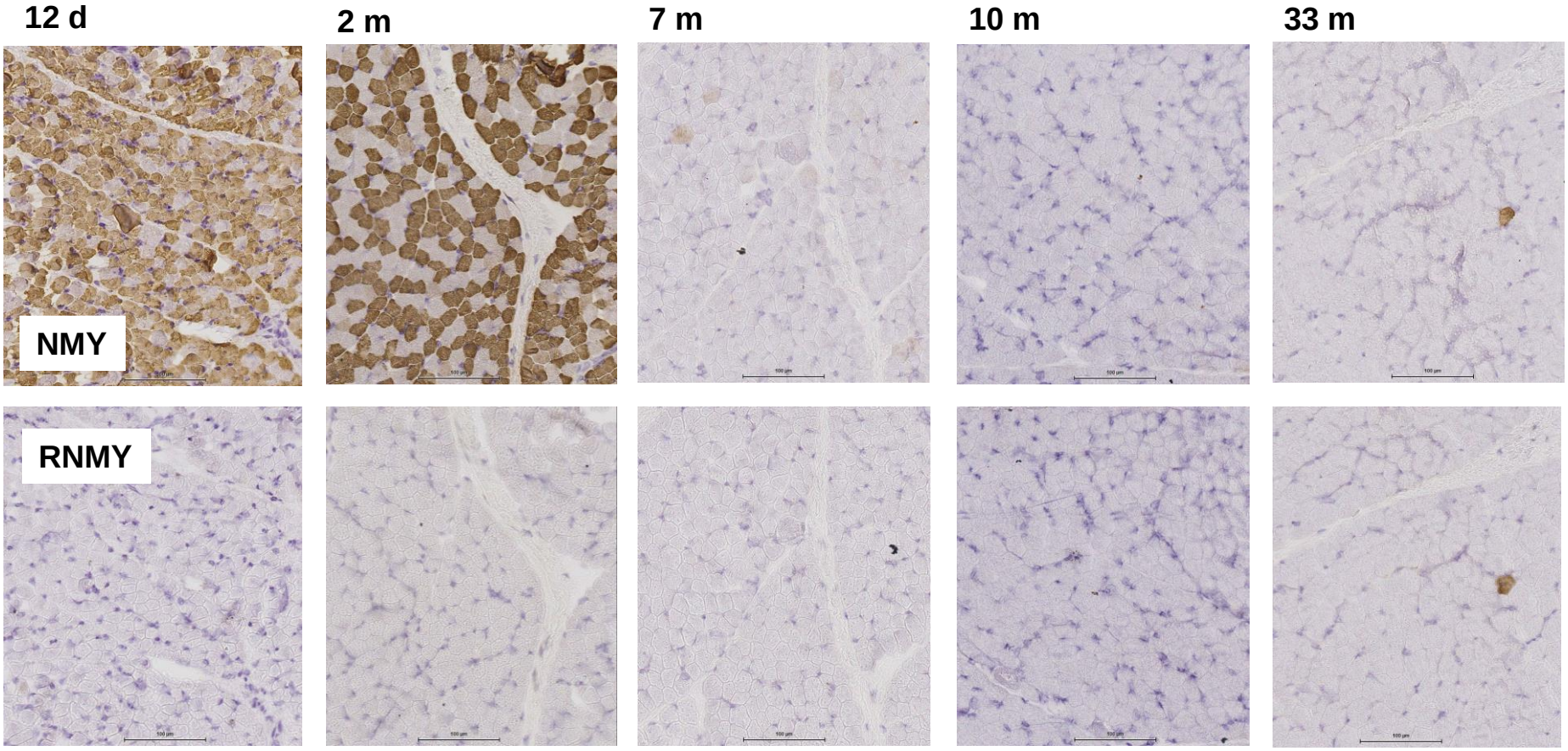






Results: controls

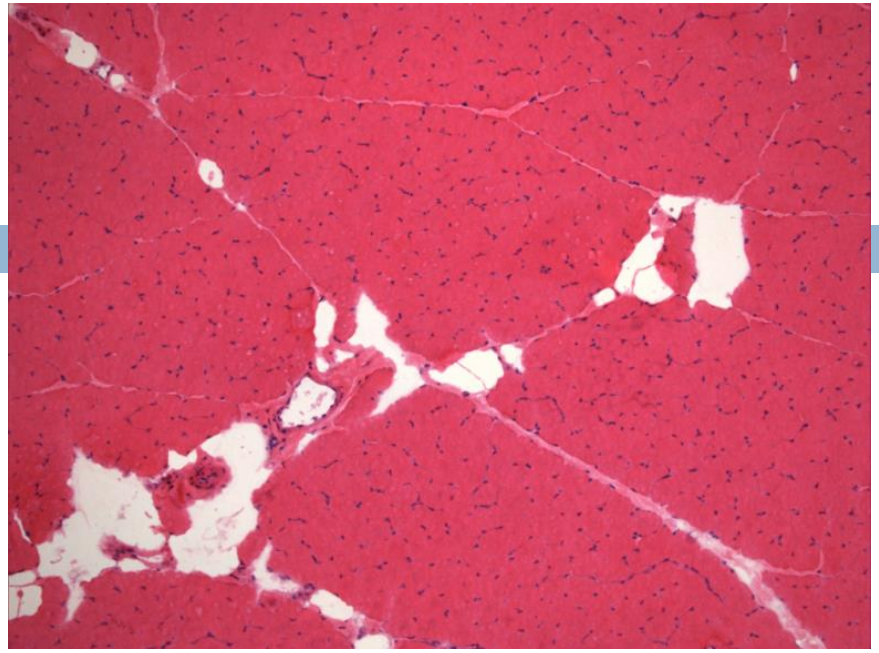
Feng, Rivas unpublished data

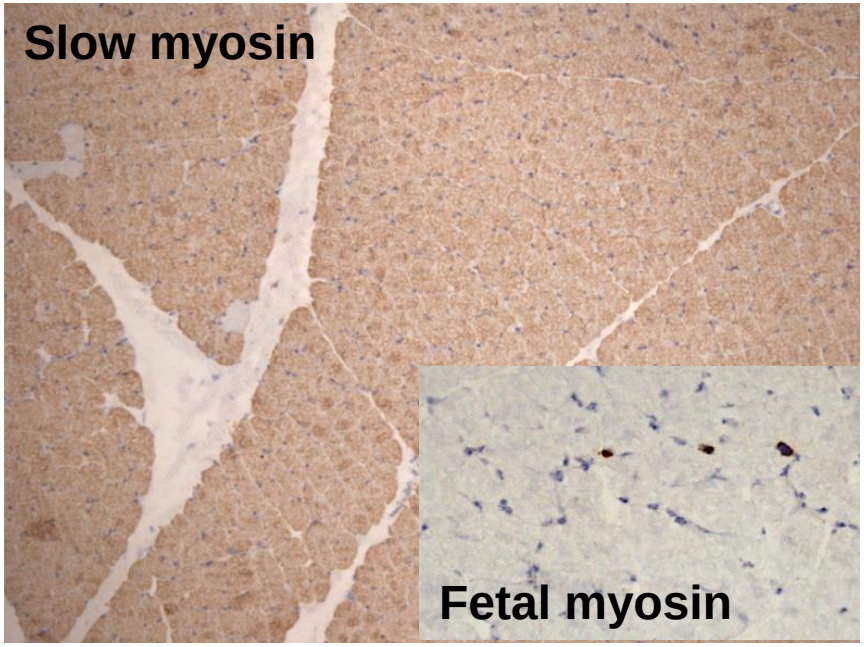


Fetal and developmental myosin heavy chain isoforms are highly developmentally regulated

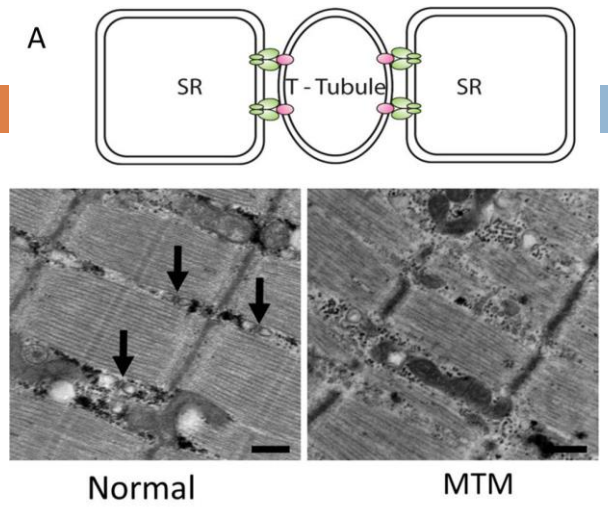
Salient pathological features

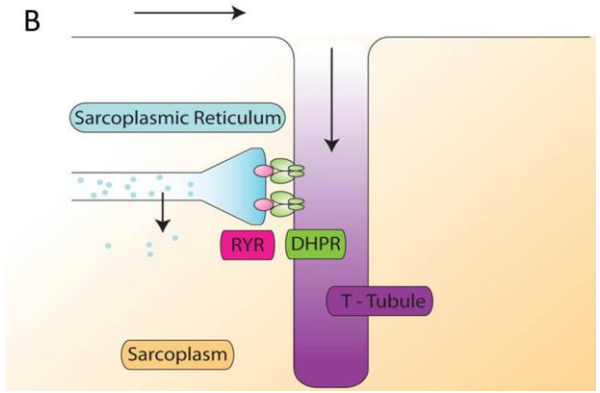
- Characteristic structural features
- Type I hypotrophy and predominance
- Size variation may reach fibre type disproportion with slow hypotrophy and fast hypertrophy
- Low numbers of fibres co-expressing myosins
- Necrosis and regeneration not typical *(MTM1, DNM2, RYR1, ACTA1)*
- Population of very small fibres diffusely dispersed expressing neonatal myosin and often co-expressing fast myosin





Pathomechanisms





Dowling JJ. Neurotheraputics 2014

Primary

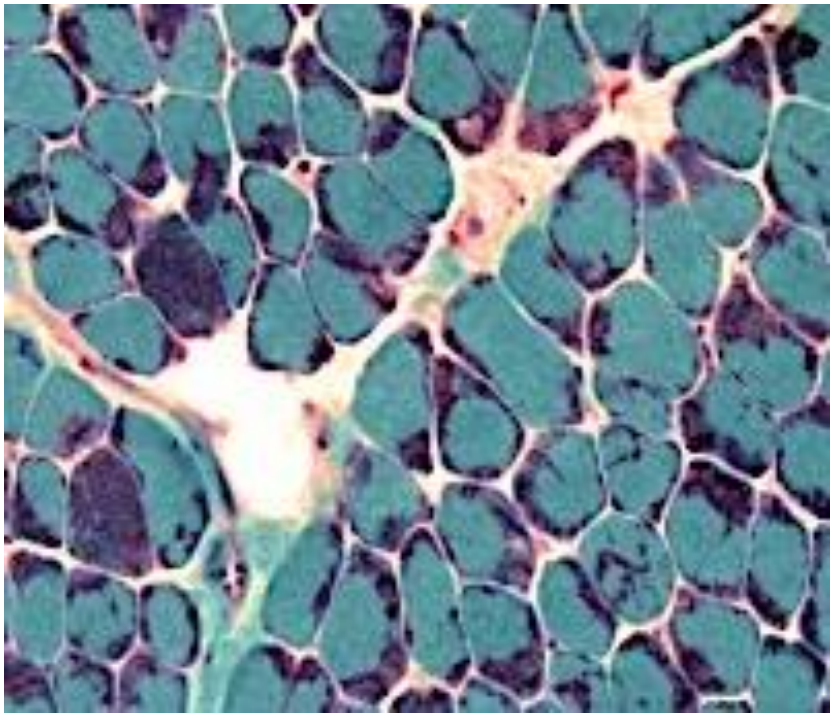
Disease	Gene	Relationship to triad
MHS	RYR1, CACN1S	Triad hypersensitivity to triggering agents; excess Ca release from RYR1
RYR1-myopathies	RYR1	Aberrant EC coupling due to reduced RYR1 function
CNMs	MTM1, DNM2, BIN1, TYT1, TTN	Abnormal triad structure and impaired EC coupling; aberrant tubulogenesis and/or abnormal membrane recycling
Native American myopathy	STAC3	Abnormal EC coupling; STAC3 may be a linker between DHPR and RYR1
Tubular aggregate myopathy	ORAI1, STIM1	Abnormal SOCE; Tas likely SR derived

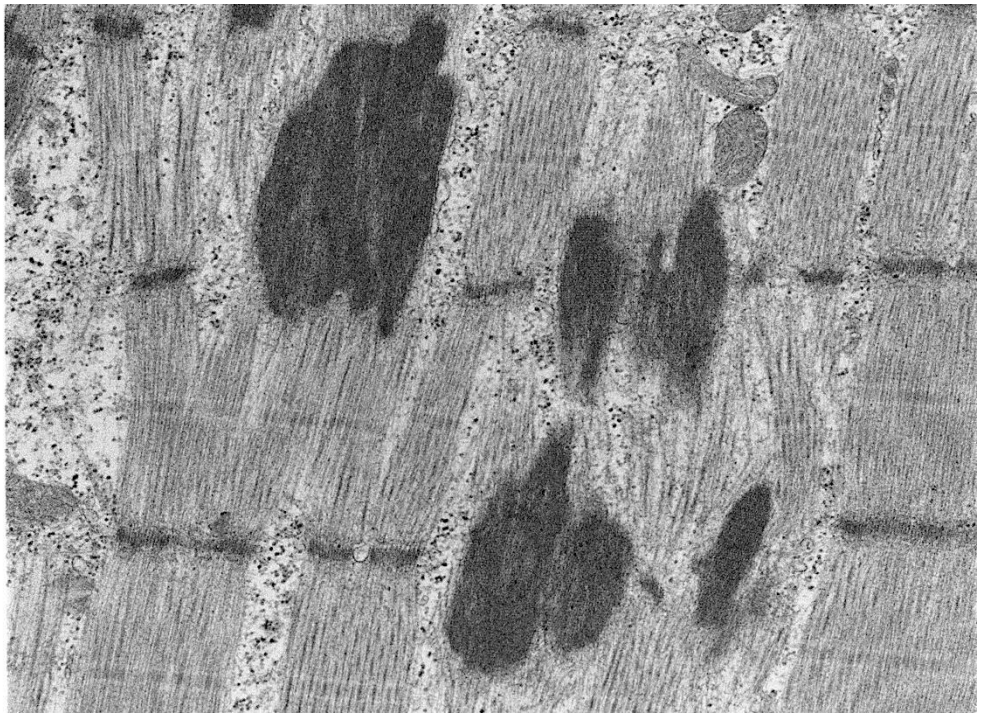
Secondary

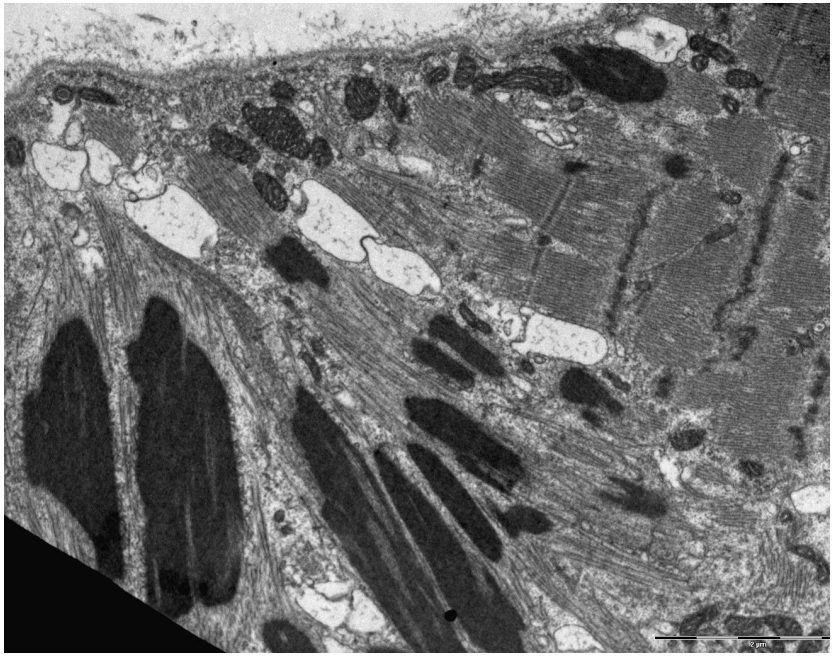
Disease	Gene	Relationship to triad
DM1	DMPK	Abnormal splicing of key triad gene products causing abnormal EC coupling
Dystrophinopathy	Dystrophin	Hypernitrosylated RYR1 causing chronic channel leakiness
Dysferlinopathy	DYSF	T tubule component; stabilise T-tubule and/or chaperone triad proteins
Calpainopathy	CAP3	Partial triad localisation; loss associated with reduced RYR1 function
SEPN1 myopathy	SEPN1	SEPN1 interacts with RYR1

