Pathology of muscular dystrophies

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EPNS 2017

Muscular dystrophies

- Definitions and current classifications
- Pathomechanisms
- Morphology: canonical features, variability and overlaps
- Immunoanalysis: methods and factors influencing interpretation
- Examples of dystrophy variants where pathology can make a useful contribution including role of immunodiagnostics

- Dystrophy – modern Latin ‘dystrophia’; Greek: ‘dys’ = hard/bad/ill + ‘trophe’ = nourishment; weakening/wasting/atrophy
- Clinically, genetically, biochemically heterogenous diseases sharing clinical features and dystrophic changes on biopsy – necrosis, regeneration and fibrosis
- Historical classifications based on clinical features: LGMDs, EDMDs, CMDs – evolution to incorporate inheritance mode and gene defect: LGMD2A and LGMD2B – one disease one gene dogma
- Modern classifications reflect better understanding of genetics and molecular pathomechanisms: clinical features-inheritance-gene-protein defect-protein localisation function

	Inheritance	OMIM number	Locus	Gene symbol	Protein	Main localisation
Duchenne or Becker muscular dystrophy	X-R	310200 (Duchenne); 300376 (Becker)	Xq21·2	*DMD*	Dystrophin	Sarcolemma-associated protein
Limb girdle muscular dystrophy						
Type 1A	AD	159000	5q31	*MYOT*	Myotilin	Sarcomere-associated protein (Z disc)
Type 1B	AD	159001	1q21·2	*LMNA*	Lamin A/C	Nuclear lamina-associated protein
Type 1C	AD	607780	3p25	*CAV3*	Caveolin-3	Sarcolemma-associated protein
Type 1D	AD	603511	7q	*DNAJB6*	Co-chaperone DNAJB6	Sarcomere-associated protein (Z disc)
Type 1E	AD	602067	6q23	*DES*	Desmin	Intermediate filament protein
Type 1F	AD	608423	7q32	Unknown	Unknown	Unknown
Type 1G	AD	609115	4p21	Unknown	Unknown	Unknown
Type 1H	AD	613530	3p23–p25	Unknown	Unknown	Unknown
Type 2A	AR	253600	15q15·1	*CAPN3*	Calpain-3	Myofibril-associated proteins
Type 2B	AR	253601	2p13	*DYSF*	Dysferlin	Sarcolemma-associated protein
Type 2C	AR	253700	13q12	*SGCG*	γ-sarcoglycan	Sarcolemma-associated protein
Type 2D	AR	608099	17q12–q21·33	*SGCA*	α-sarcoglycan	Sarcolemma-associated protein
Type 2E	AR	604286	4q12	*SGCB*	β-sarcoglycan	Sarcolemma-associated protein
Type 2F	AR	601287	5q33	*SGCD*	δ-sarcoglycan	Sarcolemma-associated protein
Type 2G	AR	601954	17q12	*TCAP*	Titin cap (telethonin)	Sarcomere-associated protein (Z disc)
Type 2H	AR	254110	9q31–q34	*TRIM32*	Tripartite motif-containing 32 (ubiquitin ligase)	Sarcomeric-associated protein (Z disc)
Type 2I	AR	607155	19q13·3	*FKRP*	Fukutin-related protein	Putative glycosyltransferase enzymes
Type 2J	AR	608807	2q31	*TTN*	Titin	Sarcomeric protein
Type 2K	AR	609308	9q34	*POMT1*	Protein-1-O-mannosyl-transferase 1	Glycosyltransferase enzymes
Type 2L	AR	611307	11p14·3	*ANO5*	Anoctamin 5	Transmembrane protein, possible sarcoplasmic reticulum
Type 2M	AR	611588	9q31	*FKTN*	Fukutin	Putative glycosyltransferase enzymes
Type 2N	AR	613158	14q24	*POMT2*	Protein-O-mannosyl-transferase 2	Glycosyltransferase enzymes
Type 2O	AR	613157	1p34	*POMGNT1*	Protein-O-linked mannose β 1,2-N-aminyltransferase 1	Glycosyltransferase enzymes
Type 2P	AR	613818	3p21	*DAG1*	Dystrophin-associated glycoprotein 1	Sarcomeric-associated protein
Type 2Q	AR	613723	8q24	*PLEC1*	Plectin 1	Sarcolemma-associated protein (Z disc)
Facioscapulohumeral muscular dystrophy						
Type 1	AD	158900	4q35	Unknown	DUX4 and chromatin rearrangement	Nuclear
Type 2	AD	158901	18	Unknown	SMCHD1	Structural maintenance of chromosomes flexible hinge domain containing 1
Emery-Dreifuss muscular dystrophy						
X-linked type 1	X-R	310300	Xq28	*EMD*	Emerin	Nuclear membrane protein
X-linked type 2	X-R	300696	Xq27·2	*FHL1*	Four and a half LIM domain 1	Sarcomere and sarcolemma
Autosomal dominant	AD	2181350	1q21·2	*LMNA*	Lamin A/C	Nuclear membrane protein
Autosomal recessive	AR	604929	1q21·2	*LMNA*	Lamin A/C	Nuclear membrane protein
With nesprin-1 defect	AD	612998	6q25	*SYNE1*	Spectrin repeat containing, nuclear envelope 1 (nesprin-1)	Nuclear membrane protein
With nesprin-2 defect	AD	5612999	4q23	*SYNE2*	Spectrin repeat containing, nuclear envelope 2 (nesprin-2)	Nuclear membrane protein
Congenital muscular dystrophy with merosin deficiency (MDC1A)	AR	607855	6q2	*LAMA2*	Laminin α2 chain of merosin	Extracellular matrix proteins
Congenital muscular dystrophy	AR	604801	1q42	Unknown	Unknown	Unknown
Congenital muscular dystrophy and abnormal glycosylation of dystroglycan (MDC1C)	AR	606612	19q13	*FKRP*	Fukutin-related protein	Putative glycosyltransferase enzymes
Congenital muscular dystrophy and abnormal glycosylation of dystroglycan (MDC1D)	AR	608840	22q12	*LARGE*	Like-glycosyl transferase	Putative glycosyltransferase enzymes
Fukuyama congenital muscular dystrophy	AR	253800	9q31–q33	*FCMD*	Fukutin	Putative glycosyltransferase enzymes

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	Inheritance	OMIM number	Locus	Gene symbol	Protein	Main localisation
Walker-Warburg syndrome						
With fukutin defect	AR	236670	9q31–q33	*FCMD*	Fukutin	Putative glycosyltransferase enzymes
With protein-O-mannosyl-transferase 1 defect	AR	236670	9q34	*POMT1*	Protein-1-O-mannosyl-transferase 1	Glycosyltransferase enzymes
With protein-O-mannosyl-transferase 2 defect	AR	236670	14q24	*POMT2*	Protein-O-mannosyl-transferase 2	Glycosyltransferase enzymes
With protein-O-linked mannose β 1,2-N-aminyltransferase 1 defect	AR	236670	1p34	*POMGNT1*	Protein-O-linked mannose β 1,2-N-aminyltransferase 1	Glycosyltransferase enzymes
With fukutin-related protein defect	AR	236670	19q13	*FKRP*	Fukutin-related protein	Putative glycosyltransferase enzymes
Muscle-eye-brain disease						
With protein-O-linked mannose β 1,2-N-aminyltransferase 1 defect	AR	253280	1p34	*POMGNT1*	Protein-O-linked mannose β 1,2-N-aminyltransferase 1	Glycosyltransferase enzymes
With fukutin-related protein defect	AR	253280	19q13	*FKRP*	Fukutin-related protein	Putative glycosyltransferase enzymes
With protein-O-mannosyl-transferase 2 defect	AR	253280	14q24	*POMT2*	Protein-O-mannosyl-transferase 2	Glycosyltransferase enzymes
Congenital muscular dystrophy due to glycosylation disorder	AR	NA	9q34·1	*DPM2*	Dolichyl-phosphate mannosyltransferase polypeptide 2	Glycosyltransferase enzymes
Congenital muscular dystrophy due to glycosylation disorder	AR	NA	1q21·3	*DPM3*	Dolichyl-phosphate mannosyltransferase polypeptide 3	Glycosyltransferase enzymes
Congenital muscular dystrophy with mitochondrial structural abnormalities	mtDNA	602541	22q13	*CHKB*	Choline kinase	Sarcolemmal and mitochondrial membrane
Congenital muscular dystrophy with rigid spine syndrome	AR	602771	1p36	*SEPN1*	Selenoprotein N1	Endoplasmic reticulum protein
Ullrich syndrome						
With collagen type VI subunit α1 defect	AR	254090	21q22·3	*COL6A1*	Collagen type VI, subunit α1	Extracellular matrix proteins
With collagen type VI subunit α2 defect	AR	254090	21q22·3	*COL6A2*	Collagen type VI, subunit α2	Extracellular matrix proteins
With collagen type VI subunit α3 defect	AR	254090	2q37	*COL6A3*	Collagen type VI, subunit α3	Extracellular matrix proteins
Congenital muscular dystrophy with integrin α7 defect	AR	613204	12q13	*ITGA7*	Integrin α7	External sarcolemmal protein
Congenital muscular dystrophy with integrin α9 defect	AR	NA	3p21·3	*ITGA9*	Integrin α9	External sarcolemmal protein
Muscular dystrophy with generalised lipodystrophy	AR	NA	17q21–q23	*PTRF*	Polymerase I and transcript release factor (cavin-1)	T tubules and sarcolemma
Oculopharyngeal muscular dystrophy	AD or AR	164300	14q11·2	PABPN1	Polyadenylate binding protein nuclear 1	Unknown

X-R=X-linked recessive. OMIM=Online Mendelian Inheritance in Man. AD=autosomal dominant. AR=autosomal recessive. NA=not assigned.

Protein defect	Locus	Gene	Phenotype
ECM proteins	6q22-23	LAMA2	Primary merosin deficiency
	21q22.3	COL6A1,A2,A3	Ullrich CMD
	2q37		
External sarcolemmal	12q13	IGTA7	Integrin alpha 7 CMD
	3p23-21	IGTA9	Integrin alpha 9 CMD
Dystroglycan and glycosyltransferase enzymes	9q34.1	POMT1	WWS, MEB, CMD with cerebellar involvement, CMD with MR and microcephaly
	1q32-34	POMGnT1	WWS, MEB, CMD with cerebellar involvement
	14q24.3	POMT2	WWS, MEB, CMD with cerebellar involvement, CMD with MR and microcephaly
	19q13.3	FKRP	WWS, MEB, CMD with cerebellar involvement, CMD with MR and microcephaly, CMD with no MR
	9q31	FCMD	Fukuyama CMD
	22q12.3-13.1	LARGE	WWS, MEB, white matter changes
	1q12-q21	DMP2/DMP3	CMD with MR and severe epilepsy
	3p21	DAG1	
	1q42	-	MDC1B
ER proteins	1p35-36	SEPN1	RSMD1
Nuclear envelope proteins	6q25	SYNE1 (nesprin1)	CMD with adducted thumbs
	1q21.2	LMNA	Congenital laminopathy
Sarcolemmal and mitochondrial membrane protein	22q13	CHKB	Mitochondrial CMD

Mercuri E, Muntoni F. The Lancet 2013

Mercuri E, Muntoni F. Annals of Neurology 2012

CMDs

Brief CMD classification overview (underlined: abbreviated nomenclature used in this paper).

Subtype and alternate nomenclatures Associated Genes	Associated phenotypic spectrum
Collagen VI related dystrophies (COL6-RD) COL6A1, COL6A2, COL6A3	■ Ullrich congenital muscular dystrophy (UCMD) – severe nonambulant and transient ambulant ■ Intermediate phenotype ■ Bethlem myopathy (BM, milder disease course)
Lamininα2 related dystrophy (LAMA2-RD, includes MDC1A, Merosin deficient CMD, LAMA2-CMD) LAMA2	■ Non-ambulant LAMA2-RD ■ Ambulant LAMA2-RD ■ Non-ambulant typically correlates with absent laminin α2 staining on muscle biopsy and ambulant with partial deficiency (with exeptions)
αDystroglycan related dystrophy (αDG-RD, also alpha dystroglycanopathy, αDGpathy) FKRP, FKTN, POMT1, POMT2, POMGnT1, LARGE, ISPD, GTDC2, DAG1,TMEM5, B3GALNT2, B3GNT1, GMPPB, SGK196 (DPM1, DPM2, DPM3, DOLK)	■ Walker–Warburg syndrome ■ Muscle–eye–brain disease; Fukuyama CMD; Fukuyama-like CMD ■ CMD with cerebellar involvement; cerebellar abnormalities may include cysts, hypoplasia, and dysplasia ■ CMD with mental retardation and a structurally normal brain on imaging; this category includes patients with isolated microcephaly or minor white matter changes evident on MRI ■ CMD with no mental retardation; no evidence of abnormal cognitive development ■ Limb-girdle muscular dystrophy (LGMD) with mental retardation (milder weakness, maybe later onset) and a structurally normal brain on imaging ■ LGMD without mental retardation (milder weakness, maybe later onset)
SEPN1 related myopathy (SEPN1-RM, also rigid spine CMD, RSMD1) SEPN1	■ Consistent rigid spine early respiratory failure phenotype ■ despite variable histological presentations as multiminicore disease, desmin positive Mallory body inclusions, congenital fiber-type disproportion, mild CMD, or nonspecific myopathy
RYR1 related myopathy (RYR1-RM, includes RYR1-CMD) RYR1	■ RYR1 related myopathies (RYR1-RM) include central core, multi-mini-core, centronuclear and nonspecific pathologies. which can assume CMD like characteristics ■ Clinically significant for early scoliosis and absent or limited ambulation
LMNA related dystrophy (LMNA-RD, includes LMNA-CMD, L-CMD, and Emery Dreifuss) LMNA	■ CMD presentation: Dropped head syndrome, axial and scapuloperoneal involvement, absent or early loss of ambulation ■ Milder presentations fuse with early-onset Emery–Dreifuss muscular dystrophy
CMD without genetic diagnosis	■ Congenital onset weakness with CMD compatible histology and variable clinical features, without confirmed genetic diagnosis, despite testing for currently known genes

- Early onset disorders; bx compatible with dystrophic process
- Gene/protein name annotated by –RD or –RM for phenotypic subclasses reflects ‘typical’ bx picture and allows for a broader clinicopathological spectrum

Bonnemann CG et. al. Neuromuscular Disorders 2014

Epidemiology

DMD - commonest inherited childhood dystrophy; myotonic dystrophy most common in adults
LGMDs - recessive more common than dominant
LGMD2A – Southern Europe; LGMD2I – Northern Europe
LAMA2-CMD, alpha-dystroglycan-CMD and Ullrich-CMD more common CMD forms
Fukuyama CMD –Japan

Audit of clinical and molecular diagnosis of CMD patients referred for molecular testing in the UK 2001-2013

Maria Sframeli *DNC* and Marta Bertoli *Walton Centre*

Total of 3734 referrals

-1042 for *LAMA2, POMT1, POMT2, POMGNT1, FKRP, FKTN, LARGE, ISPD, GMPPB, B3GALNT2, COL6A1, COL6A2, COL6A3* and *SEPN1* gene testing

-2692 for LMNA and FKRP gene testing

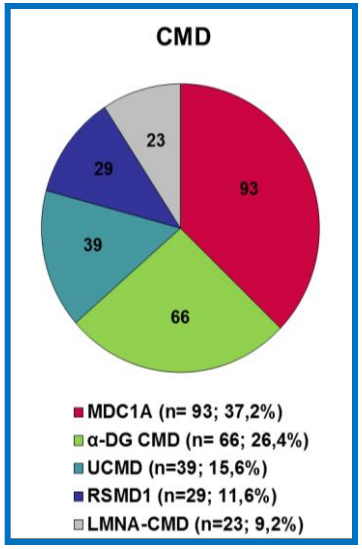
Clinic subgroups:

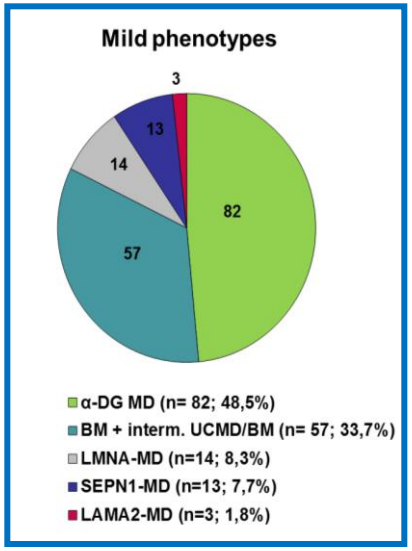
a)CMD (1) presentation before 2 years of age with hypotonia, weakness, contractures, delayed motor milestones or characteristic eye or brain abnormalities; 2) dystrophic or myopathic changes on the muscle biopsy, with exclusion of other specifically identifiable neuromuscular disorders.

b)milder phenotype.

Confirmed genetic diagnosis: 441/3734 patients (12%)

363 mutations, 181 novel.





- **MDC1A being the most common CMD subtype (37,2%) in the UK**
- **In CMD with A-DG reduction, mutations in the *POMGnT1* gene are the most common and MEB disease is the prevalent phenotype (16 pts, 27%);**
- **In milder phenotypes, *FKRP* gene mutations are the most common (49.1%), followed *COL6* gene (33,7%).**
- **A multiple gene approach(NGS panel), or targeted/whole exome gene sequencing, will possibly better approach the diagnosis of this complex group of patients.**

Clinical features

Onset varies from birth to childhood to adulthood
Several variants show distinctive patterns of muscle weakness
Scoliosis; stiffness; contractures; respiratory impairment
Extra-muscular involvement: CNS, ocular cardiac
Serum CK can be normal – Ullrich, FSHD
Muscle imaging: differential patterns of muscle involvement

	Motor function	Distribution of weakness	Rigid spine	Cardio-myopathy	Respiratory impairment	Disease course	Increased CK	Other signs
Congenital-onset muscular dystrophy								
Congenital muscular dystrophy with merosin deficiency	Independent ambulation generally not achieved in patients with absent merosin	Upper limbs>lower limbs	–	Not frequent	++	Slowly progressive	++	White matter changes on brain MRI
Congenital muscular dystrophy and abnormal glycosylation of dystroglycan (Walker-Warburg syndrome, muscle-eye-brain disease, congenital muscular dystrophy type 1C, etc)	Independent ambulation generally not achieved	Upper limbs>lower limbs	–	Not frequent	+	Slowly progressive	++	Frequent structural brain changes
Congenital muscular dystrophy with rigid spine syndrome type 1 (SEPN1)	Ambulation achieved	Axial muscles>limbs	++	–	Early respiratory failure	Progression of respiratory signs>motor signs	N or +	Scoliosis
Ullrich syndrome	Ambulation achieved in ~50% but lost by middle teens	Proximal and axial	++	–	Early respiratory failure	Progression of respiratory and motor signs	N or +	Distal laxity

