Unità di Pediatria ad Alta Intensità di Cura
Università degli Studi di Milano
Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico

34 Congresso Nazionale di
ANTIBIOTICOTERAPIA
in età pediatrica

Le distrofie muscolari

M. Moggio, Milano

Le distrofie muscolari

Maurizio Moggio
UOD Malattie Neuromuscolari e Rare
Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico Milano

34° Congresso Nazionale di Antibioticoterapia in età pediatrica
Milano, 11/13 novembre 2015

FONDAZIONE IRCCS CA' GRANDA
OSPEDALE MAGGIORE POLICLINICO
Sistema Sanitario Regione Lombardia

ASSOCIAZIONE ITALIANA
MIOLOGIA
ITALIAN ASSOCIATION OF MYOLOGY

Malattie Muscolari - Nosografia

Geneticamente determinate (AD, AR, X-linked, Matrilineari) / Acquisite

Distrofie: Distrofinopatie di Duchenne e Becker, congenite, dei cingoli, distrofia Facio Scapolo Omerale, Emery-Dreifuss.

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Canalopatie: Malattia di Steinert (DM1, DM2)

Infiammatorie: Polimiositi, dermatomiositi, miopatie a corpi inclusi

Iatrogene, tossiche.

Alterazioni della giunzione neuromuscolare: miastenia, Eaton Lambert.

Prevalence of muscle disease investigated by muscle biopsy

Dystrophinopathies: 8.46/100000

FSHD: 3.95/100000

Inflammatory Myopathies: 2.4-33.8/100000

LGMDs: 2.27/100000

DM1: 10.4/100000

GSD: 2.0/100000

SMA: 1.87/100000

DM2: 0.17/100000

MFM: 0.17/100000

Prevalence of genetic muscle disease in Northern England: in-depth analysis of a muscle clinic population

Fiona L. M. Norwood,[1,2] Chris Harling,[1] Patrick F. Chinnery,[3] Michelle Eagle,[1] Kate Bushby[1] and Volker Straub[1]

Brain 2009: 132; 3175–3186 | 3175

Hum Genet. 1999 Jul-Aug;105(1-2):151-6.

The frequency of lysosomal storage diseases in The Netherlands.
Poorthuis BJ, Wevers RA, Kleijer WJ, Groener JE, de Jong JG, van Weely S, Niezen-Koning KE, van Diggelen OP.

Rheumatology. 2014 Jul 26.

Incidence and prevalence of inflammatory myopathies: a systematic review.

Meyer A, Meyer N, Schaeffer M, Gottenberg JE, Geny B, Sibilia J.

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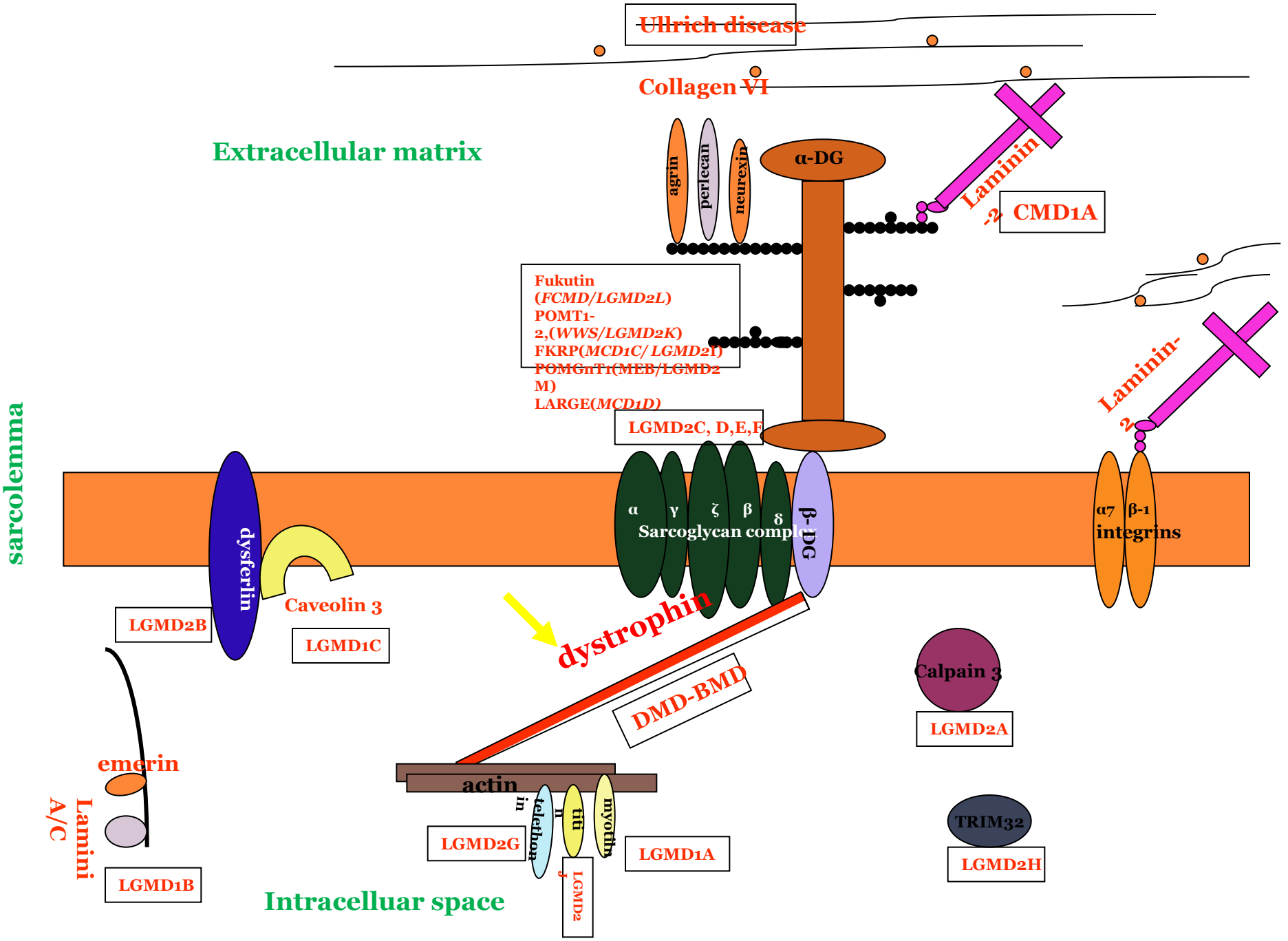

Ullrich disease
Collagen VI
Extracellular matrix
agrin
perlecan
neurexin
α-DG
Laminin -2
CMD1A
Fukutin (FCMD/LGMD2L)
POMT1-2,(WWS/LGMD2K)
FKRP(MCD1C/ LGMD2I)
POMGnT1(MEB/LGMD2M)
LARGE(MCD1D)
LGMD2C, D,E,F
Laminin-2
sarcolemma
dysferlin
Caveolin 3
LGMD2B
LGMD1C
α γ ζ β δ
Sarcoglycan complex
β-DG
α7 β-1 integrins
dystrophin
DMD-BMD
Calpain 3
LGMD2A
emerin
Lamini A/C
LGMD1B
actin
sarcoglycan
telethonin
titin
myotilin
LGMD2G
LGMD1A
LGMD2J
TRIM32
LGMD2H
Intracelluar space

Duchenne, G.B.A. (1855). De l'Electrisation Localisee et de son Application a la Physiologie, a la Pathologie et a la Therapeutique. Paris: J.-B. Baillière et Fils.

Duchenne, G.B.A. (1868). Recherches sur la paralyse musculaire pseudo-hypertrophique ou paralyse myo-sclerosique. Arch Gen Med. 11, 5-25, 179-209, 305-321, 421-443, 552-588.

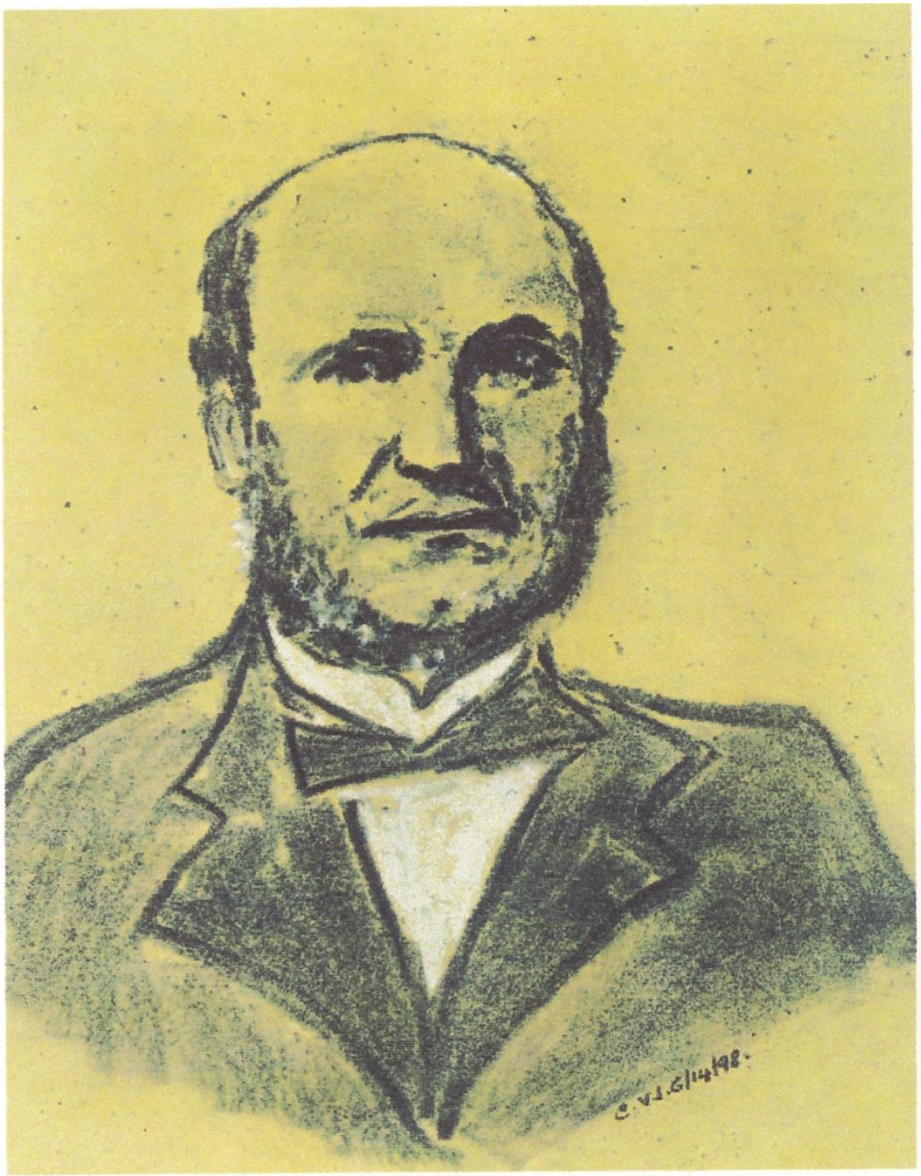


Figure 2: **G B A Duchenne**
Illustration by Venita Jay.

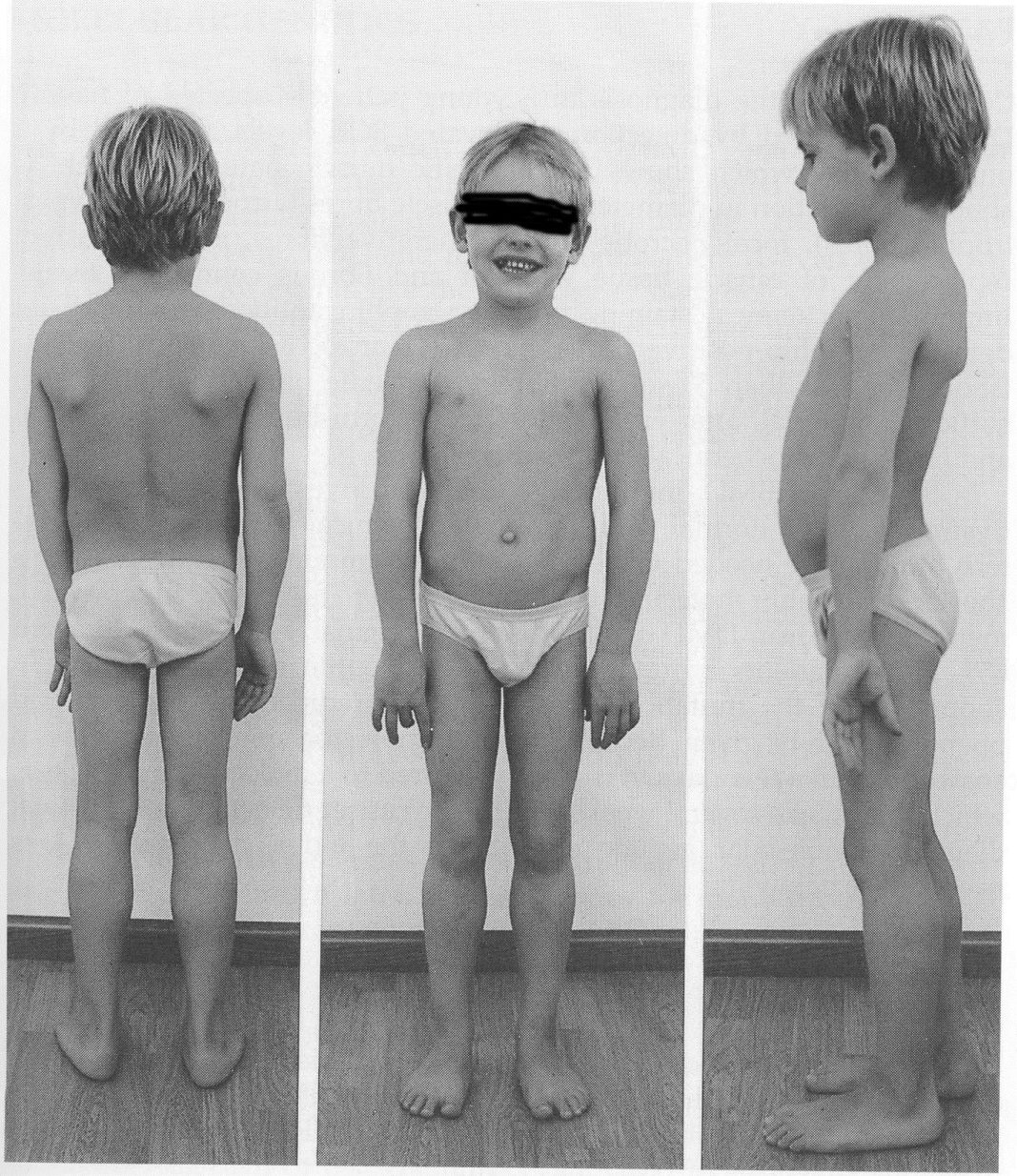


Figure 3.1. Duchenne muscular dystrophy patient (seven years), with symmetrical weakness of the proximal limb muscles and mild calf hypertrophy