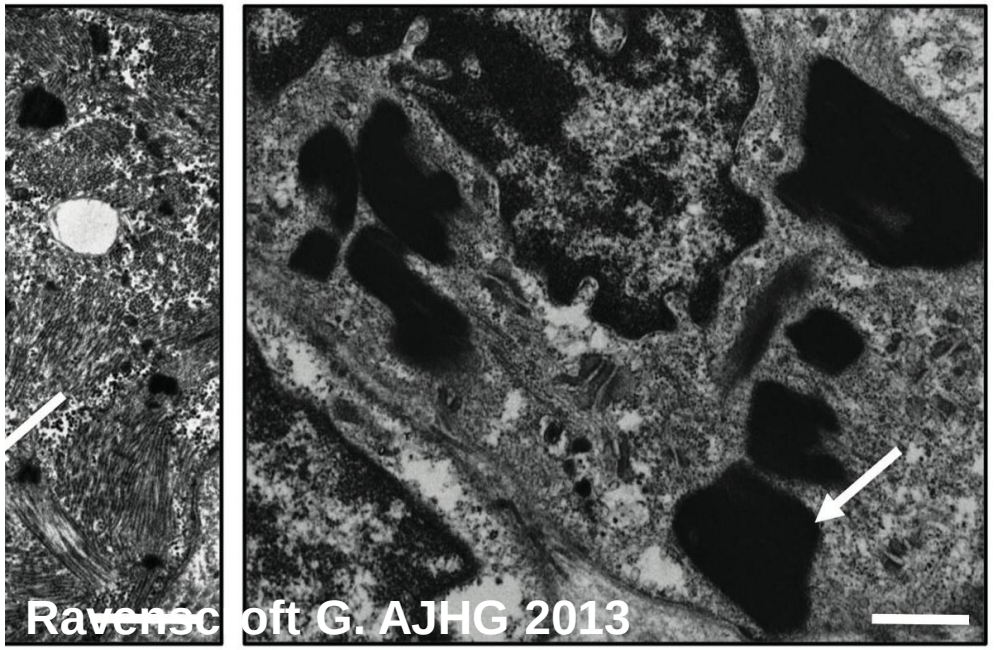
Nemaline myopathies

- Defects in 10 genes; ACTA1, NEB, TPM3, TPM2, TNNT1, CFL2; KBTBD13, KLHL40, KLHL41, LMOD3
  ACTA1 (dominant) and NEB (recessive) mutations common
- New genes: Kelch protein family – KBTBD13, KLHL40, KLHL41; LMOD3
- Mostly AD, often de-novo, rare recessive homozygous null mutations show absent skeletal actin
- Rods are thread-like inclusions that stain red in the modified GT stain; absent in extrafusal fibres, smooth muscle
- EM: dense, osmiphilic rod like or ovoid often in continuity with Z line; similar lattice structure
- Alpha actinin major constituent; other Z line proteins associated: actin and myotilin, desmin at periphery
- Variable number and distribution between fibres and between muscles; sometimes Type I restricted (TPM3)
- No correlation with clinical severity
- Difficult to predict genotype; exception is intranuclear rods and cytoplasmic actin accumulation resulting from ACTA1 mutations and brick-like rods in KHL40 mutations
- Rods are unspecific: present in normal myotendinous junctions, normal EOM, ageing muscle, minor secondary feature in other myopathies
- No rods: distal Nebulin, rare ACTA1 family, ACTA1 with cores








Ravensc oft G. AJHG 2013

Nemaline myopathy: ACTA1

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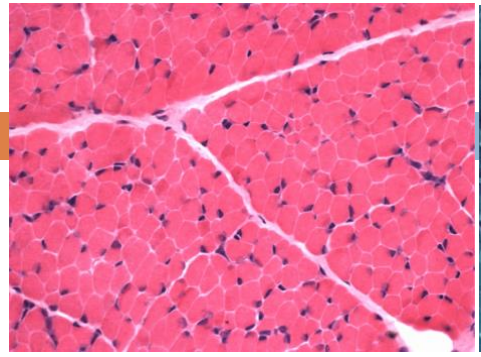
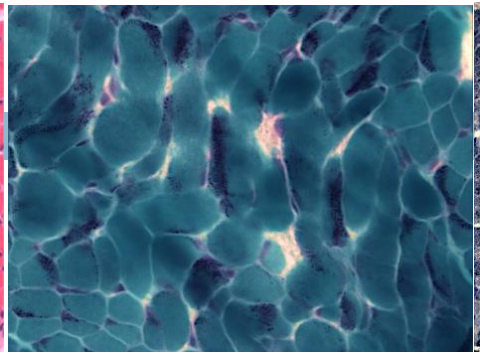
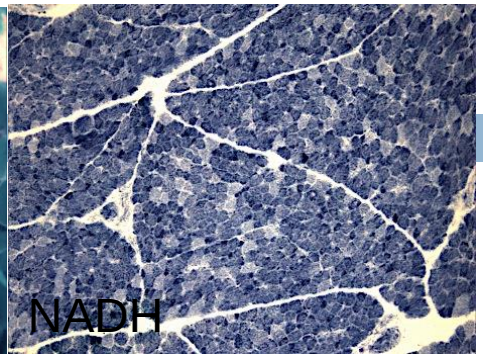

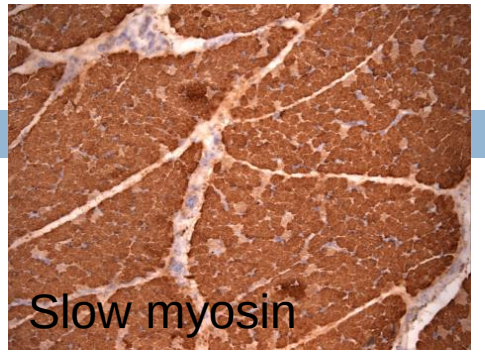

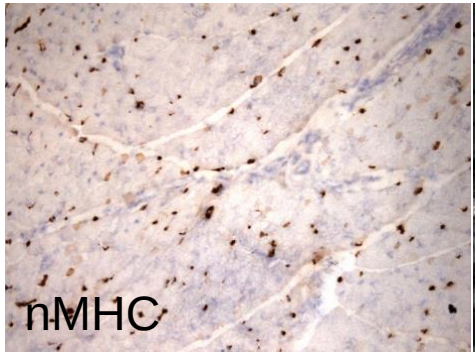

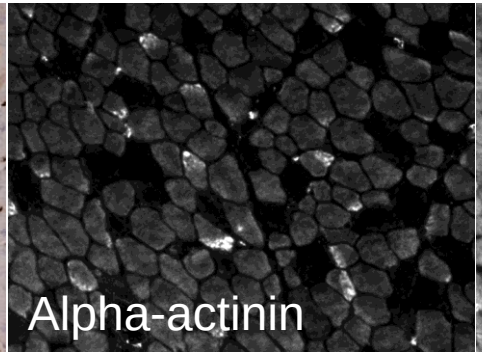

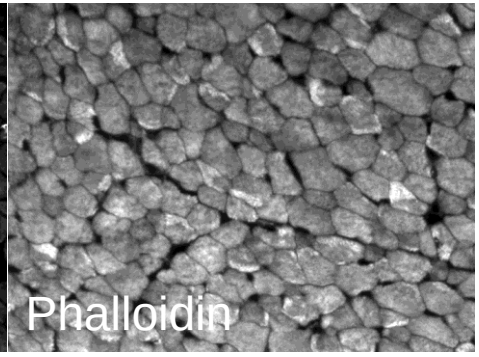

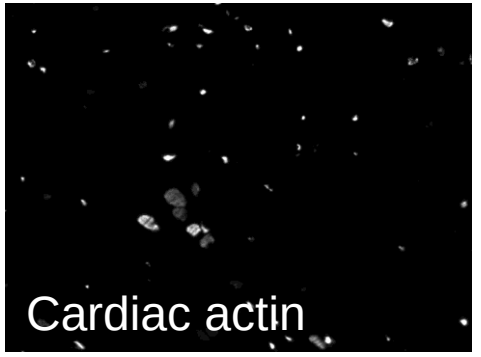

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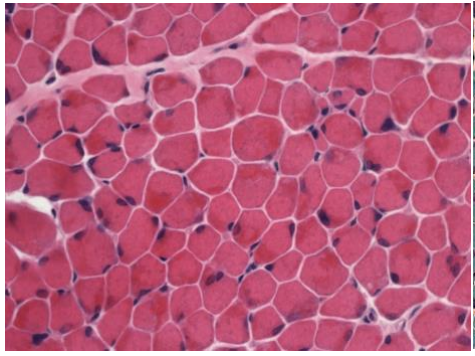
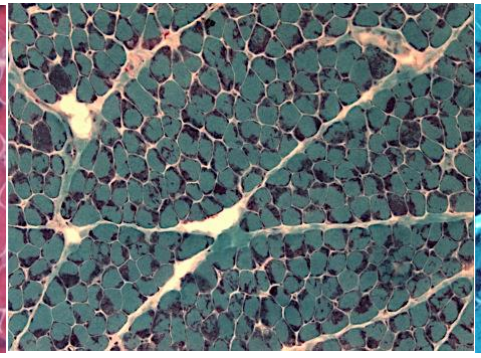
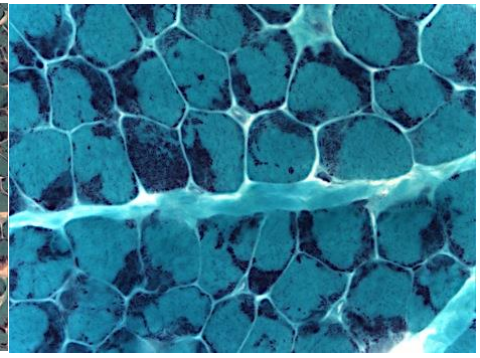
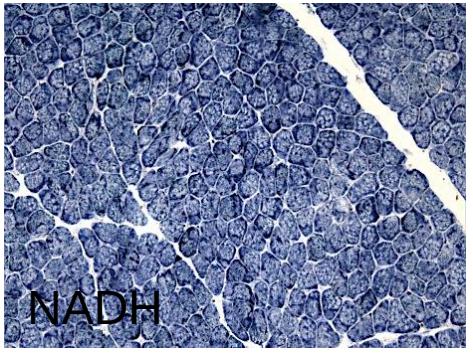
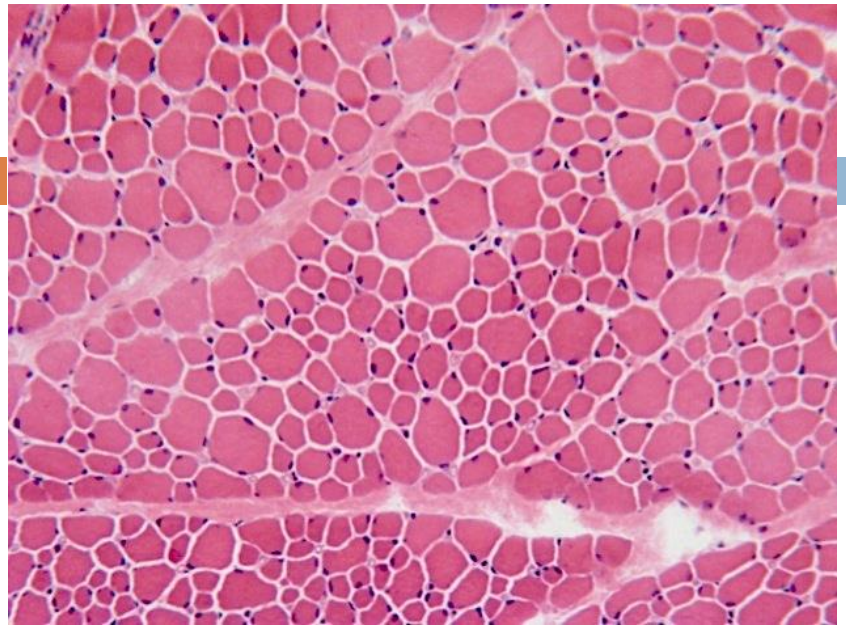
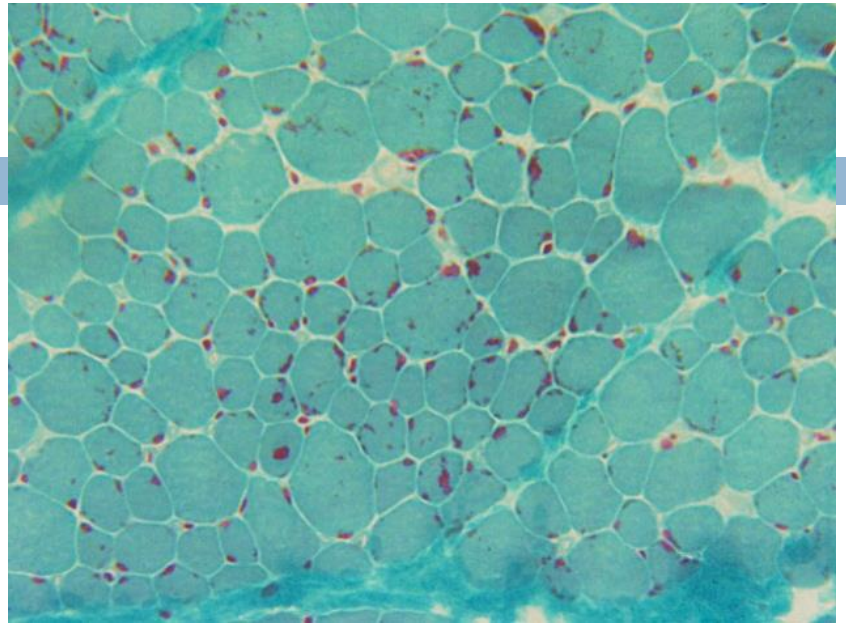
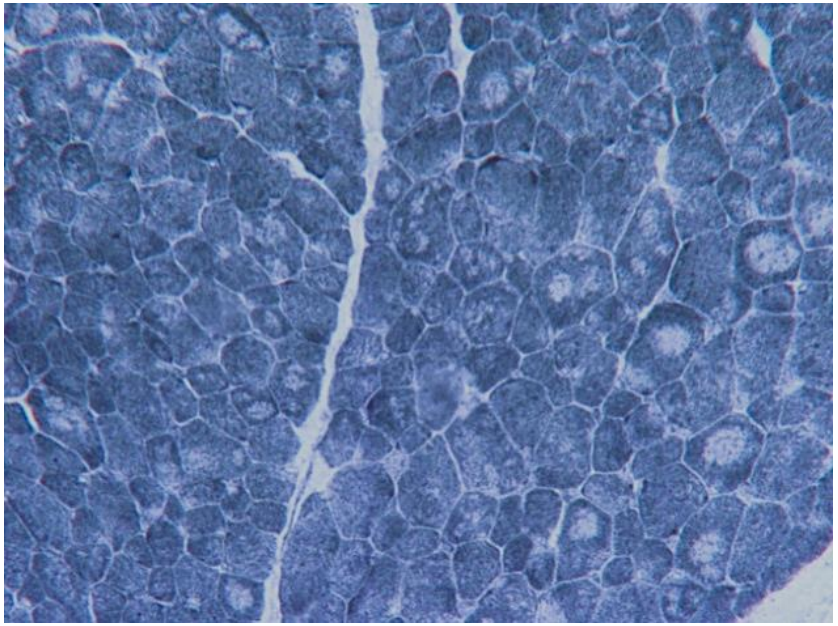
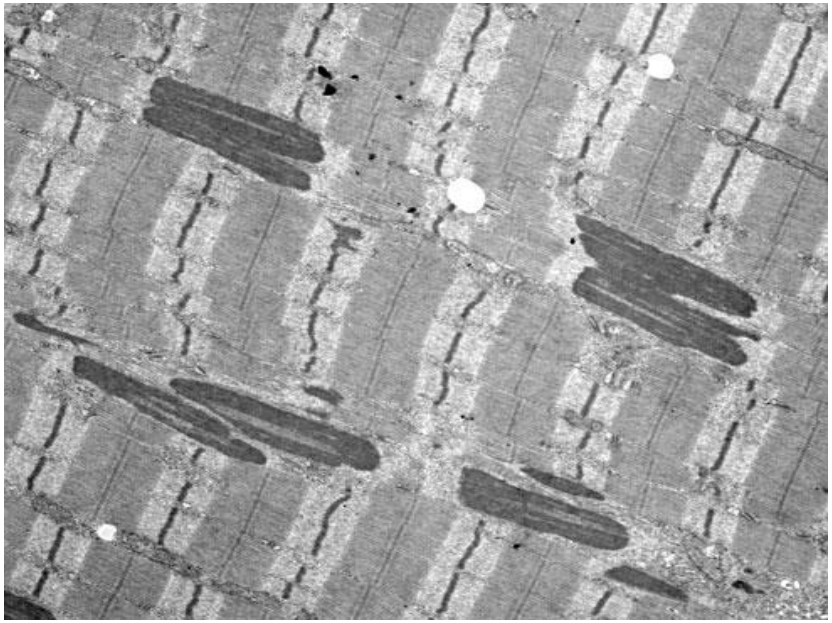