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	Motor function	Distribution of weakness	Rigid spine	Cardio-myopathy	Respiratory impairment	Disease course	Increased CK	Other signs
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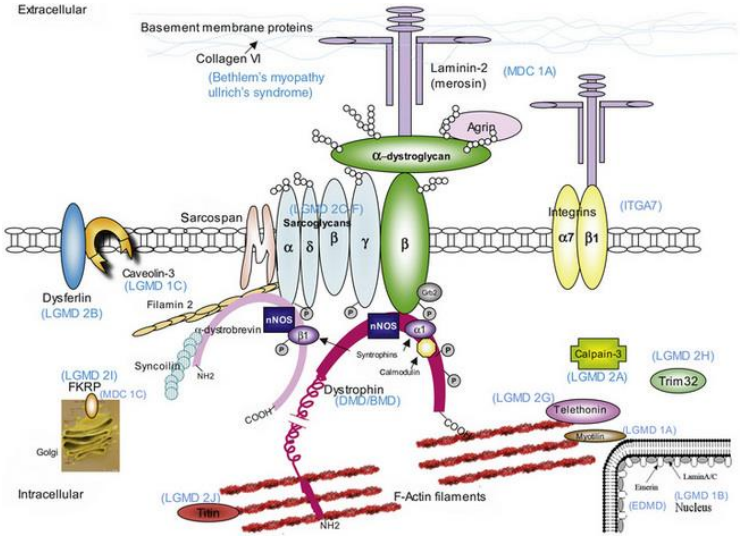
From early-onset to childhood-onset muscular dystrophy								
Duchenne muscular dystrophy	Independent ambulation achieved, but lost before age of 13 years	Proximal>distal (pattern A)	–	++	++	Progression of motor, cardiac, and respiratory signs	++	Mental retardation in 30%
Emery-Dreifuss muscular dystrophy with lamin AC deficiency (type 2)	Ambulation achieved in all cases except for rare cases with congenital onset	Scapulo peroneal (pattern B)	++	++	In adulthood in the typical form, but also in childhood (congenital variants)	Slowly progressive	+ (+)	Frequent association with Dunningham type lipodystrophy
Limb girdle muscular dystrophy with lamin AC deficiency (type 1B)	Independent ambulation achieved, variable progression	Proximal>distal (pattern A)	+	++	In adulthood	Progression of cardiac signs>motor signs	+ (+)	None
Limb girdle muscular dystrophy with calpain deficiency (type 2A)	Ambulation achieved	Proximal>distal (pattern A)	+	–	Not frequent	Slow progression	++	None
Childhood-onset and adulthood-onset muscular dystrophy								
Becker muscular dystrophy	Independent ambulation achieved, variable progression	Proximal>distal (pattern A)	–	++	Not frequent	Progressive with substantial variability	++	None
Limb girdle muscular dystrophy with sarcoglycan deficiency (type 2C, 2D, 2E, 2F)	Independent ambulation achieved, generally lost in the second decade	Proximal>distal (pattern A)	–	++	++	Progression of motor, cardiac and respiratory signs	++	None
Limb girdle muscular dystrophy with abnormal glycosylation of dystroglycan (type 2I, 2K, 2L, 2M, 2N, 2O)	Independent ambulation achieved, variable progression	Proximal>distal (pattern A)	–	++	+(+)	Progressive	++	Mental retardation reported in some cases
Limb girdle muscular dystrophy with dysferlin deficiency (type 2B)	Independent ambulation always achieved	Both pattern A and pattern E	–	–	–	Progressive in adulthood	++	None
Limb girdle muscular dystrophy with telethonin deficiency (type 2G)	Independent ambulation achieved, generally lost in the fourth decade	Proximal>distal (pattern A); in some pattern B	–	+	+	Progressive in adulthood	+ (+)	None
Limb girdle muscular dystrophy with titin deficiency (type 2J)	Independent ambulation achieved	Proximal>distal (pattern A) but also pattern E	–	–	–	Roughly half lose ambulation in adulthood	++	None
Facioscapulohumeral dystrophy	Independent ambulation achieved, variable progression	Pattern D	–	–	Uncommon and mild	Slowly progressive	N or +	Neurosensory hearing loss and retinal degeneration
Emery-Dreifuss muscular dystrophy with merin deficiency (type 1)	Independent ambulation achieved, variable progression	Scapuloperoneal (pattern B)	+	++	Not frequent	Progression of cardiac signs>motor signs	+ (+)	None
Adult-onset muscular dystrophy								
Limb girdle muscular dystrophy with anoctamin deficiency (type 2L)	Onset in adulthood, 8:1 ratio of men:women	Mainly lower limbs pattern A, rarely pattern E	–	–	–	Slowly progressive in adulthood	++	None
Limb girdle muscular dystrophy type 1A (myotilin)	Independent ambulation achieved	Proximal>distal (pattern A)	–	–	–	Generally slowly progressive in adulthood	+	Dysarthria in some cases
Limb girdle muscular dystrophy with caveolin deficiency (type 1C)	Independent ambulation achieved; rippling might be seen before weakness	Proximal and distal	–	+	–	Slowly progressive, variable	++	Cramps, rippling, percussion-induced repetitive contractions

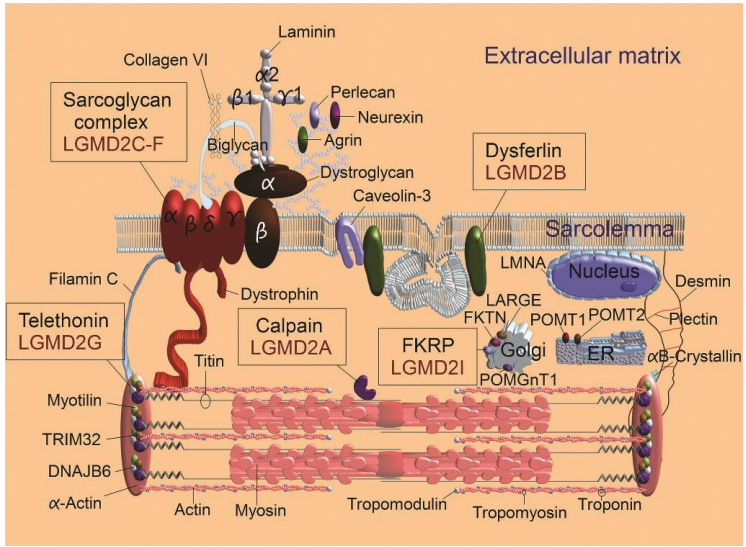
Mercuri E, Muntoni F. The Lancet 2013

Pathomechanisms

Escolar DM, Leshner RT. Swaimans Pediatric Neurology Principles and Practice



Cotta A. et. al. Arq Neuropsiquiatr 2014

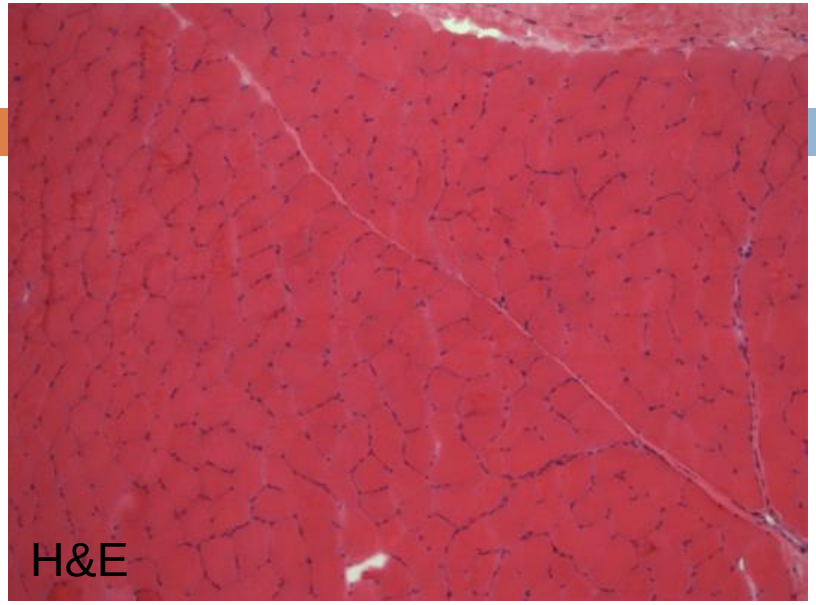


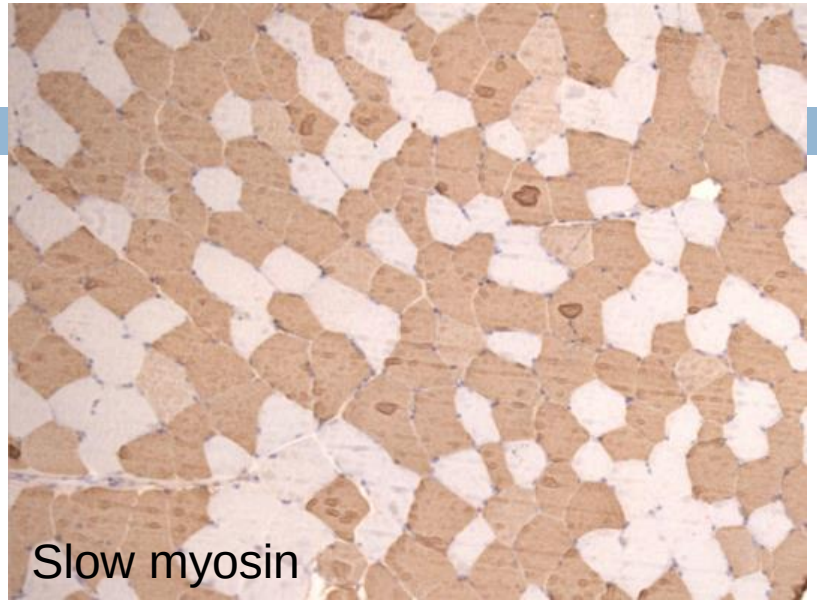
Sarcolemmal DAPC – links the intracellular cytoskeletan to the ECM
Mechanical stability – shock absorber and protection against contraction mediated injury
N-Dys has actin binding domains
Rod region has gamma-actin binding domain and NNOS domain
C-terminus has BDG binding site
BDG – ADG – ECM LA2 –IIH6 glycosylated epitope
Extreme C terminal interacts with syntrophins
Signal transduction via NNOS and SGC

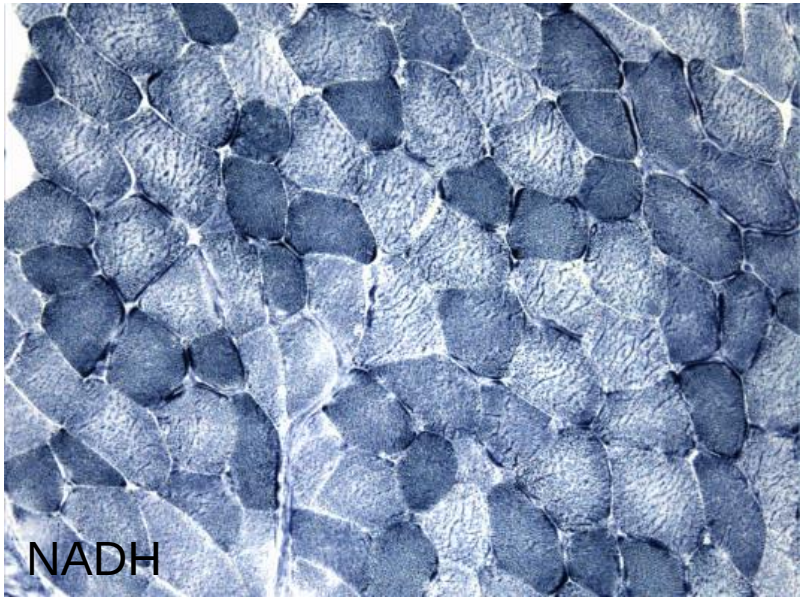
Loss of DAPC – leaky, fragile sarcolemma
Increased Ca flux, ROS damage, protease cascade
Necrotic cell death; inflammatory cytokine response; regeneration; fibrosis
Satellite cell, mitochondrial dysfunction; impaired membrane repair

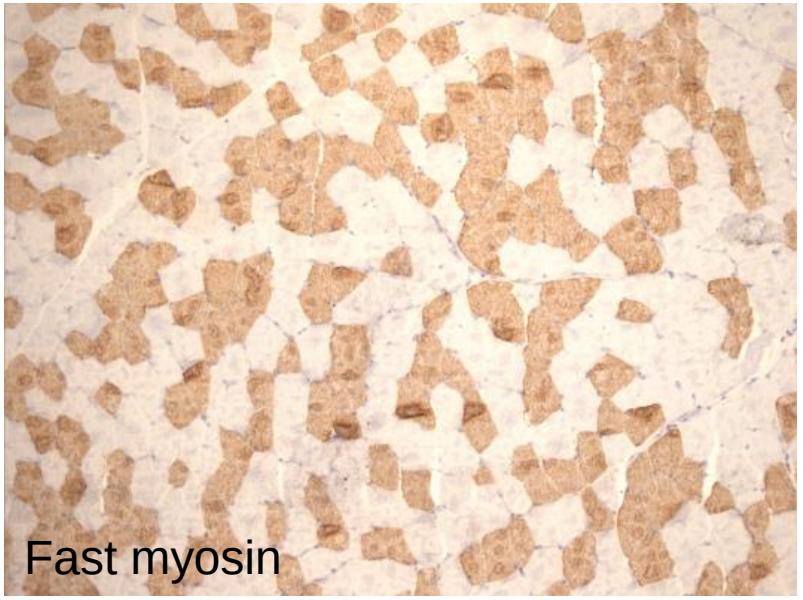
Necrosis, regeneration and fibrosis are key morphological indicators of a dystrophic process

Histology of normal muscle



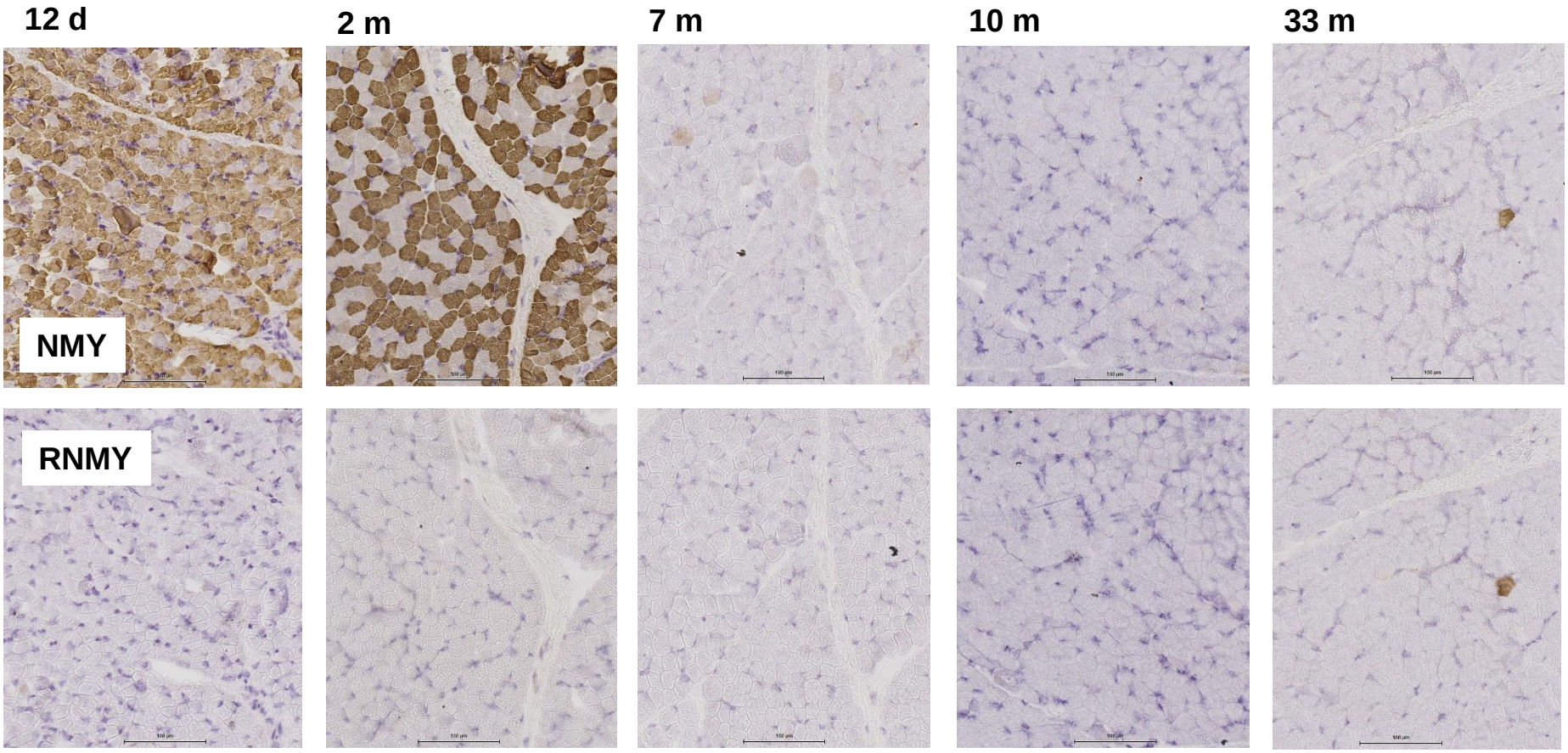






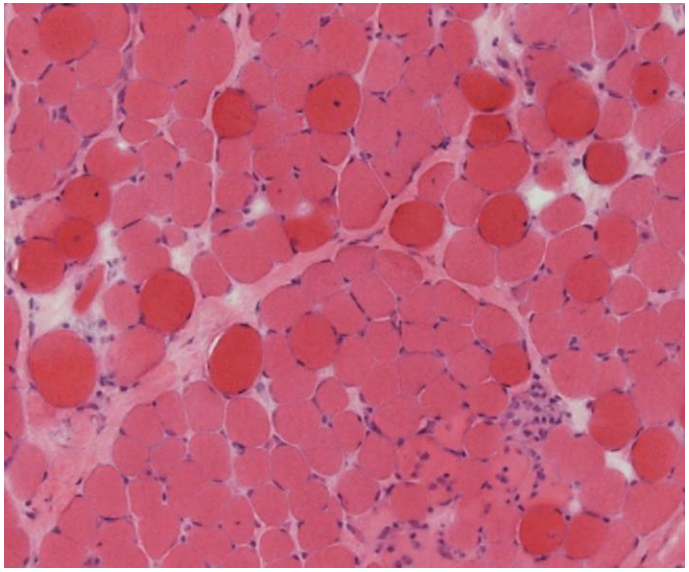
Minimal change/normal histology controls

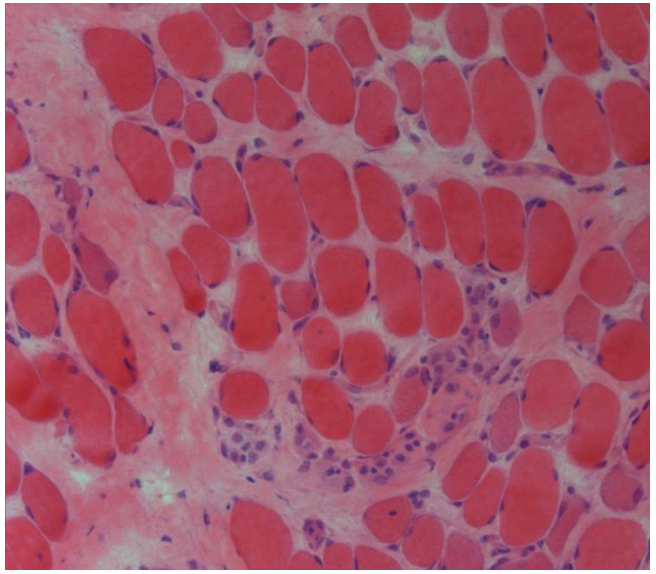
Feng, Rivas unpublished data

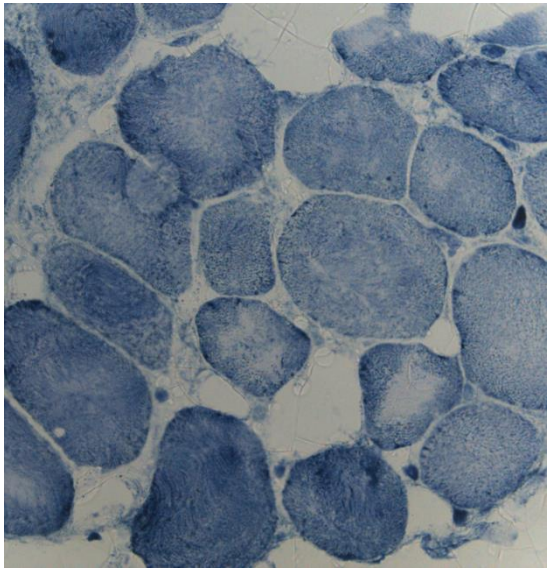


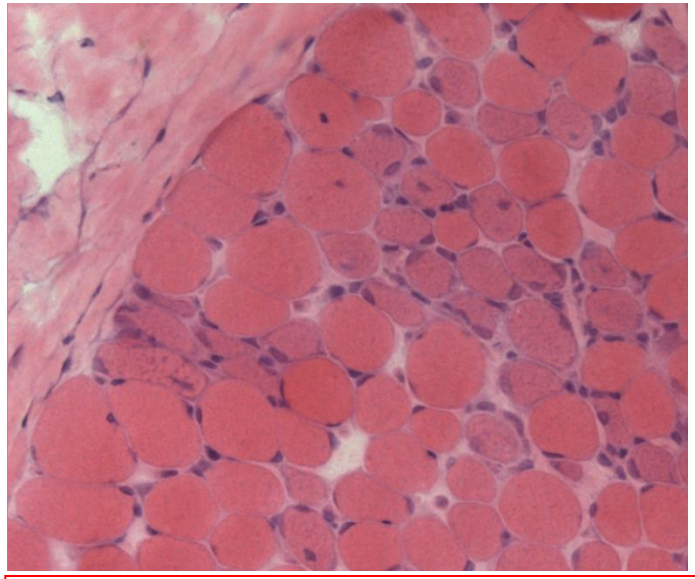
Fetal and developmental myosin heavy chain isoforms are highly developmentally regulated and reappear in regenerating fibres