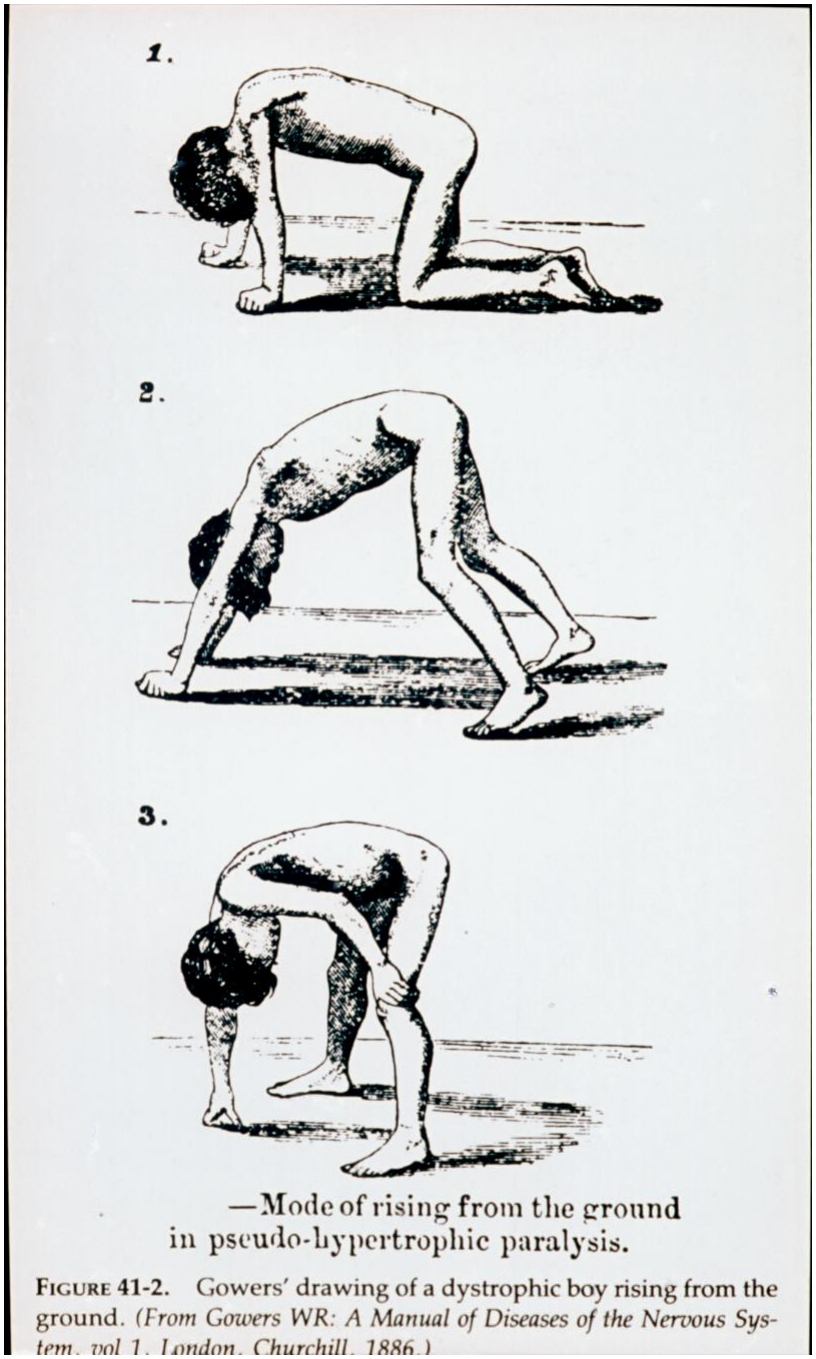
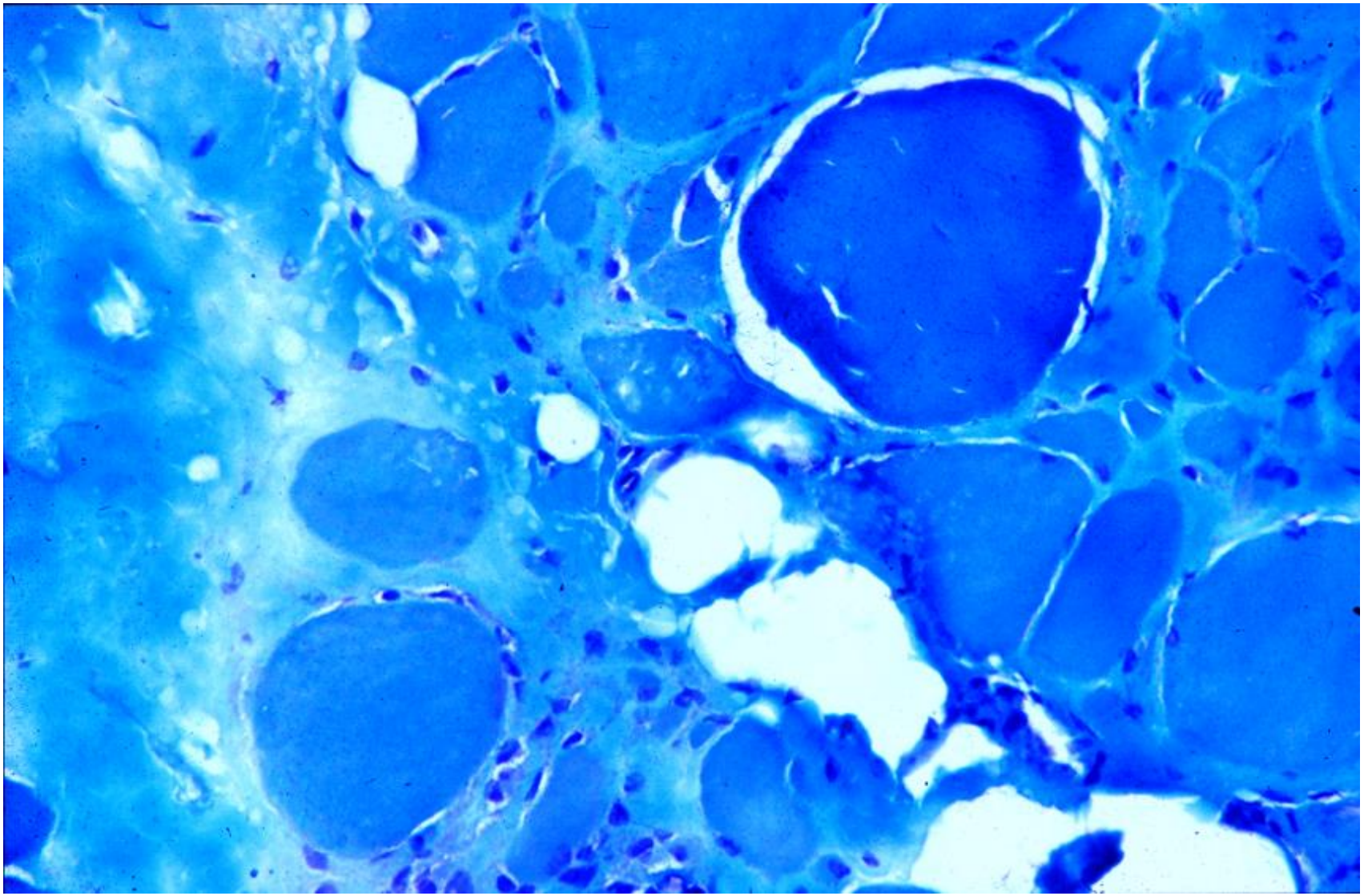
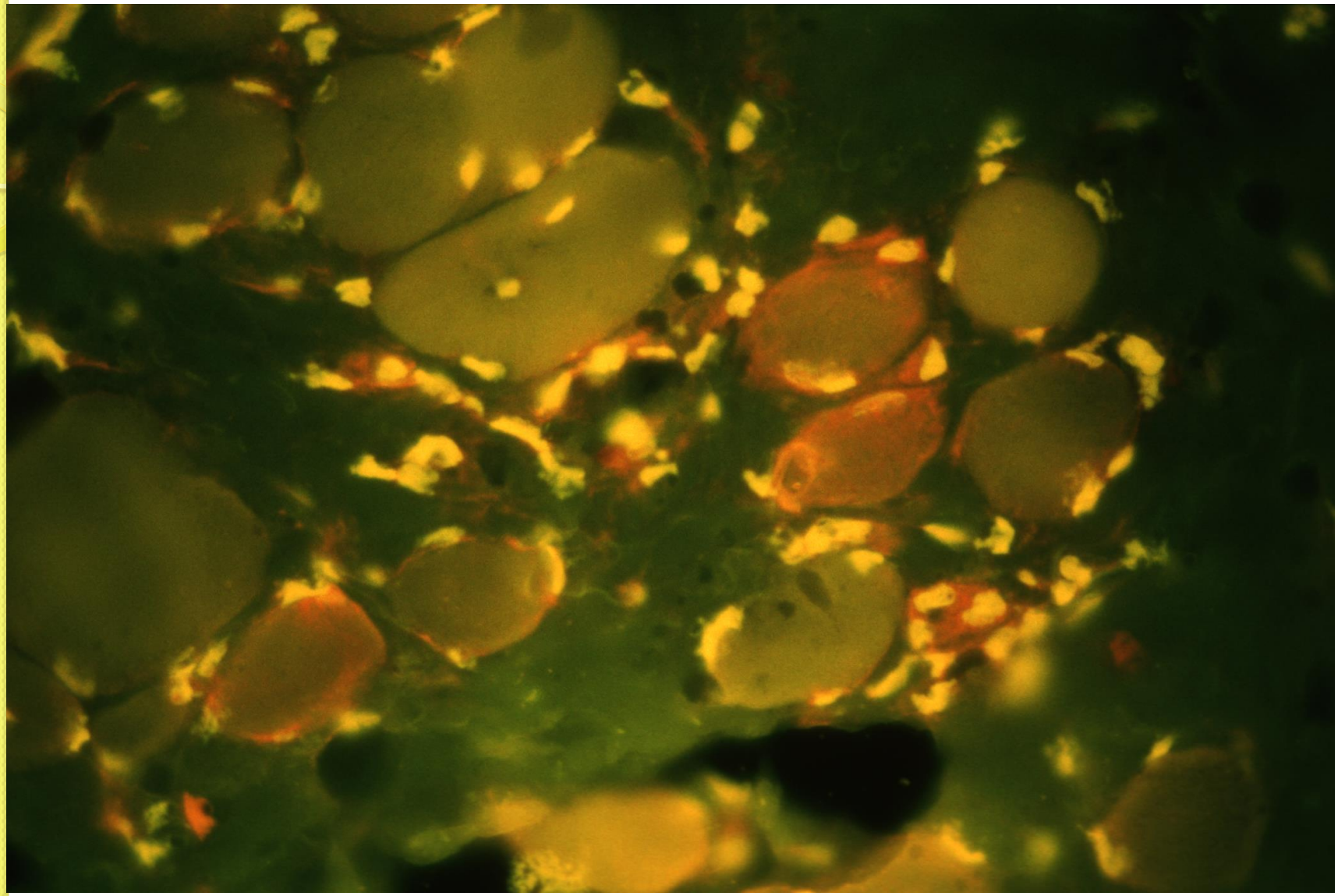
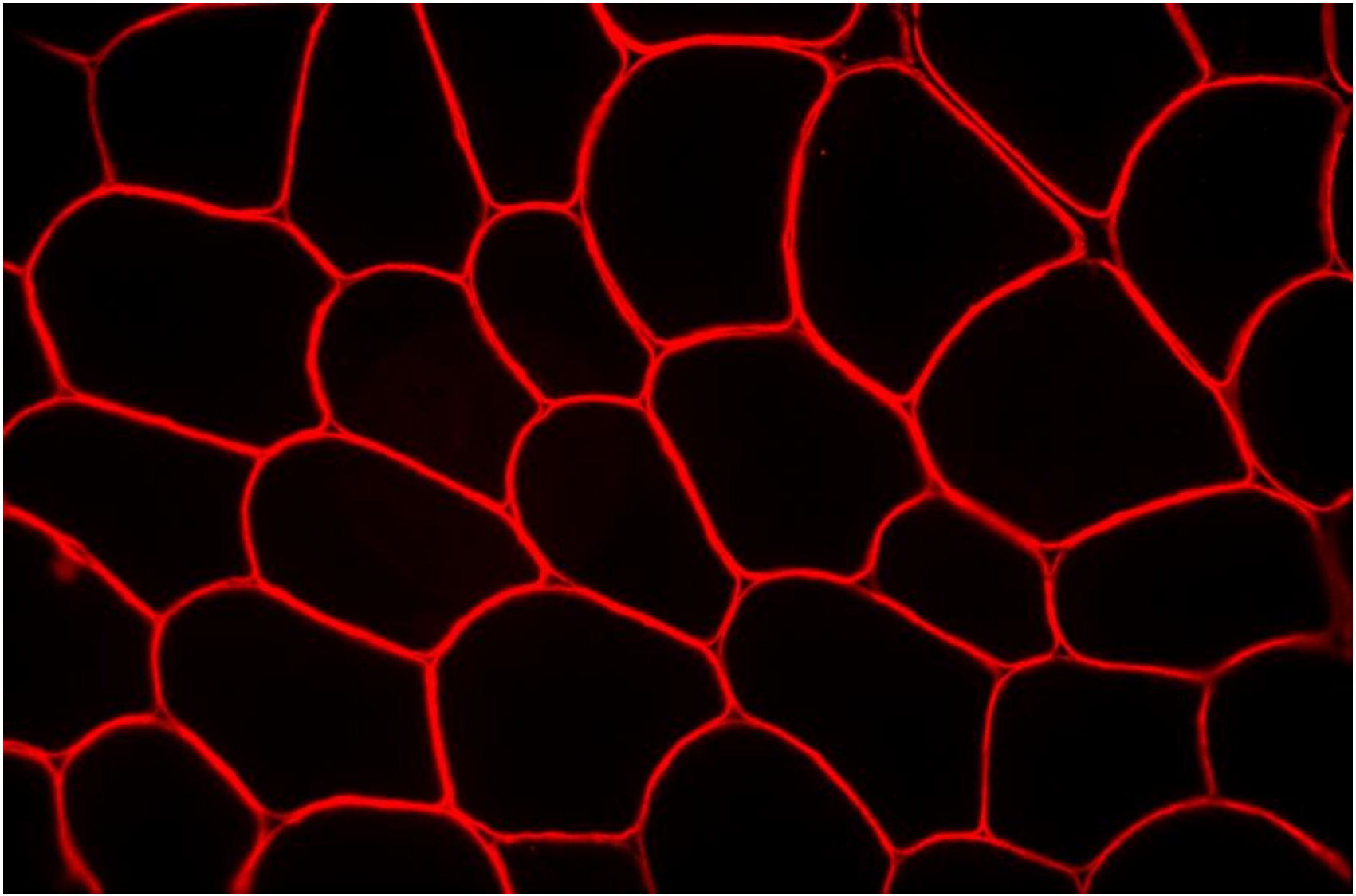
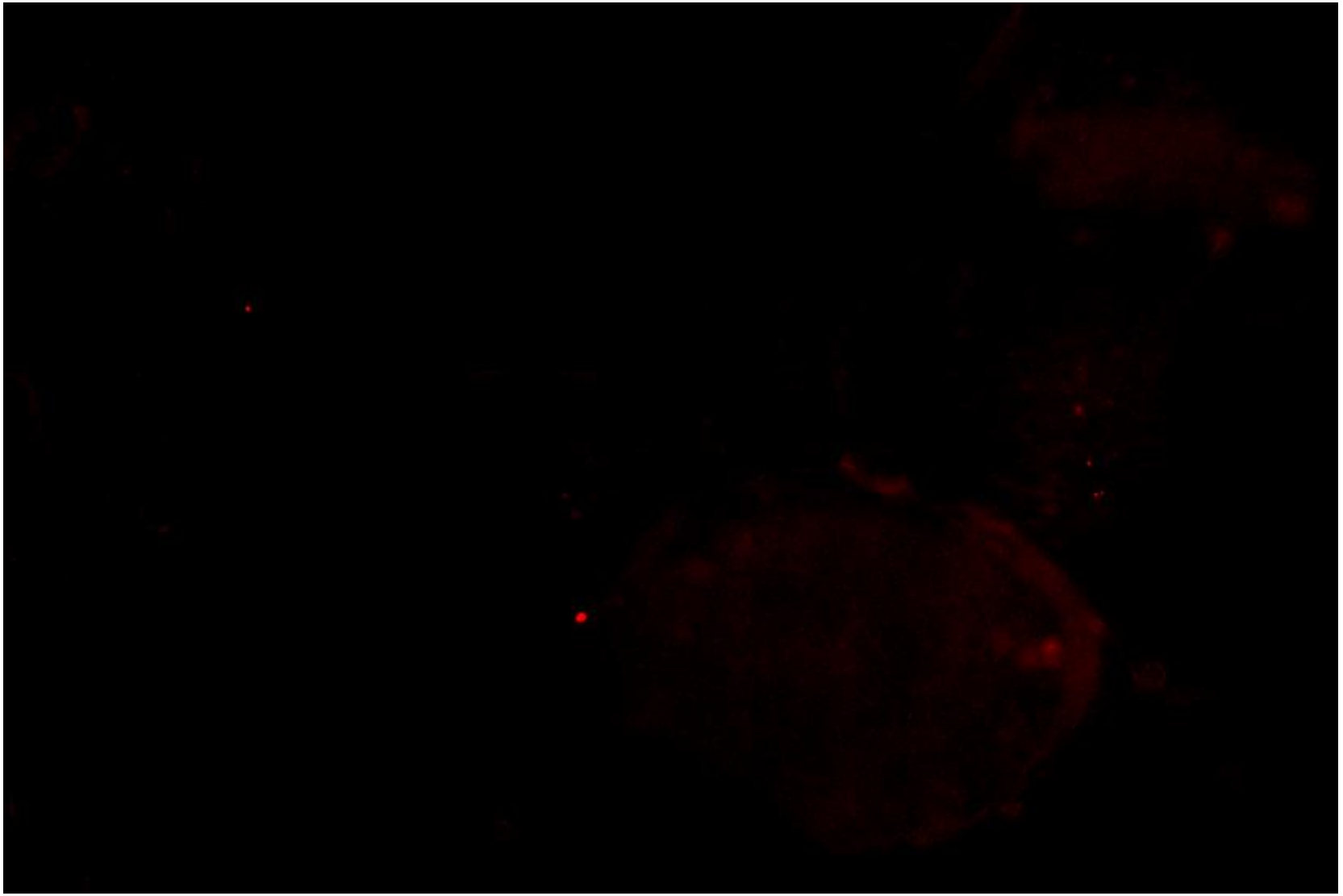