Rod-Core myopathy: Nebulin





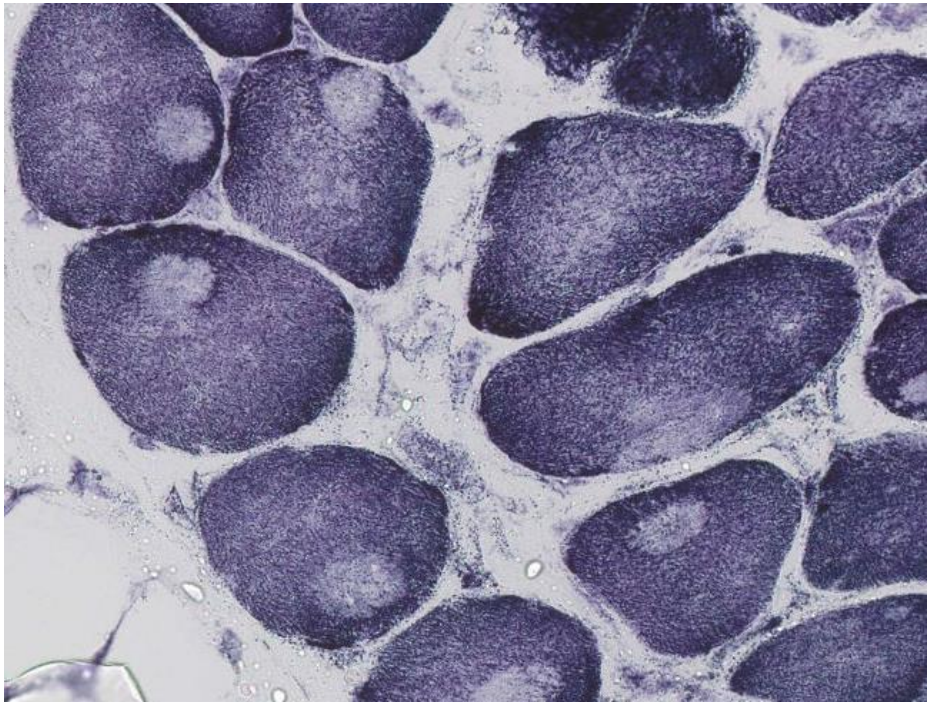


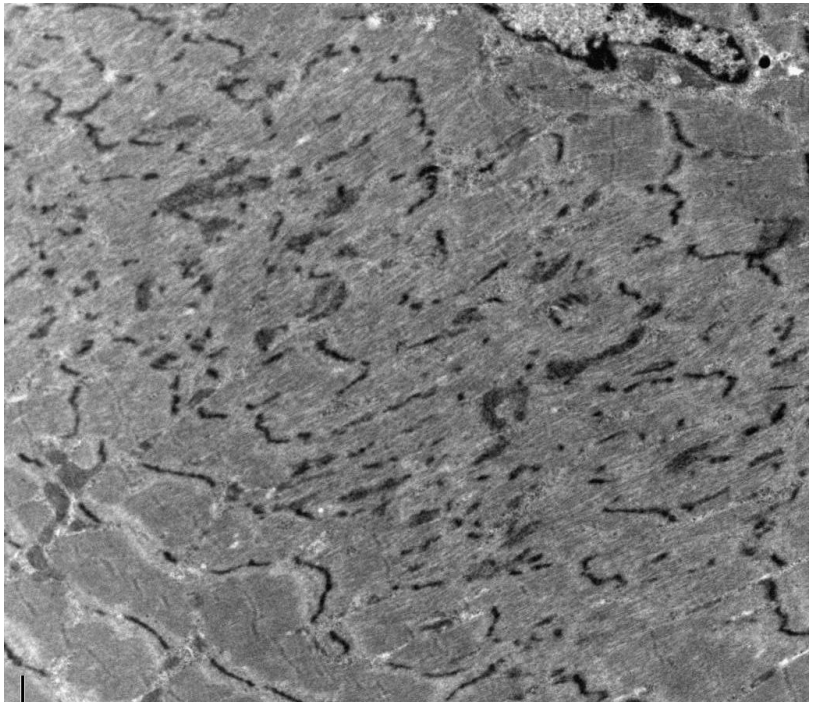


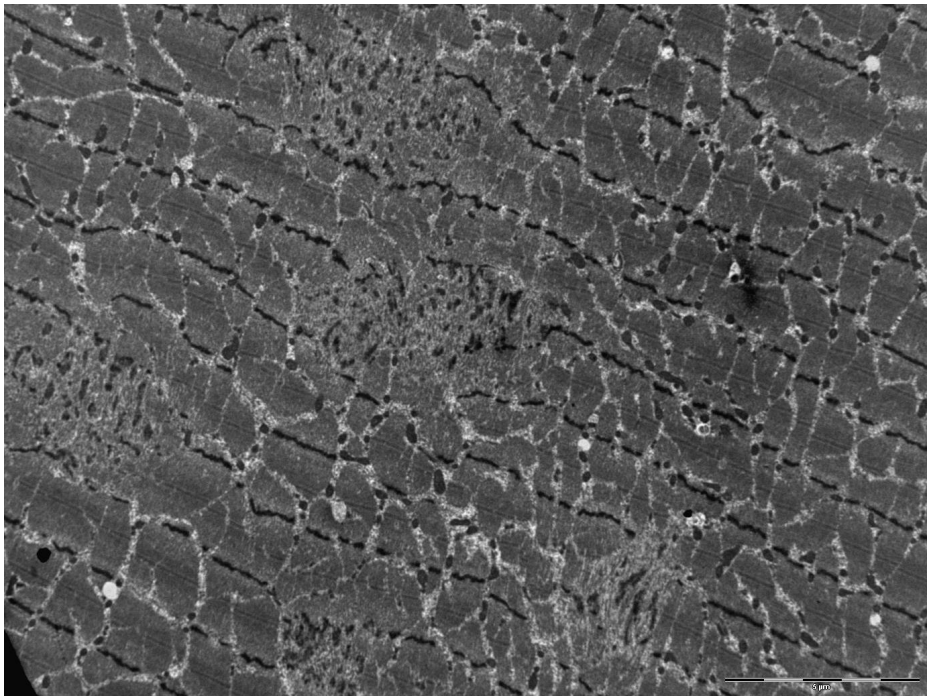
Other associated mutations: RYR1, KBTBD13

Congenital myopathies with cores

- Cores are devoid of mitochondria with variable accompanying myofibrillar disruption
- Large cores, central or peripheral involving a long length of a fibre, predominantly slow type define CCD due to RYR1 mutations
- Some RYR1 cases show no cores, or indistinct, multiple cores or evolution over time
- Marked fatty infiltration and fibrosis in some RYR1 cases mimicking a dystrophy
- Internal nuclei in RYR1, can be central
- Cores observed in cases with CFL2, TTN, MYH7, ACTA1 and KBTBD13 mutations
- Variety of neurogenic and myopathic conditions feature cores
- RYR1 mutations are common in several populations, increasing possibility of dual trouble
- Multicore disease – SEPN1 and RYR1; also ACTA1, MYH7, MYH2, CFL2, TTN, CCDC78 and KBTBD13
- Greater pathological than clinical heterogeneity with SEPN1 mutations; multiminicores with and without dystrophic features, former similar to allelic RSMD1; mallory bodies and desmin accumulation and CFTD







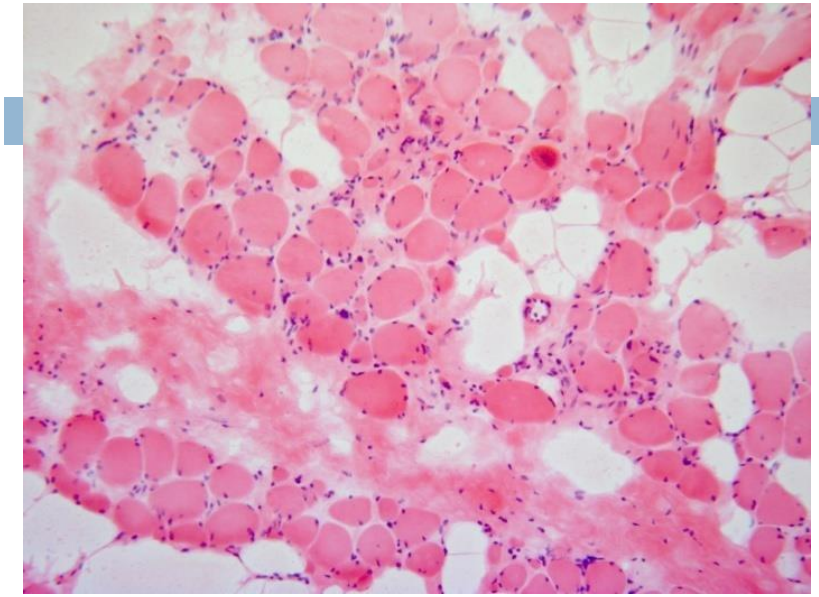
Cores and mini-cores

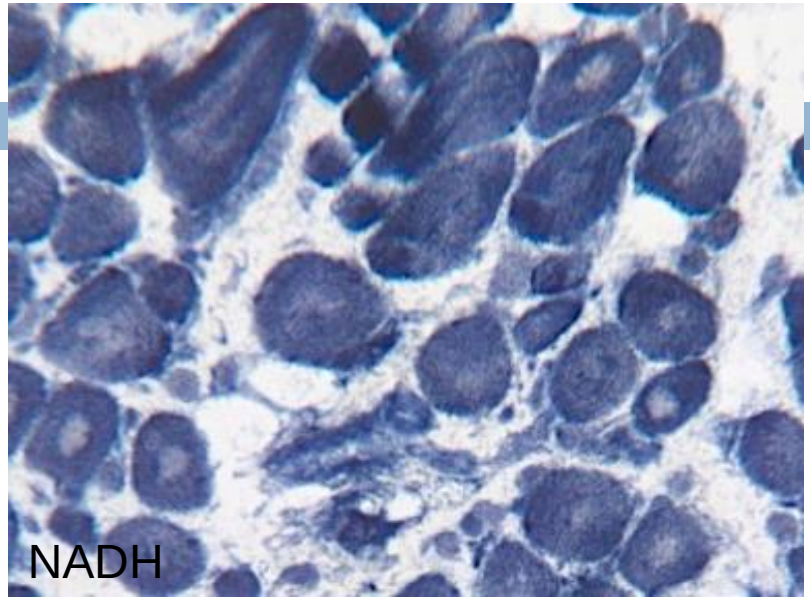
Pathological distinction: RYR1 versus SEPN1

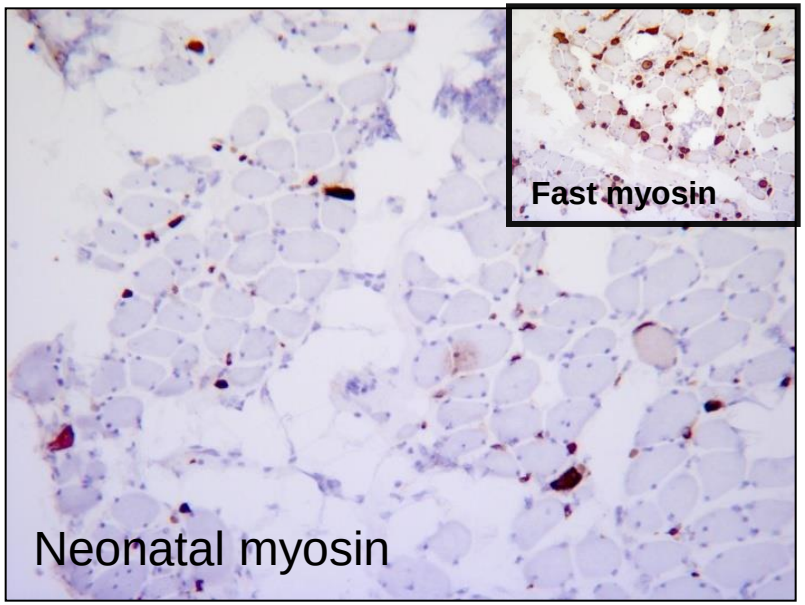
- RYR1
  1. *Fibre type uniformity or marked slow predominance*
  2. *Predominance of cores in slow fibres*
  3. *Very small neonatal myosin expressing fibres common*

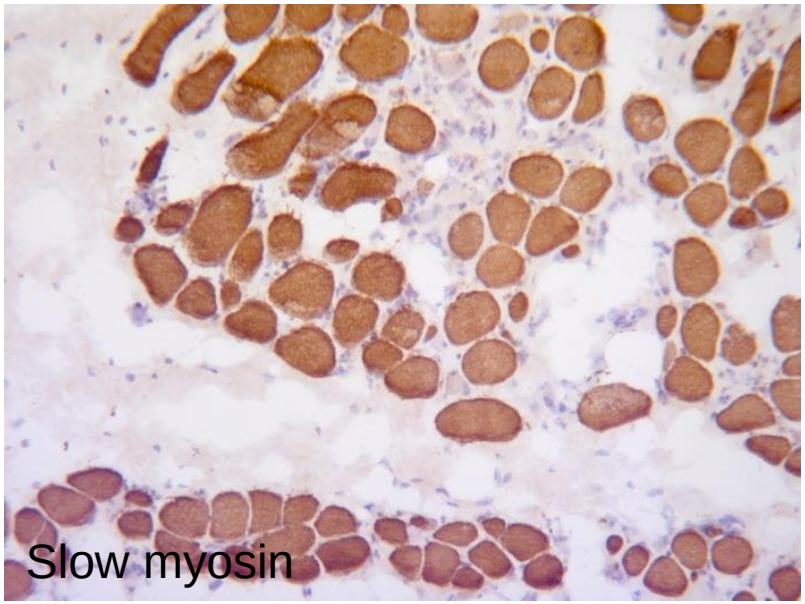
- SEPN1
  1. *Mixed fibre typing retained*
  2. *Cores present in both fibre type*
  3. *Little neonatal myosin expression*
  4. *Profuse internal nuclei in some cases*