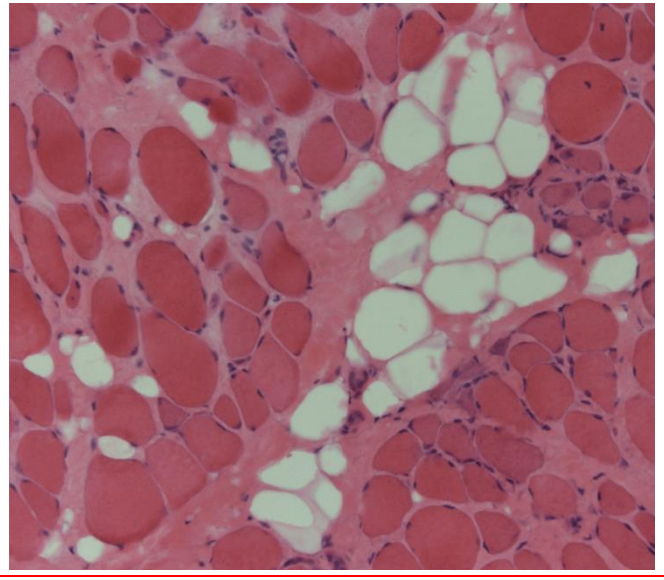
Morphology: canonical features

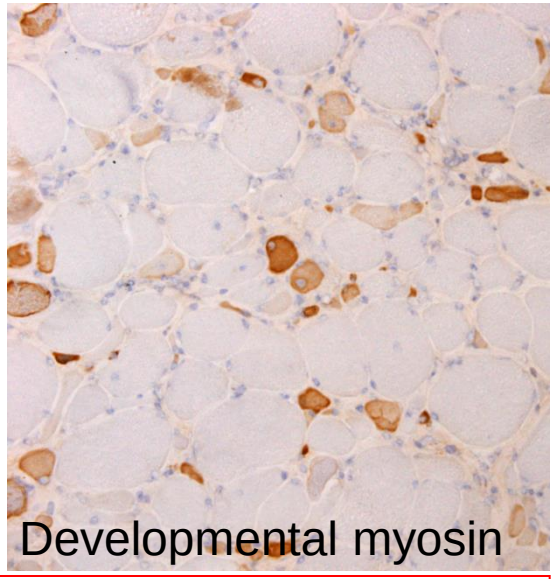










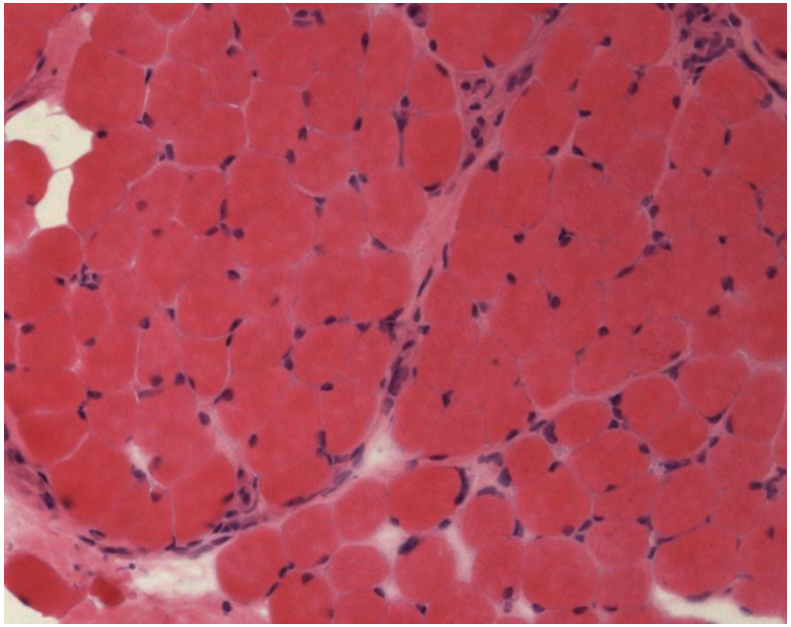


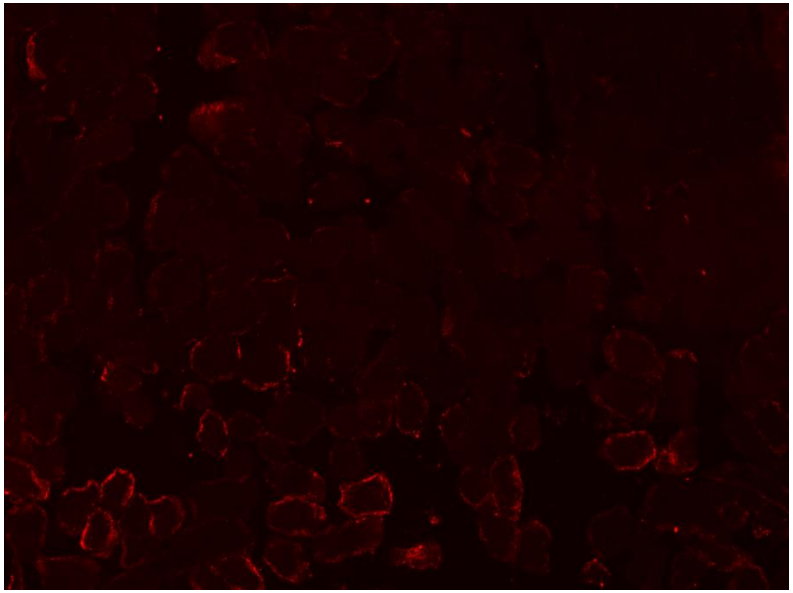
Fibre size variation; clustered and/or scattered necrosis +/- phagocytosis and regeneration; internal nuclei; fibrosis; fatty replacement; architectural abnormalities; polymorphic f/dMHC+ fibres; inflammation; vacuoles

Confounding factors in muscle pathology interpretation

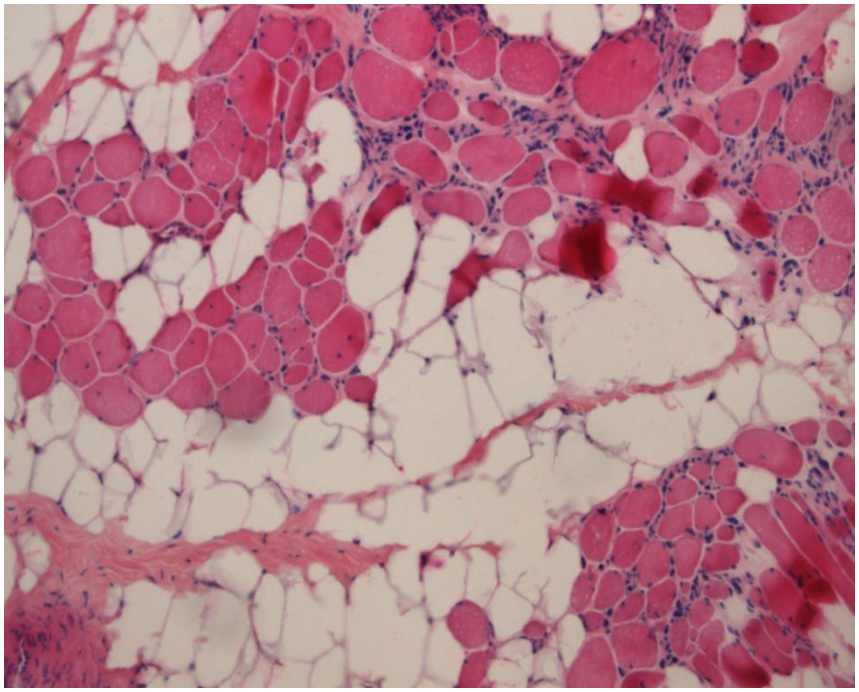
- Clinico-genetic-pathologic heterogeneity
- Morphology overlap between different inherited and acquired diseases
- One gene – different morphologies, one morphology across genetic backgrounds
- Range of pathological severity
- Age related disease progression – acquisition of new features
- Poor correlation between clinical and pathological severity
- Secondary changes
- Dual pathology
- Biopsy site, focal pathology and sampling bias

Diagnostic confounders: there is a range of severity



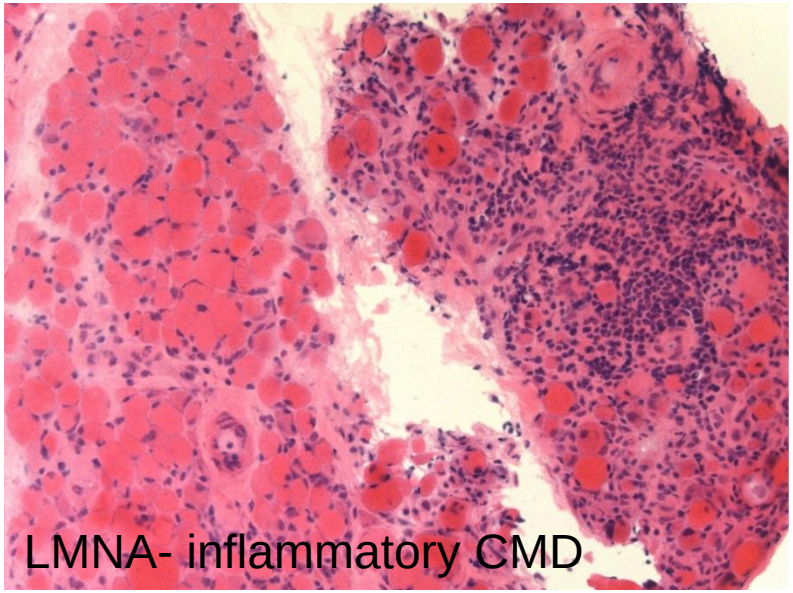


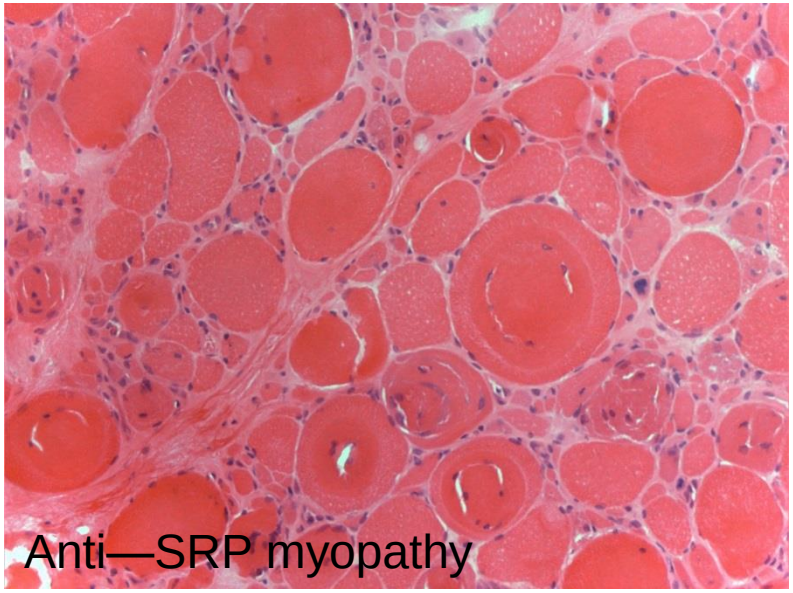
2 years ; MEB phenotype; CK 450; Muscle US normal; Homozyous mutation exon 16 POMGnT1

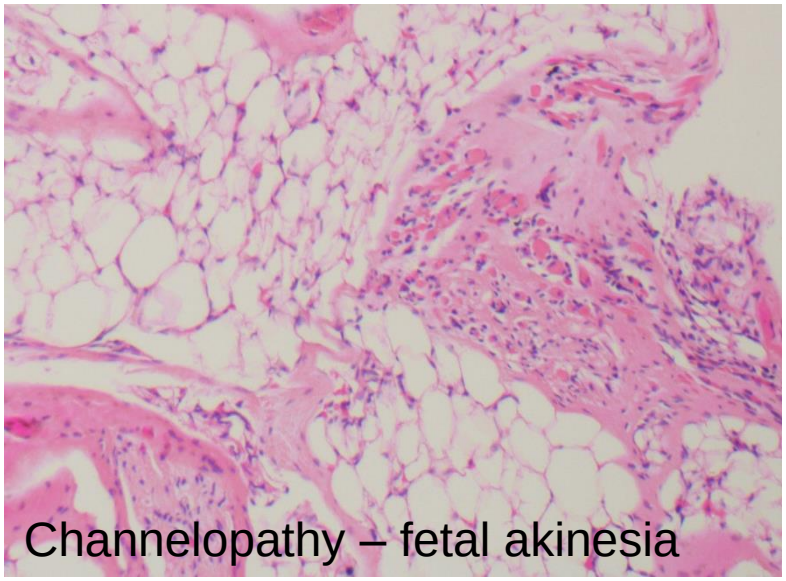


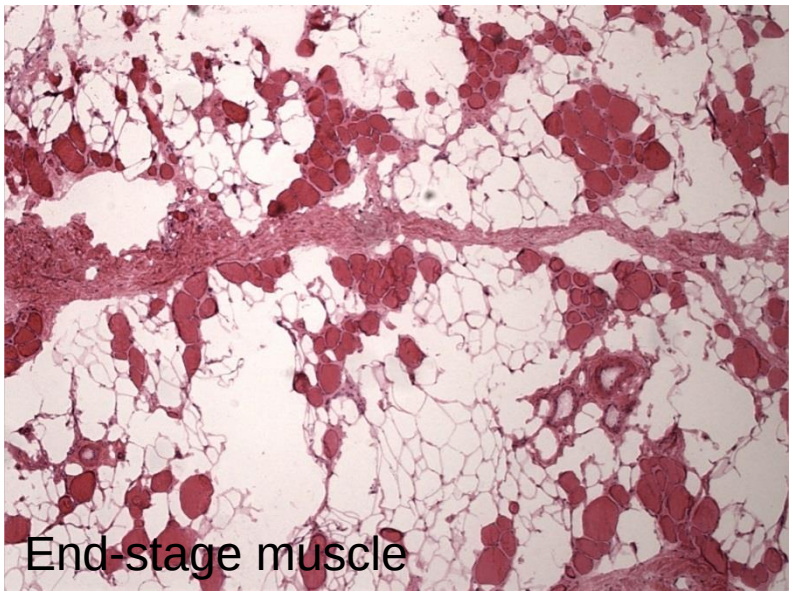
18 years ; Ullrich spectrum phenotype; marked pathology without necrosis or regeneration; mild Col VI depletion in sections, moderate in fibroblasts; dominant COLVIA1 mutation

Morphological mimics


LMNA- inflammatory CMD


Anti—SRP myopathy


Channelopathy – fetal akinesia


End-stage muscle

IHC: broad principles

- IHC on sections relies on semi-quantitative analysis of intensity as a surrogate label of 'quantity' and spatial localisation as indicator of a molecular defect – multitude of barriers to standardisation
- Molecular genetic defect determines the protein abnormality

  Recessive null/truncating/loss-of-function mutations are easier to assess

  Dominant mutations are generally difficult to assess except some dominant negative changes

  Missense changes are difficult to assess

  Unequivocal protein abnormality can signal a 'missing mutation' scenario – rearrangements or deep intronic changes
- Developmental changes influence interpretation of results
- Large proteins like dystrophin, laminin-alpha2 and titin require multiple domain-specific antibodies for assessment
- Antibody repertoire must take into account development/tissue-specific isoforms – plectin, integrins, channel proteins
- Secondary changes are of diagnostic value

Developmental regulation of proteins

- **Change of isoform**

Actin: cardiac to skeletal

Myosin: embryonic to neonatal to slow/fast

- **Low expression on immature/regenerating fibres**

Beta spectrin

C terminal dystrophin

Dystrophin associated proteins

Neuronal NOS

Laminin beta 2

Integrin alpha 7

- **High expression on regenerating fibres**

Utrophin

Laminin alpha 5

NCAM

Vimentin

Desmin

MHC Class I

Protein alteration in muscular dystrophies

- Primary

Dystrophin

Sarcoglycans

Calpain-3

Dysferlin

Caveolin-3

Laminin alpha 2

Collagen VI

Integrin alpha 7

Emerin

Plectin

Myotilin

Telethonin and Titin

- Secondary

Dystrophin

Sarcoglycans

Utrophin

Neuronal NOS

Laminin alpha 2

Laminin beta 1

Laminin alpha 5

Integrin alpha 7

Alpha dystroglycan

Dysferlin, calpain-3 and caveolin-3

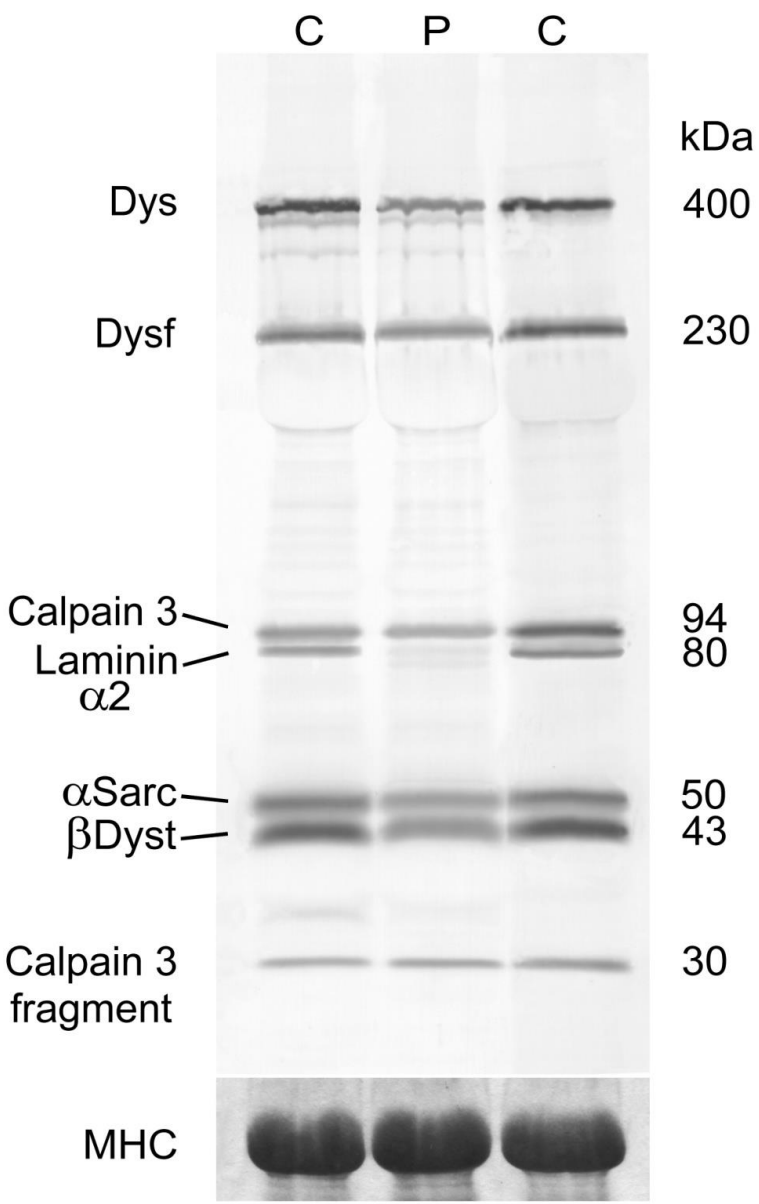
MHC Class I

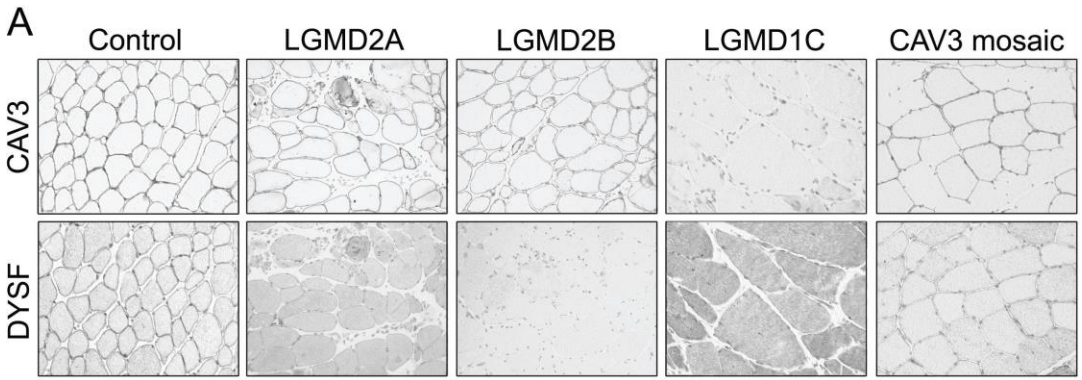
Myosin isoforms

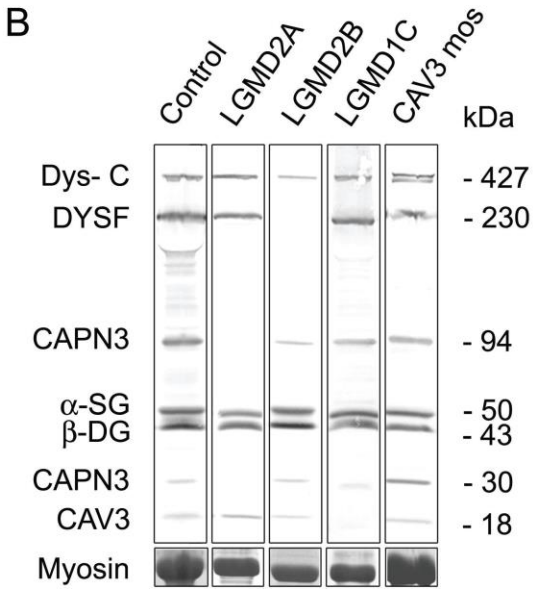
Immunoblots

- Quantative technique – assessment of protein molecular mass and quantity
- Multiplex blots – simultaneous multiprotein analysis for primary and secondary changes e.g. calpain-3/dysferlin/caveolin-3
- BMD – equivocal IHC; IB may detect shift in molecular mass depending on the size of the mutation; reduction in amount regardless of the size of mutation
- IB is more reliable in some instances – calpain-3, dysferlin