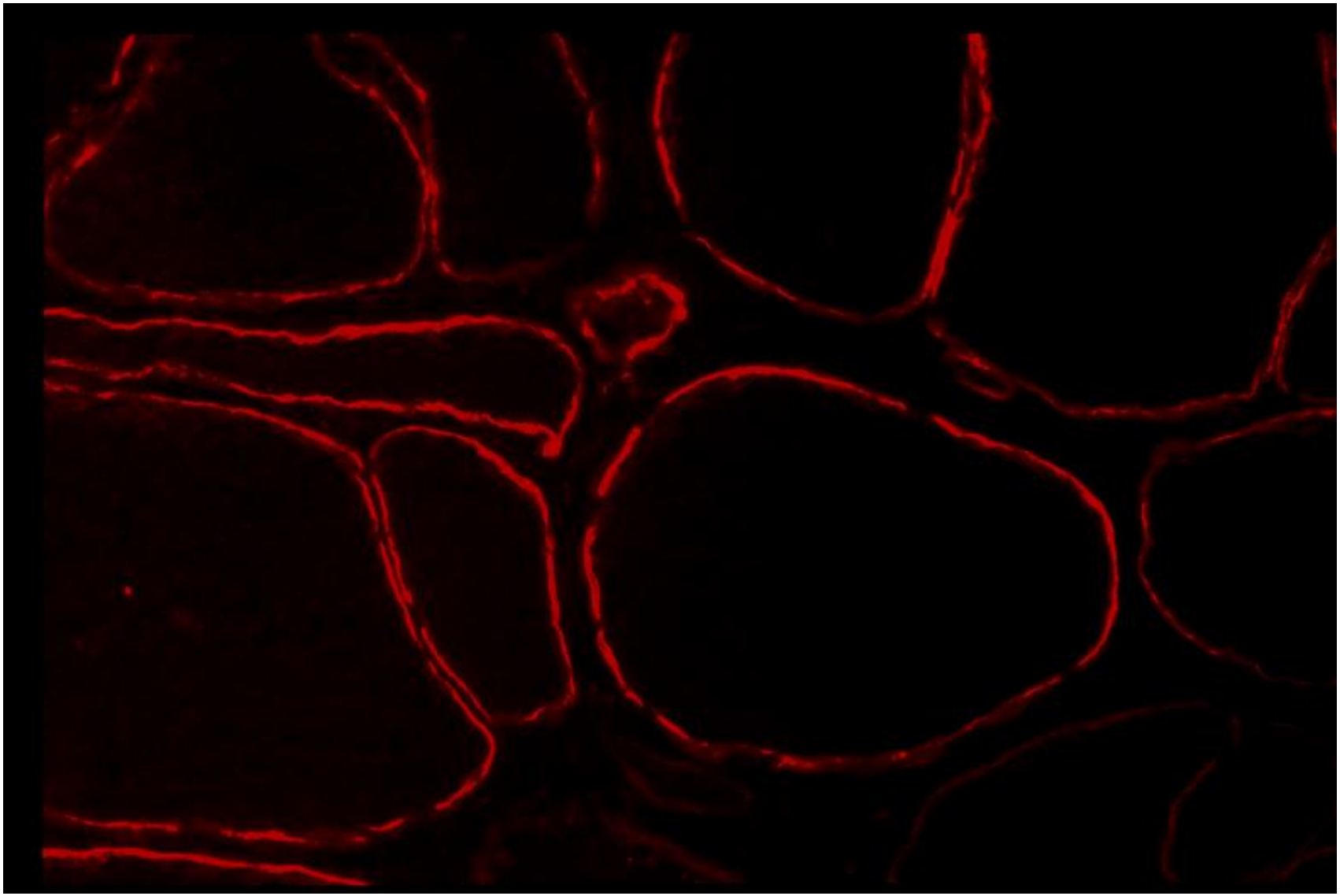
FIGURE 41-2. Gowers' drawing of a dystrophic boy rising from the ground. (*From Gowers WR: A Manual of Diseases of the Nervous System, vol 1, London, Churchill, 1886.*)



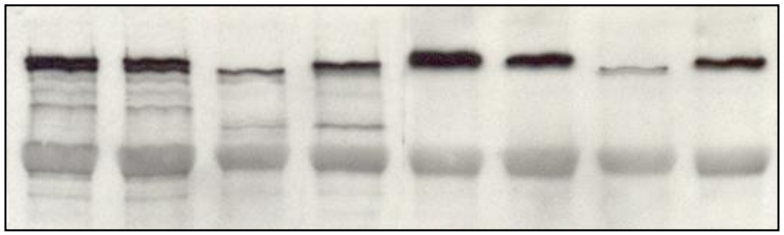




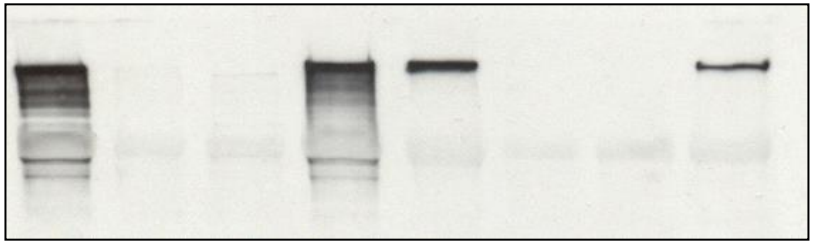




Becker muscular dystrophy (BMD)

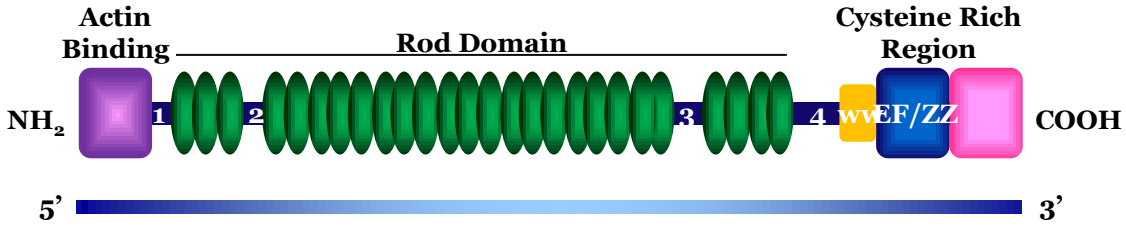


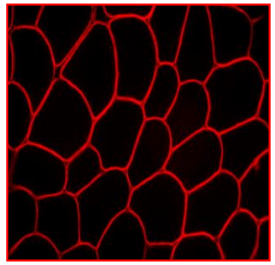
Duchenne muscular dystrophy (DMD)



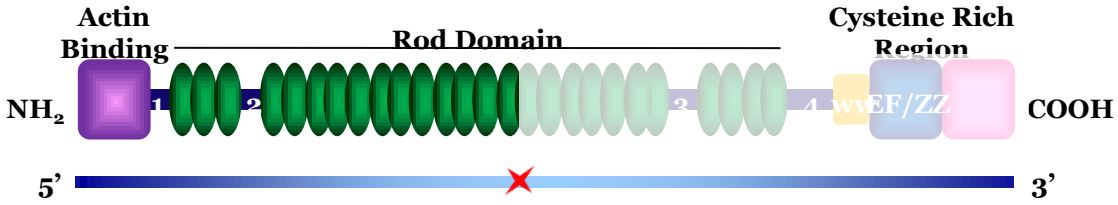
Basi Molecolari del Fenotipo

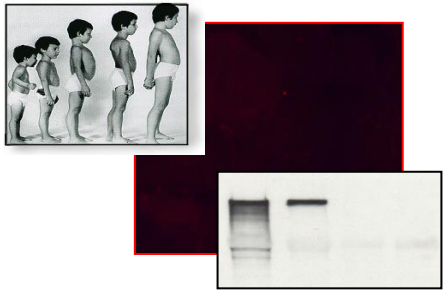
wild type



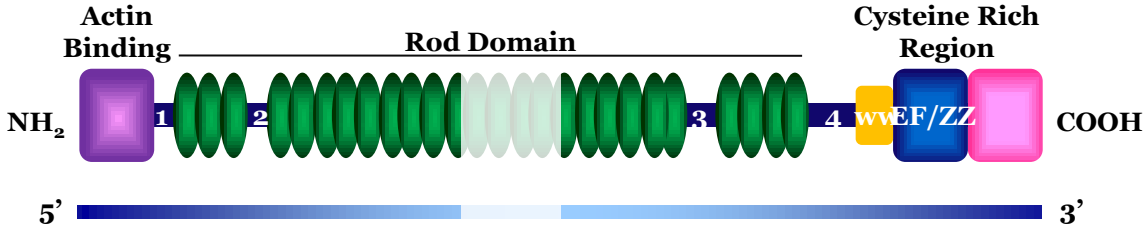


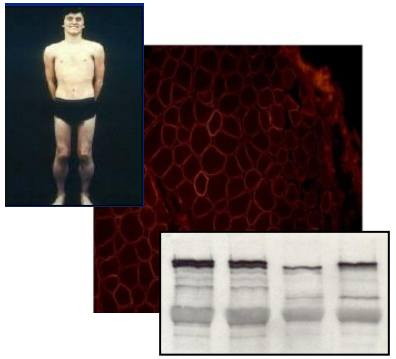
Duchenne





Becker





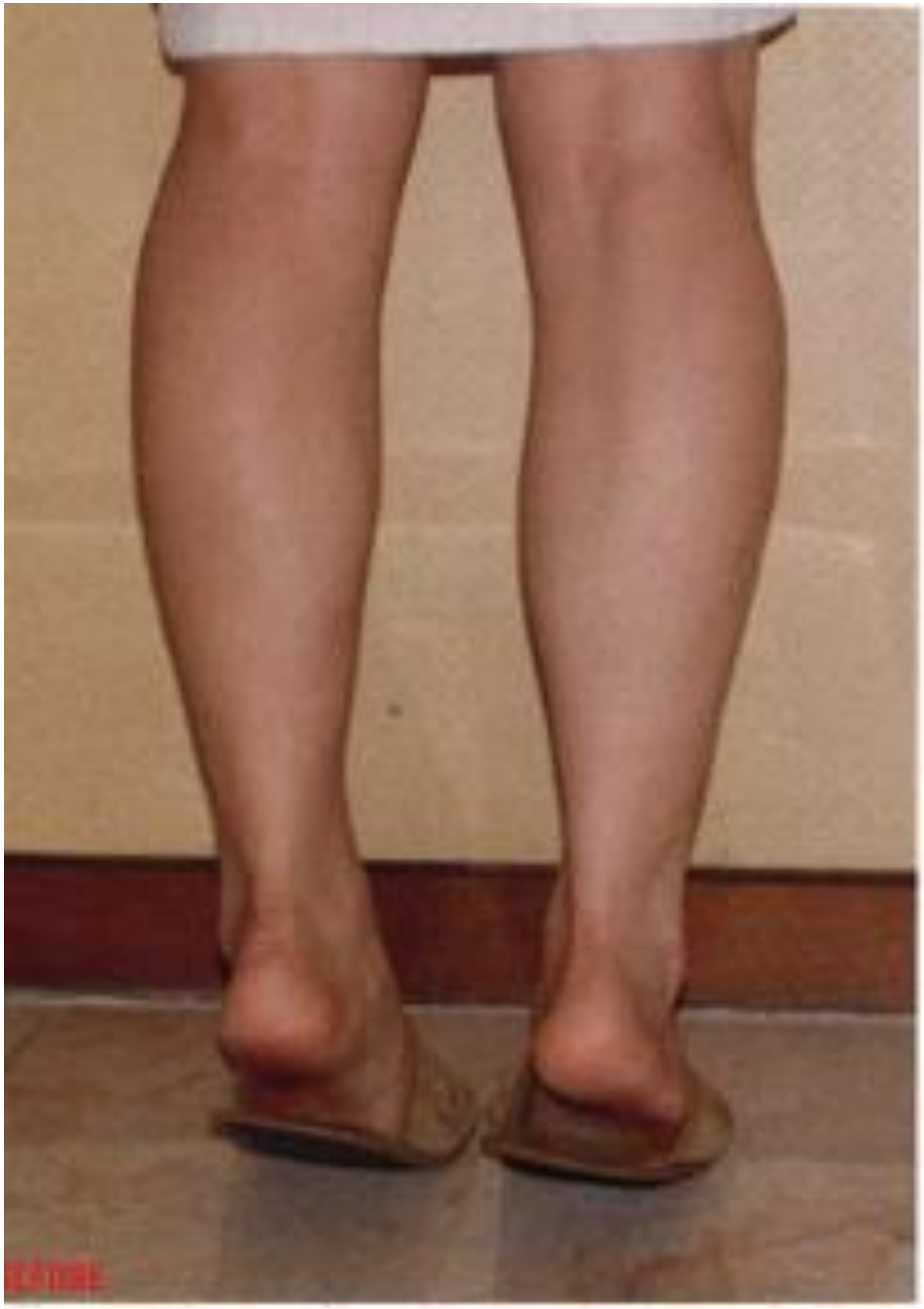
expedited publication

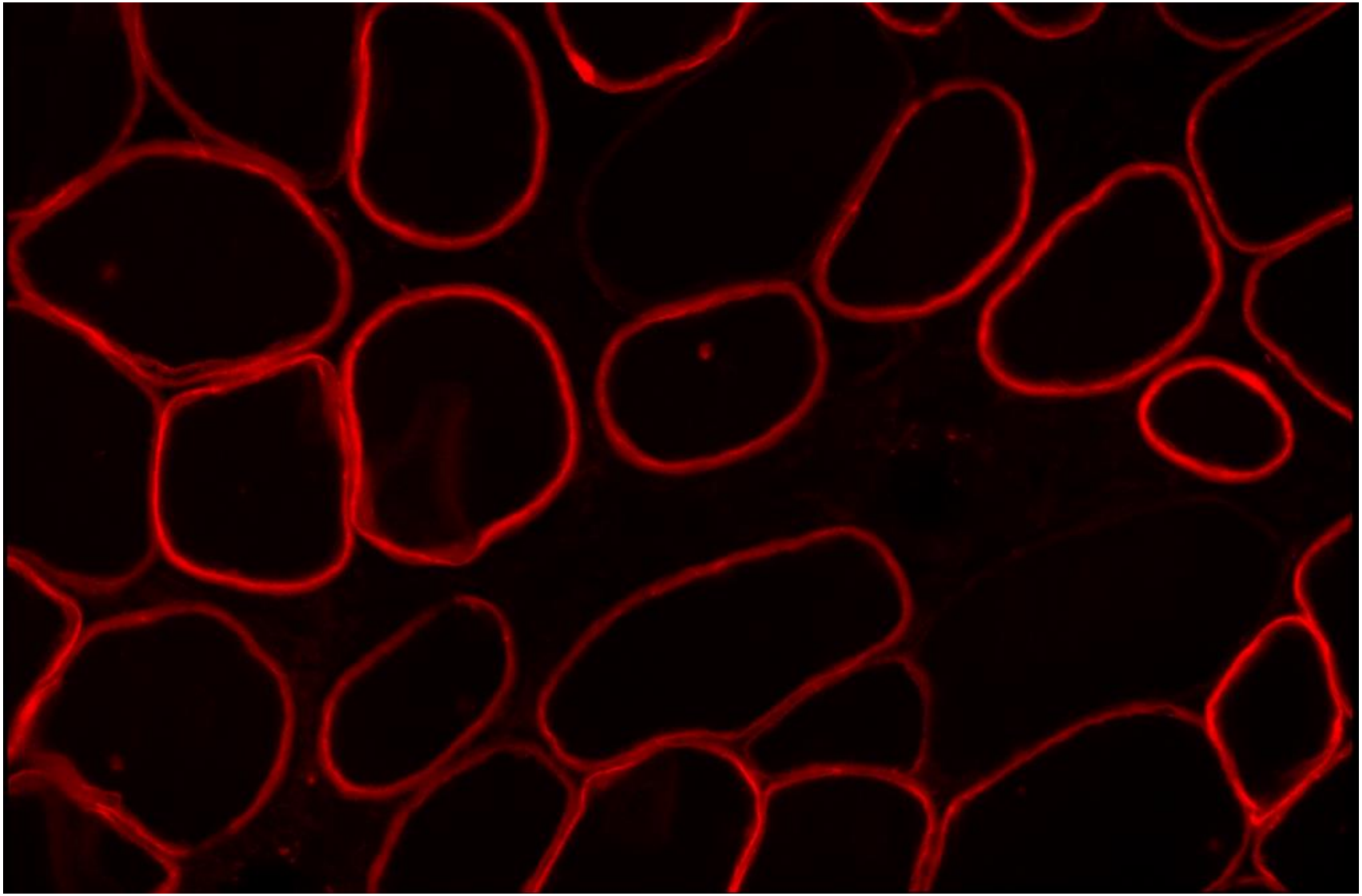
Dystrophinopathy in isolated cases of myopathy in females

E.P. Hoffman, PhD[1]; K. Arahata, MD[2]; C. Minetti, MD[3]; E. Bonilla, MD[3]; L.P. Rowland, MD[3]; and Co-Authors[*†]

Article abstract—X-linked dystrophinopathy is the most common cause of isolated cases of myopathy in males. To investigate dystrophin abnormalities as a cause of myopathy in girls and women, we used dystrophin immunocytochemistry to study muscle biopsies from 505 girls and women with neuromuscular disease. Forty-six muscle biopsies showed a combination of fibers containing or lacking dystrophin; this mosaic immunostaining pattern denoted a carrier status. Twenty-one of 46 (45.6%) had a family history of Duchenne muscular dystrophy in males. Twenty-five of 46 (54.3%) were isolated cases, with no previous family history of neuromuscular disorder. The laboratory findings of the isolated cases were consistent with the familial cases; all showed myopathic histopathology and abnormal elevations of serum CK. The clinical presentations of the isolated cases varied but were consistent with the familial cases: 40% (10/25) of isolated cases showed proximal limb weakness before age 10, 24% (6/25) presented with myalgias or cramps, 24% (6/25) presented with incidental findings of grossly elevated CK levels, 8% (2/25) noted easy fatigue, and 4% (1/25) had slowly progressive proximal limb weakness beginning at age 45. From our data, the clinical criteria for consideration of an underlying dystrophinopathy in isolated female cases of myopathy are CK levels greater than 1,000 IU/l and myopathic histopathology. About 10% of the isolated cases of hyperCKemic myopathy (25/210) were proven by dystrophin analysis to have a dystrophinopathy as the cause of their disease (manifesting carriers of Duchenne dystrophy). However, we feel that this may be an underestimate. The correct diagnosis in these patients is imperative for appropriate genetic counseling to the patients and their families.