Core myopathy: RYR1

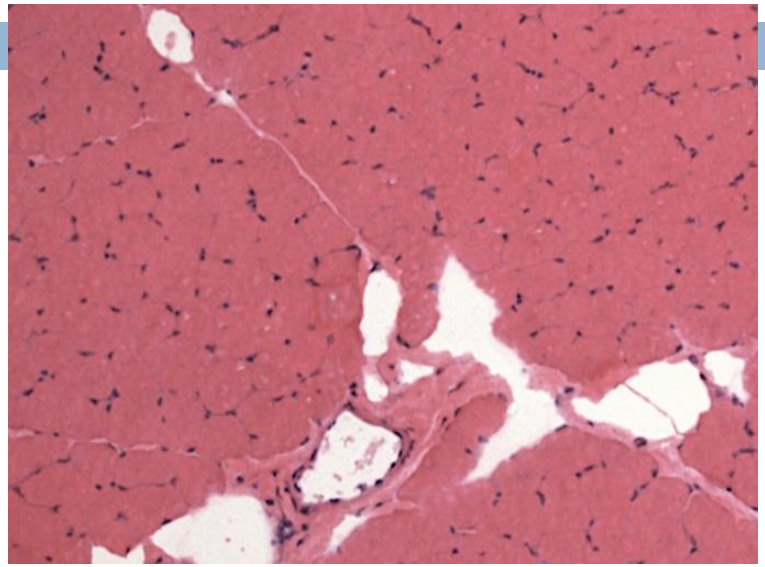


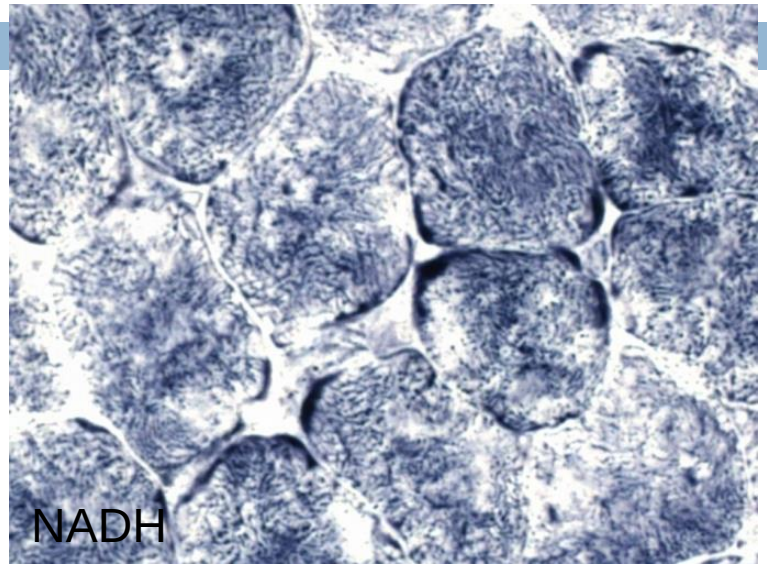


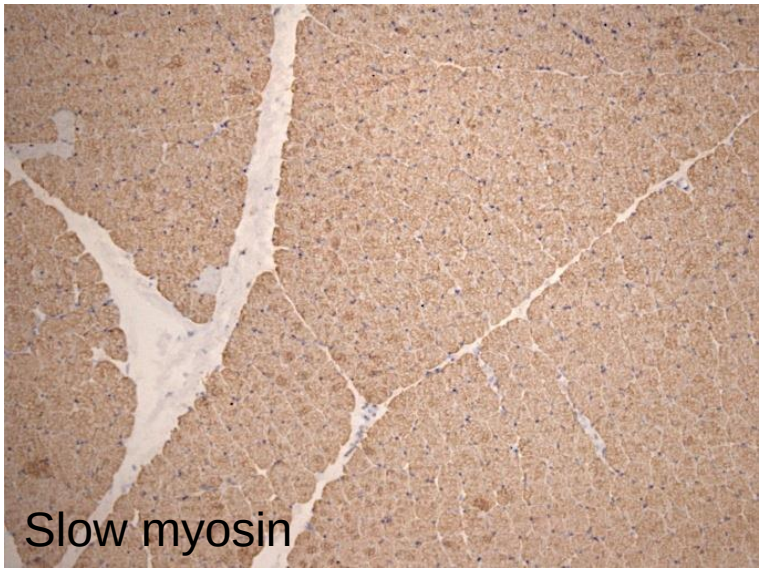


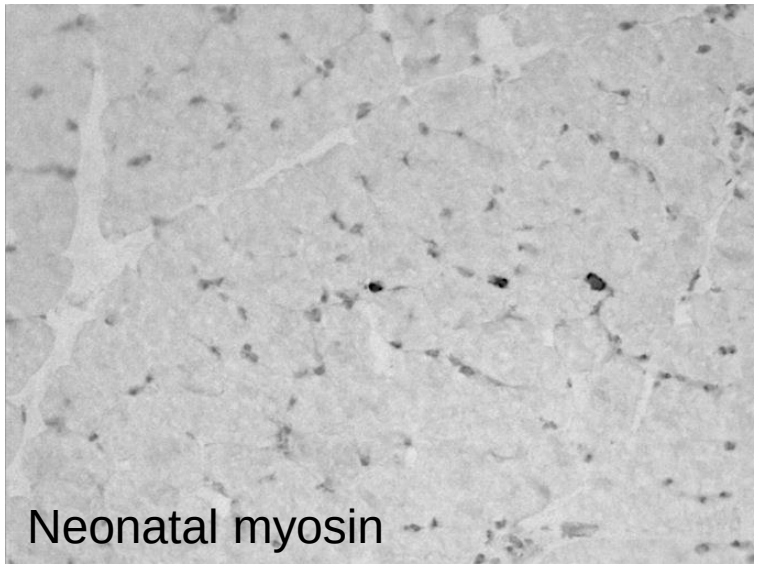


Core myopathy: RYR1

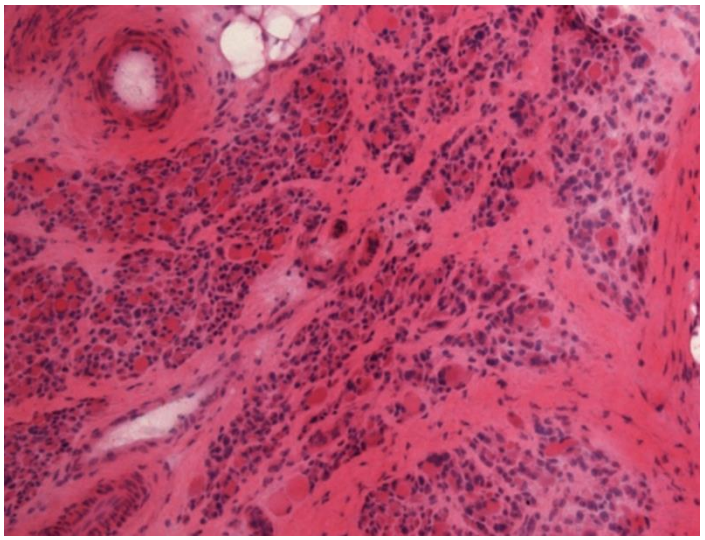


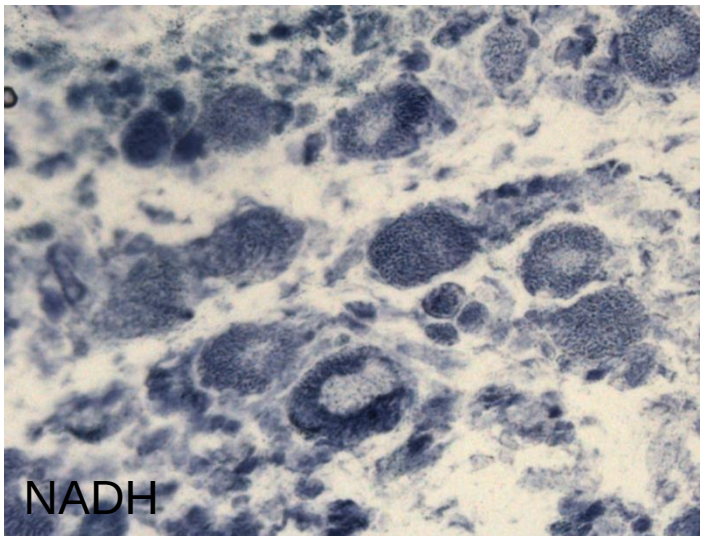


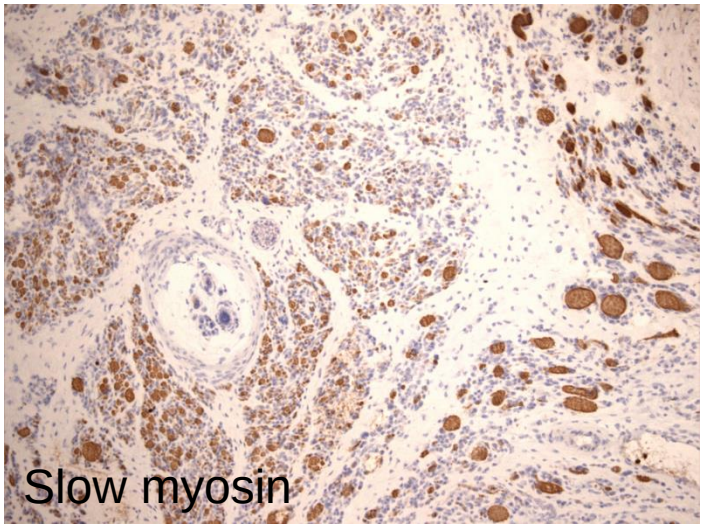


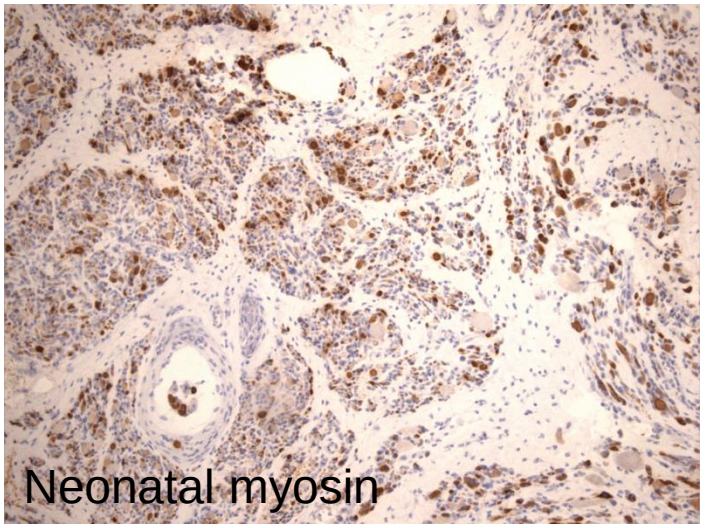


Core myopathy: RYR1 with dystrophy-like changes

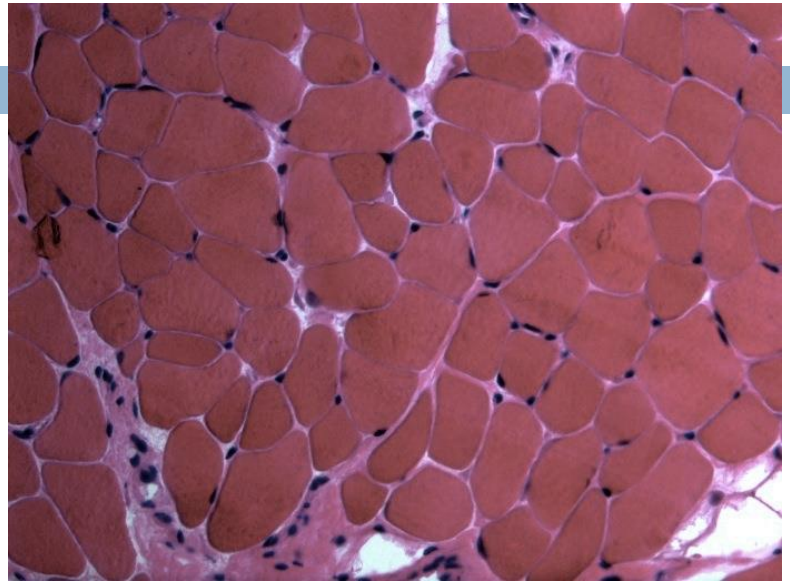


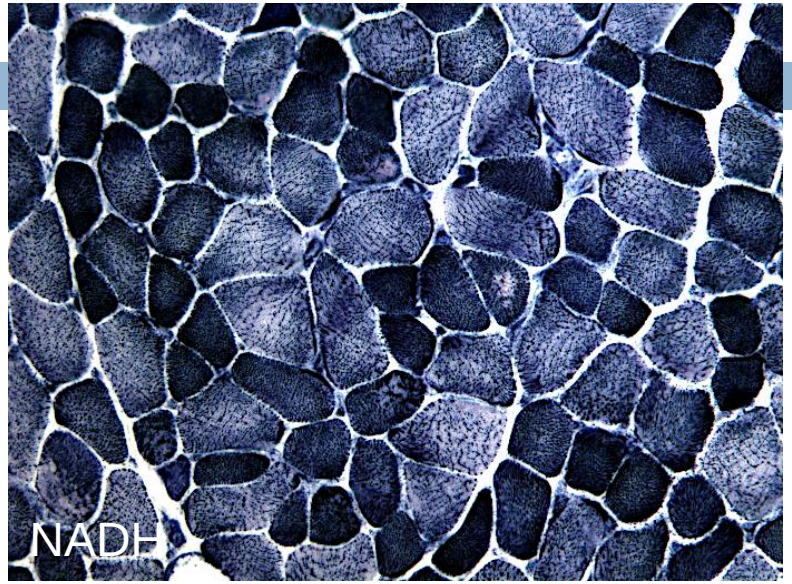


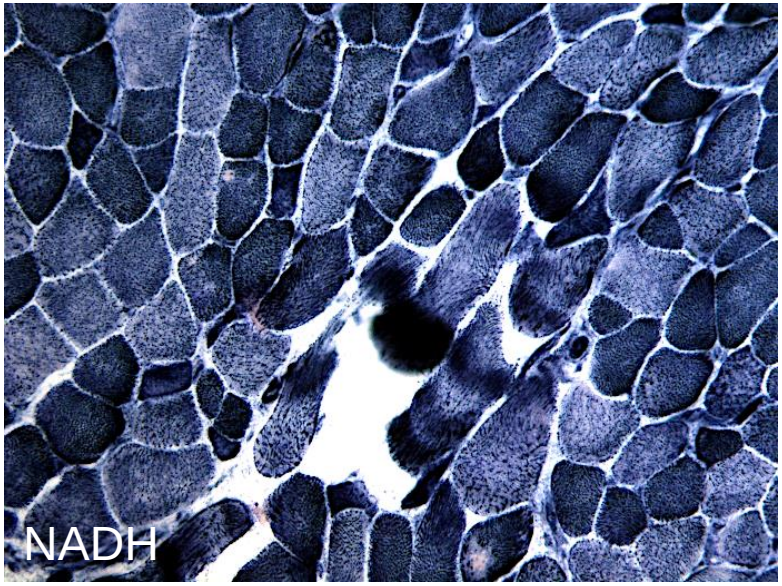


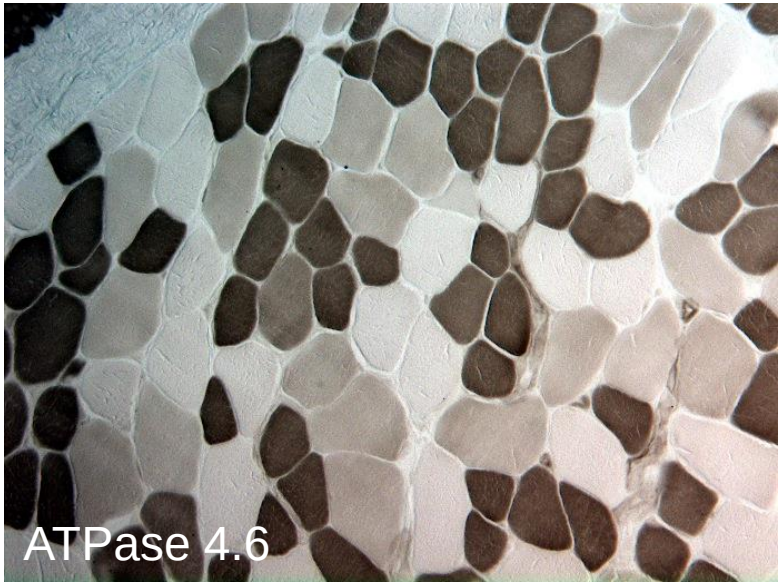


Multiminicore myopathy: SEPN1

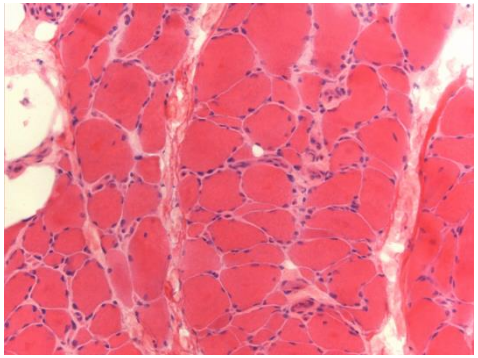


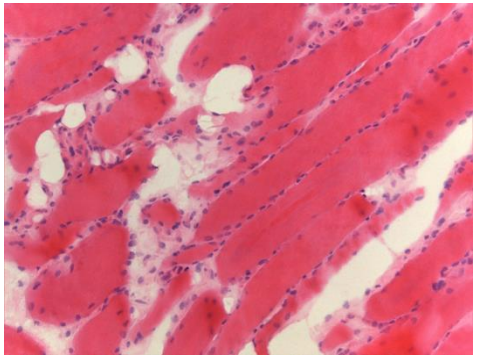


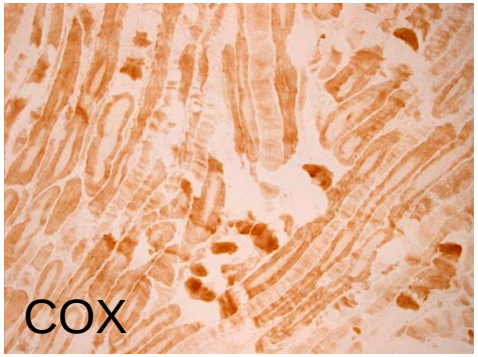


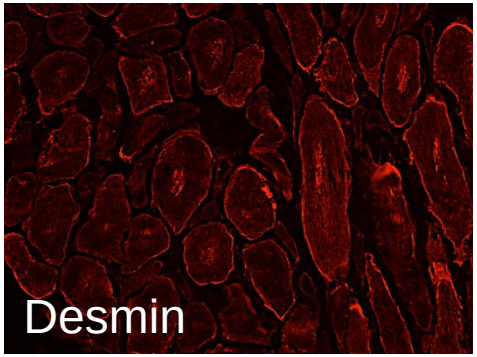


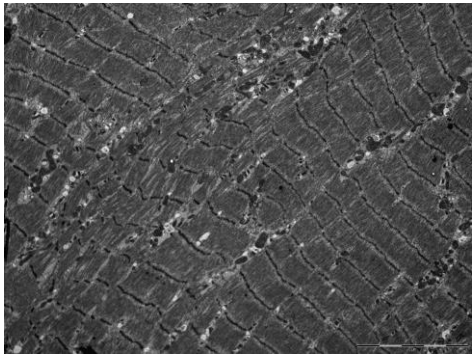
Cores and targets

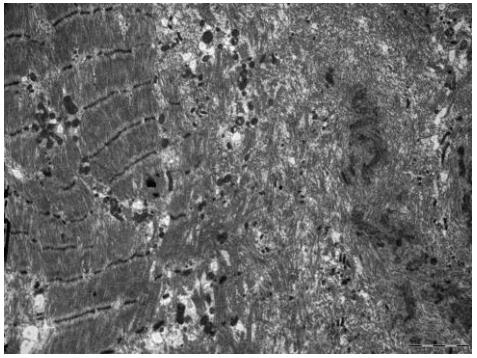


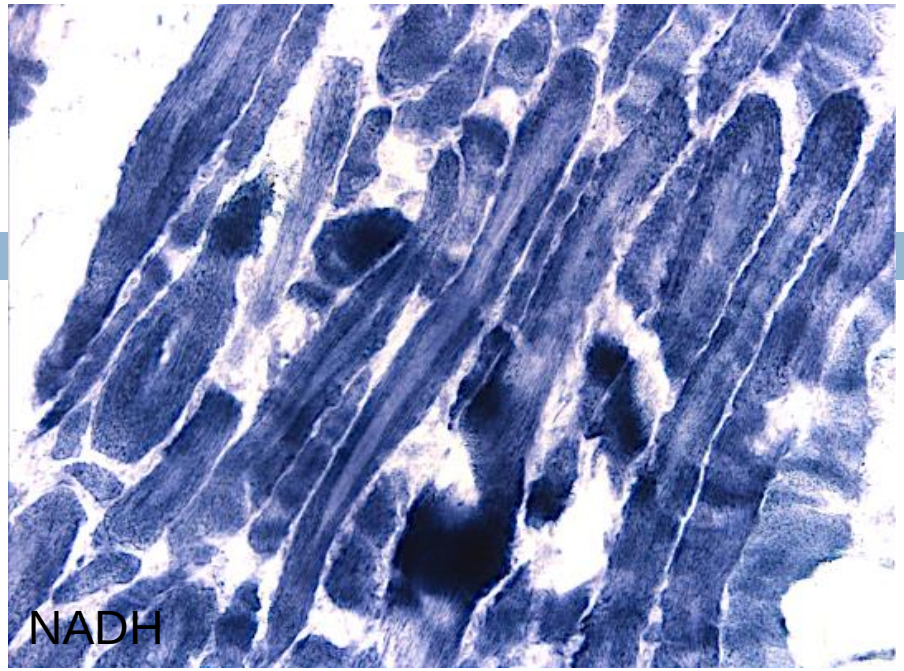


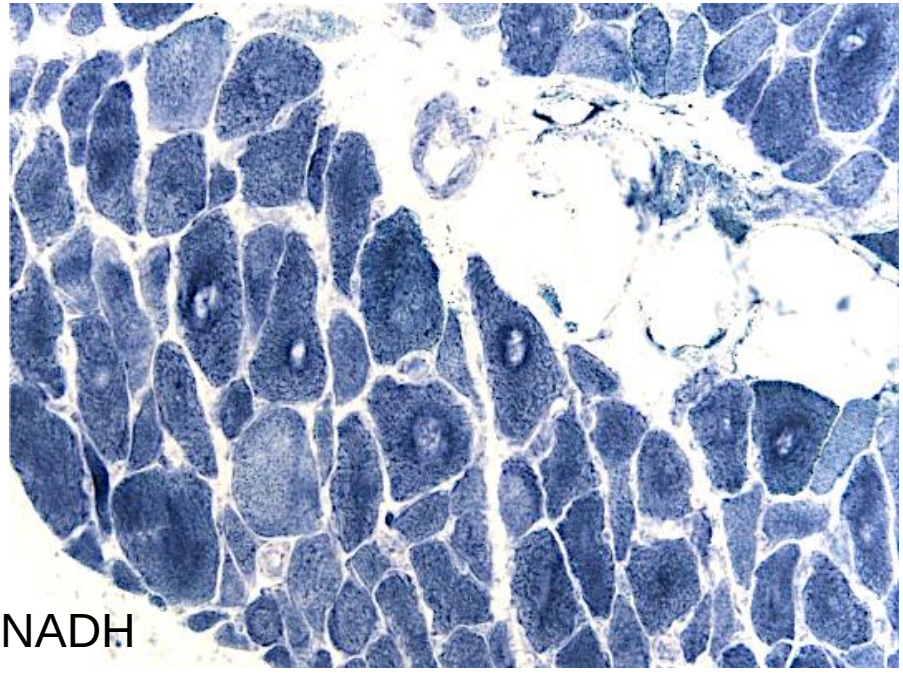










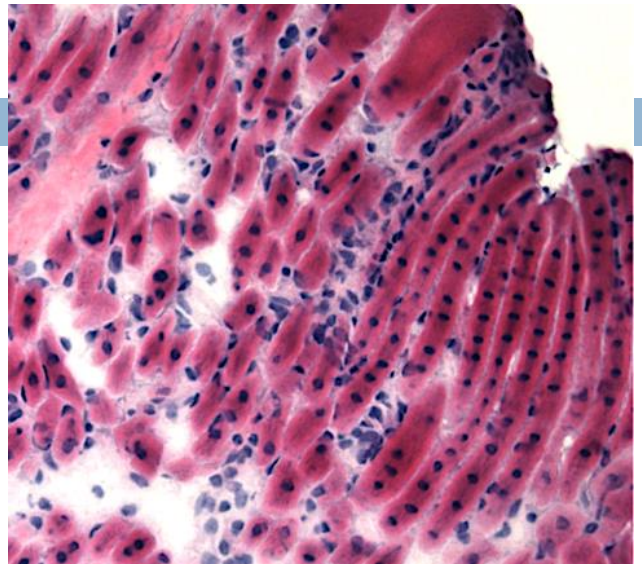


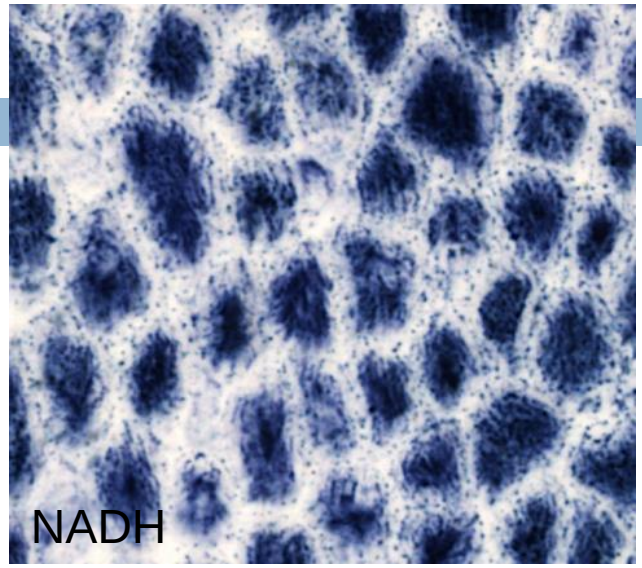
Central nuclei, centronuclear and myotubular myopathies

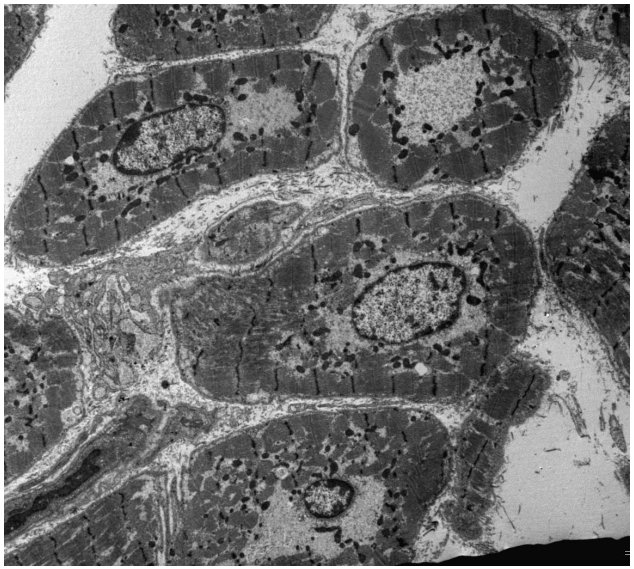
- Misplacement of myonuclei from subsarcolemmal to a central position
- Myotubular myopathy: term reflects resemblance of fibres to myotubes, now reserved for severe X linked disorder due to MTM1 mutations
- MTM1 – severe phenotype; fatal respiratory failure