Multiplex blot

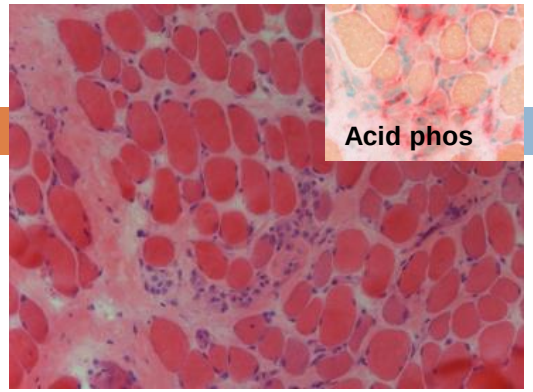


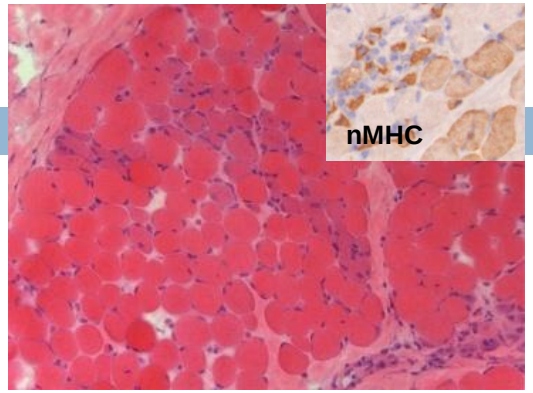


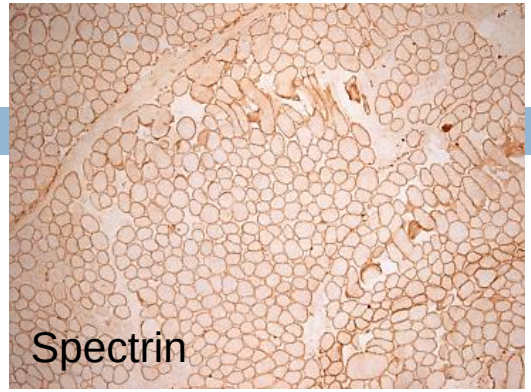


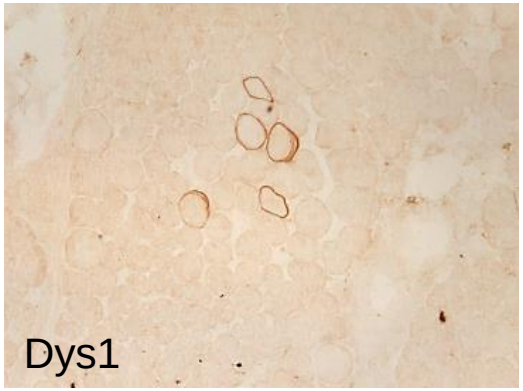
Barresi R. Skeletal muscle 2011

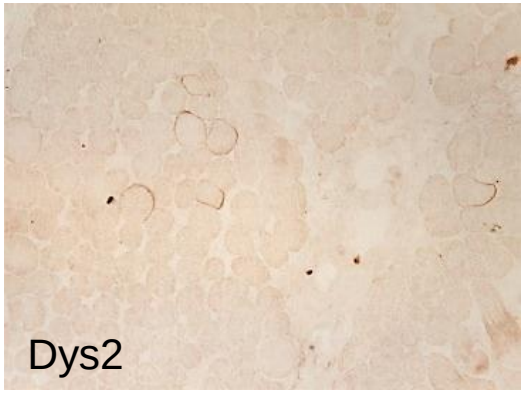
Xp21 dystrophies: Duchenne

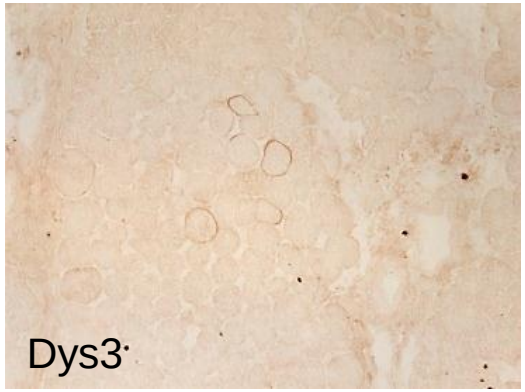


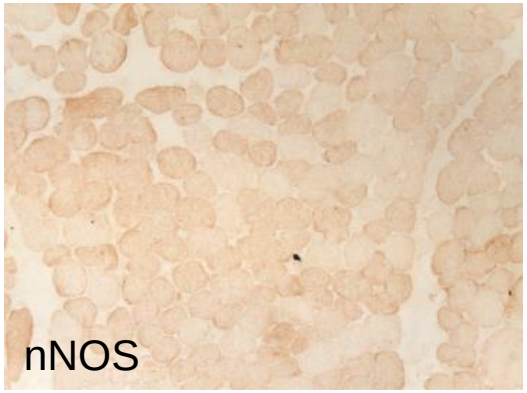


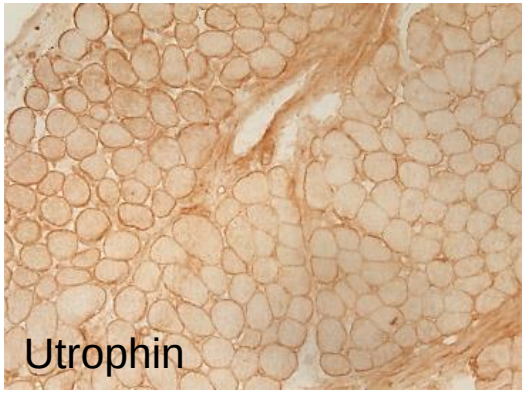


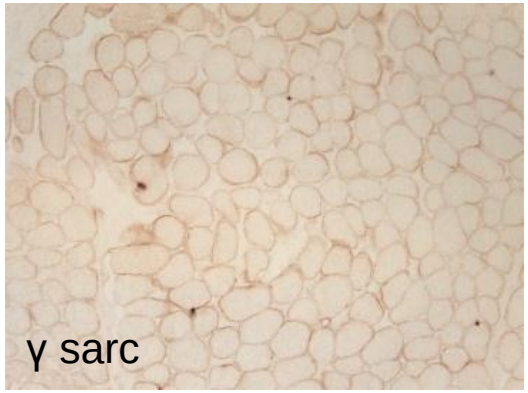








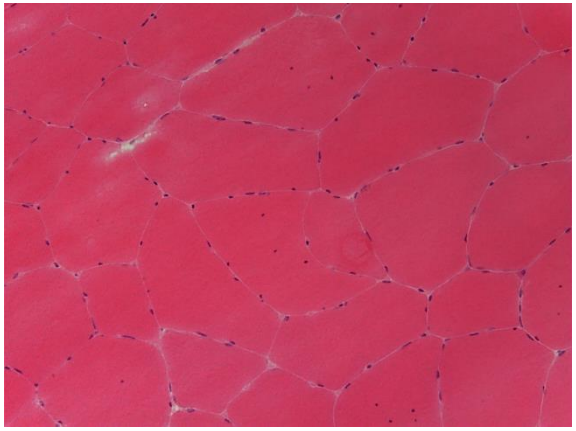


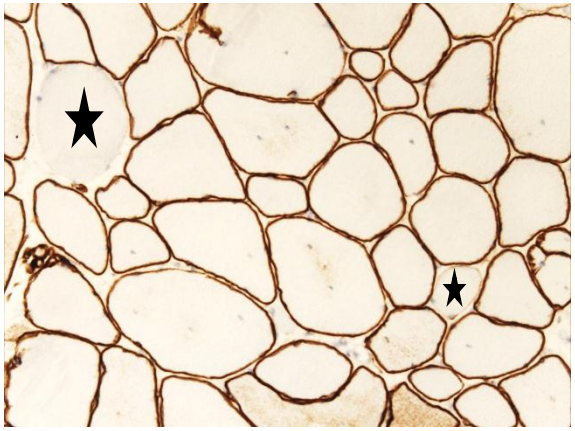


- ***3 year male***
- ***Gross motor delay***
- ***Speech and language delay***
- ***CK 15,189***
- ***Total absence of dystrophin except few revertants***
- ***Secondary changes in DAG***
- ***Exon 32 point mutation***

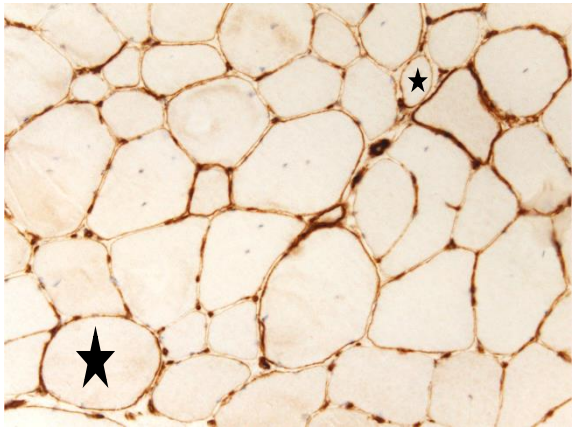
Xp21: Duchenne manifesting carrier

- Normal to minimal pathology
- Mosaic pattern of dystrophin labeling
- Utrophin upregulation on fibres with and without dystrophin
- Distinction from other forms of LGMD





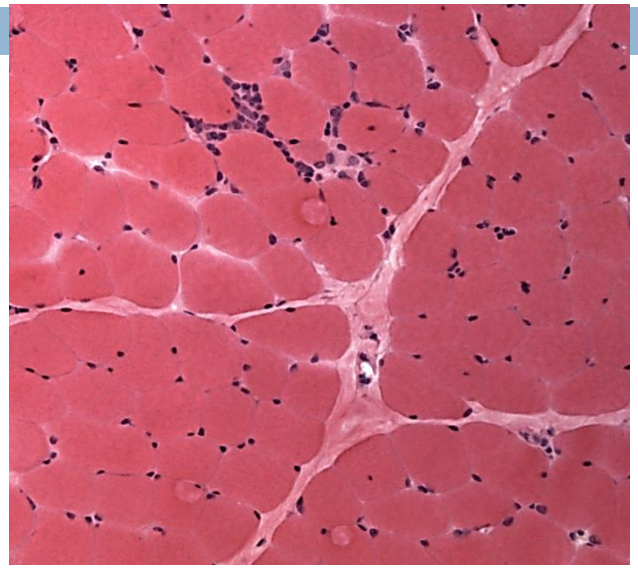
Dys1

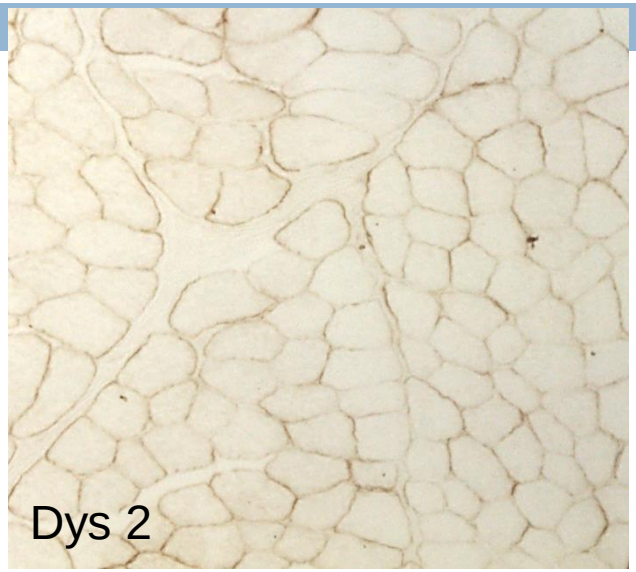


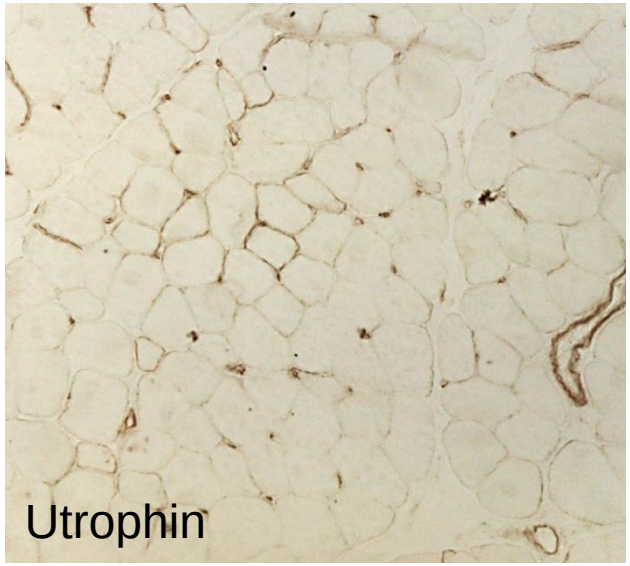
Utrophin

Xp21: Becker muscular dystrophy

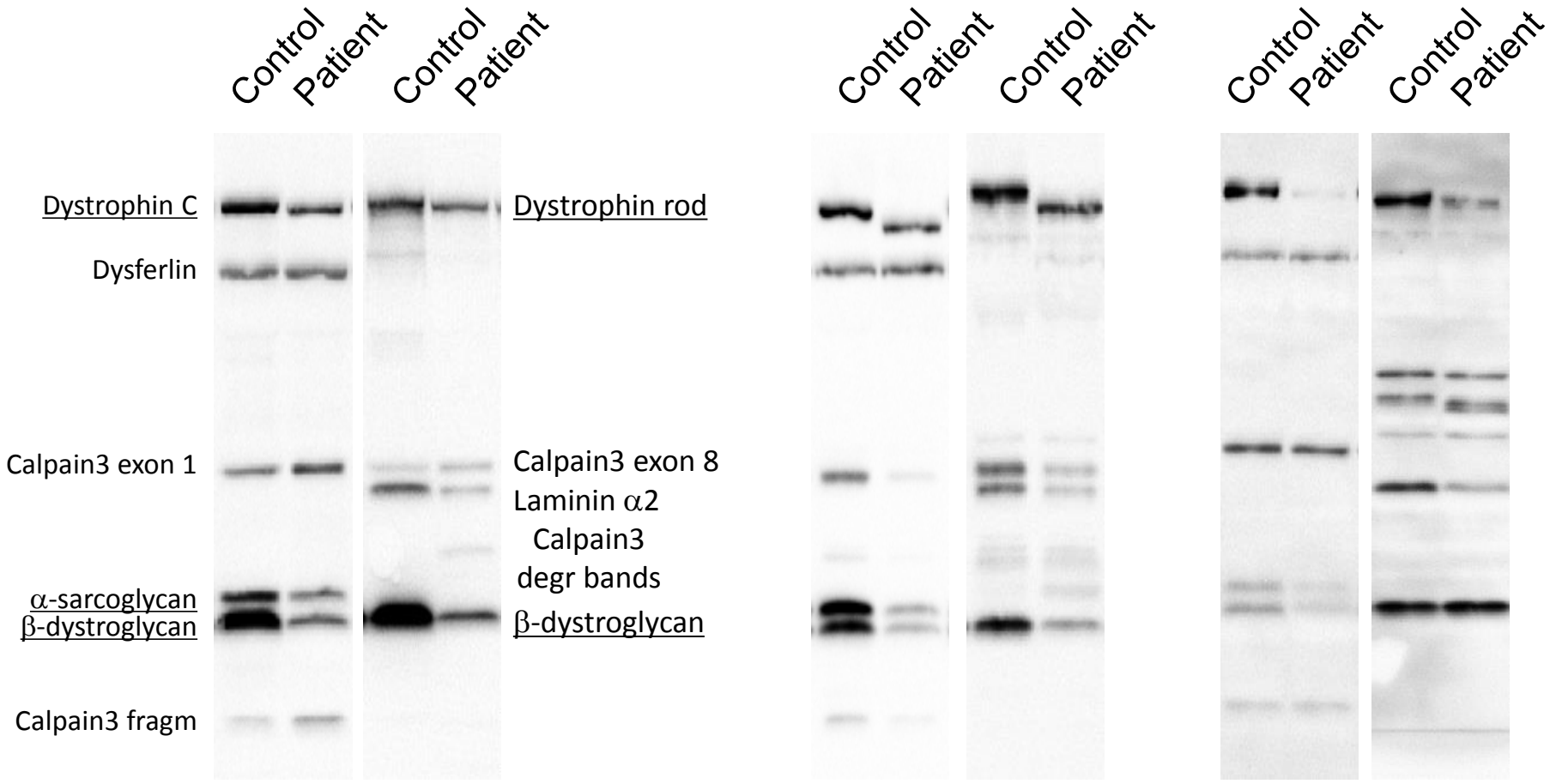
- 6 year male
- Splicing mutation in exon 41 – in-frame deletion
- Mild dystrophic changes
- Overall reduced dystrophin expression
- Mild utrophin upregulation







Becker Muscular Dystrophy



Small in frame deletion
(1 exon)

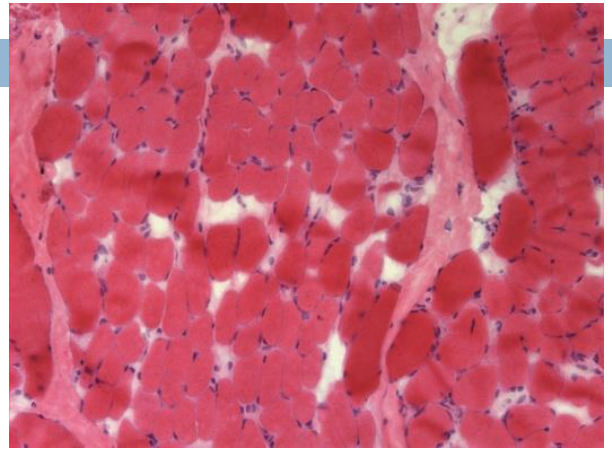
Large in frame deletion
(10 exons)

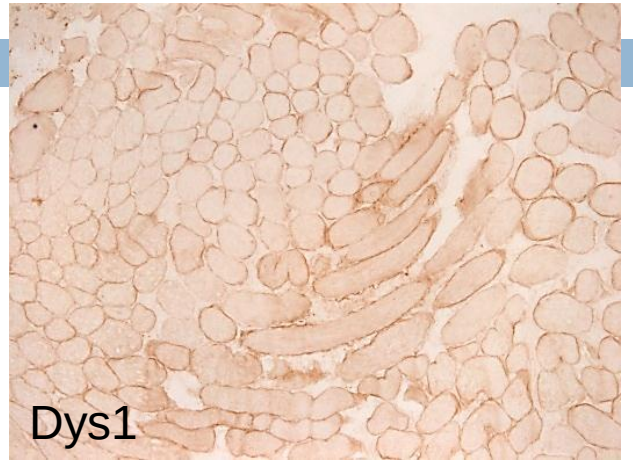
? Point mutation

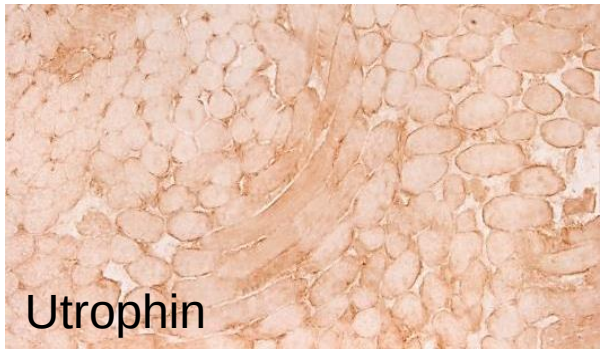
Dr Rita Barresi, Newcastle Immunoanalysis Unit

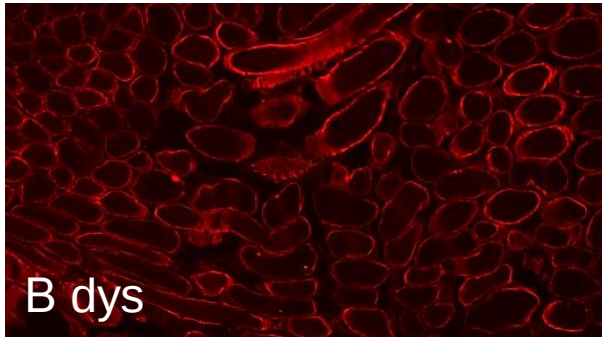
BMD versus LGMD2I

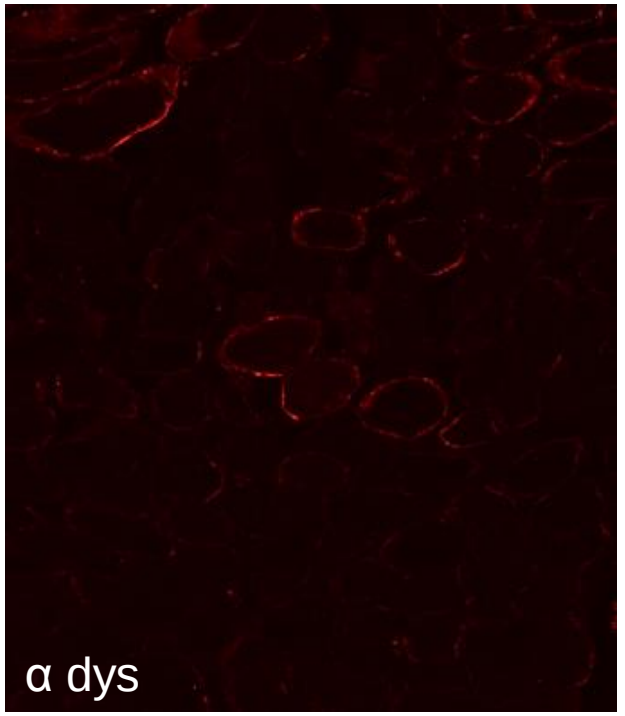
- Ambulent LGMD2I adults can resemble Becker
- Normal dystrophin labeling in biopsies
- Overlap in utrophin upregulation
- No specific FKRP antibodies
- Secondary changes aid distinction: reduced alpha dystroglycan and laminin alpha 2