NEUROLOGY 1992;42:967-975





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Neurotherapeutics: The Journal of the American Society for Experimental NeuroTherapeutics

Muscular Dystrophies Due to Glycosylation Defects

Francesco Muntoni, Silvia Torelli, and Martin Brockington

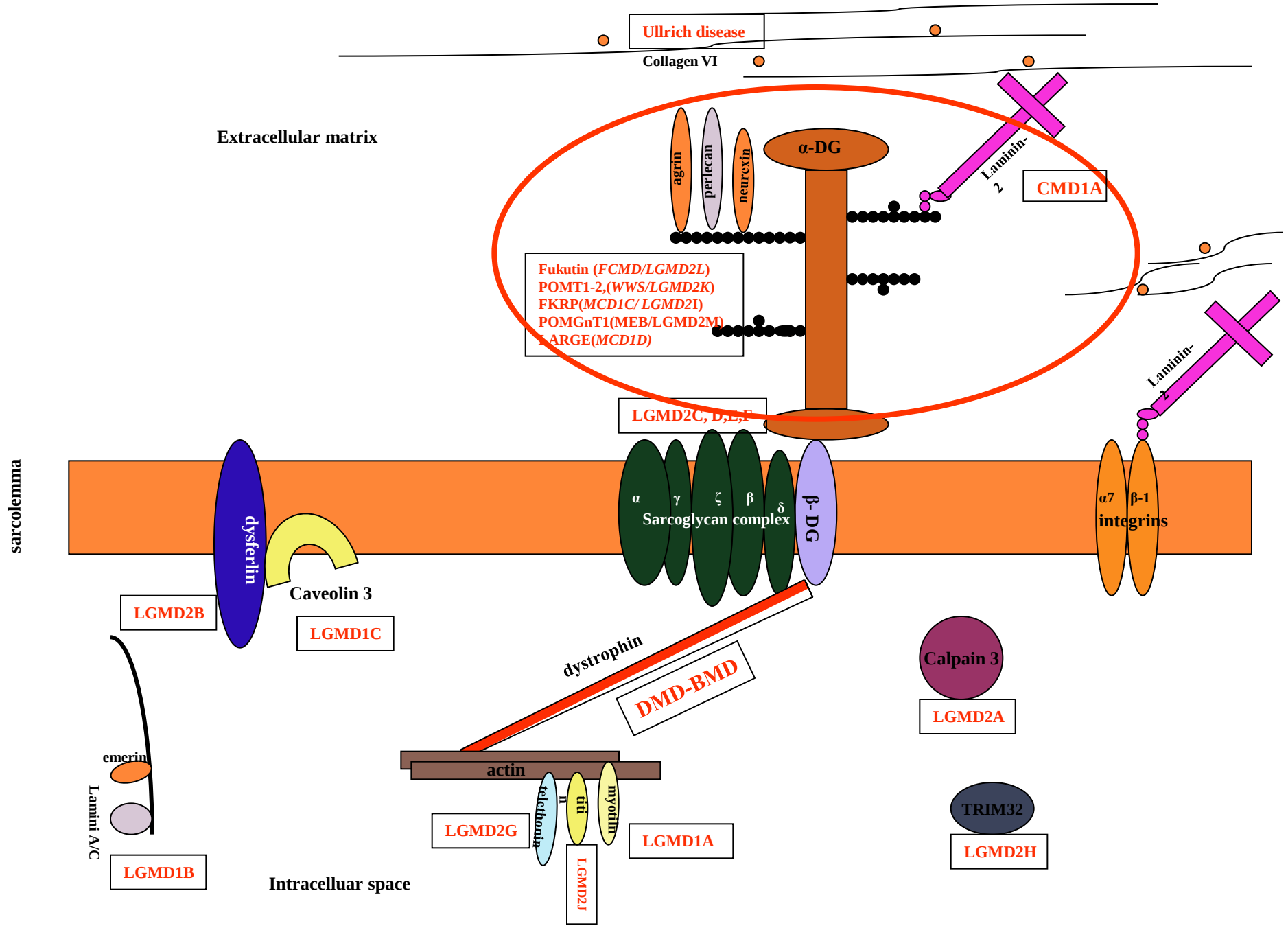
Department of Neuroscience, Dubowitz Neuromuscular Centre, UCL Institute of Child Health & Great Ormond Street Hospital, London, United Kingdom

Summary: In the last few years, muscular dystrophies due to reduced glycosylation of alpha-dystroglycan (ADG) have emerged as a common group of conditions, now referred to as dystroglycanopathies. Mutations in six genes (*POMT1, POMT2, POMGnT1, Fukutin, FKRP* and *LARGE*) have so far been identified in patients with a dystroglycanopathy. Allelic mutations in each of these genes can result in a wide spectrum of clinical conditions, ranging from severe congenital onset with associated structural brain malformations (Walker Warburg syndrome; muscle-eye-brain disease; Fukuyama muscular dystrophy; congenital muscular dystrophy type 1D) to a relatively milder congenital variant with no brain involvement (congenital muscular dystrophy type 1C), and to limb-girdle muscular dystrophy (LGMD) type 2 variants with onset in childhood or adult life (LGMD2I, LGMD2L, and LGMD2N).

ADG is a peripheral membrane protein that undergoes multiple and complex glycosylation steps to regulate its ability to effectively interact with extracellular matrix proteins, such as laminin, agrin, and perlecan.

Although the precise composition of the glycans present on ADG are not known, it has been demonstrated that the forced overexpression of LARGE, or its paralog LARGE2, is capable of increasing the glycosylation of ADG in normal cells. In addition, its overexpression is capable of restoring dystroglycan glycosylation and laminin binding properties in primary cell cultures of patients affected by different genetically defined dystroglycanopathy variants.

These observations suggest that there could be a role for therapeutic strategies to overcome the glycosylation defect in these conditions via the overexpression of LARGE. **Key Words:** Alpha dystroglycan, glycosylation, O-mannosylation, laminin binding, pharmacological upregulation.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LARGE(MCD1D)
LGMD2C, D,E,F
Laminin-2
sarcolemma
dysferlin
Caveolin 3
LGMD2B
LGMD1C
α
γ
ζ
β
δ
Sarcoglycan complex
β- DG
α7
β-1
integrins
dystrophin
DMD-BMD
Calpain 3
LGMD2A
emerin
Lamini A/C
LGMD1B
actin
filamin
titin
myotilin
LGMD2G
LGMD1A
LGMD2J
TRIM32
LGMD2H
Intracelluar space

α – distroglicano (AD)

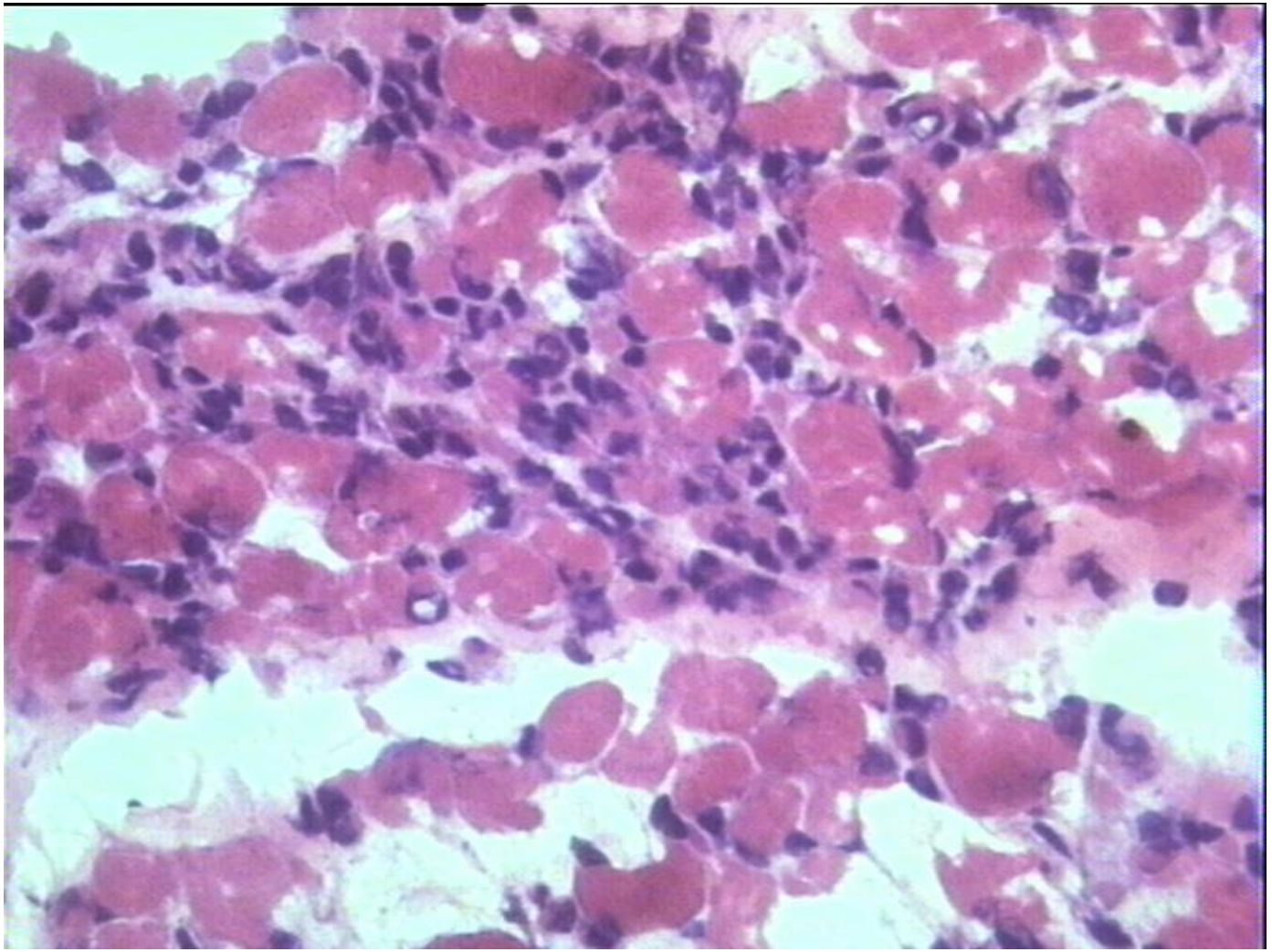
- Componente del complesso distrofina-glicoproteine.

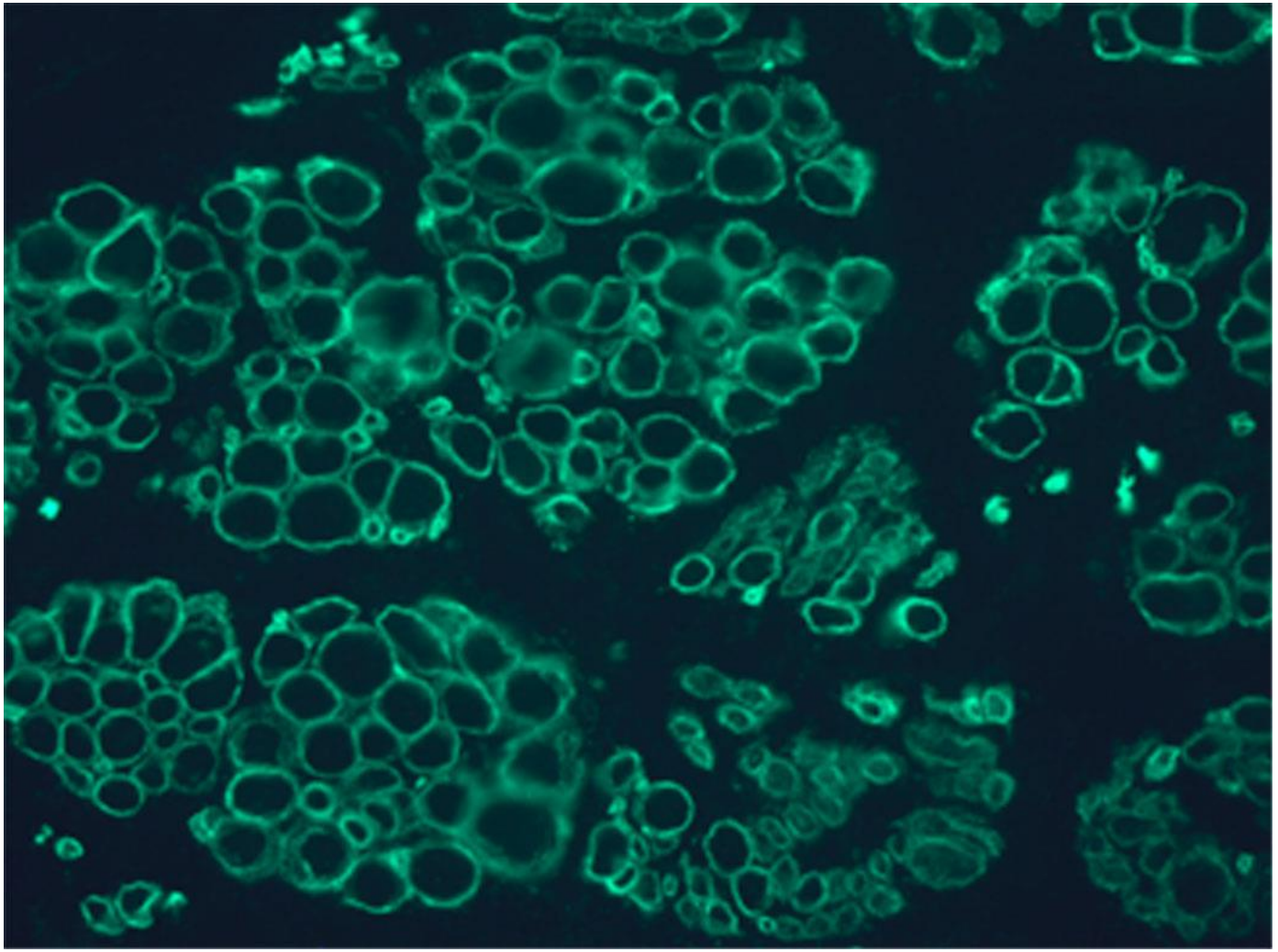
  (questo complesso lega l'actina associata al citoscheletro alla matrice extracellulare attraverso la distrofina e la catena laminina α2 della merosina).