- Majority of female carriers are asymptomatic except in cases with skewed X-inactivation
- Pathology in cases of congenital myotonic dystrophy (DM1) and some RYR1 cases can be identical; slow fibre depletion in DM1 useful marker
- Autosomal CNMs rarer, dominant or recessive inheritance age of onset and severity variable – DNM2, BIN1, RYR1, MTMR14, CCDC78 (CN + cores)
- DNM2 – adolescence/early adult presentation; preceding distal weakness; ptosis, ophthalmoplegia; axonal neuropathy (CMT2B) in some
- DNM2 - radiating sarcoplasmic strands; ??? gene specific or age related; DNM2 – CN in chains; rare BIN1- subsarcolemmal vacuoles, radial strands less common
- Necklace fibres - late onset MTM1 linked cases; also some neonatal cases; atypical NFs reported in DNM2
- MTM1, DNM2, BIN1 – abnormal triad morphology
- Homozygous mutations in C terminal kinase domain of TTN – central/internal nuclei, cores, slow uniformity; hypotonia, proximal+distal weakness; spine rigidity, contractures and dilated

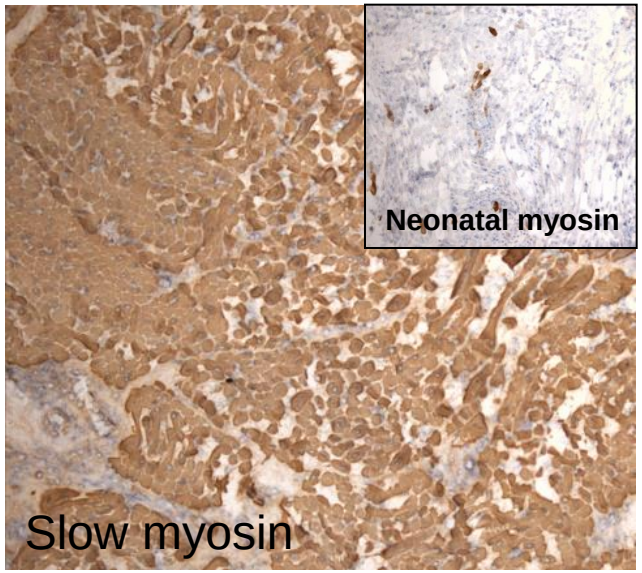
Myotubular myopathy – MTM1

- Regular spacing of central nuclei down the fibre
- Central region devoid of myofibrils and organelles or aggregates mitochondria and glycogen
- Peripheral part of the fibre appears as a halo
- Central nuclei occur in both fibre types and in fibres with and without neonatal myosin
- Maturation with regard to myosin isoforms occurs