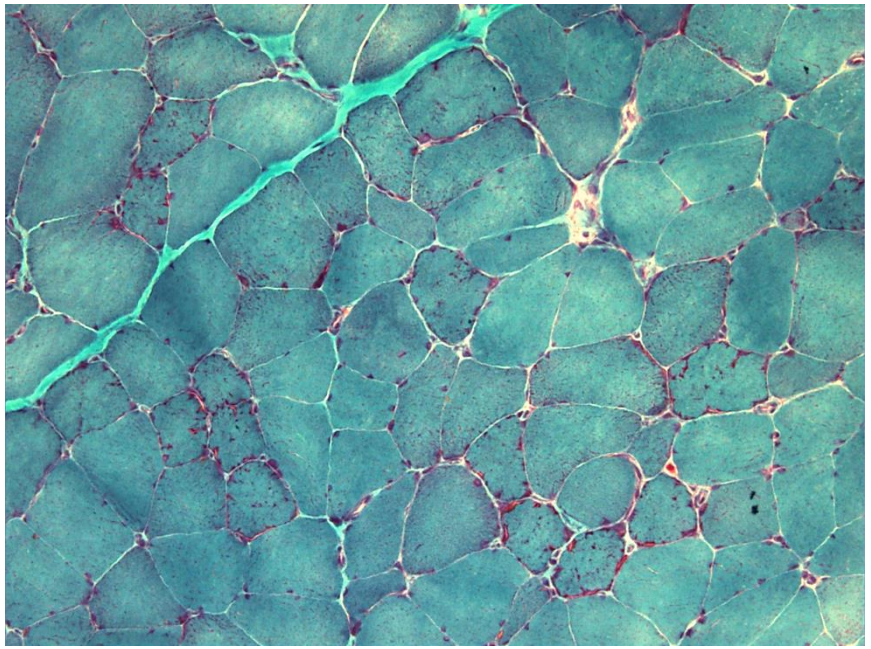






LGMD2A

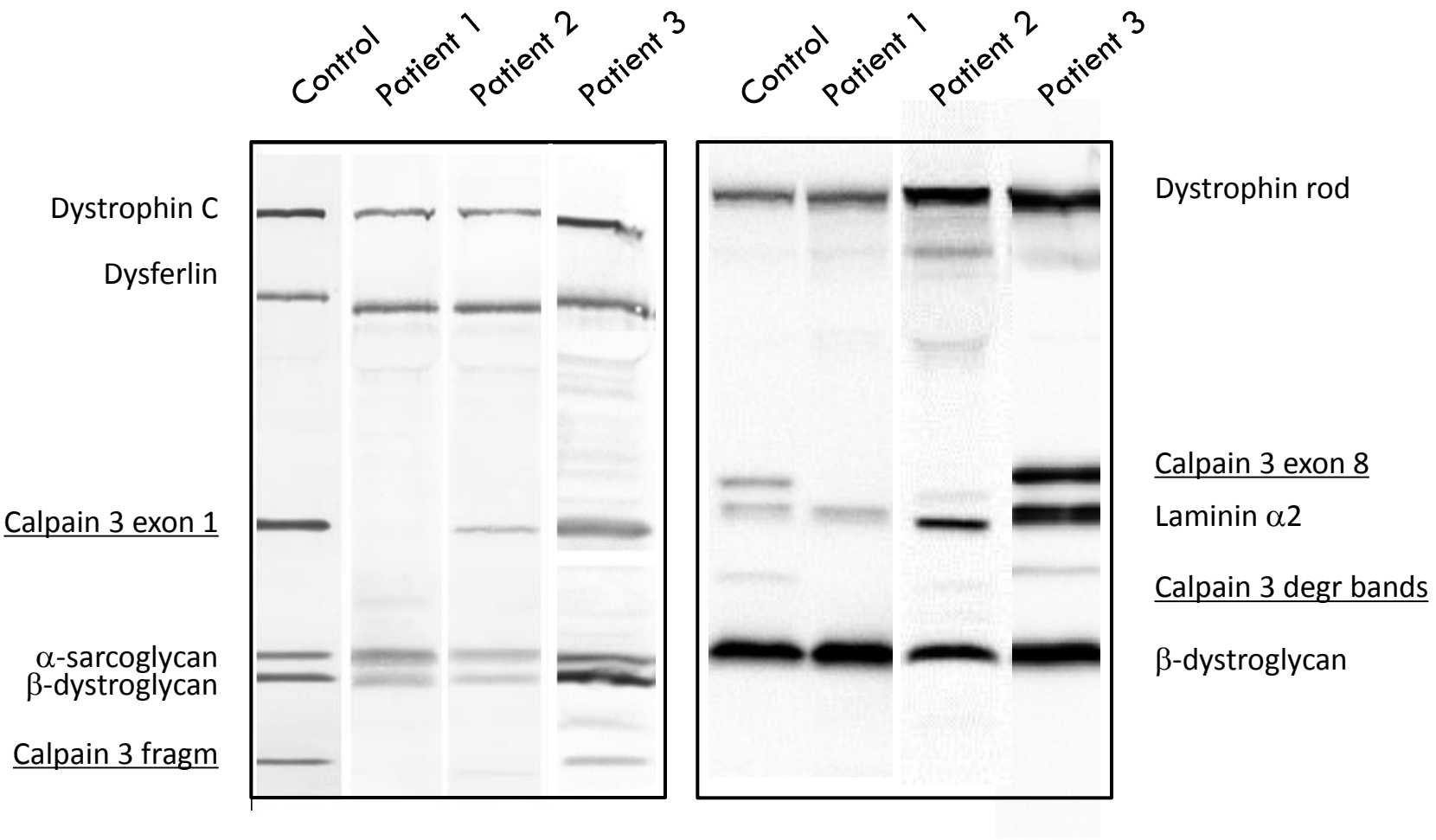
- Variable dystrophic changes
- Lobulated fibres (non-specific)
- Eosinophilic myositis
- Immunoblots superior for assessing calpin-3 reduction
- Normal quantity does not exclude a defect
- Secondary reduction in dysferlin (immunoblots)



Muscle diseases with eosinophilic infiltrates:

- *Becker dystrophy*
- *Sarcoglycanopathies*
- *Myopathies associated with drugs, parasites, toxic-oil syndrome and eosinophilic fasciitis*

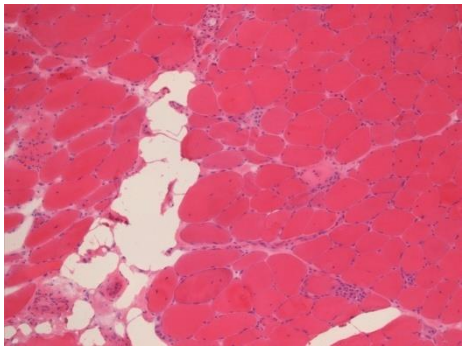
LGMD2A

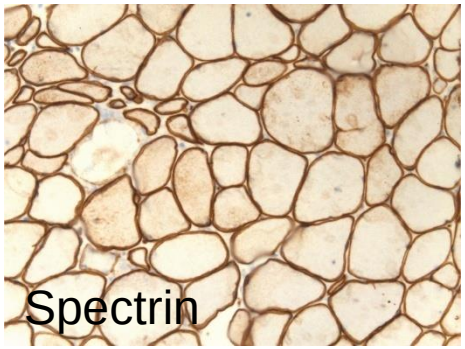


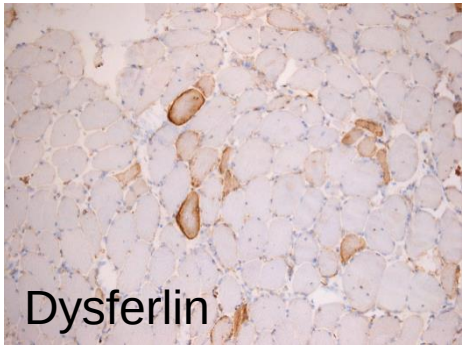
Dr Rita Barresi, Newcastle Immunoanalysis Unit

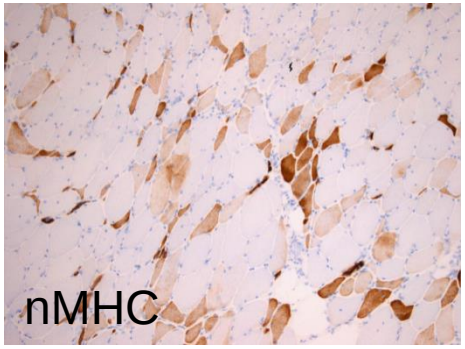
LGMD2B

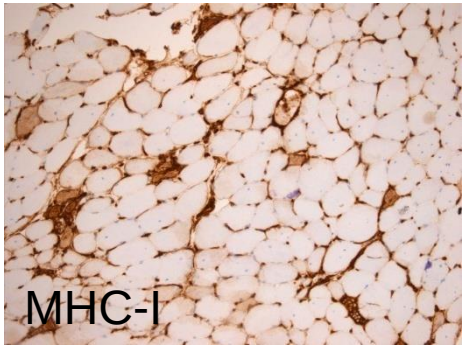
- High CK levels
- Variable dystrophic and degenerative changes
- Internal labeling in regenerating fibres
- Sarcolemmal granular deposition of C5b-9 complement in some cases
- Prominent inflammatory exudates and MHC I upregulation can mimic inflammatory myositis
- Absence or reduction detected on sections and immunoblots (two antibodies)
- Immunoblots to assess secondary reduction in defects in calpain-3 and caveolin-3

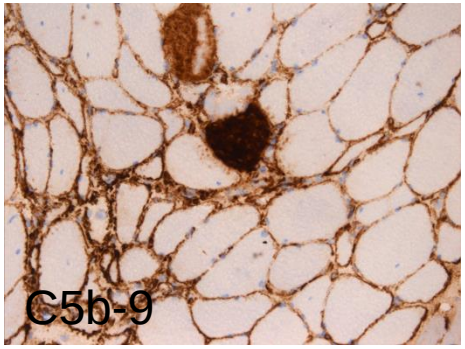










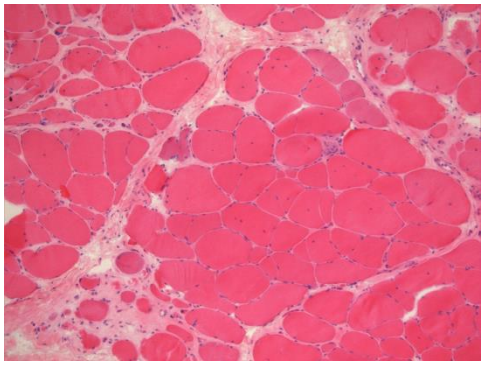


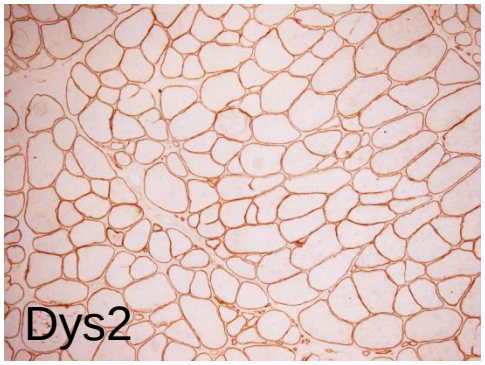
LGMD2C – 2F (Sarcoglycanopathies)

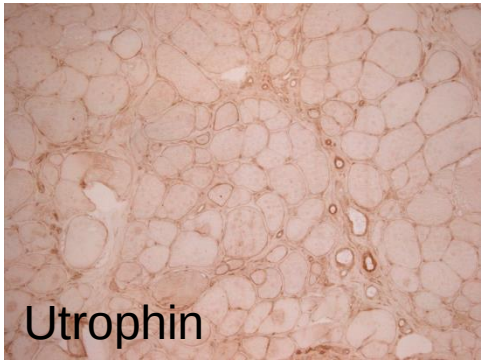
- Expression of each sarcoglycan varies from trace, to mild to severe reduction/absence
- Variable secondary reduction in other members
- Total absence of the entire complex indicates likely primary defect in beta sarcoglycan
- Secondary reduction in dystrophin may occur (beta sarcoglycan)
- Secondary reduction in dystrophinopathy
- Utrophin upregulation rare, but may occur
- Careful correlation with normal immunocontrols essential

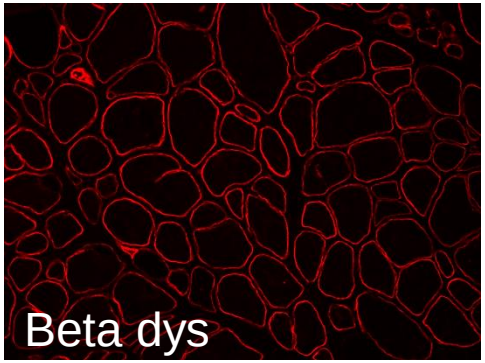
LGMD2I

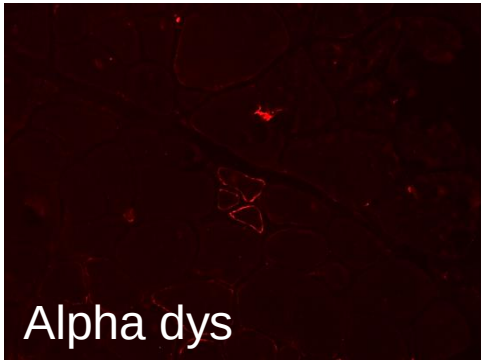
- Most common form in the UK Caucasian population with wide clinical spectrum
- Variable dystrophic features in biopsies
- No specific FKRP antibodies
- Dystrophin levels usually normal
- Hypoglycosylation of alpha dystroglycan, normal beta dystroglycan
- Utrophin may be upregulated
- Secondary reduction of laminin alpha 2 and beta 1 in some cases (immunoblots)
- Combination of the above changes can aid distinction from BMD

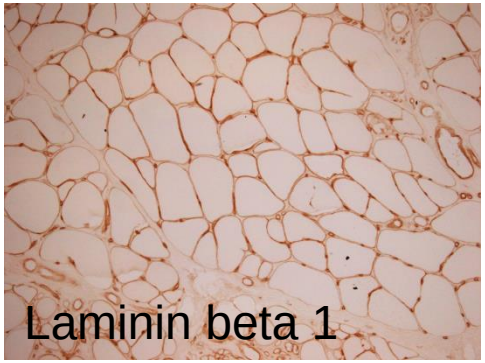












Other Dominant LGMDs

- LGMD1A (myotilin): overlap with myofibrillar myopathy; rimmed vacuoles; myotilin accumulation
- LGMD1B (Lamin A/C): no specific markers identifying the primary protein defect
- LGMD1C (Caveolin-3): Reduction in sarcolemmal protein demonstrable on sections and immunoblots in patients with a mutation due to dominant negative effect; internal labeling in regenerating fibres; mosaic pattern in cases without mutation
- LGMD1D-1G: mutant proteins yet unidentified

Other recessive LGMDs

- LGMD2G: rare originally described in Brazil; defect in telethonin; rimmed vacuoles reported with absent telethonin labeling
- LGMD2H: rare, described in Hutterite Canadians; no reported studies of TRIM32 expression (putative E3 ubiquitin ligase)
- LGMD2J: rare form in Finnish; distal leg anterior compartment restricted; recessive titin mutations; commercial antibodies detect titin; secondary calpain-3 reduction; dominant mutations in titin cause isolated cardiomyopathy
- LGMD2K-2O: mutations in POMT1, POMT2, POMGnT1 and Fukutin (also cause severe forms of CMD); variably reduced alpha dystroglycan labeling
- LGMD2L: late onset proximal MD; recessive mutations in anoctamin 5 (ANO5); commercial antibodies unhelpful

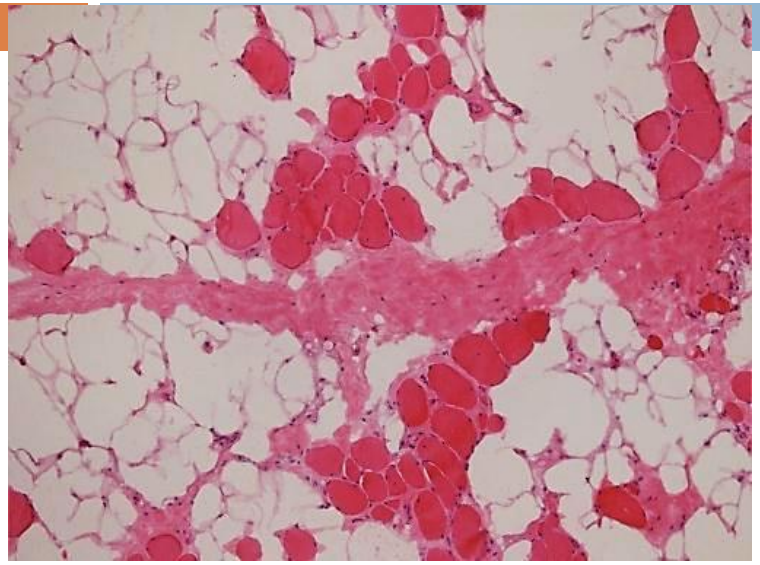
Congenital muscular dystrophies

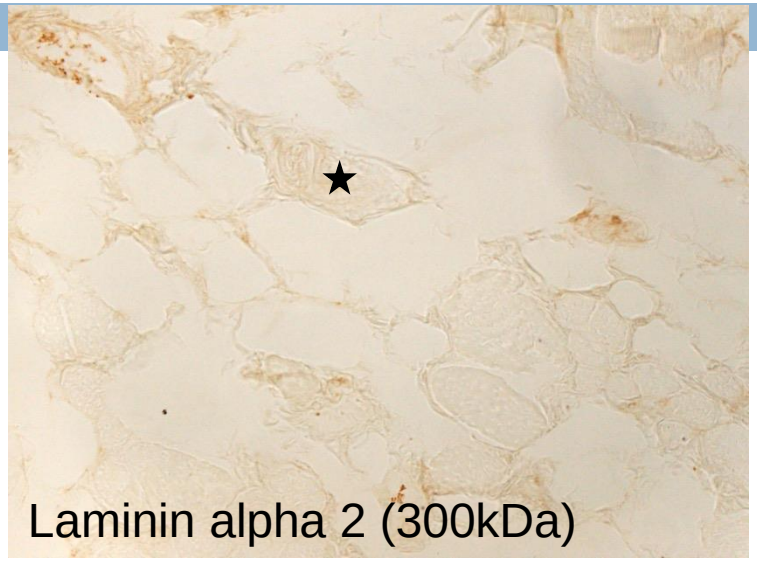
- Over 20 variants with known genetic defects identified with well characterised phenotypes
- Recent identification of several new genes
- Wide clinical spectrum from severe early onset cases with muscle weakness and hypotonia in the first few weeks to milder LGMD phenotype
- Contractures are common; CK elevated but not in all sub-types
- Muscle pathology ranges from minimal to myopathic to dystrophic (necrosis and/or regeneration) depending on the age and muscle biopsied
- All variants share common dystrophic pathological features; pathology may appear worse than the clinical picture
- Areas of mitochondrial depletion (cores), aggregation and myofibrillar disruption may occur; necrosis and regeneration may not be striking and the overall picture may resemble a myopathy
- Clinical and pathological overlap with congenital myopathies

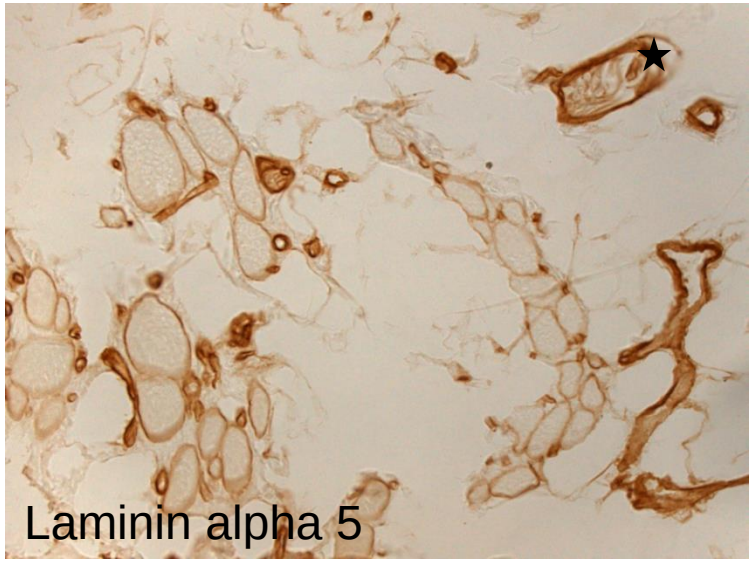
MDC1A (LAMA-2 related MD)