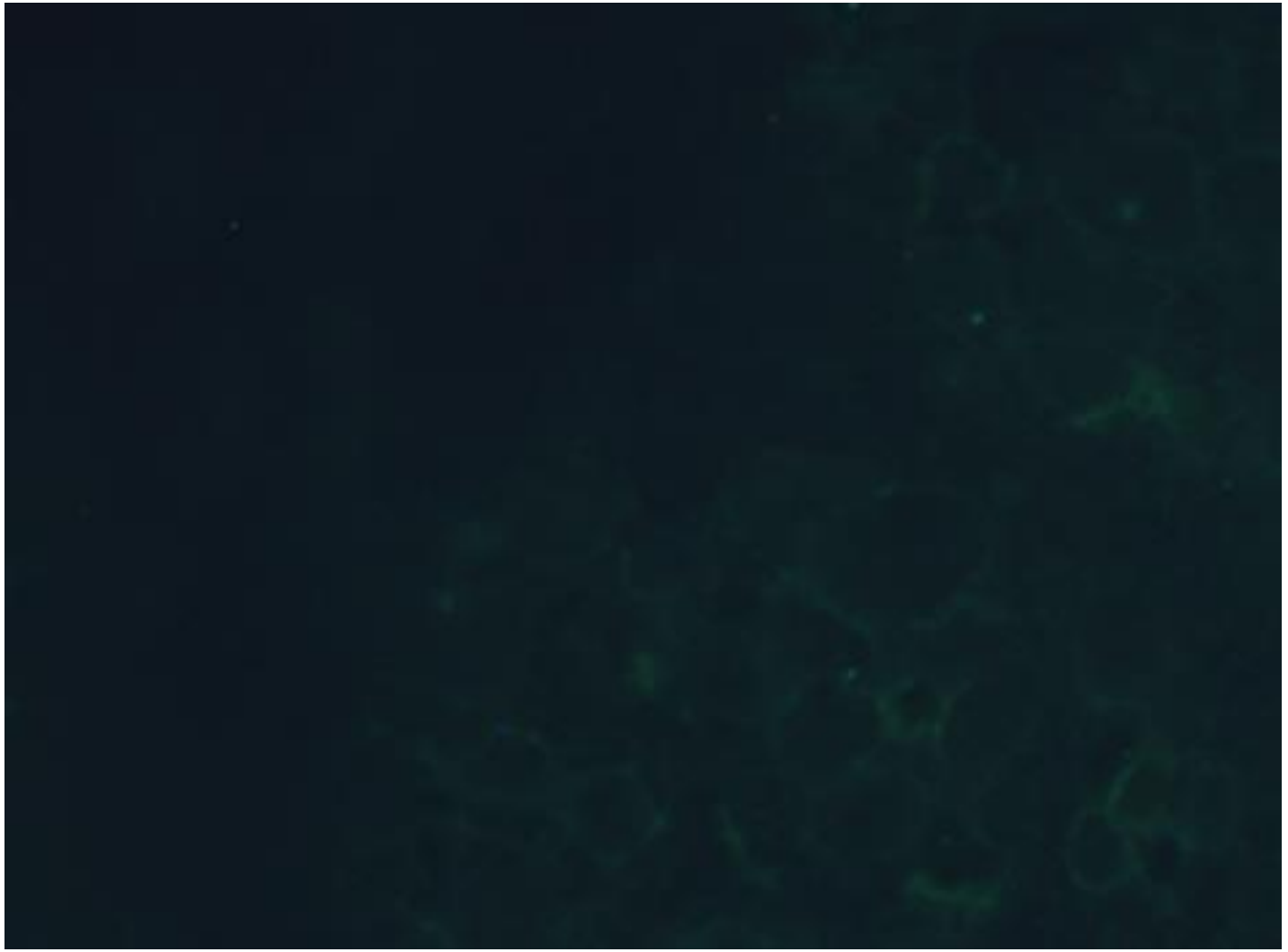
- AD è coinvolto nell'aggregazione degli Ach-R attraverso la proteina Agrina della lamina basale
- AD è coinvolto nella migrazione neuronale grazie al suo legame con la laminina e la neurexina nel cervello
- AD è il recettore extracellulare per il virus della choriomeningite linfocitaria e per molti arena virus

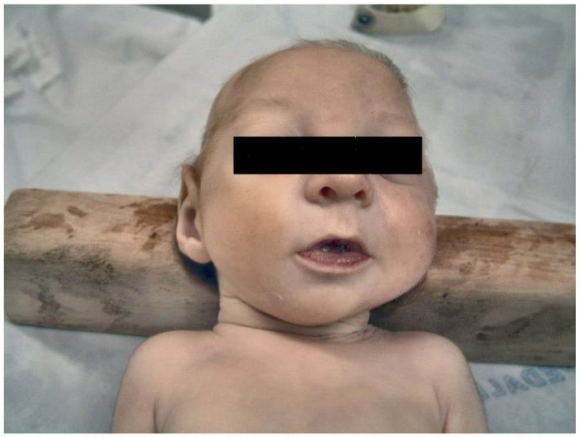
Distrofie congenite

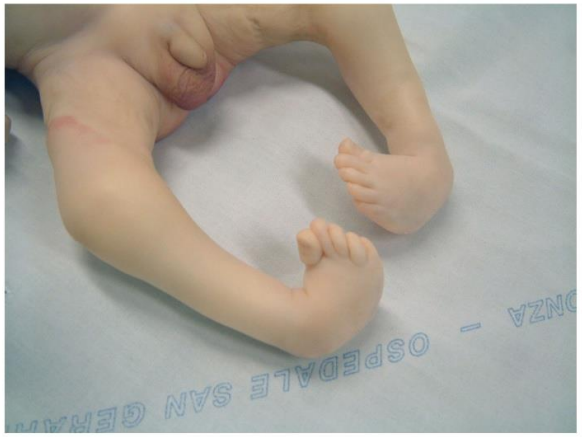
- Insorgenza nella prima infanzia
- Autosomiche recessive
- Spesso associate ad alterazioni maggiorni del sistemai nervoso centra con malformazioni .
- Spesso portano a morte nei primi anni di vita.
- Forme alleliche sono le distrofie dei cingoli dell'adulto
- Geni coinvolti: FKRP, Fuktin, POMT1-2, POGnT1-2, LARGE

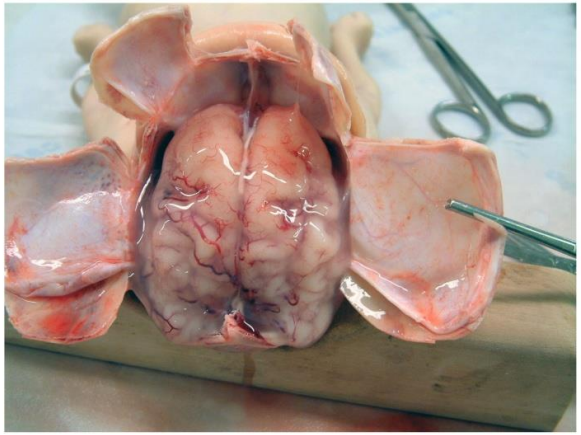


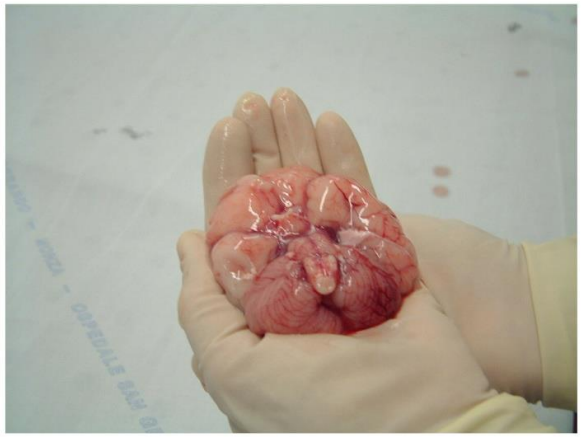


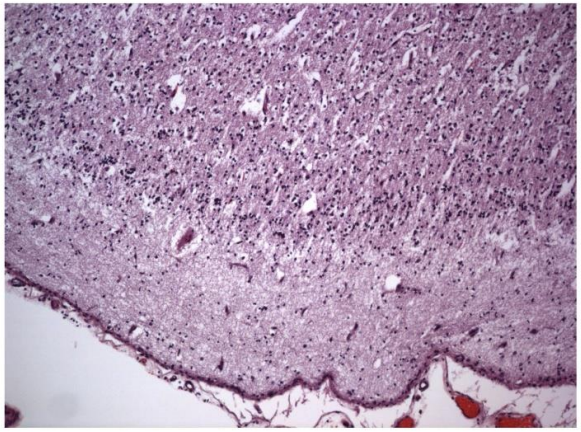


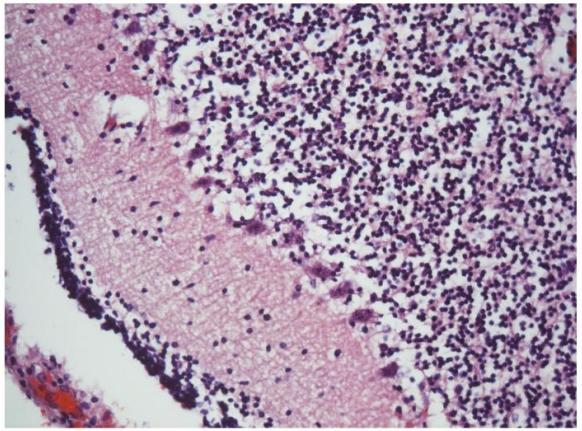


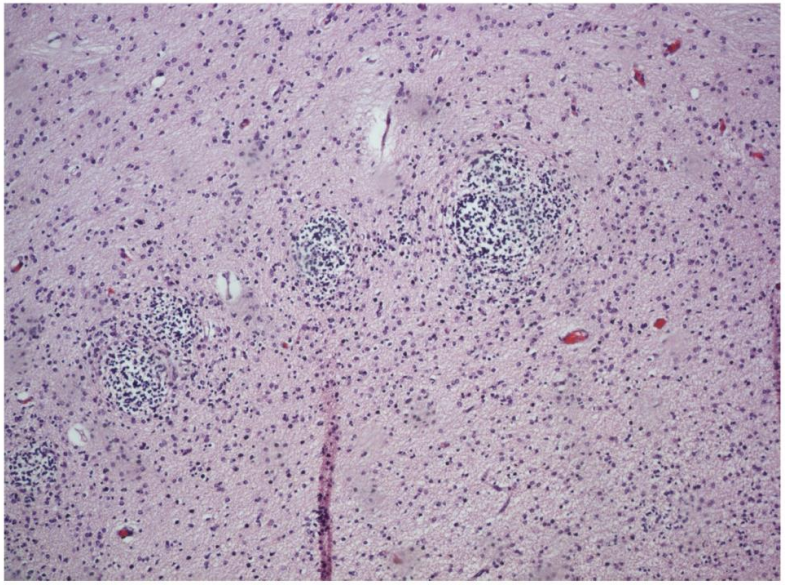


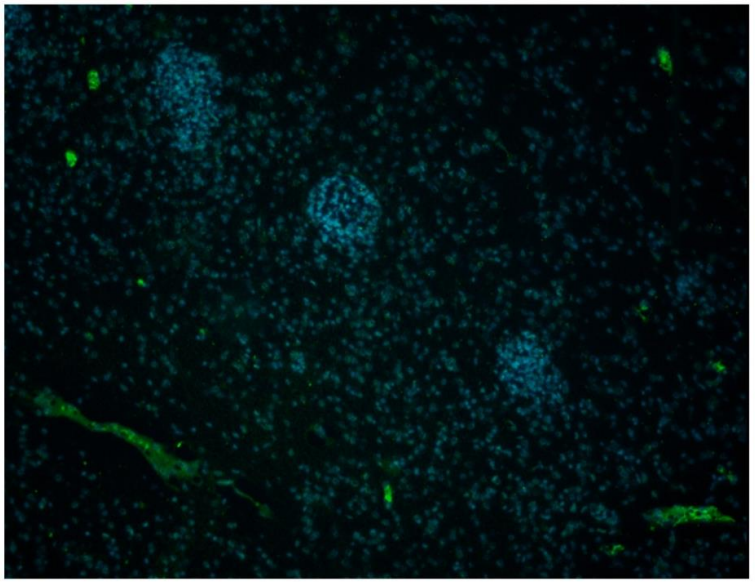


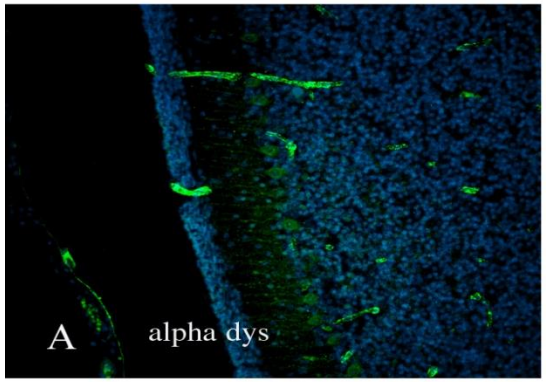


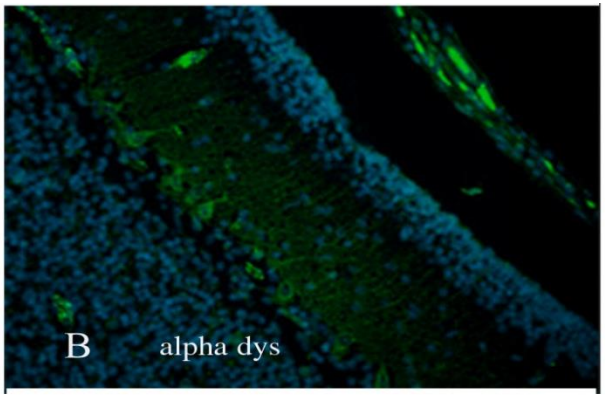


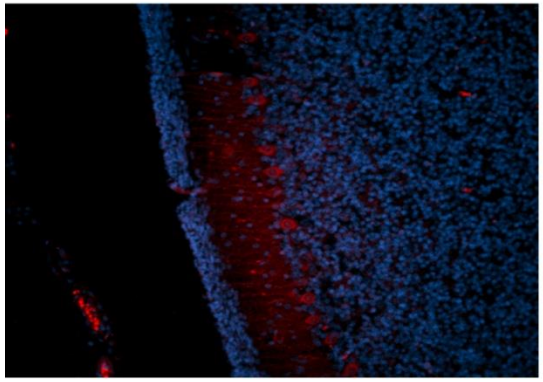


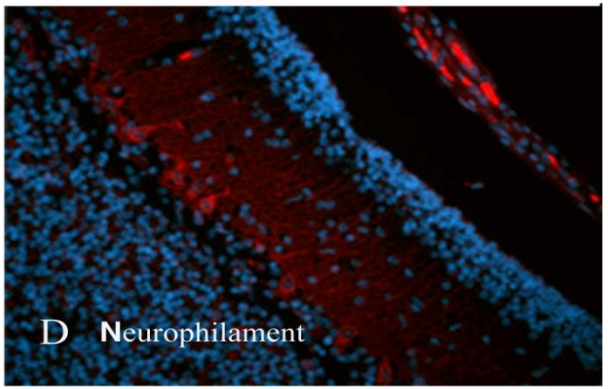


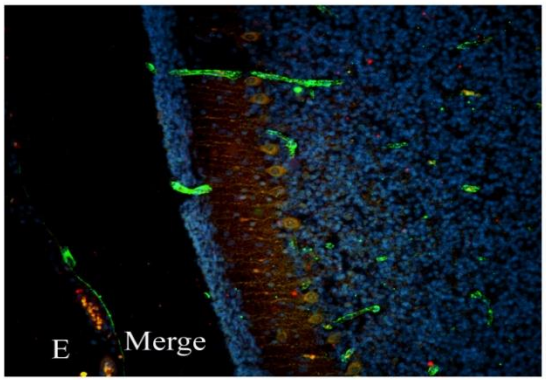


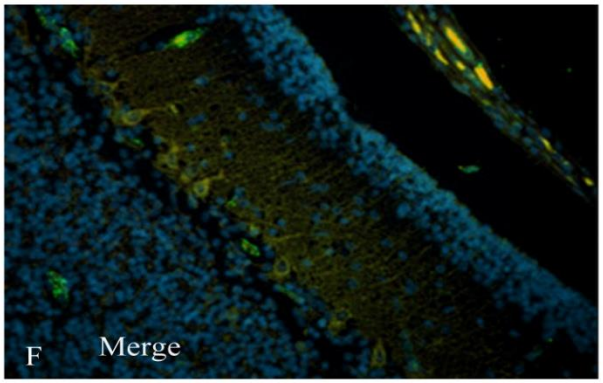












CTR **PZ**

Malattie Muscolari - Nosografia

Geneticamente determinate (AD, AR, X-linked, Matrilineari) / Acquisite

Distrofie: Distrofinopatie di Duchenne e Becker, congenite, dei cingoli, distrofia Facio Scapolo Omerale, Emery-Dreifuss.

Miopatie congenite / Miopatie miofibrillari.

Miopatie metaboliche: Miopatie mitocondriali, da accumulo lipidico o glucidico, miopatie lisosomiali.