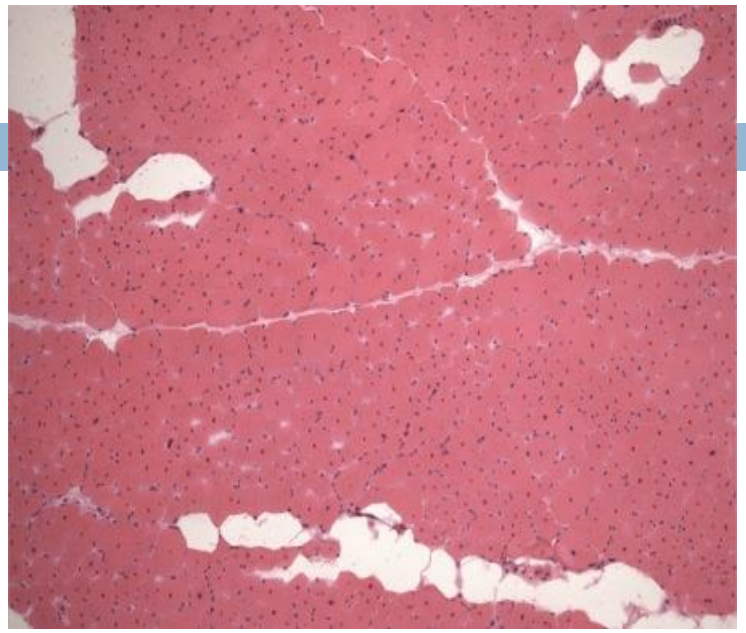


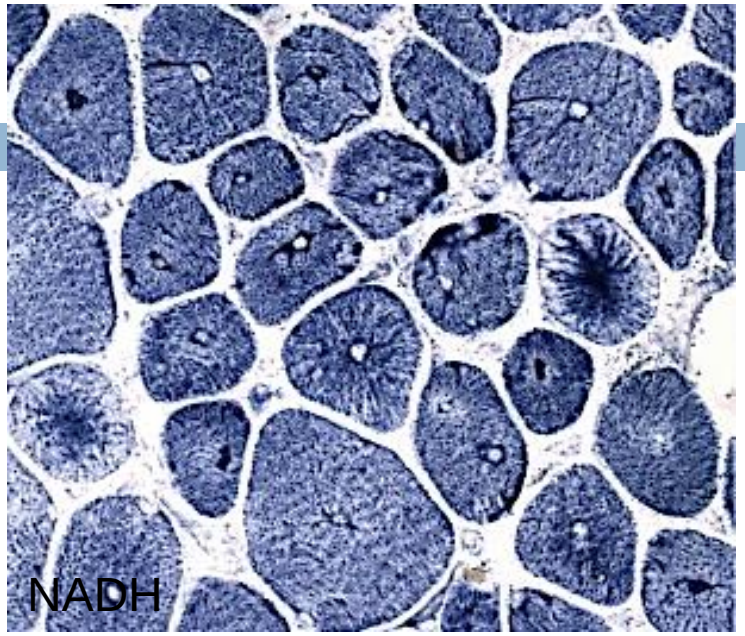


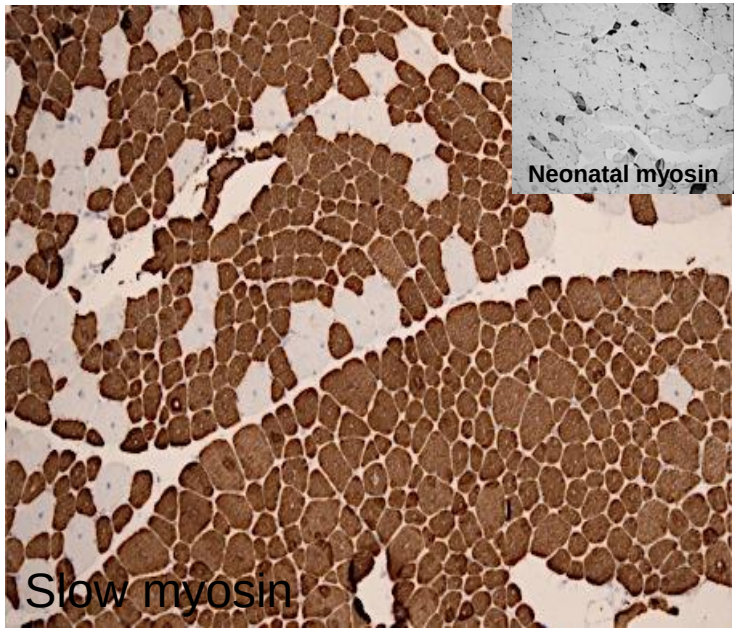


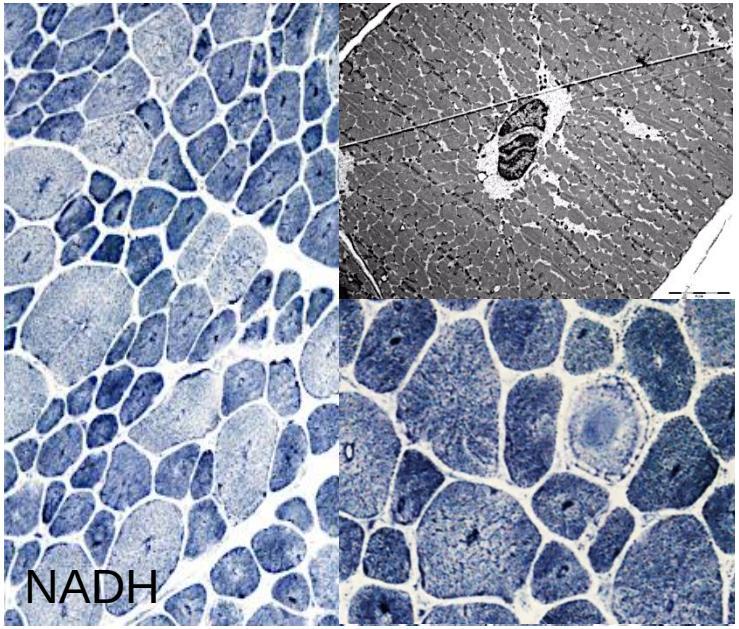
Centronuclear myopathy – DNM2

- **Marked variation in fibre size**
- **Type I hypotrophy and Type II hypertrophy approaching FTD in some fascicles**
- **Increased perinuclear oxidative staining around enlarged nuclei, with radial sarcoplasmic strands**



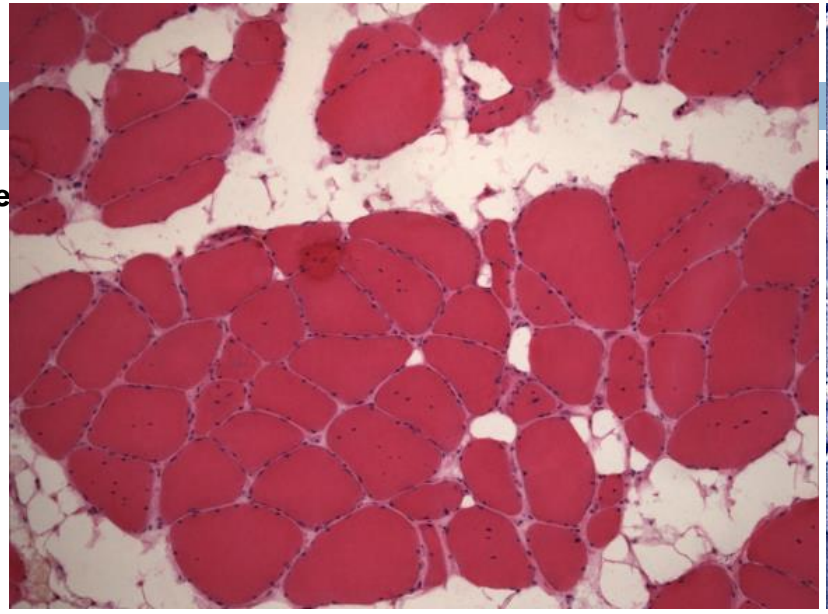


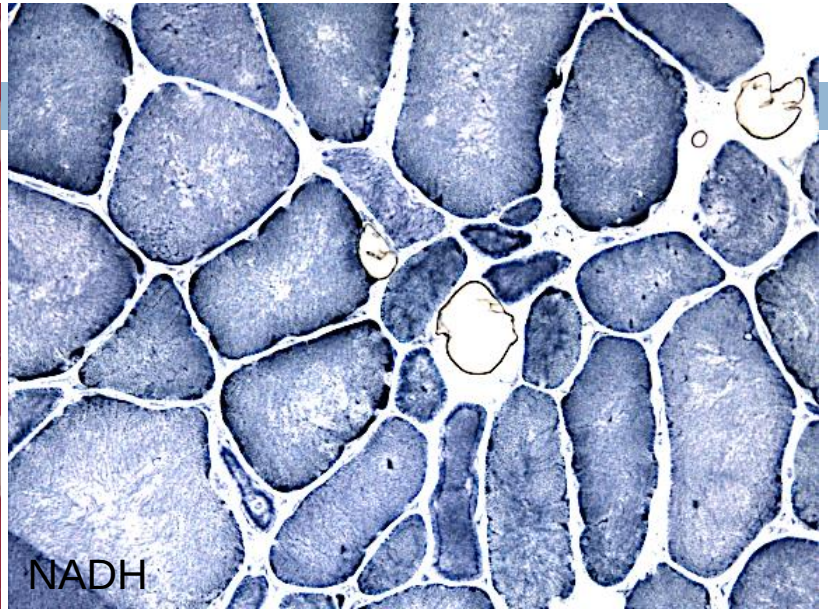


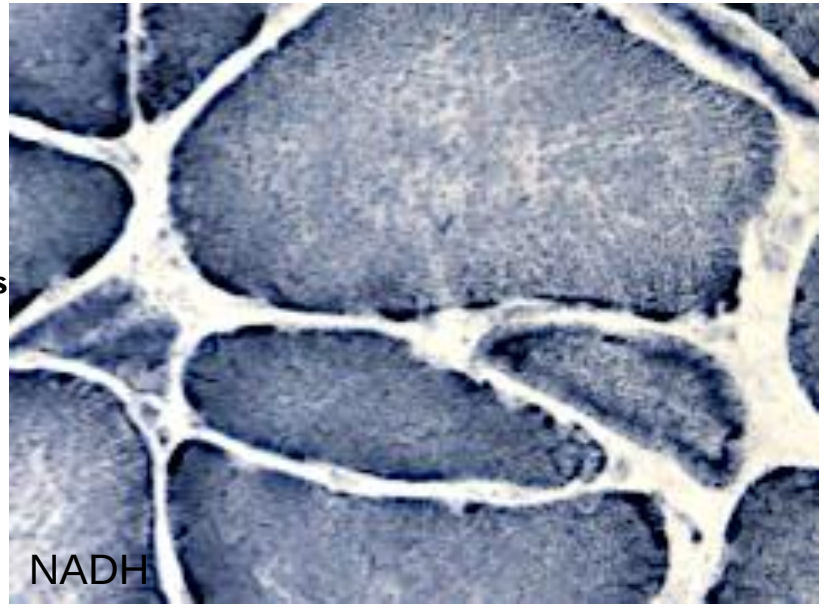


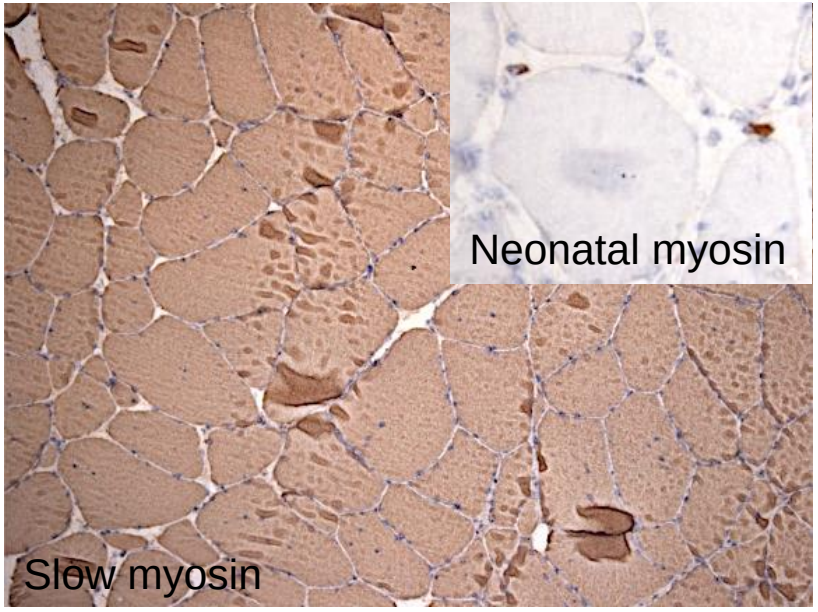
Necklace myopathy – MTM1 linked

- **Fibre size variation**
- **Slow predominance**
- **Uneven staining with few cores**
- **Several 'necklace' fibres; increased rim of oxidative staining at a distance from the sarcolemma**
- **Few neonatal myosin positive fibres**



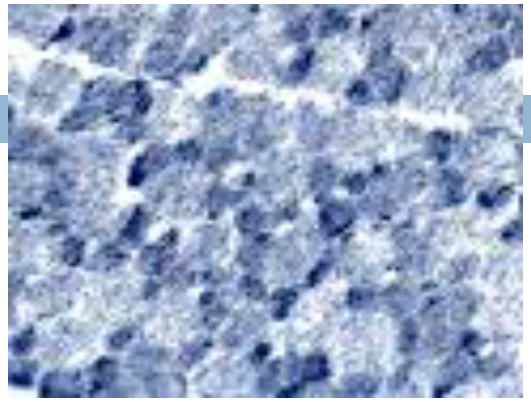


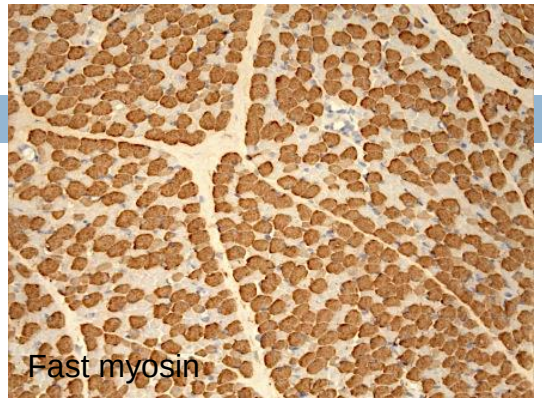


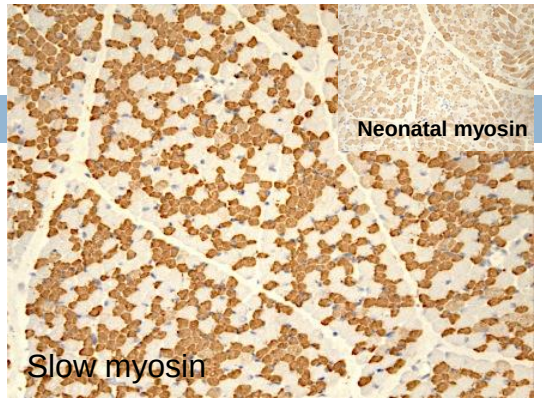


Congenital fibre type disproportion

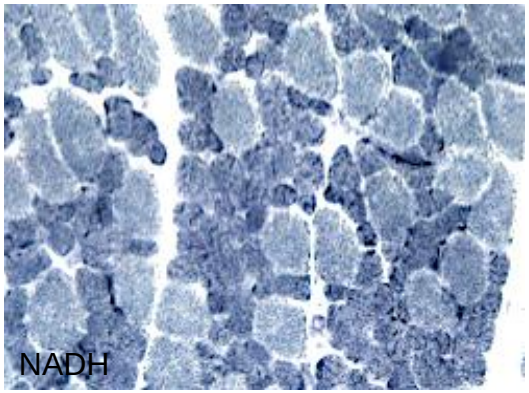
Type I fibres at least 12% smaller (later revised to 25%) than type II fibres with no other structural defect

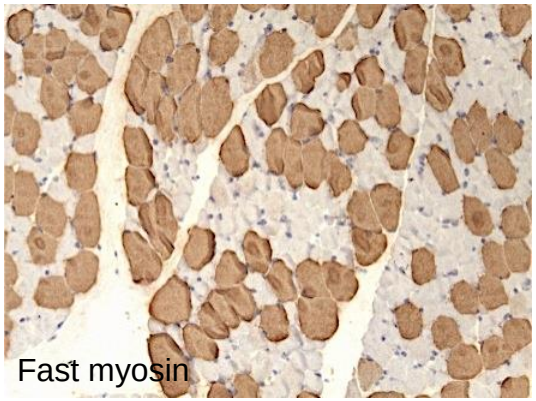


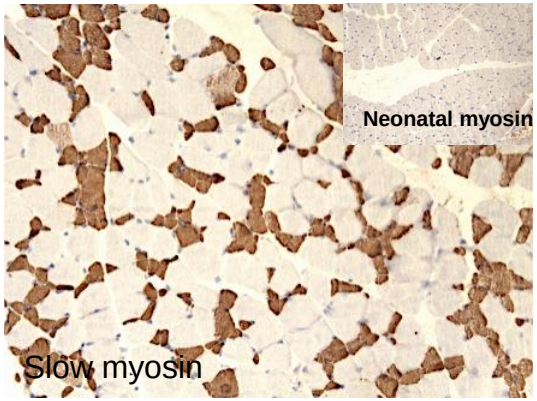




ACTA1







TPM3

CFTD linked genes – ACTA1, SEPN1, RYR1, TPM2, TPM3 MYH7
? Specific entity

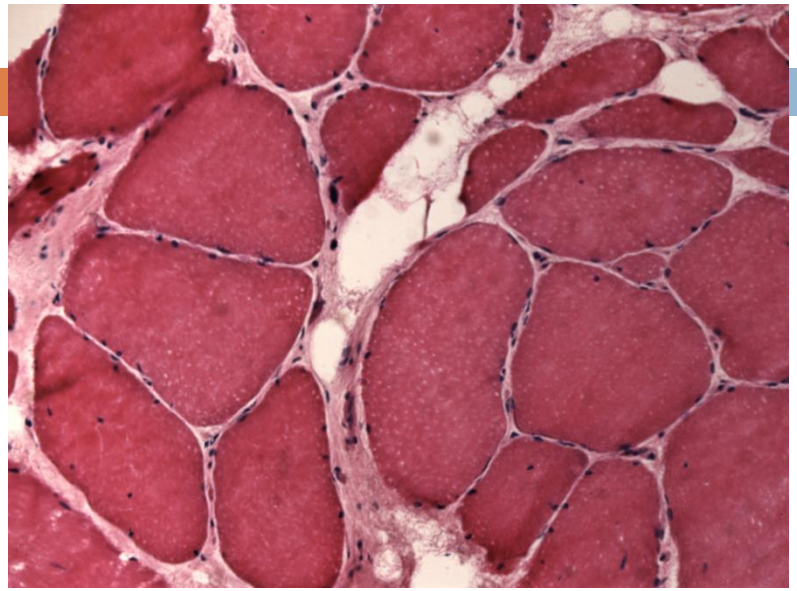
Gene defects associated with other early onset structural myopathies