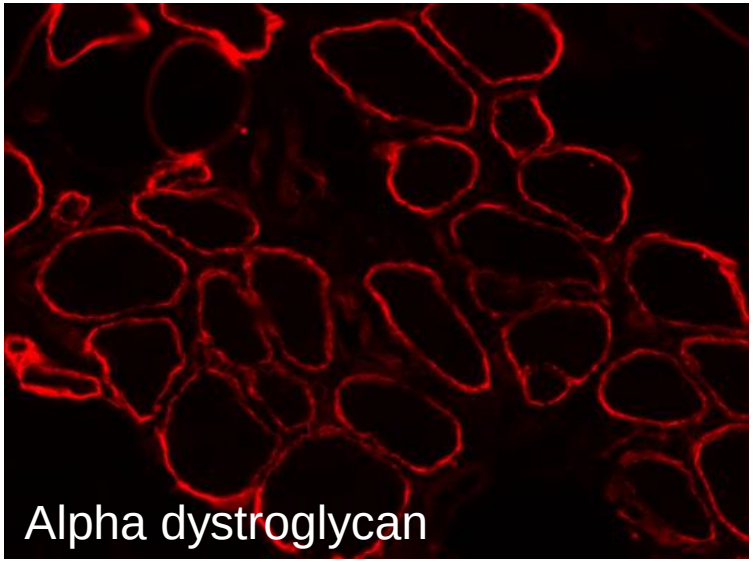
- Range of severity
- High CK, white matter changes on T2 weighted brain MR by 6 months
- Most mutations in the LAMA2 gene result in complete absence or traces of laminin alpha 2, associated with severe phenotype
- Some LAMA2 mutations result in partial protein reduction, usually with milder LGMD-like phenotype
- Partial reduction is better recognised by N terminal 300kDa Alexis and C terminal NCL antibodies compared to 80kDa C terminal Chemicon antibody
- Laminin beta 2, integrin alpha 7, Alpha dystroglycan secondarily reduced, laminin alpha 5, alpha 4 overexpressed
- Laminin alpha 2 secondarily reduced in secondary alphadystroglycanopathy
- Distinction between primary versus secondary CMD difficult in cases with partial laminin alpha 2 reduction
- Laminin alpha 2 absent from nerves in MDC1A

MDC1A: complete deficiency



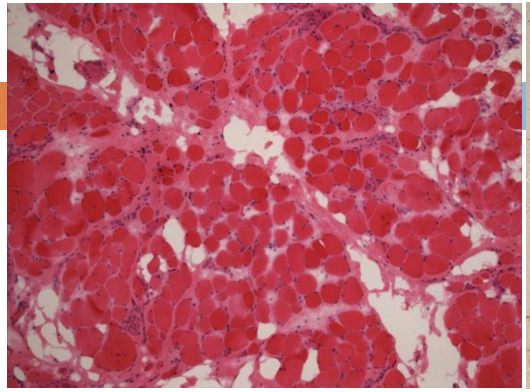


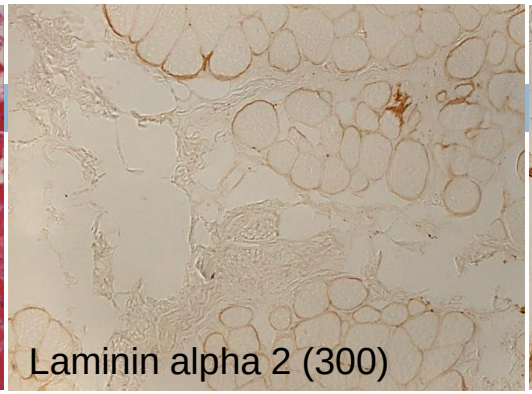


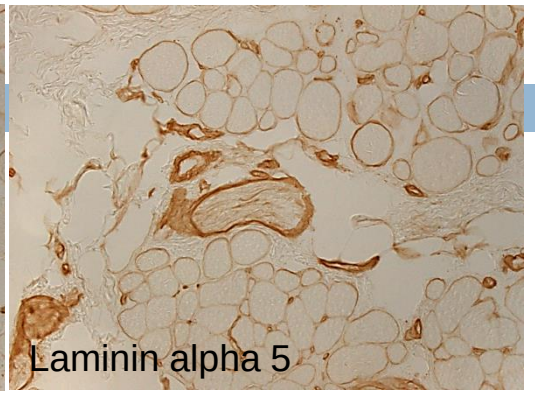


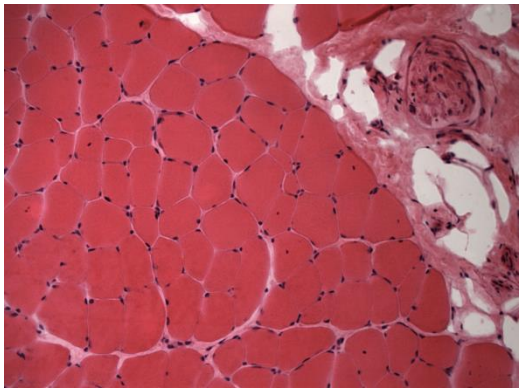
- ***13 year male***
- ***Neonatal onset hypotonia and muscle weakness***
- ***Delayed motor milestones***
- ***Contractures***
- ***CK 2579 at one month***
- ***NCS 2008: slowing of sensorimotor conduction velocities***
- ***Investigated for peripheral neuropathy***
- ***Hz pathogenic mutation exon 9 c.1303C>T(p.Arg435X) exon 9 and Hz VUCS exon 2 LAMA2***

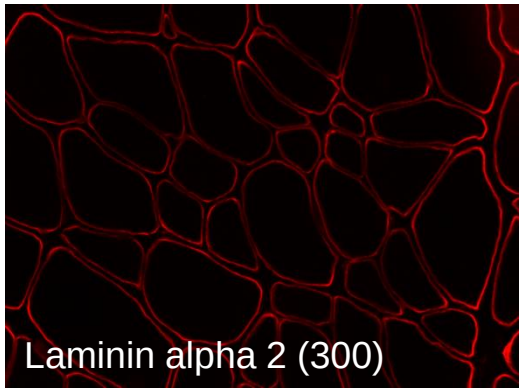
MDC1A: partial deficiency

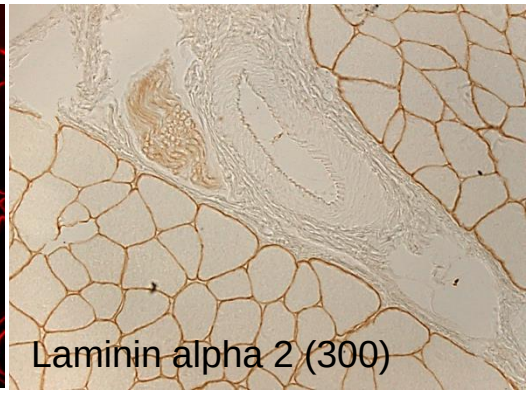


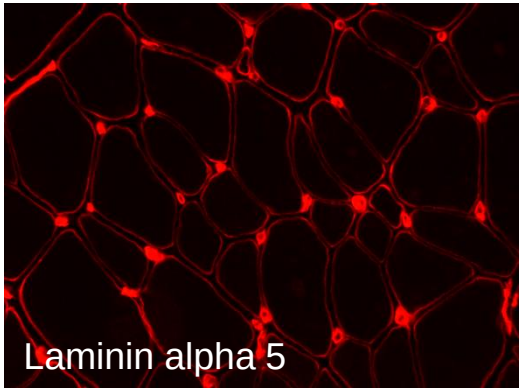


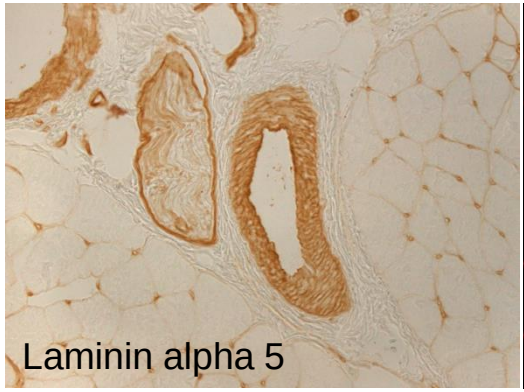


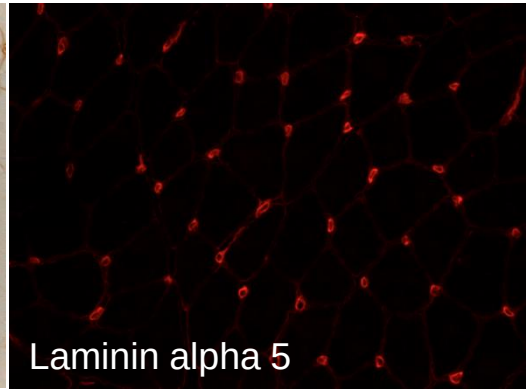












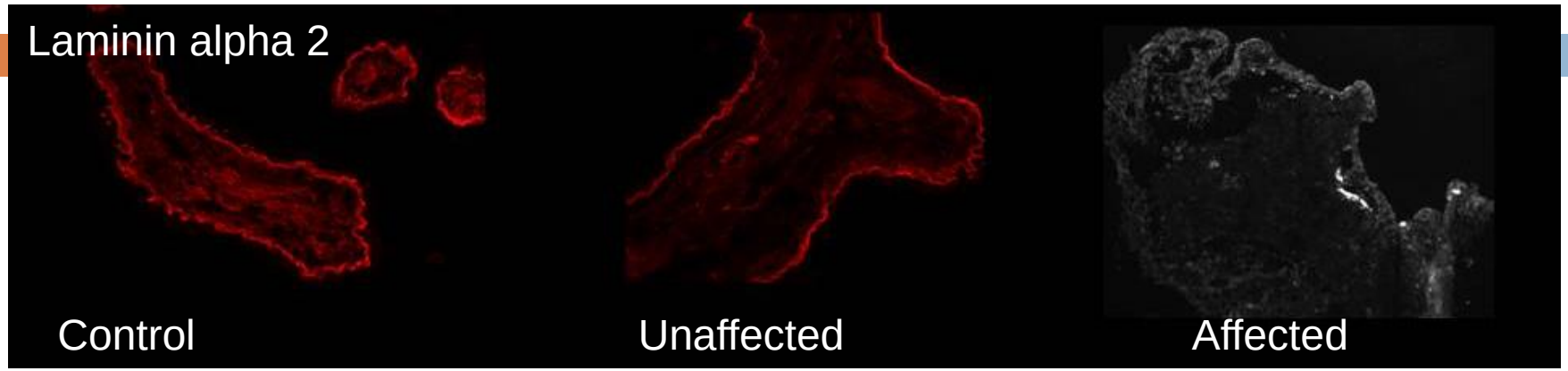
Case 1

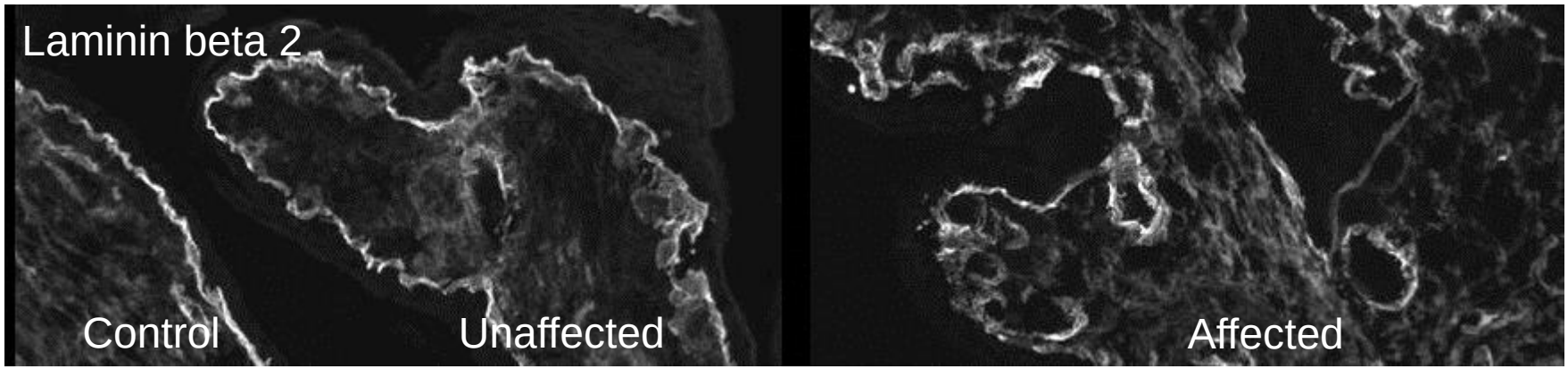
- ***18 months female***
- ***Floppy since birth***
- ***Axial, proximal weakness***
- ***CK 2318***

Case 2

- ***7 years female***
- ***Frequent falls***
- ***Proximal >> distal weakness***
- ***Mild axonal motor-sensory neuropathy***
- ***CK 733***
- ***C.391C>T (P.Gln131X) Hz nonsense mutation exon 3 and 2 Hz VUCS in exons 4 and 31***

MDC1A prenatal diagnosis: CVS linkage analysis plus IHC (LA2 absent in proband, no pathogenic mutations identified in parents)

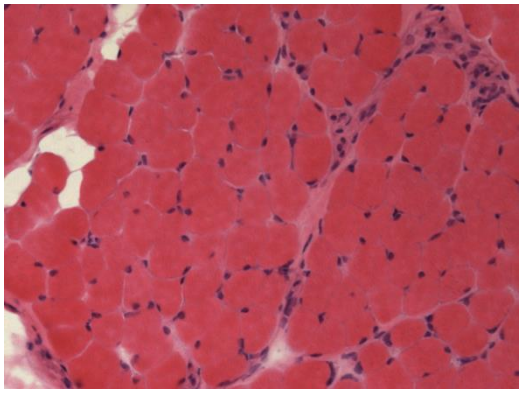


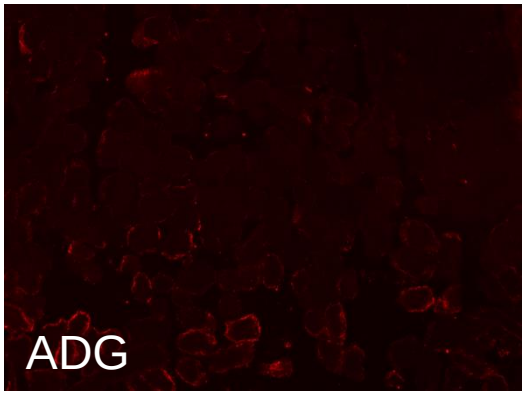


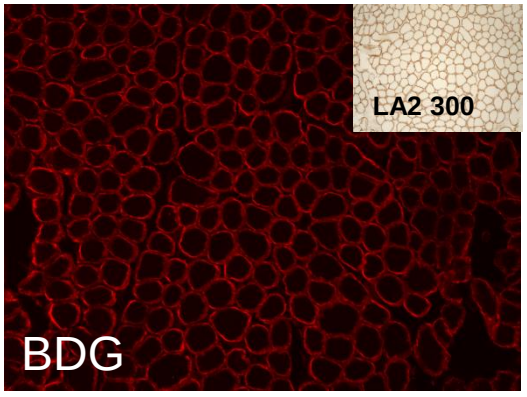
- Laminins expressed on basal lamina beneath trophoblast
- Absence of laminin alpha 2 from trophoblast is highly suggestive of the foetus affected by MDC1A accompanied by reduction in laminin beta 2
- Reliability of CVS studies in primary partial or secondary deficiency unknown
- Important to establish laminin alpha 2 status in the proband before CVS studies

Dystroglycanopathies

- Range of clinical severity with varying degrees of brain and eye involvement
- Mutations in 13 genes identified
- Immunohistochemistry and immunoblotting show reduced labeling of glycolysated epitopes of alpha-dys with often normal beta dys in contrast to DMD/BMD
- IIH6 and VIA4-1 show significant variation between batches requiring carefully controlled studies; immunofluorescence labeling identifies subtle alterations
- Reduction of alpha dystroglycan variable within and between cases (?epitope masking)
- Some correlation in clinical severity and alpha dys reduction with POMT1, POMT2 and POMGnT1 mutations
- Secondary reduction of laminin alpha 2, but never complete

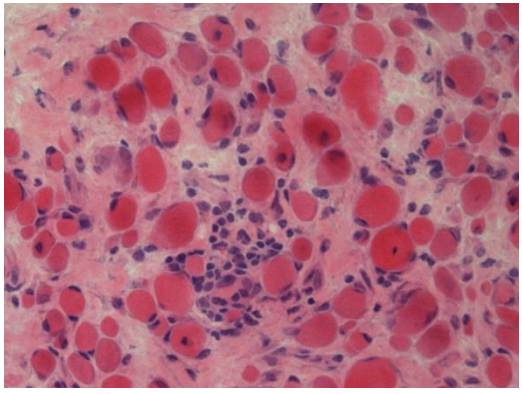


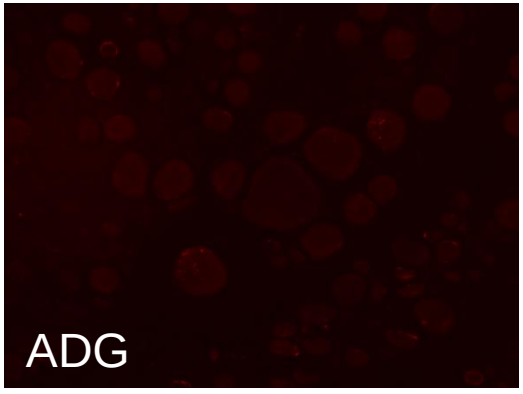


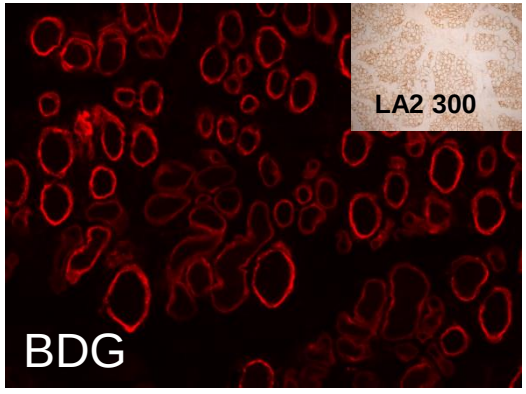


Case 1

- *2Y F; developmental delay; MEB phenotype*
- *CK 450*
- *Muscle US normal*
- *Homozy mutation exon 16 POMGnT1*

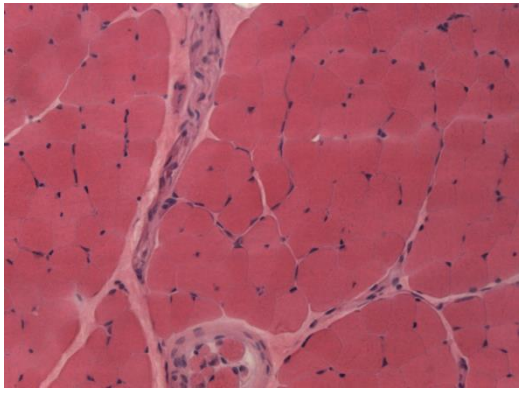


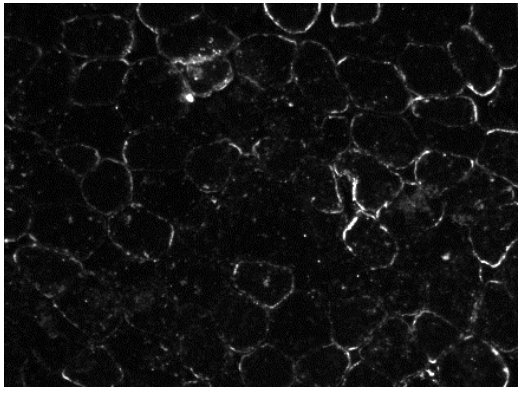


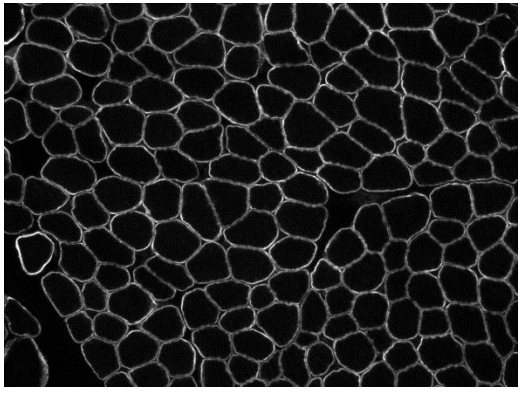


Case 2

- *11m, F; developmental delay; high CK*
- *Muscle US increased echogenicity*
- *Pathogenic homozygous mutation exon 20 POMT1*