Canalopatie: Malattia di Steinert (DM1, DM2)

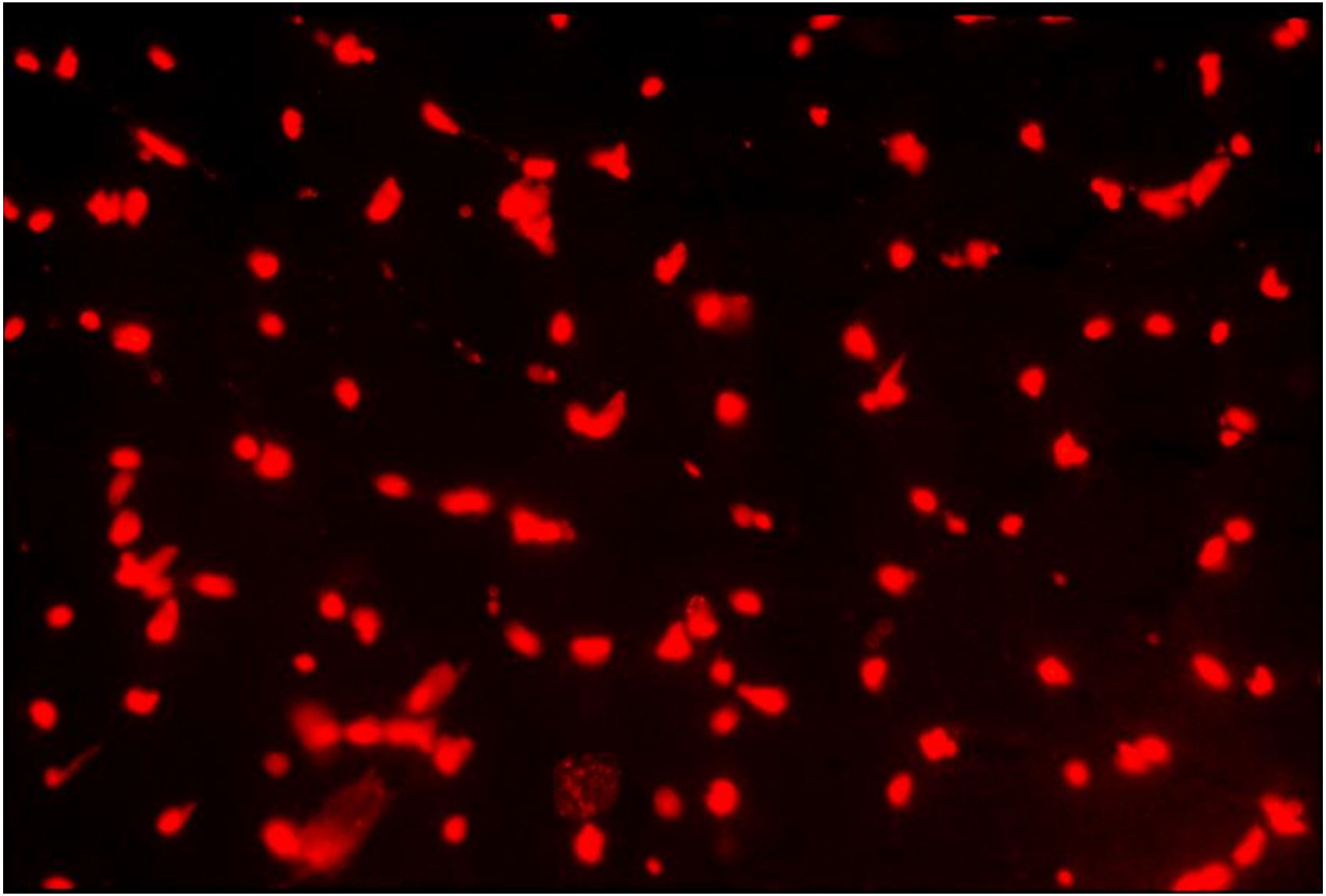
Infiammatorie: Polimiositi, dermatomiositi, miopatie a corpi inclusi

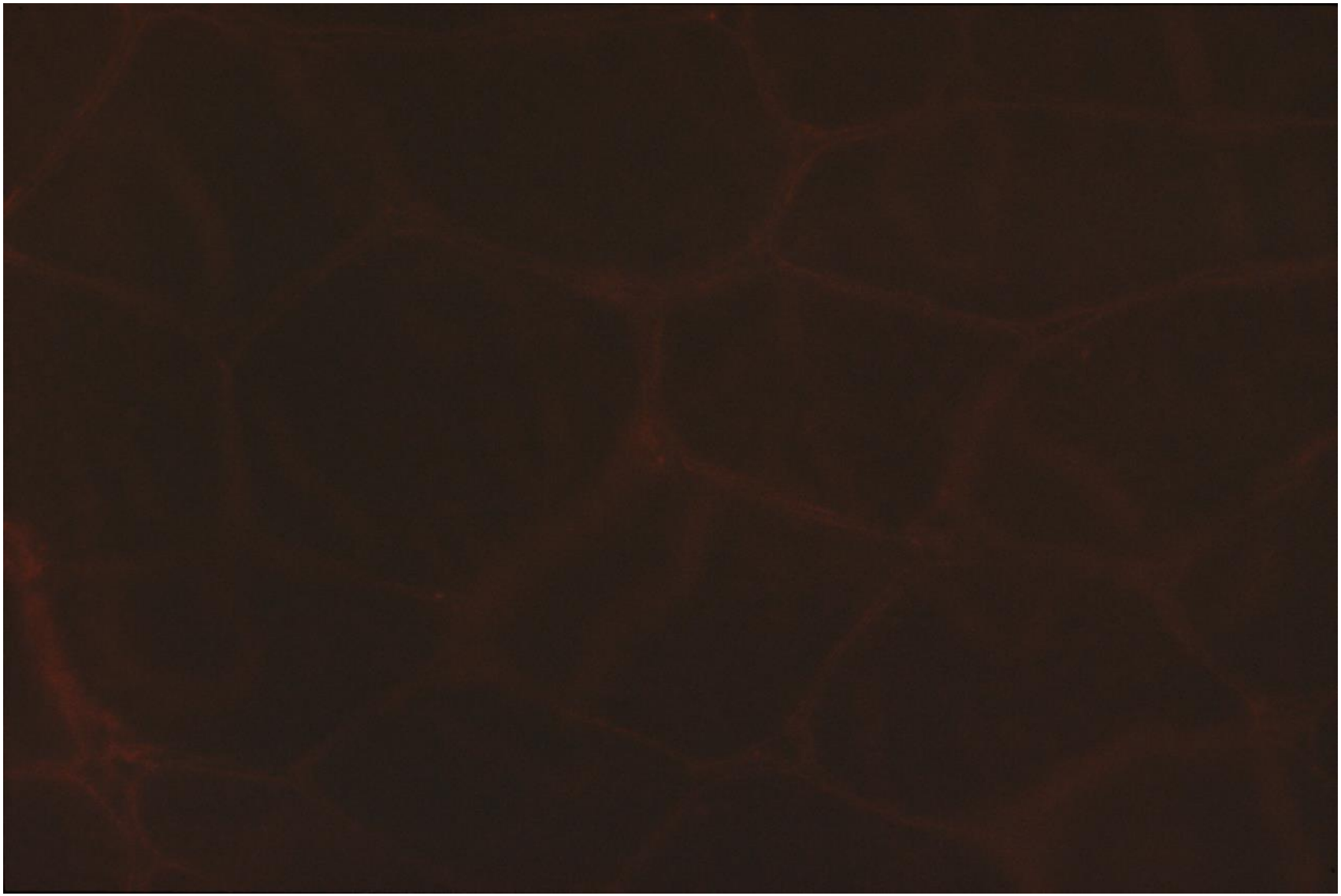
Iatrogene, tossiche.

Alterazioni della giunzione neuromuscolare: miastenia, Eaton Lambert.

Emery - Dreifuss

- Insorgenza nella prima infanzia
- X- linked
- Precoce ipostenia con distribuzione omero peroneale
- Precoci contratture/anchilosi gomiti, retrazione t. Achille, m. cervicali
- Interessamento cardiaco (blocco di branca)





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Canalopatie: Malattia di Steinert (DM1, DM2)

Infiammatorie: Polimiositi, dermatomiositi, miopatie a corpi inclusi

Iatrogene, tossiche.

Alterazioni della giunzione neuromuscolare: miastenia, Eaton Lambert.

Distrofie dei cingoli

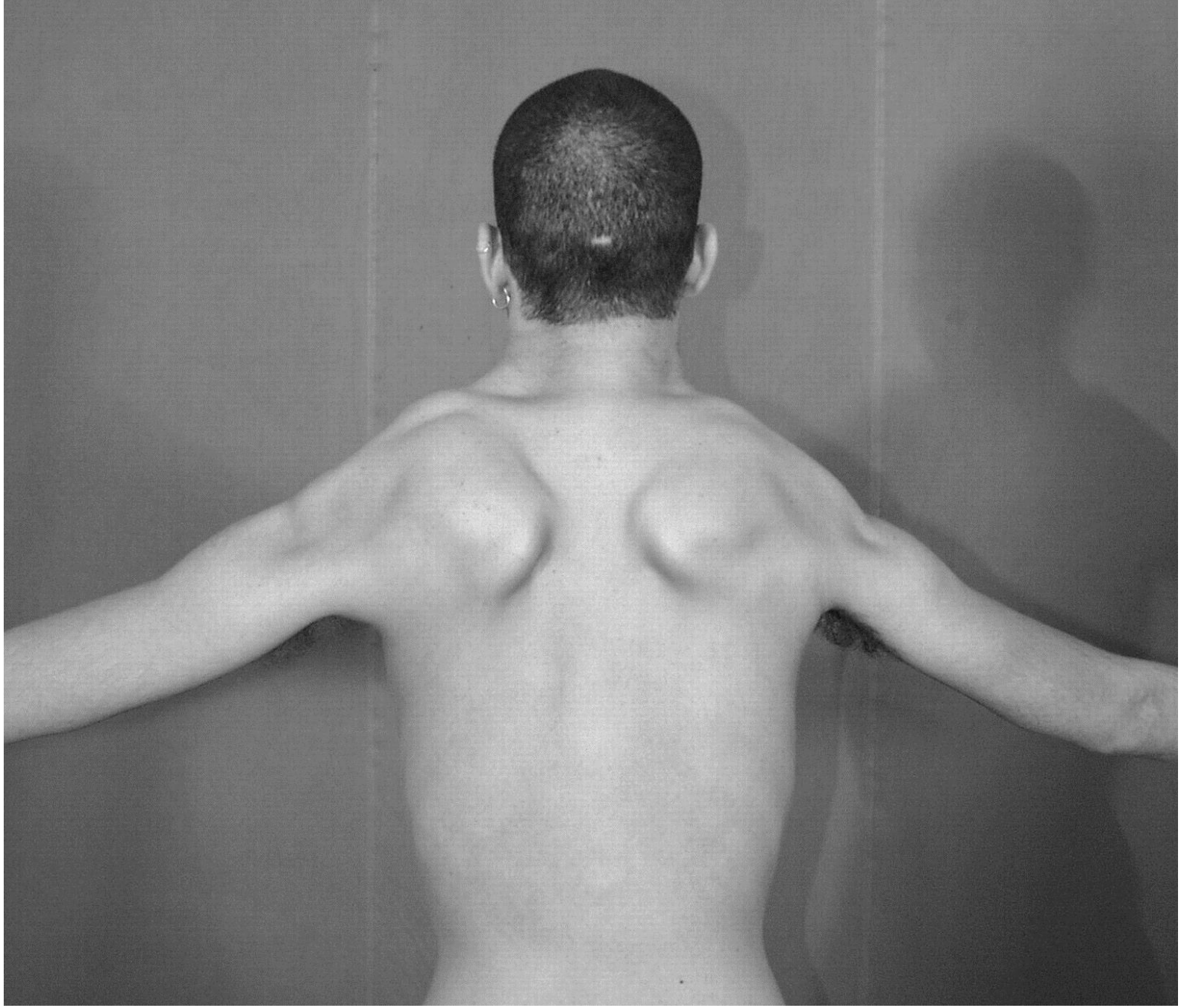
Distrofie muscolari dei cingoli - Limb-Girdle Muscular Dystrophies: (LGMD)

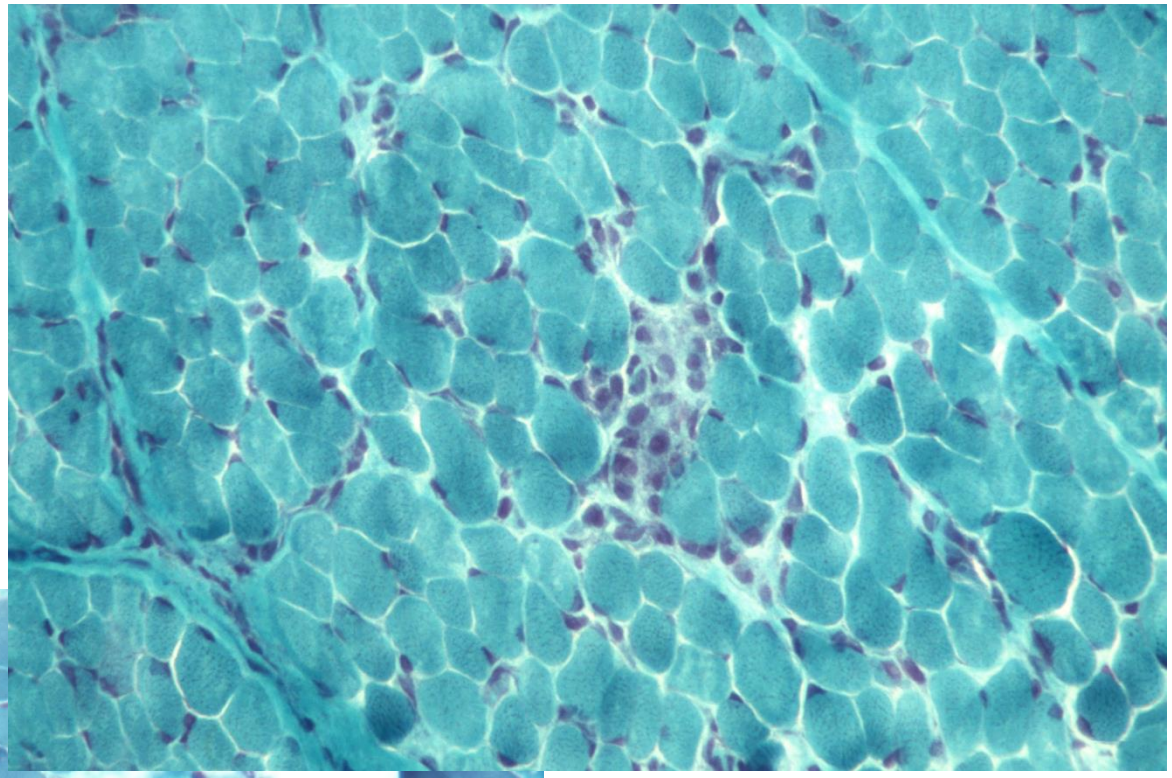
Ereditarie caratterizzate da progressiva debolezza ed atrofia muscolare a carico del cingolo scapolare e pelvico e della muscolatura prossimale degli arti.

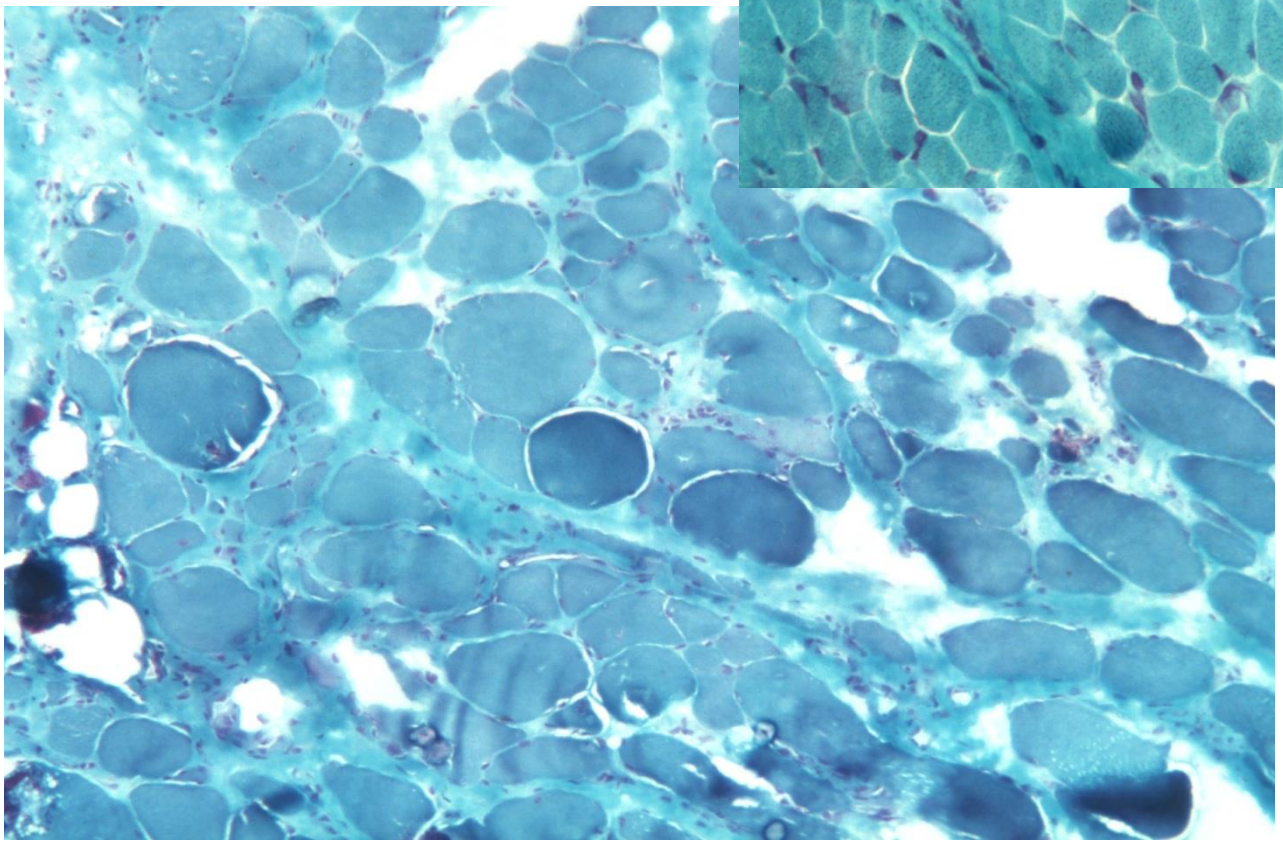
Elevato grado di eterogeneità per quanto attiene all'età di esordio, al pattern di interessamento muscolare, alla presenza di danno cardiaco, alla velocità di progressione e alle modalità di trasmissione.