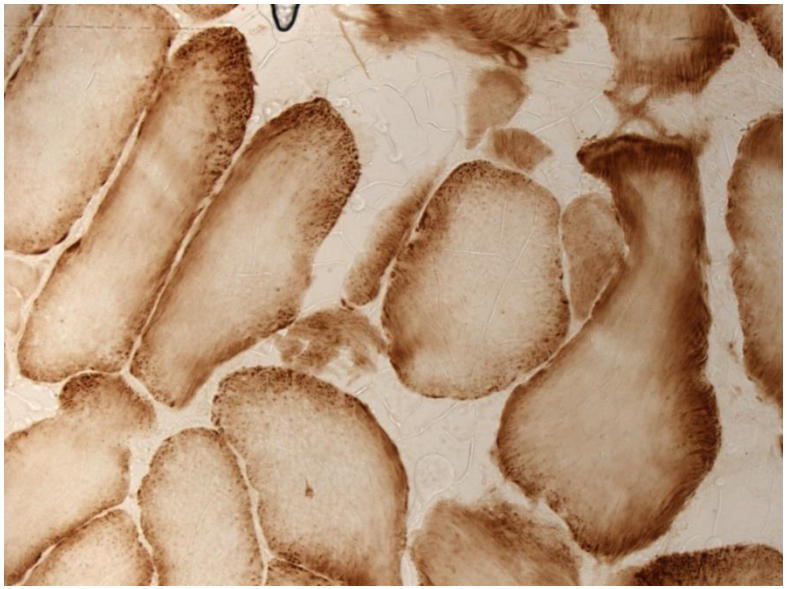
- Myosinopathies: MYH2, MYH3, MYH7 and MYH8; variable clinical presentation; pathological overlap with congenital myopathies; MYH7: myosin storage in type I fibres
- Reducing body myopathy: FHL1 gene mutation; aggregates reduced by menadione NBT; not all cases especially late onset show reducing bodies; severe X-linked dominant form with early onset and rapid clinical progression; milder adult limb girdle form
- Cap disease: Focal peripheral hyaline areas with disrupted myofibrillar material; stain with NADH, but not COX, SDH and unstained in myosin ATPase reaction; TPM2, TPM3, ACTA1 mutations; dominant mutations also form nemaline rods
- Zebra bodies: striped structures observed recently in ACTA1 null cases suggests a link
- Distal arthrogrypotic syndromes: mutations in genes encoding myosin isoforms (MYH3, MYH8) and other sarcomeric proteins (NEB, TPM2, TNNT3, TNNI2, MYBPC1) can cause DAS and some also present as congenital myopathy

Myosinopathies: Clinicopathological spectrum (Oldfors, Neuromuscular disorders 2007)

Protein and Gene	Disease	Clinical phenotype	Pathology
MYHC IIa **MYH2**	AD myopathy	Congenital joint contractures and ophthalmoplegia; mild in children, progressive in adults	Small and few type IIa fibres with structural changes in children; dystrophic with rimmed vacuoles in adults
	AR myopathy	Early onset weakness and ophthalmoplegia; very selective muscle imaging	Myopathic with absent IIa fibres
Embryonic MYHC **MYH3**	Freeman-Sheldon & Sheldon-Hall syndromes	Distal arthrogryposis and facial dysmorphism	Mild myopathic
Perinatal MYHC **MYH8**	Trismus and pseudocamptodactyly syndrome	Congenital contractures of hands, feet, jaws; trismus; hand and foot deformities; pseudocamptodactyly	Not described
MYHC I (Beta-Cardiac) **MYH7**	Familial hypertrophic/dilated cardiomyopathy	Cardiac failure; sudden death	Irregular staining with NADH in cardiac muscle
	Laing early onset distal myopathy	Childhood onset; slow progression; weakness of ankle dorsiflexors and hanging big toe sign	Myopathic; cores; type I hypotrophy; dystrophic with rimmed vacuoles in some cases
	Myosin storage myopathy	Childhood to middle age	Subsarcolemmal hyaline material in type I fibres immunoreactive for slow myosin but not desmin; NADH negative

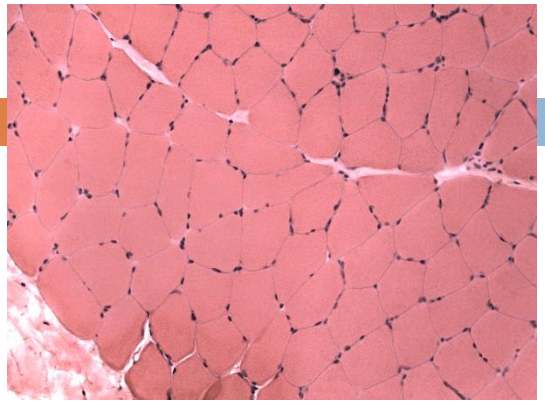
Myosinopathy: MYH7





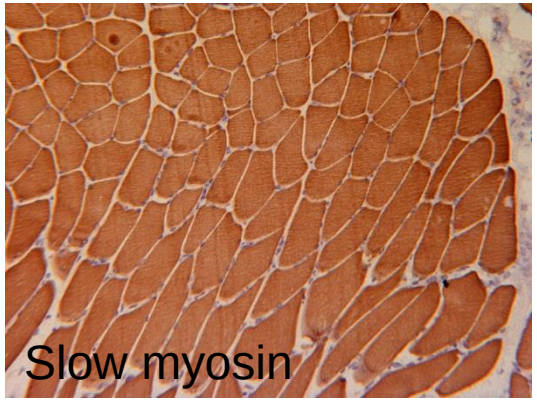
40 male
Scapuloperoneal syndrome
Heterozygous missense mutation p.Arg1845Trp
Previously described in MSM and dominant myopathy with scapuloperoneal distribution without hyaline bodies

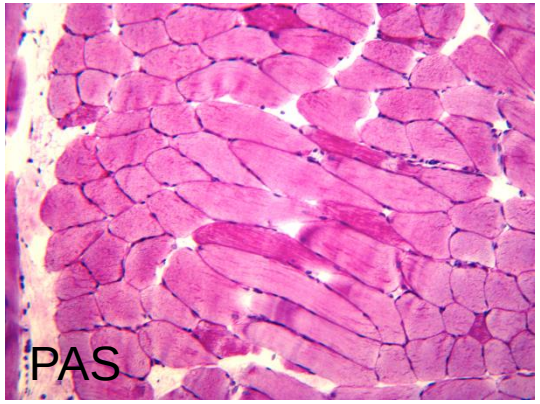
Myosinopathy: MYH2

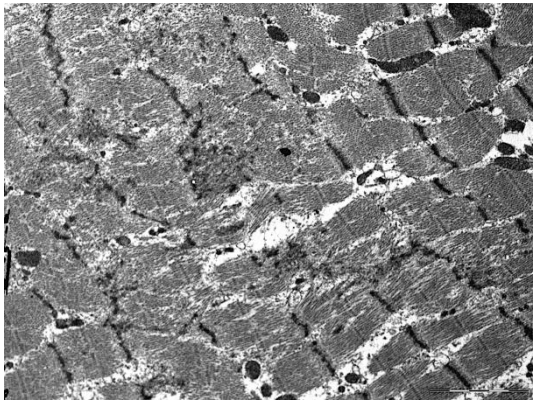


Case 1

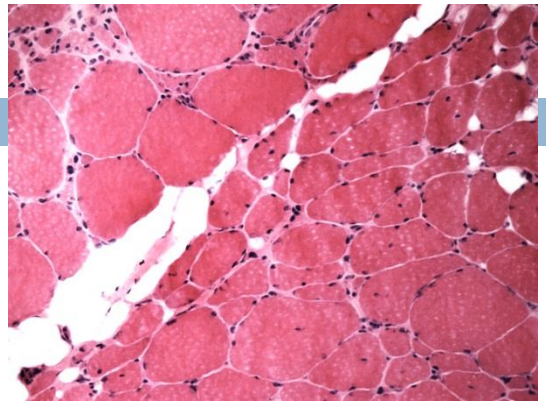
- *6 years male*
- *Axial, mild proximal weakness*
- *Facial weakness*
- *High arched palate*
- *Feeding difficulties*
- *Severe ophthalmoplegia*
- *Ptosis*

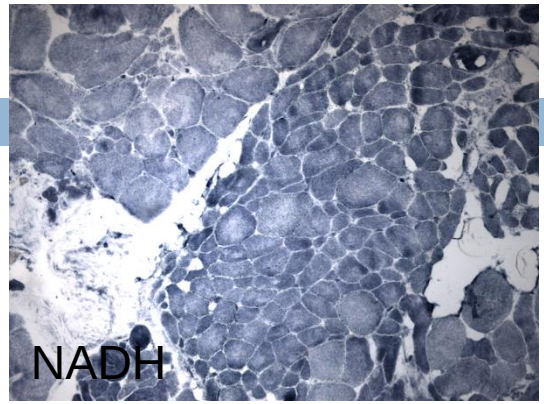


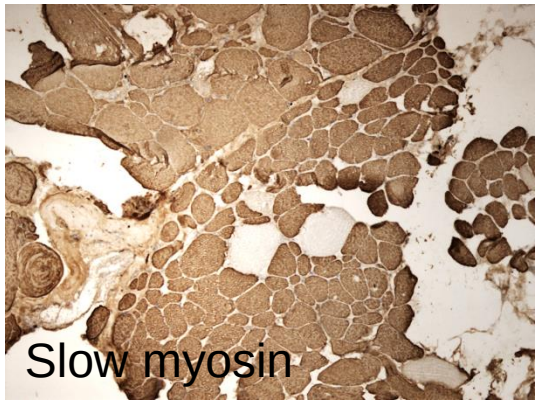


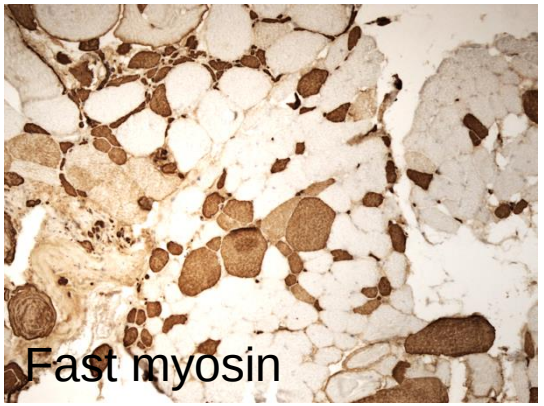


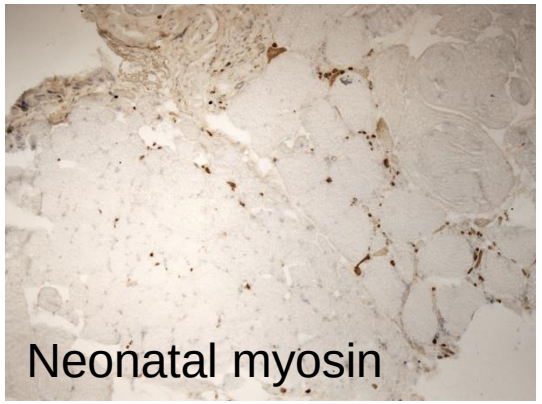
Case 2











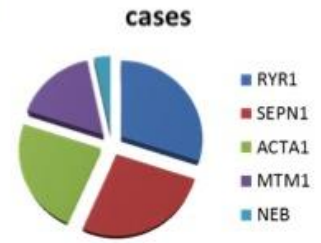
Pinprick fibres: a useful marker of CMs

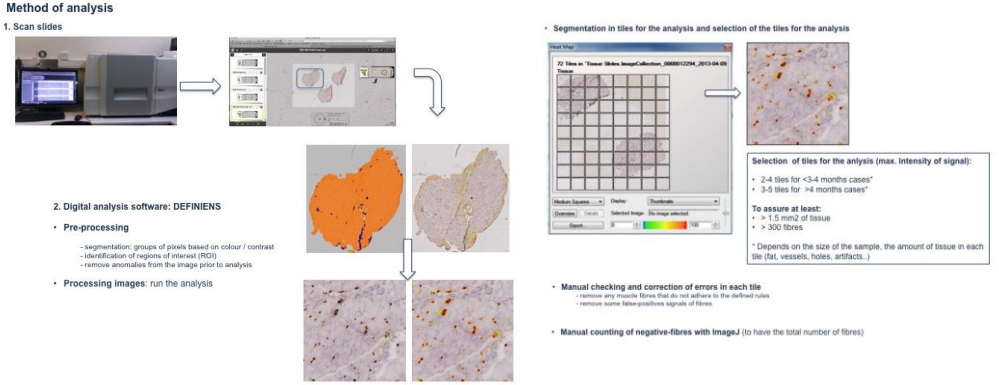
Cases recruited:

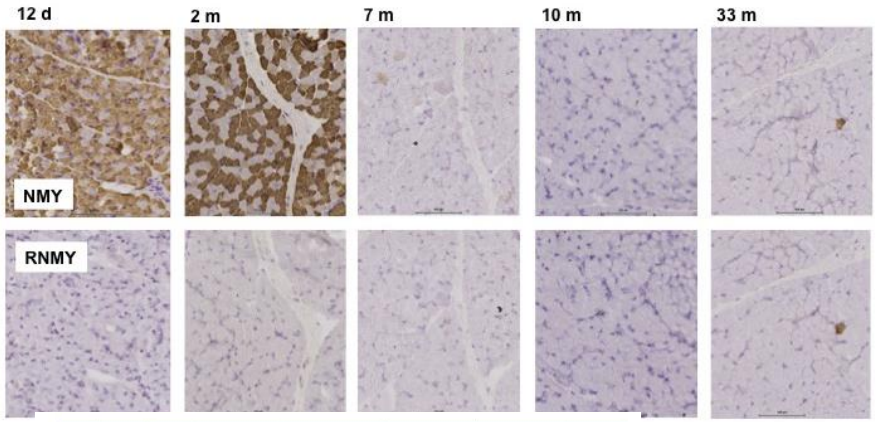
- ***30 Congenital Myopathies (52m [0-211]):***
  - RYR1: 9 (89 m [1-211])
  - SEPN1: 8 (63 m [25-151])
  - Acta1: 7 (28 m [0-129])
  - MTM1: 5 (40 m [2-111])
  - NEB: 1 (83 m)

  + 20 CM cases
  (12 RYR1, 2 ACTA1, 2 MTM1, 3 NEB, 2 TMP3)

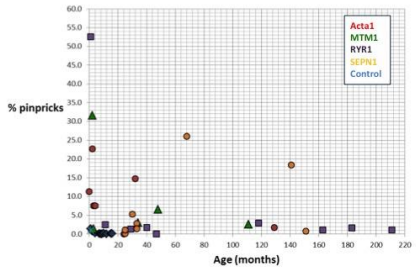
- ***10 Controls (8 m [1-16]):*** muscle *with "mild/minimal inespecific myopathic changes"*