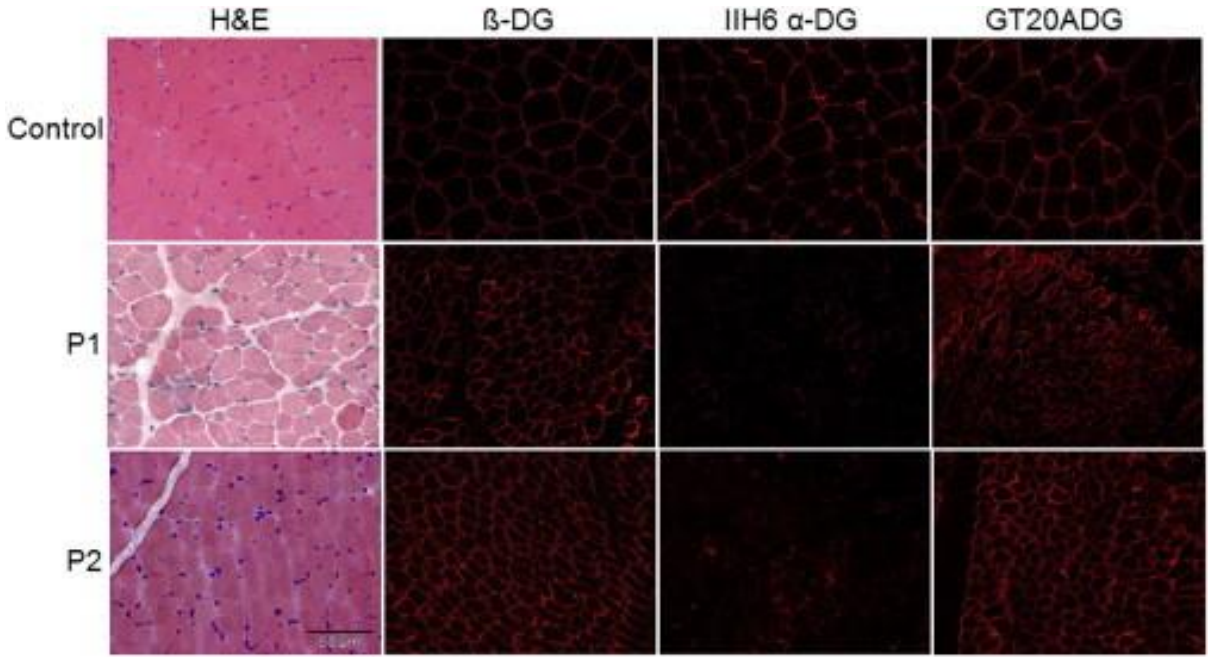




Case 3

- *4Y, M; proximal limb girdle weakness*
- *CK 140,876*
- *?? DMD/Sarco-glycanopathy*
- *FKRP homozygous mutation*

Alpha-dystroglycanopathy - mutations in *B3GalNT2*

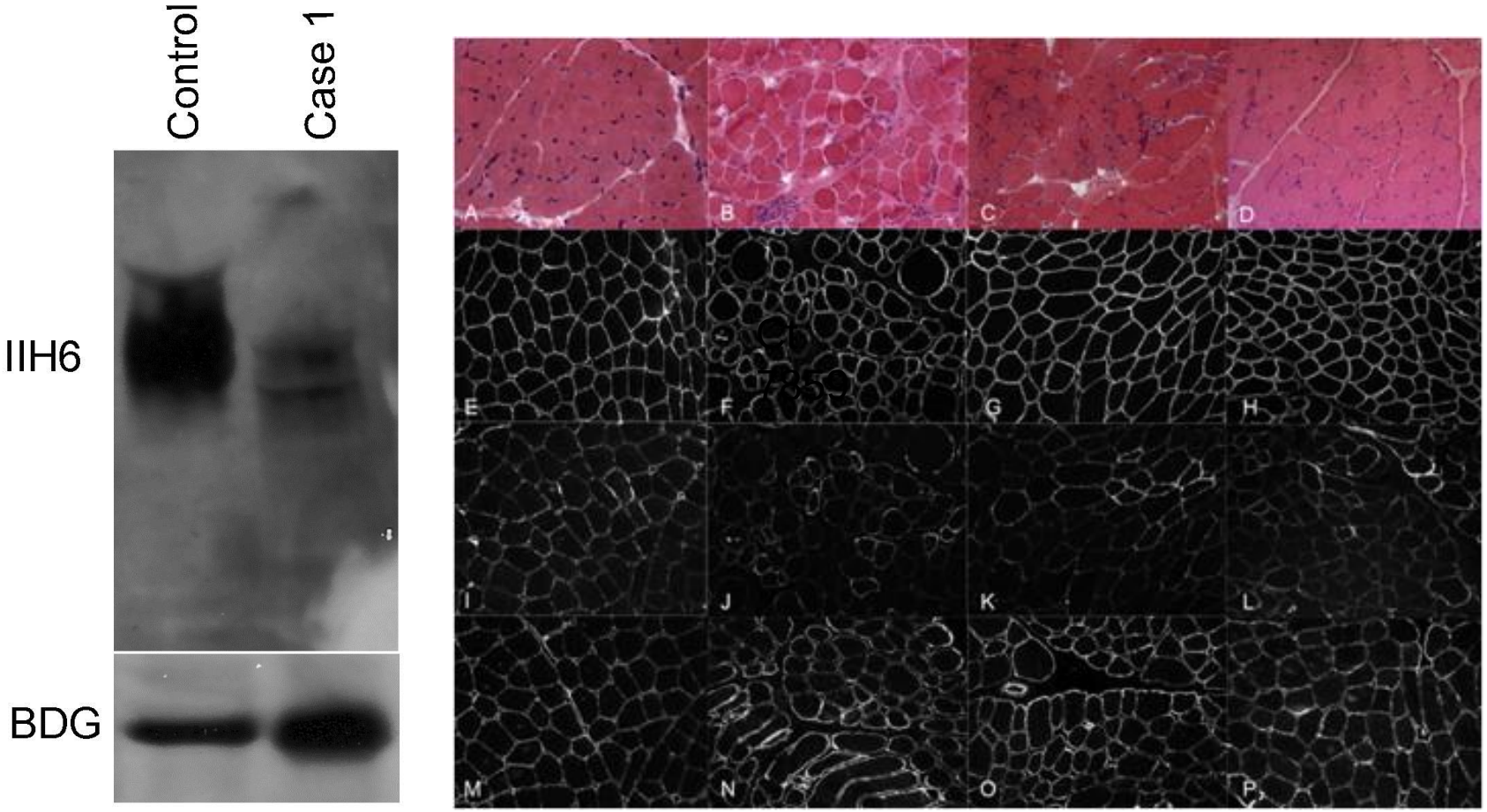


WES/Sanger identified cohort of patients with recessive mutations with MD and brain involvement

Functional ADG glycosylation reduced in muscle and fibroblasts, and in zebrafish knockout

Stevens E. AJHG 2013

Carss KJ. AJHG 2013



Cohort of patients with clinical severity ranging from classic CMD to LGMD

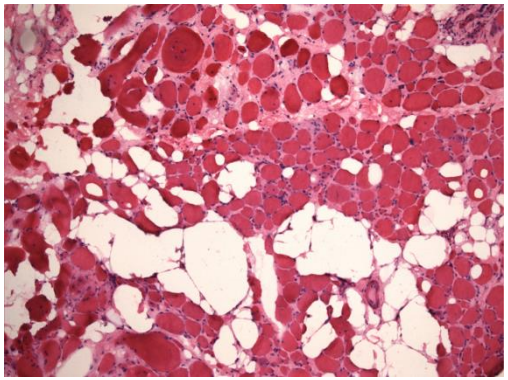
WES/Sanger – recessive mutations in GMPPB

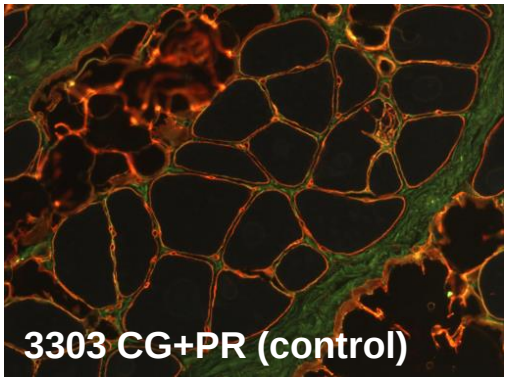
Functional ADG glycosylation reduced in muscle and fibroblasts, and in zebrafish knockout

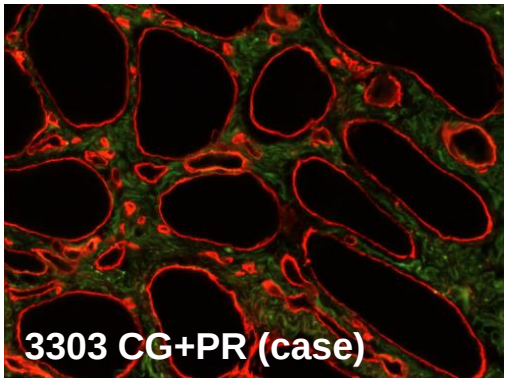
Collagen VI disorders (Ullrich and Bethlem)

- UCMD is one of the most common forms of CMD; mutations in one of the 3 Col VI genes (A1, A2, A3)
- Col VI normally localises to the perimysium and endomysium with enhanced basal lamina labeling
- Reduced labeling in UCMD but normal labeling does not exclude a defect
- Bethlem cases show mild non-specific alterations in muscle biopsies with Col VI staining indistinguishable from normal
- Double labeling with another basal lamina protein such as perlecan, Col IV, V or nidogen required to ensure basal lamina integrity and identify subtle reduction
- Flow cytometric quantitative assessment of Col VI in cultured skin fibroblasts is more sensitive

Ullrich CMD

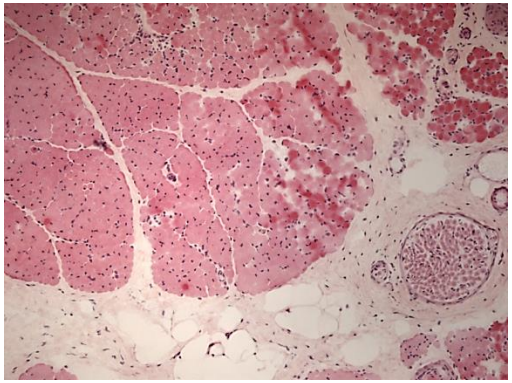


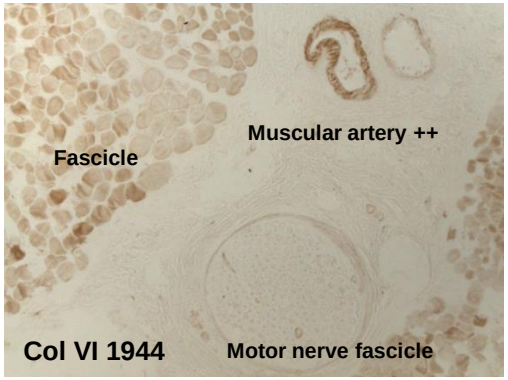


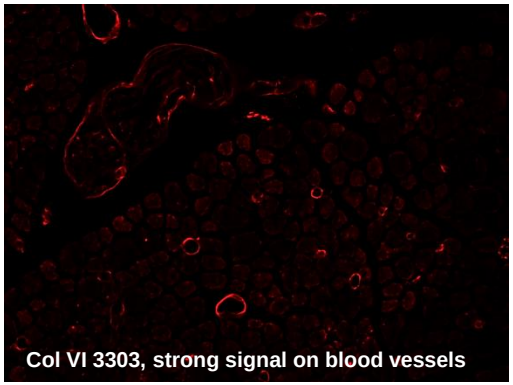


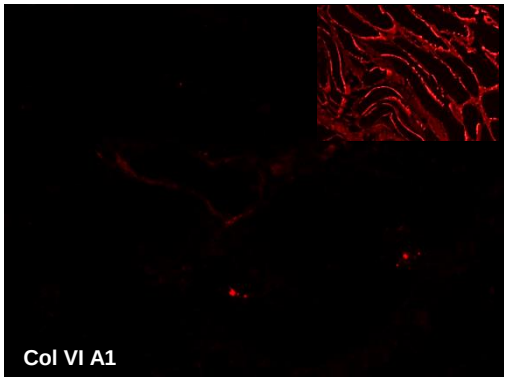
Case 1

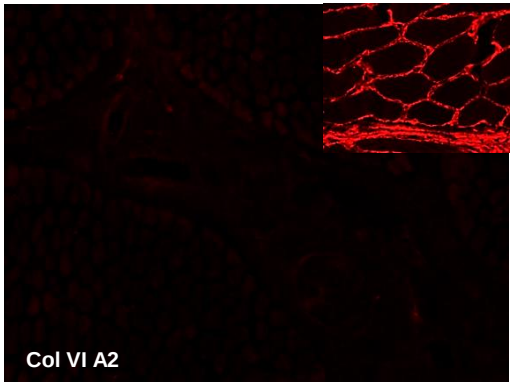
- *3 years male*
- *Torticollis few weeks after birth*
- *Delayed motor milestones*
- *Unable to run*
- *Elbow and wrist contractures*
- *Finger laxity*
- *Normal CK*
- *Muscle US increased echo*
- *Homozy pathogenic mutation COL6A2 c.2329T>C(p. Cys777Arg)*

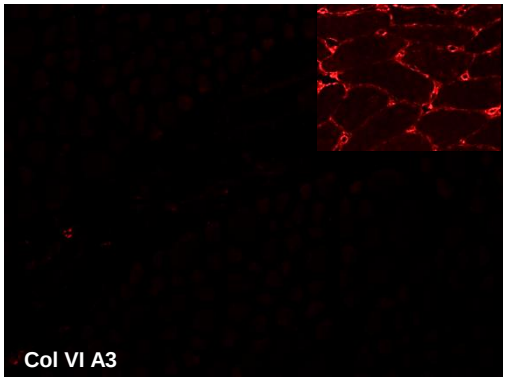








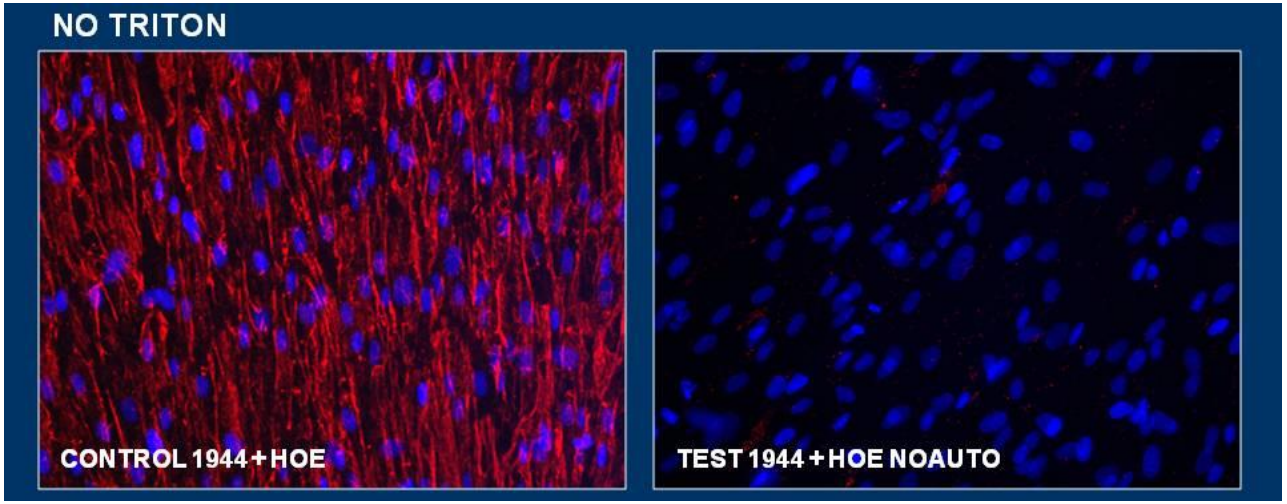




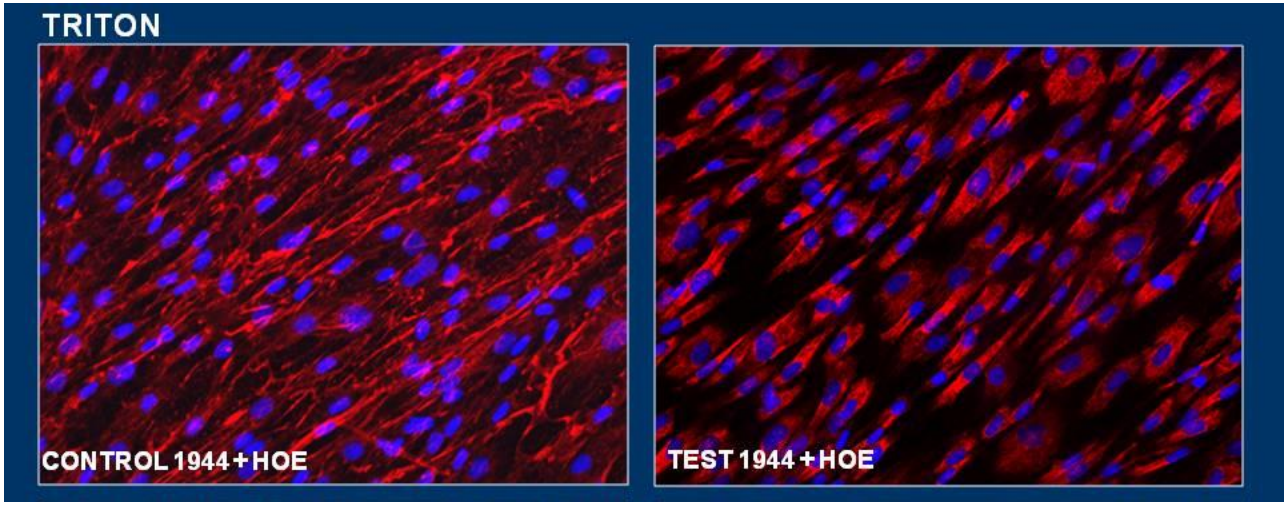
Case 2

6 months female; floppiness and hypotonia ar birth; bilateral CHD; distal laxity; motor developmental delay; muscle US increased echo; normal CK compound heterozy: c.1770+1delG intron 23 and c.2386A>T(p.Lys796X) COL6A2 pathogenic mutations, one causing abberant splicing and other causing truncated alpha 2 polypeptide; no COL VI in muscle and nerves (ColVI null)

Ullrich CMD: cultured skin fibroblasts

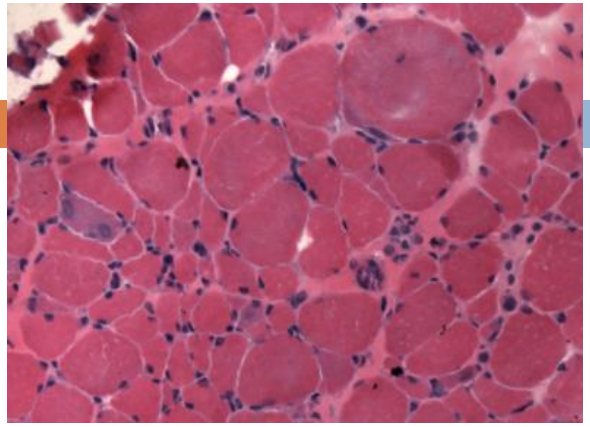


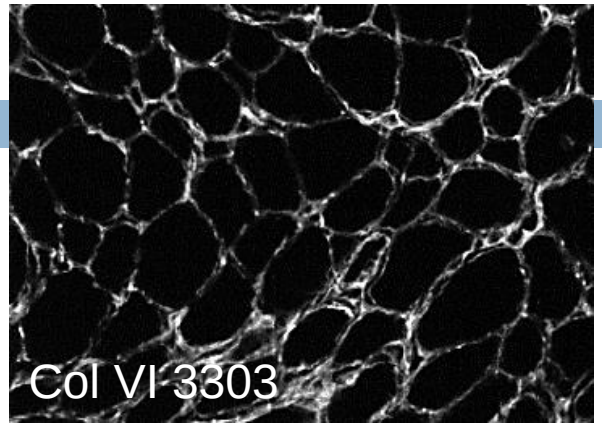
Reduced Col VI labeling in ECM in Ullrich compared to control cells

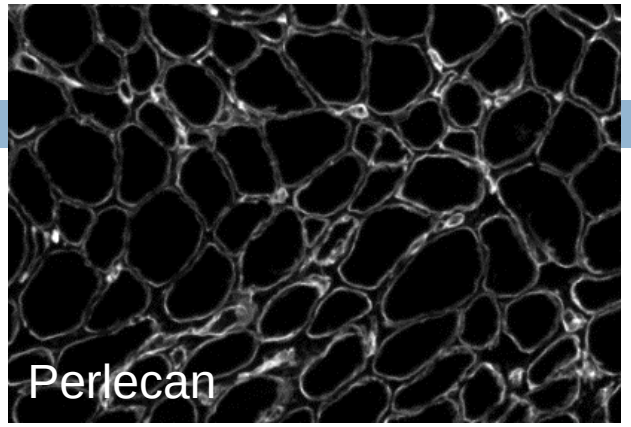


Increased intracellular Col VI labeling and overall reduced labeling in ECM after permeabilisation in Ullrich compared to control cells