Sulla base della modalità di trasmissione si distinguono:

- **forme autosomico dominanti (LGMD1)**
- **forme autosomico recessive (LGMD2)**







Distrofie dei cingoli - LGMD

	Gene	Locus	Proteina
LGMD 1A	*MYOT*	5q31	Miotilina
LGMD 1B	*LMNA*	1q21.2	Lamina A/C
LGMD 1C	*CAV3*	3p25	Caveolina-3
LGMD 1D	*?*	?	?
LGMD 1E	*DNAJB6*	7q36	DNAJB6
LGMD 1F	*TNPO3*	7q32	Transportina-3
LGMD 1G	*?*	4p21	?
LGMD 1H	*?*	3p23-p25	?

	Gene	Locus	Proteina
LGMD 2A	*CAPN3*	15q15.1-q21.q	Calpaina-3
LGMD 2B	*DYSF*	2p13.3-13-1	Disferlina
LGMD 2C	*SGCG*	13q12	γ-sarcoglicano
LGMD 2D	*SGCA*	17q12-q21.33	α-sarcoglicano
LGMD 2E	*SGCB*	4q12	β-sarcoglicano
LGMD 2F	*SGCD*	5q33	δ-sarcoglicano
LGMD 2G	*TCAP*	17q12	Teletonina
LGMD 2H	*TRIM32*	9q31-9q34	TRIM32
LGMD 2I	*FKRP*	19q13.3	Fukutin Related Protein
LGMD 2J	*TTN*	2q14.3	Titina
LGMD 2K	*POMT1*	9q34.1	O-Mannosiltransferasi-1
LGMD 2L	*ANO5*	11p12-p13	Anoctamina-5
LGMD 2M	*FKTN*	9q31-q99	Fukutina
LGMD 2N	*POMT2*	14q24	O-Mannosiltransferasi-2
LGMD 2O	*POMGnT1*	1p34	POMGnT1
LGMD 2P	*DAG1*	3p21	DAG
LGMD 2Q	*PLEC1*	8q24.3	Plectina-1
LGMD 2R	*LAMA2*	6q22	Merosina
LGMD 2S	*ISPD*	7p21	ISPD

LGMD relazioni genotipo fenotipo

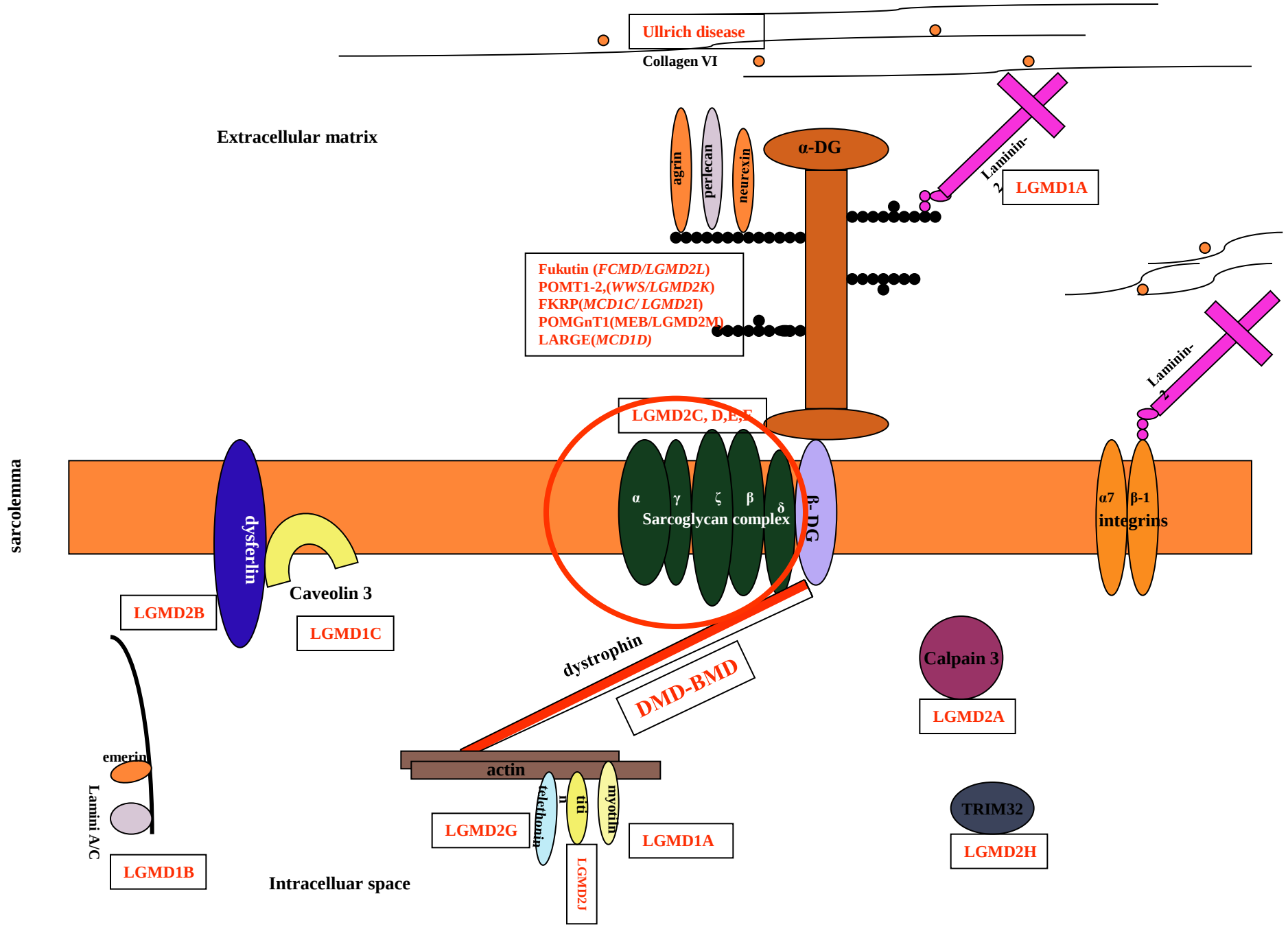
Age at onset	→	Earlier onset in LGMD2C-2E, dystroglycanopathies
CPK levels	→	High levels in LGMD2B, LGMD2I
Selective muscle involvement and MRI pattern	→	LGMD2I, LGMD2L, LGMD2N, LGMD2C-2F
Cardiac involvement	→	LGMD2E , LGMD2C, LGMD2F, LGMD2I, LGMD2M
Respiratory involvement	→	LGMD2A, LGMD2D, LGMD2M
Muscle biopsy analysis	→	LGMD1C, 2A, 2B, sarcoglycanopathies, dystroglycanopathies
Brain MRI	→	Merosin deficiency, dystroglycanopathies

Deficiency of a glycoprotein component of the dystrophin complex in dystrophic muscle

James M. Ervasti, Kay Ohlendieck, Steven D. Kahl, Mitchell G. Gaver & Kevin P. Campbell*

Howard Hughes Medical Institute and Department of Physiology and Biophysics, University of Iowa College of Medicine, Iowa City, Iowa 52242, USA

Dystrophin, the protein encoded by the Duchenne muscular dystrophy (DMD) gene, exists in a large oligomeric complex. We show here that four glycoproteins are integral components of the dystrophin complex and that the concentration of one of these is greatly reduced in DMD patients. Thus, the absence of dystrophin may lead to the loss of a dystrophin-associated glycoprotein, and the reduction in this glycoprotein may be one of the first stages of the molecular pathogenesis of muscular dystrophy.

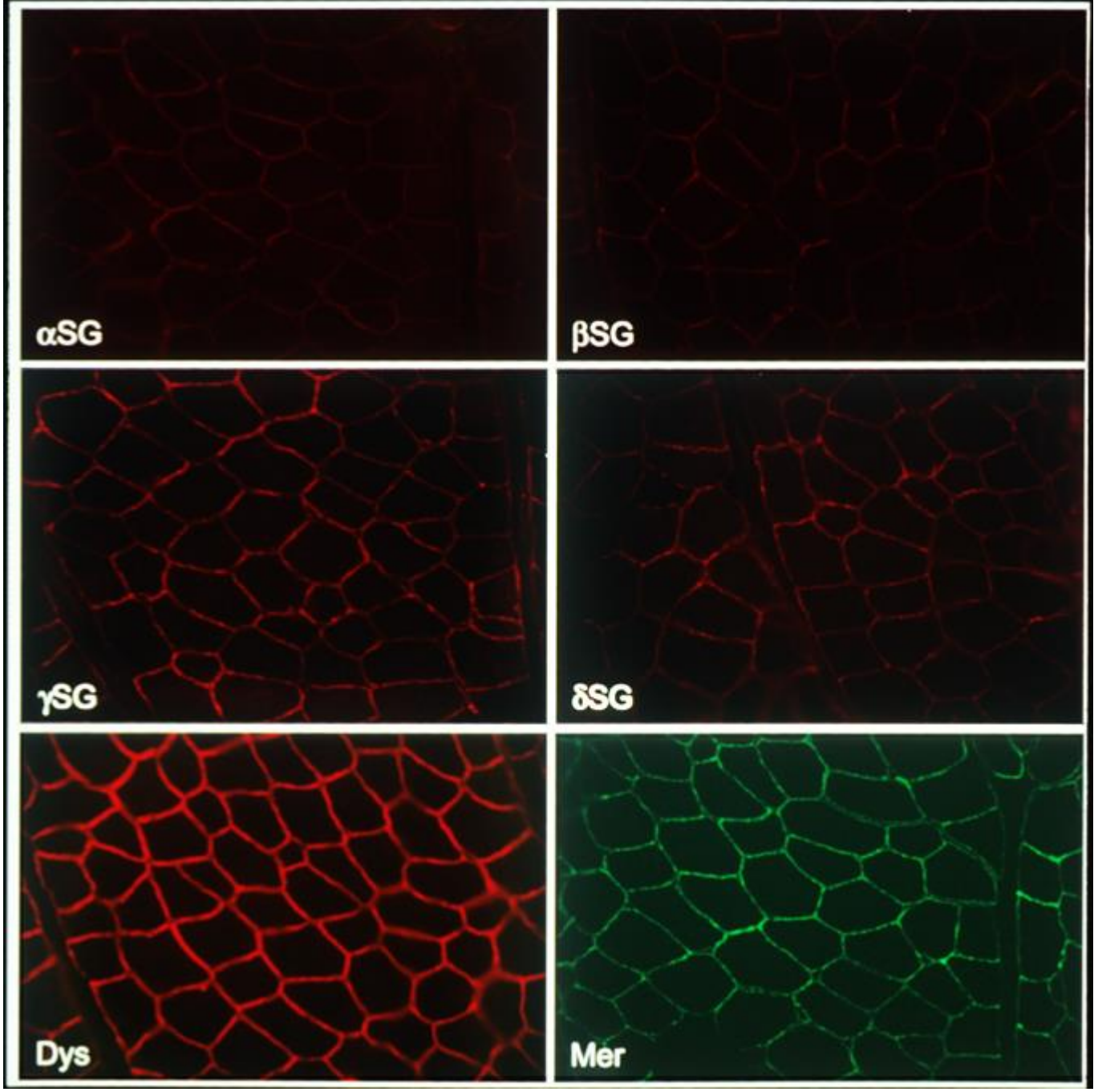

Ullrich disease
Collagen VI
Extracellular matrix
agrin
perlecan
neurexin
α-DG
Laminin-2
LGMD1A
Fukutin (FCMD/LGMD2L)
POMT1-2,(WWS/LGMD2K)
FKRP(MCD1C/ LGMD2I)
POMGnT1(MEB/LGMD2M)
LARGE(MCD1D)
LGMD2C, D,E,F
sarcolemma
dysferlin
Caveolin 3
LGMD2B
LGMD1C
α
γ
ζ
β
δ
Sarcoglycan complex
ß- DG
α7
β-1
integrins
Laminin-2
dystrophin
DMD-BMD
Calpain 3
LGMD2A
emerin
Lamini A/C
LGMD1B
actin
telethonin
titi
myotilin
LGMD2G
LGMD1A
LGMD2J
TRIM32
LGMD2H
Intracelluar space

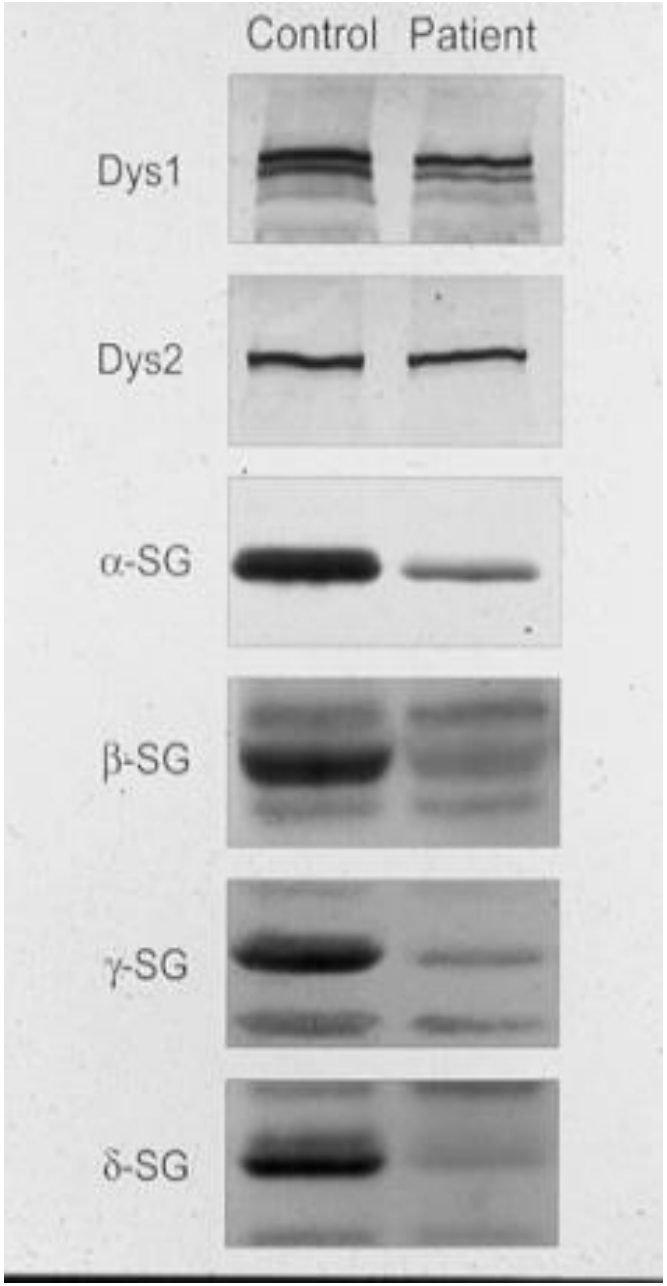
Sarcoglicanopatie LGMD 2C,D,E,F

- Ereditarietà autosomica recessiva
- Grande eterogeneità, da forme a-paucisintomatiche con CK elevate a forme con grave ipostenia cingolare e prossimale artuale. Esordio in età giovanile e in molti casi perdita deambulazione fra la terza e quinta decade di vita.
- Compromissione cardiaca
- CK elevate

Sarcolgycanolpathies

LGMD	Gene	Protein	Age at onset	Loss of ambulation in most cases	Cardiac involvement	Immunoistochemestry
LGMD2C	13q21	γ- SG	I-III decade	II-III decade	common	γ↓ β↓ δ↓ α ↓ dys↓
LGMD2D	17q21	α-SG	I-III decade	II-V decade	rare	γ↓ β↓ δ↓ α ↓ dys↓
LGMD2E	4q21	β-SG	I-III decade	II-IV decade	common	γ↓ β↓ δ↓ α↓ dys↓
LGMD2F	5q33	δ-SG	I decade	II decade	common	γ↓ β↓ δ↓ α↓ dys↓


αSG
βSG
γSG
δSG
Dys
Mer


Control
Patient
Dys1
Dys2
α-SG
β-SG
γ-SG
δ-SG

Mutations in the caveolin-3 gene cause autosomal dominant limb-girdle muscular dystrophy

Carlo Minetti[1], Federica Sotgia[1,2], Claudio Bruno[1,3], Paolo Scartezzini[4], Paolo Broda[1], Massimo Bado[1], Emiliana Masetti[1], Michela Mazzocco[4], Aliana Egeo[4], Maria Alice Donati[5], Daniela Volonté[6], Ferruccio Galbiati[6], Giuseppe Cordone[1], Franca Dagna Bricarelli[2], Michael P. Lisanti[6] & Federico Zara[2]

Limb-girdle muscular dystrophy (LGMD) is a clinically and genetically heterogeneous group of myopathies, including autosomal dominant and recessive forms[1–3]. To date, two autosomal dominant forms have been recognized[2,3]: LGMD1A, linked to chromosome 5q, and LGMD1B, associated with cardiac defects and linked to chromosome 1q11–21. Here we describe eight patients from two different families with a new form of autosomal dominant LGMD, which we propose to call LGMD1C, associated with a severe deficiency of caveolin-3 in muscle fibres. Caveolin-3 (or M-caveolin) is the muscle-specific form of the caveolin protein family, which also includes caveolin-1 and -2 (refs 4–9). Caveolins are the principal protein components of caveolae (50–100 nm invaginations found in most cell types) which represent appendages or sub-compartments of plasma membranes[10,11]. We localized the human caveolin-3 gene (*CAV3*) to chromosome 3p25 and identified two mutations in the gene: a missense mutation in the membrane-spanning region and a micro-deletion in the scaffolding domain. These mutations may interfere with caveolin-3 oligomerization and disrupt caveolae formation at the muscle cell plasma membrane.