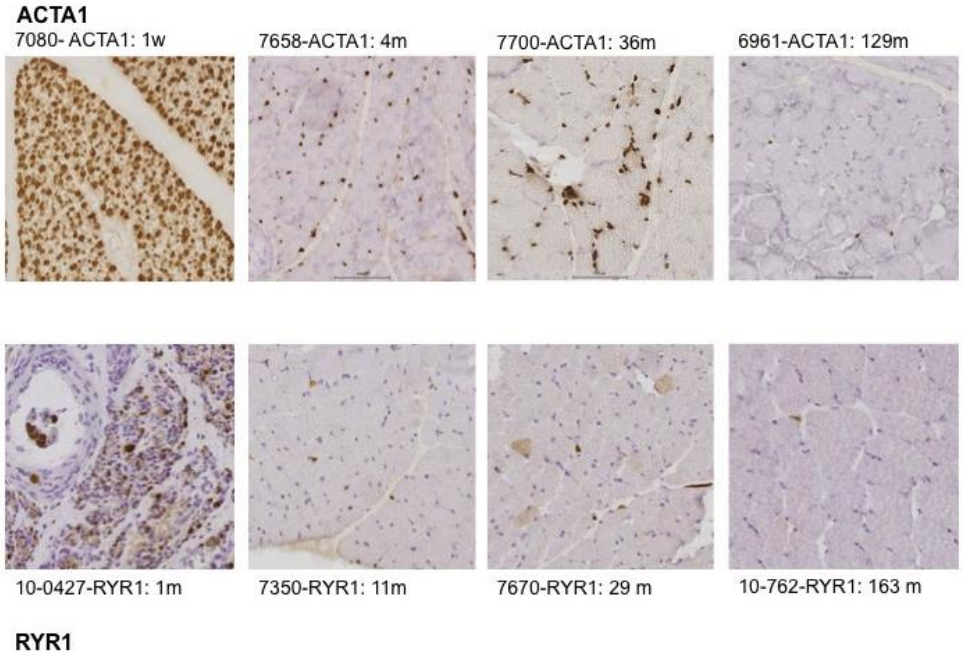






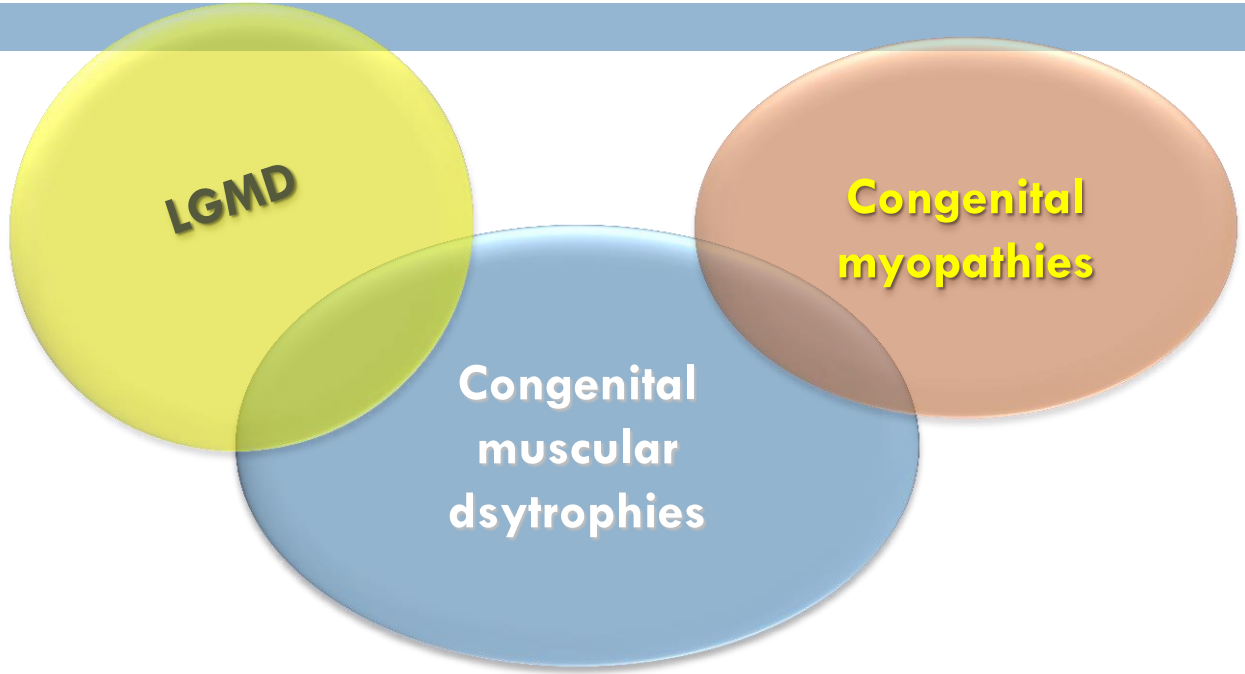
	n cases	Age (m)	Total number fibres	Total number positive fibres	Total Positive Fibres (%)	Total number pinpricks	Pinprincks (%)
ACTA1	7	28	19792	6830	25.7	2335	9.4
Neb	1	83	1811	10	0.6	5	0.3
MTM1	5	40	9943	1528	9	450	2.6
RYR1	9	89	8476	1168	7.2	562	3.3
SEPN1	8	63	5541	386	7	107	1.9
Control	10	8	18005	1807	14.1	47	0.3





Rivas, unpublished data

Overlapping phenotypes…



Genetic diagnosis allow us to

- counsel families appropriately
- Apply anticipatory care
- Plan adequate service provision
- Establish baselines for future interventions

Gene test as diagnostic standard

1. Direct sequencing of single→panel of genes
2. MLPA for single genes-deletions/duplications
3. Next Generation sequencing

- Panel sequencing of all known/relevant Cmyo-CMD genes

- **Phenotype driven**
- Better coverage
- ↓ variants of unknown significance
- ↓incidental findings

- Whole exome/genome sequencing

- >3million variants/analysis
- ↑↑ UCVs and incidental findings
- Clinical context is paramount

NHS

Diagnostic and Advisory Service for Rare Neuromuscular Diseases

National Service Referral Form for
Congenital Muscular Dystrophies and Myopathies

If possible, muscle biopsy slides and MRI images should be forwarded at the time of referral so that they can be reviewed in advance of the consultation. This facilitates the diagnostic process for families.

📖 **DETAILED CLINIC LETTER (mandatory)**

📖 MUSCLE MRI on CD or via the GOSH Image Exchange Portal* (if available/indicated)

📖 BRAIN MRI on CD or via the GOSH Image Exchange Portal (if available/indicated)

📖 CLINICAL PHOTOGRAPHS (if available/indicated)

📖 MUSCLE BIOPSY REPORT (if available/indicated)

*Muscle and brain MRI images may be forwarded via the GOSH Image Exchange Portal. Clinical photographs may aid diagnosis if the patient is not being seen here.

NSCT Referral Gatekeeping

CMv1	CMv2: new genes
ACTA1	CCDC78
BIN1	KLHL41
CFL2	KLHL40
DNM2	DNA2
ECEL1	SLC35A3
KBTBD13	MYBPC1
KBTBD13	PIEZO2
MTM1	ZC4H2
MYH2	VPS33B
MYH3	LAMP2
MYH7	VMA21
MYH8	STAC3
NEB	LMOD3
ORAI1	MEGF10
RYR1	EPG5
SEPN1	
STIM1	
STIM2	
TNNI2	
TNNT1	
TNNT3	
TPM2	
TPM3	
TTN	

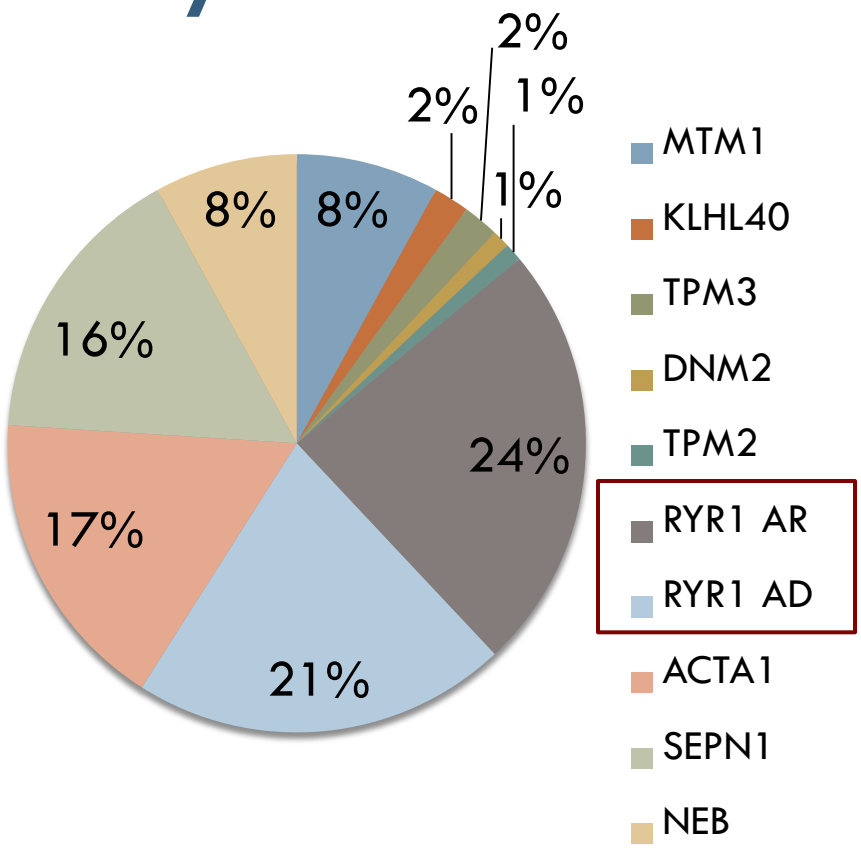
CMD

B3GALNT2	GMPPB
B3GNT1	GTDC2
CHKB	ISPD
COL12A1	ITGA7
COL4A1	ITGA9
COL4A2	LAMA2
COL6A1	LARGE
COL6A2	MICU1
COL6A3	PLEC
DAG1	POMGNT1
DOLK	POMT1
DPM1	POMT2
DPM2	SGK196
DPM3	SIL1
FKRP	TMEM5
FKTN	

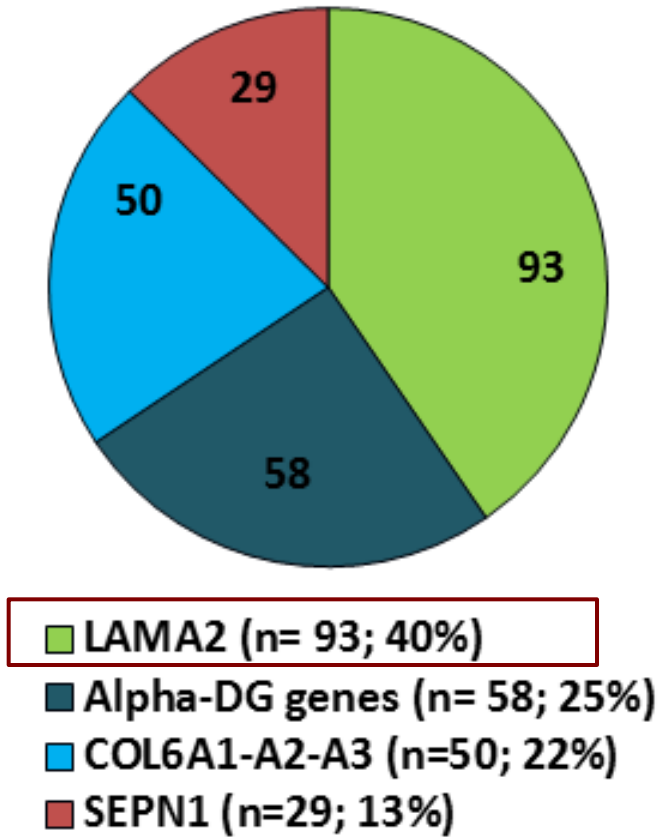
Anna Sarkozy, Rachael Mein

Frequencies in the UK

CMyo



CMD



[Colombo et al, 2015]

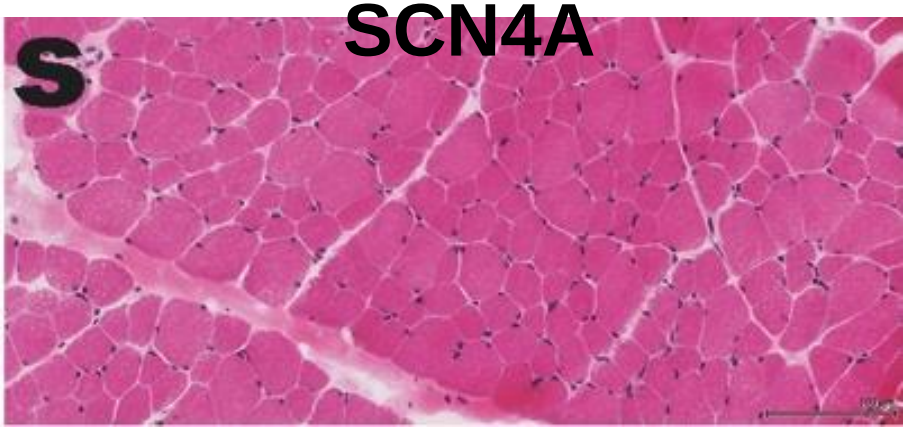
[Sframeli et al. WMS 2014]

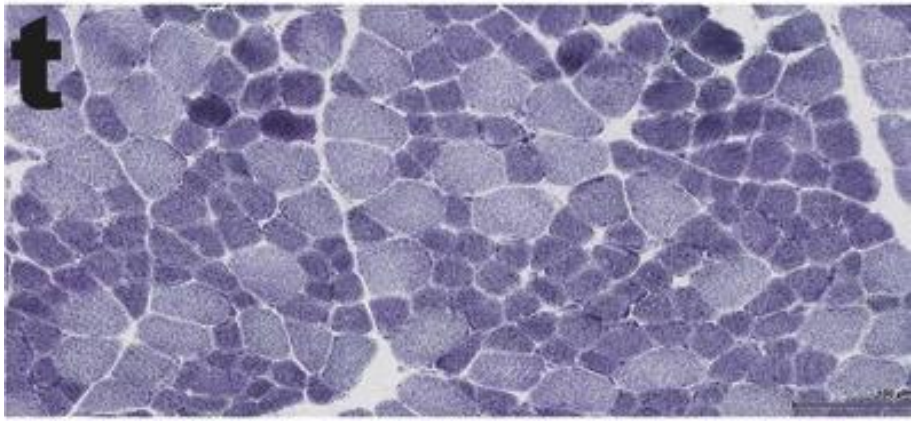
Novel CMYo genes/diseases

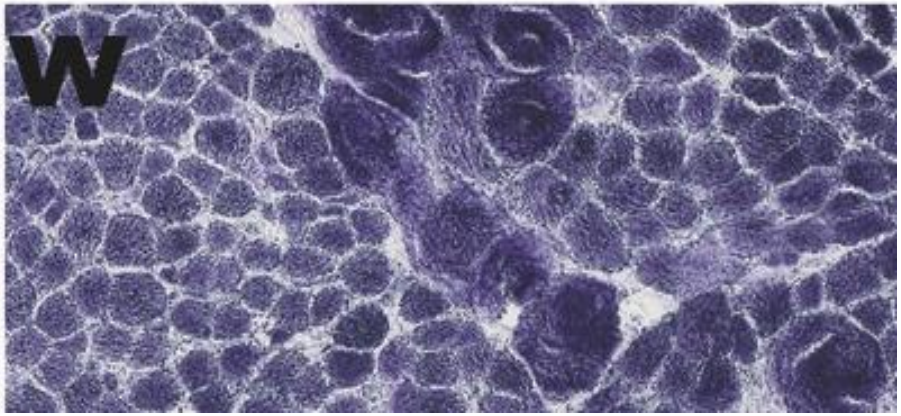
Gene/protein	Inheritance	Clinical phenotype	Pathology
CACNA1S/DHPR	AR/AD	Hypotonia; axial++; PP; MHS	NSM; slow predominance; minicores
TRDN/triadin	AR (null)	Arrhythmia + myopathy	Dilated/degenerating SR lateral cisterns
STIM-1/ORAI-1	AD (gain/loss of function)	Allelic disorders; multisystem involvement with myopathy	TAM; NSM; minicores
PYROXD1	AR	Moderately severe early onset myopathy	NSM
ECEL1	AR	DA spectrum contractures and myopathy	Cores and internal nuclei
PIEZO2	AD	DA spectrum contractures and myopathy	Cores and internal nuclei
SCN4A	AR	Fetal akinesia to early onset myopathy spectrum	End-stage to NSM; fibre type disproportion

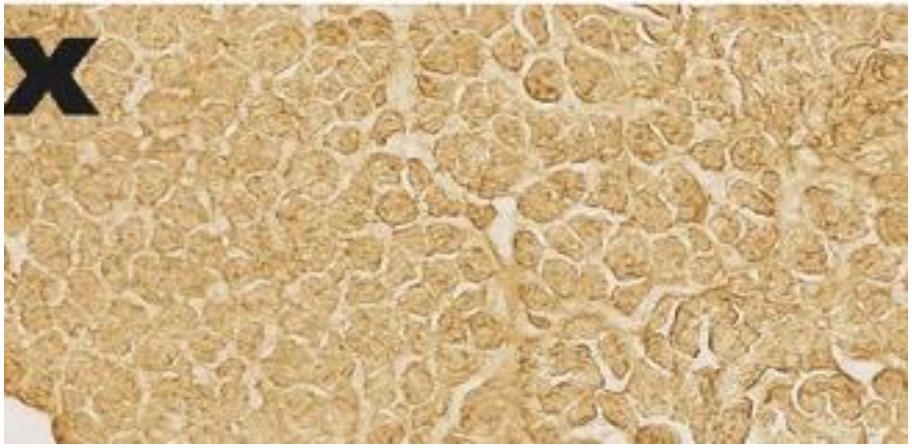
SCN4A

CACNA1S









Summary

- Specific structural abnormalities coupled with classic clinical phenotype; broad spectrum and overlap
- Discovery of new genes; expanding conventional pathological phenotypes
- Slow fibre predominance with very small fMHC+ fibres is a common signature across CMs
- Refinement of gene panels will likely reduce the need for muscle biopsy as a first line investigation; however systematic clinical assessment, muscle pathology and functional studies will be key in assessing pathogenicity of new variants and refining novel gene phenotypes

Acknowledgements

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