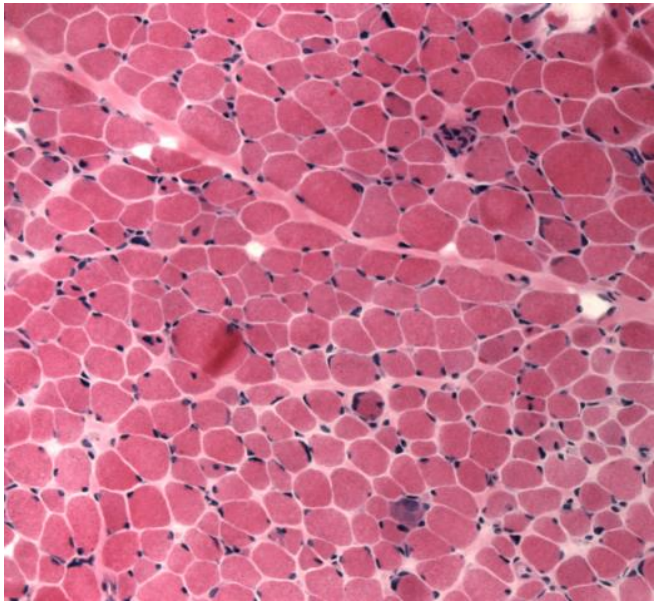
Bethlem myopathy and Intermediate phenotype

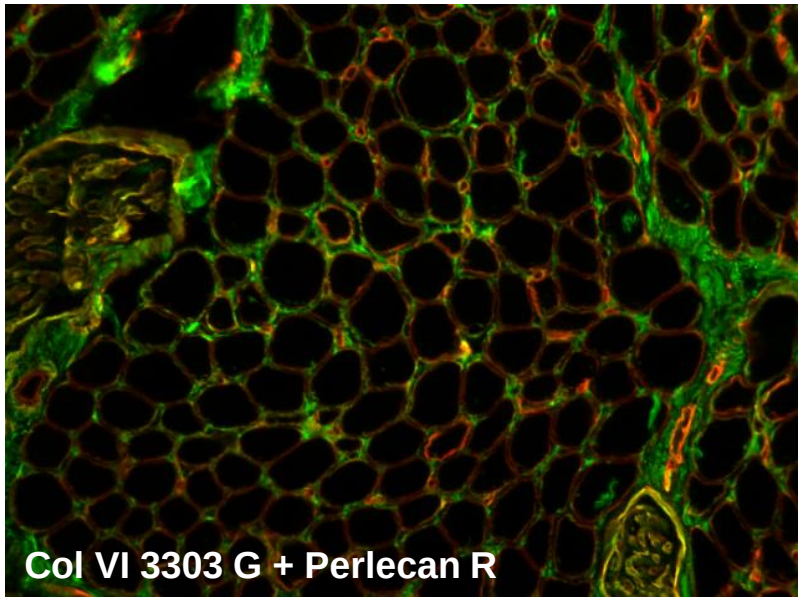




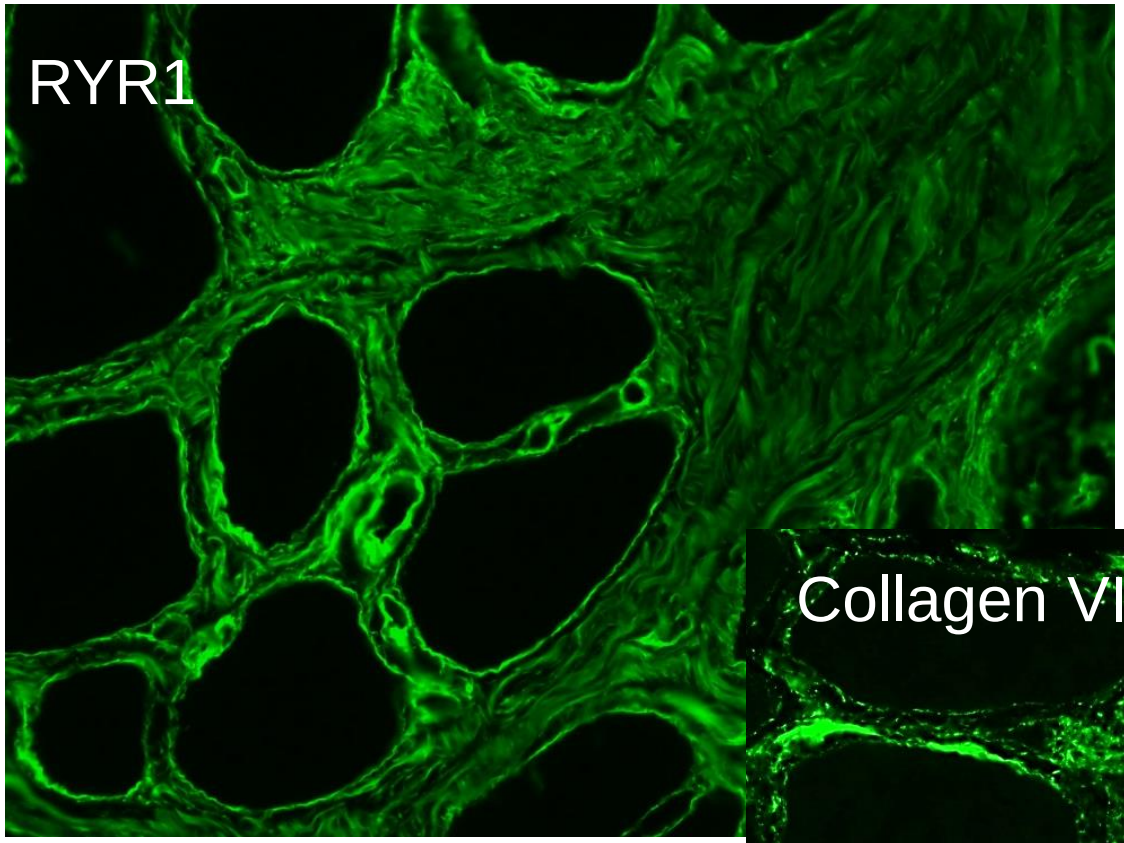


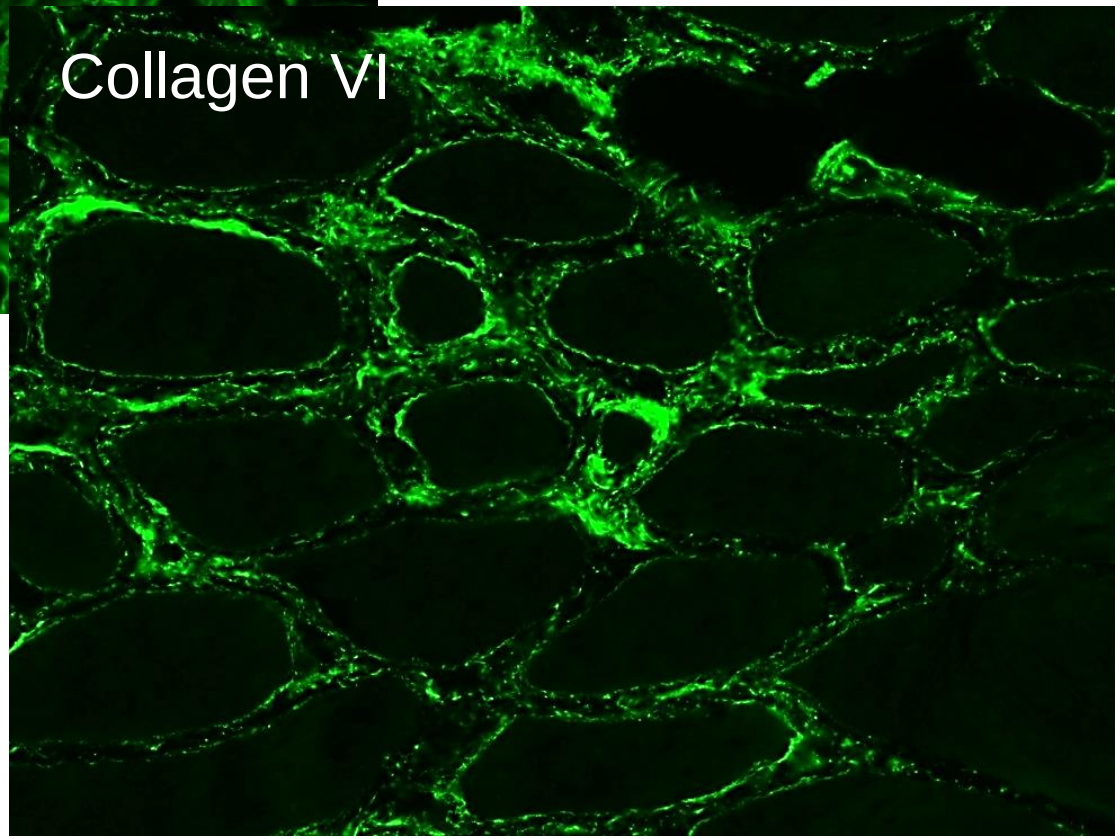
Case 1 (Bethlem): *4 years male; delayed motor milestones; lax ligaments; mother had contractures (elbows, long FFs); dystrophic muscle; subtle col VI reduction in muscle; Heterozy mutation in exon 11 COL6A3*





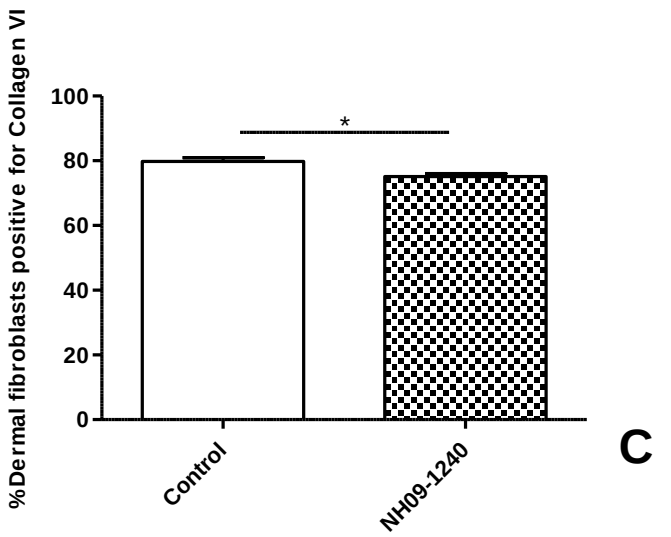
Case 2 (Intermediate):
3 years female; delayed motor milestones; waddling gait; normal CK; mildly dystrophic biopsy; reduced Col VI in muscle and skin fibroblasts Heterozygous deletion exons 6-10 COL6A2



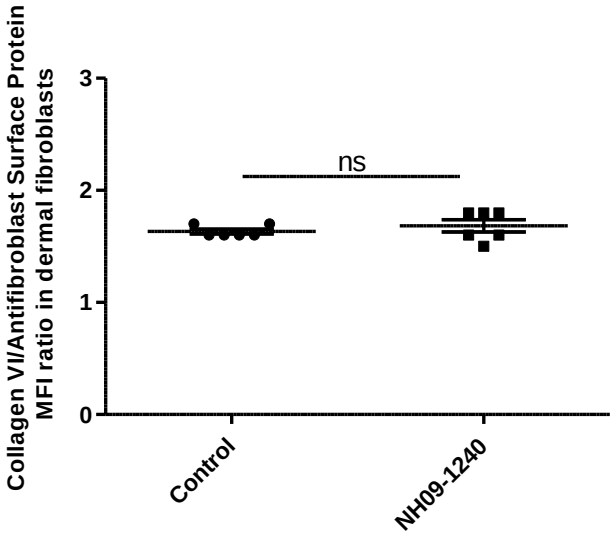


Residual basal lamina and interstitial collagen is qualitatively abnormal and ‘lumpy bumpy’ in many cases

Flow cytometric analysis of cultured dermal fibroblasts in suspected collagen VI-RD

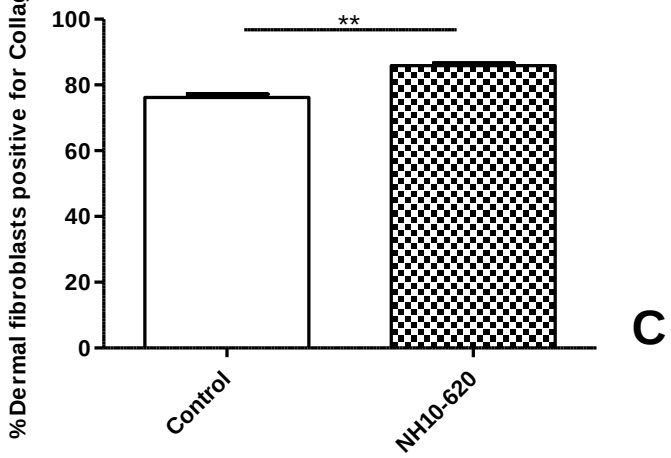


C



% positive cells

P1



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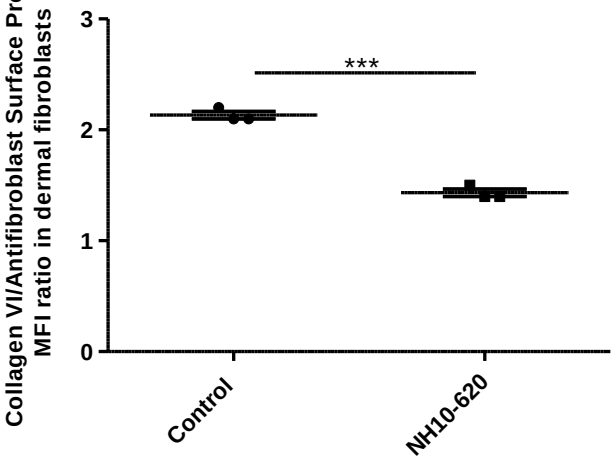


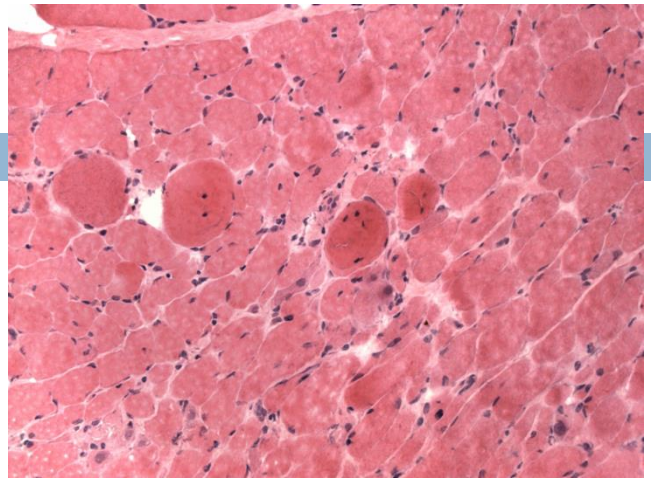
MFI

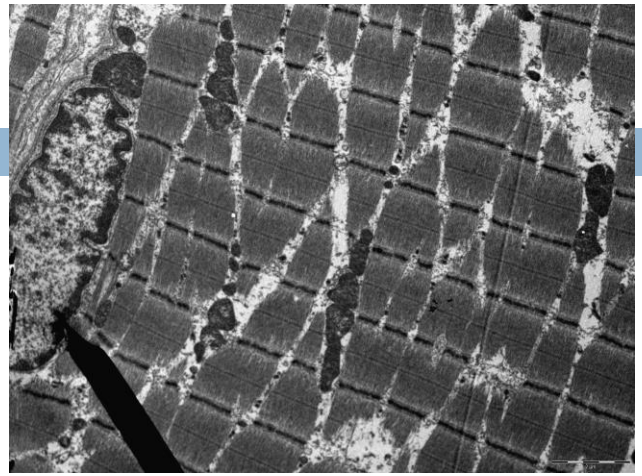
P2

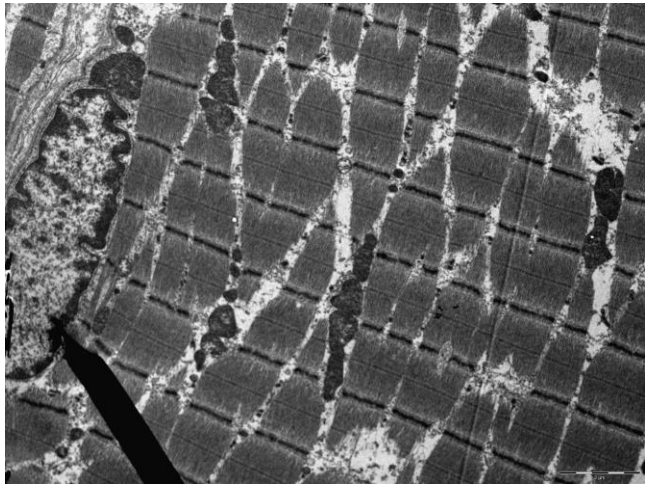
Pierpaolo Ala. ICH

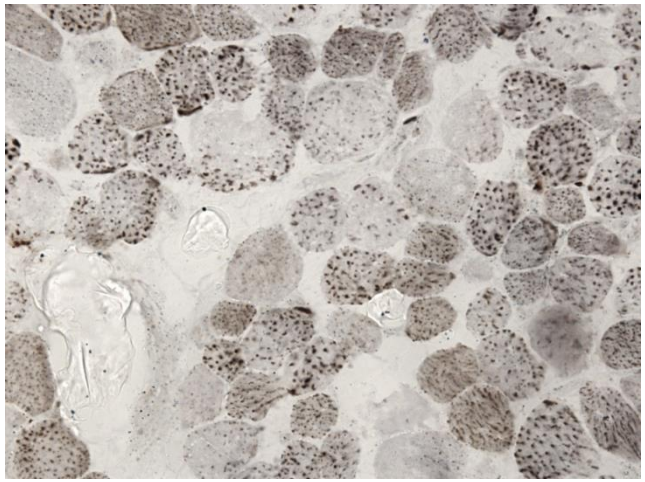
CHKB dystrophy

- ***Early onset muscle wasting***
- ***Mental retardation***
- ***Characteristic giant mitochondria prevalent in a peripheral distribution***
- ***Defective de novo phosphatidylcholine biosynthesis***
- ***Homozygous or compound heterozygous CHKB mutations***
- ***Absent or reduced muscle choine kinase activity***
- ***Mitsuhashi S et. Al. Am J Hum Gen 2011***





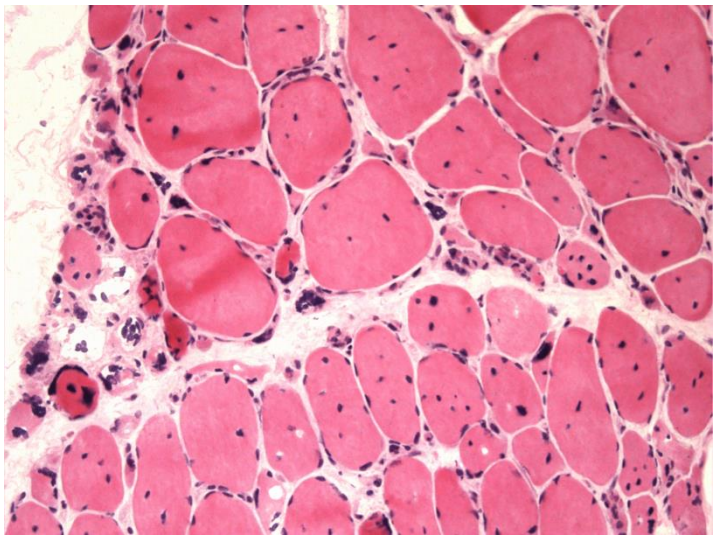




30 months male at biopsy
Learning difficulty with expressive speech delay
Gross motor delay
Elevated CK
Pathogenic homozygous missense mutations in CHKB

Disorders with deletions or expansions of repeated sequences

- FSHD: non-specific features; rimmed vacuoles
- Myotonic dystrophies (DM1 and DM2): marked internal nucleation; early atrophy of slow fibres in DM1; nuclear clumps and fast fibre atrophy in DM2
- OPMD: acid phsophatase reactive rimmed vacuoles in slow fibres; characteristic ultrastructural intranuclear filamentous inclusions



Emery-Dreifuss muscular dystrophy

- X-linked and autosomal forms
- Mutations in lamin A/C gene cause wide clinical spectrum
- X-linked forms with emerin mutations result in absence of protein demonstrable immunohistochemically
- AD EDMD with mutations in LMNA gene associated with normal labeling of emerin and lamin A/C
- Reduced sarcolemmal laminin beta 1 in some cases, but not a specific finding
- Other nuclear envelope proteins such as LAP2, SUN1 and nesprins are of interest

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

NSCT Referral Gatekeeping

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.

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	

Acknowledgements

HSS Rare Neuromuscular Disorders Diagnostic and Advisory Service for Congenital Muscular Dystrophies and Myopathies

Dubowitz Neuromuscular Centre GOSH, ION, ICH and UCL, London, UK

Clinical
Francesco Muntoni
Anna Sarkozy
Stephanie Robb
Adnan Manzur
Pinki Munot
Ros Quinlivan
Joanna Poulton
Ruth Barratt
Marion Main
Ruth Haslett
Bella Summers

Pathology
Caroline Sewry
Lucy Feng
Darren Chambers
Cathy Timson
PierPaolo Ala
Silvia Torelli
Narinder Janghra
Eloy Rivas
Lee Martindale

Genetics
GSTS/Viapath DNA laboratory Guy's Hospital
Rachael Mein
Nicholas Parkin
Sophie Marks
Natalie Chandler
Michael Yau