Caveolins are thought to play an important role in the formation of caveolae membranes, acting as scaffolding proteins to organize and concentrate specific caveolin-interacting lipids and proteins within caveolae microdomains[12,13]. Immunostaining of caveolin-3 in skeletal-muscle fibres revealed that it is localized to the sarcolemma, coinciding with the localization of dystrophin, the protein product of the Duchenne muscular dystrophy (*DMD*) gene[14,15]. Subcellular fractionation studies revealed that caveolin-3 cofractionates with members of the dystrophin-associated protein complex[14,16]. Caveolin-3 is immunoprecipitated by antibodies directed against dystrophin, suggesting that both proteins are part of a discrete complex[14].

We examined the expression of caveolin-3 in muscle biopsies from 44 patients with dystrophinopathies, 12 patients with sarcoglycanopathies, 137 patients with LGMDs (including 12 patients with a dominant form) and 12 normal controls. Caveolin-3 was detected at the sarcolemma of normal human muscle fibres; the cell surface of each muscle fibre showed a thin and continuous layer of immunofluorescence, and there was no immunostaining of intracellular components (Fig. 1*a*). In muscle biopsies of eight patients from two different families with a dominant form of LGMD, the intensity of the staining of caveolin-3 at the cell surface was reduced (Fig. 1*b*). This reduction in caveolin-3 levels in patients was confirmed by western-blot analysis (Fig. 1*c*). Quantitative analysis by immunohistochemistry and immunoblotting showed that caveolin-3 expression in patients was reduced by 92% and 95%, respectively, compared with normal controls. In contrast, other membrane proteins involved in different forms of muscular dystrophies (dystrophin, adhalin, β-, γ- and δ-sarcoglycan and merosin[1,15–17]) were detected at normal levels in the muscle biopsies of these patients (Fig. 2). Muscle biopsies from patients with different forms of muscular dystrophy showed normal caveolin-3 staining pattern. Caveolin-1 and caveolin-2 were not detected in muscle from patients or controls (data not shown).

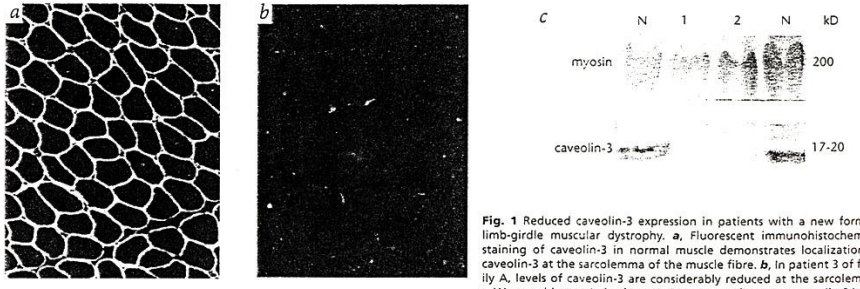


Fig. 1 Reduced caveolin-3 expression in patients with a new form of limb-girdle muscular dystrophy. ***a***, Fluorescent immunohistochemical staining of caveolin-3 in normal muscle demonstrates localization of caveolin-3 at the sarcolemma of the muscle fibre. ***b***, In patient 3 of family A, levels of caveolin-3 are considerably reduced at the sarcolemma. ***c***, Western-blot analysis of muscle biopsies confirms that caveolin-3 levels are reduced in patient 3 of family A (lane 1) and in patient 3 of family B (lane 2), compared with controls (N). Myosin was stained with Ponceau S as an internal control; the amount of myosin does not differ significantly between control and patient samples.

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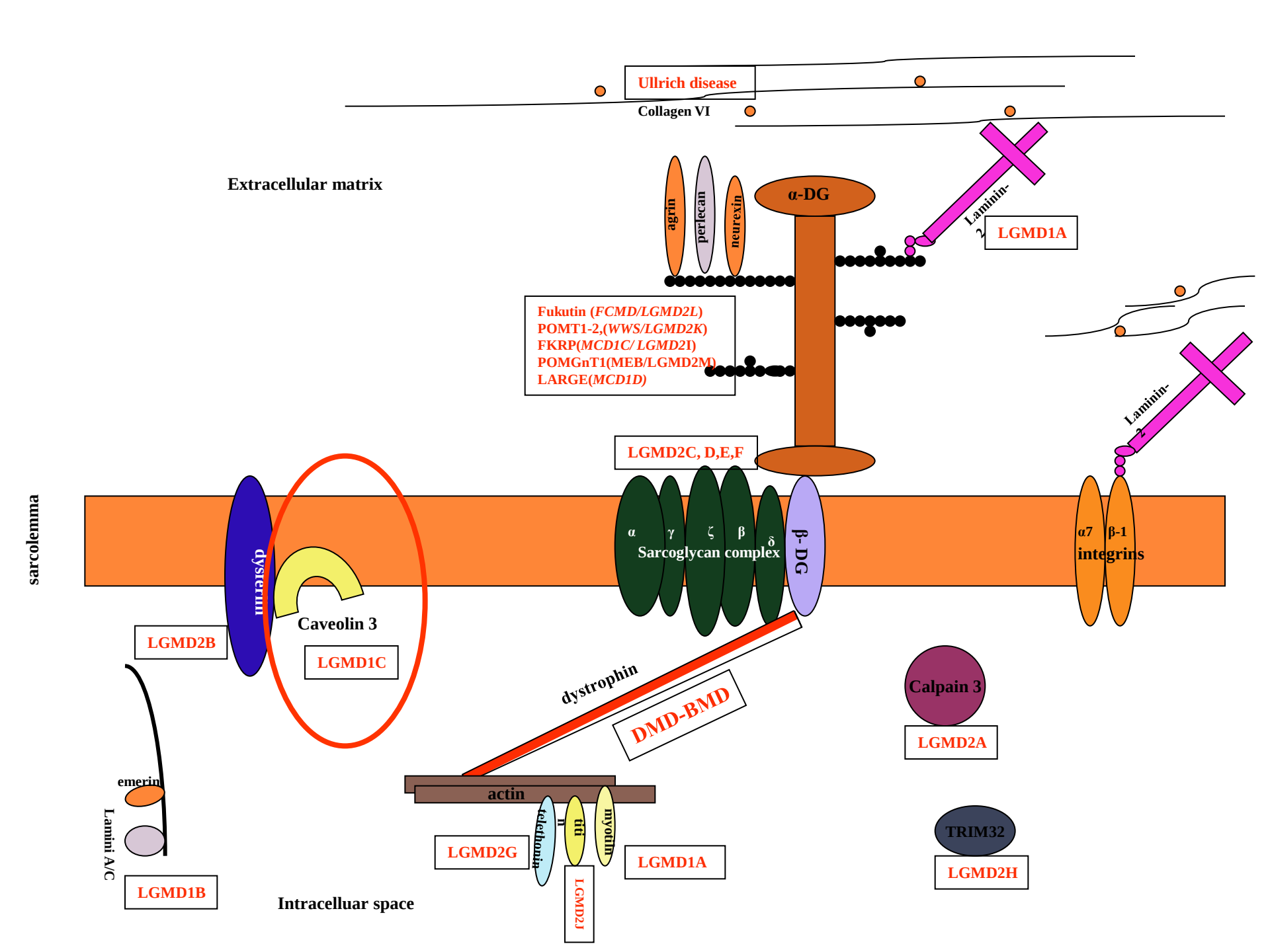
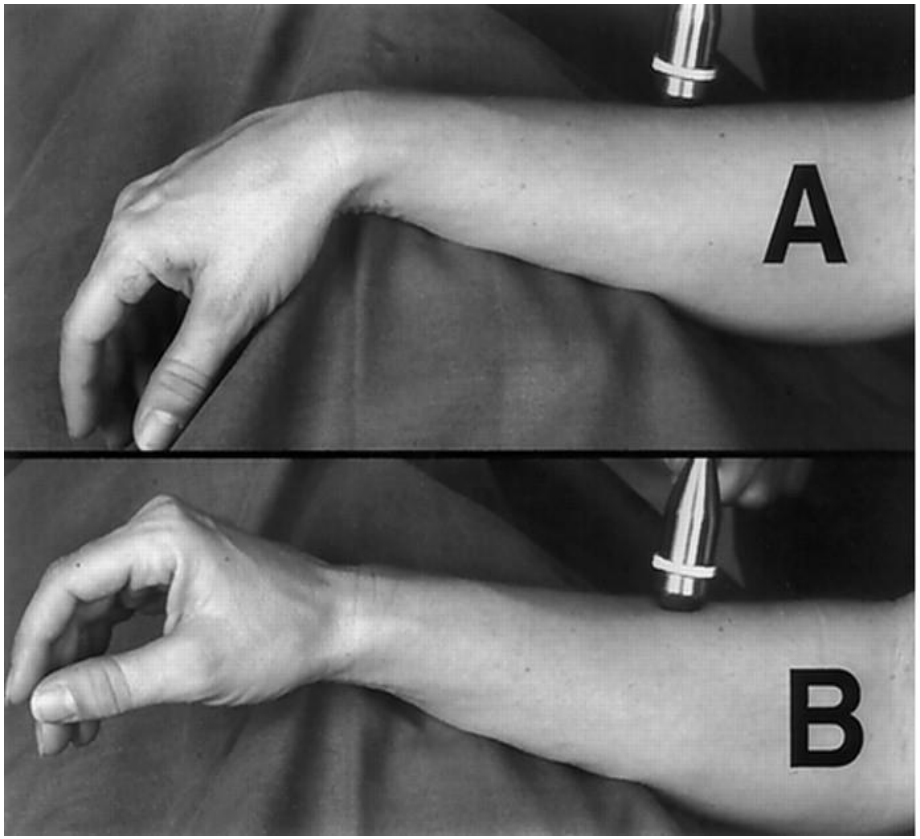
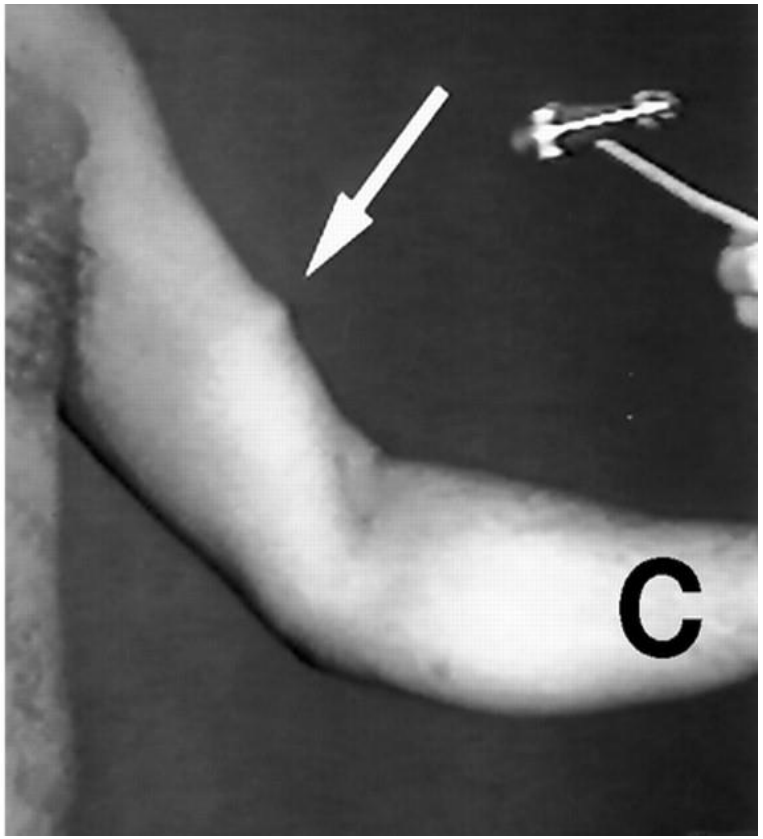

Ullrich disease
Collagen VI
Extracellular matrix
agrin
perlecan
neurexin
α-DG
Laminin-2
LGMD1A
Fukutin (FCMD/LGMD2L)
POMT1-2,(WWS/LGMD2K)
FKRP(MCD1C/ LGMD2I)
POMGnT1(MEB/LGMD2M)
LARGE(MCD1D)
LGMD2C, D,E,F
Laminin-2
sarcolemma
dysferlin
Caveolin 3
LGMD2B
LGMD1C
α
γ
ζ
β
δ
Sarcoglycan complex
β- DG
α7
β-1
integrins
dystrophin
DMD-BMD
Calpain 3
LGMD2A
emerin
Lamini A/C
LGMD1B
actin
telethonin
titi
myotilin
LGMD2G
LGMD1A
LGMD2J
TRIM32
LGMD2H
Intracelluar space

Caveolinopatie LGMD 1C

- Ereditarietà autosomica dominante
- Ipertrofia dei polpacci
- Può essere presente rippling o pseudomiotonia
- Miopatia distale
- Raramente vi è un coinvolgimento cardiaco
- CK poco aumentate – molto aumentate


A
B


C

A *CAV3* microdeletion differentially affects skeletal muscle and myocardium

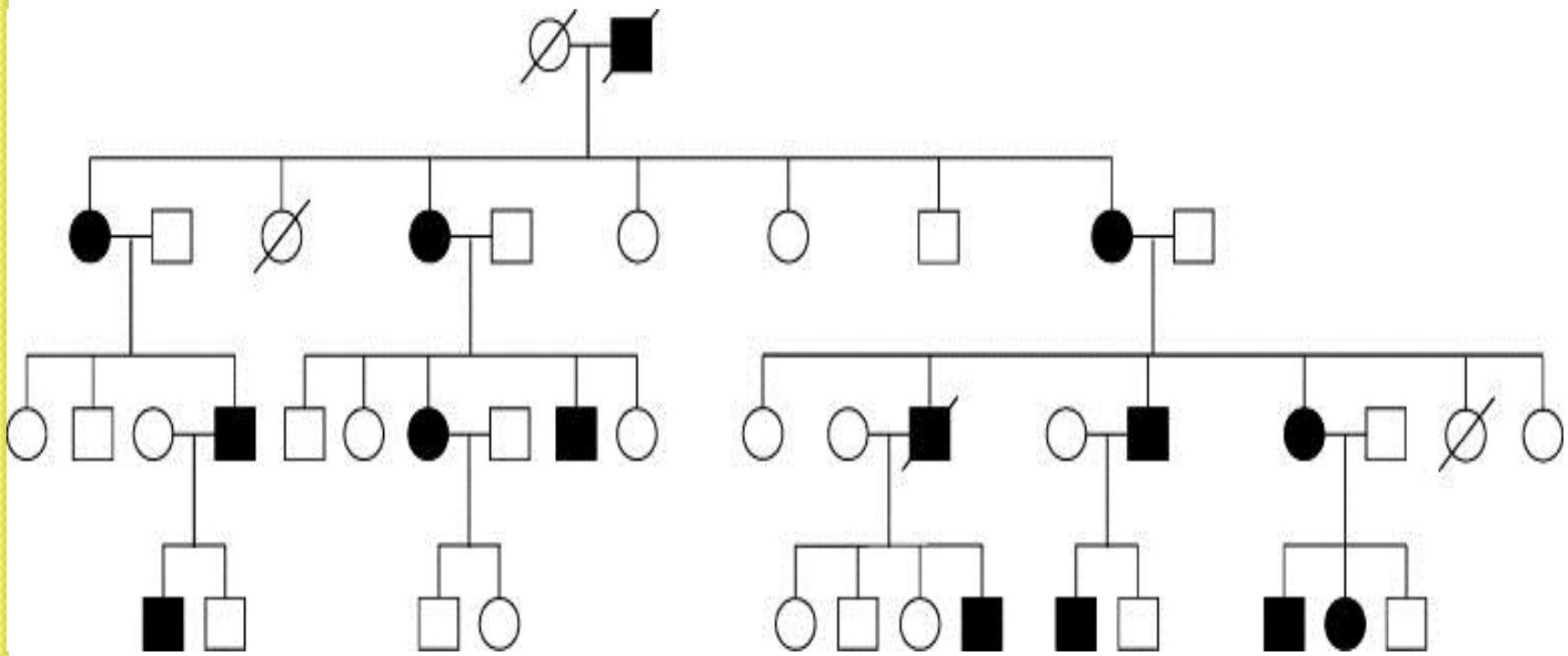
R. Cagliani, PhD; N. Bresolin, MD; A. Prelle, MD; A. Gallanti, MD; F. Fortunato, BS; M. Sironi, PhD; P. Ciscato, BS; G. Fagiolari, PhD; S. Bonato, MD; S. Galbiati, MD; S. Corti, MD; C. Lamperti, MD; M. Moggio, MD; and G.P. Comi, MD

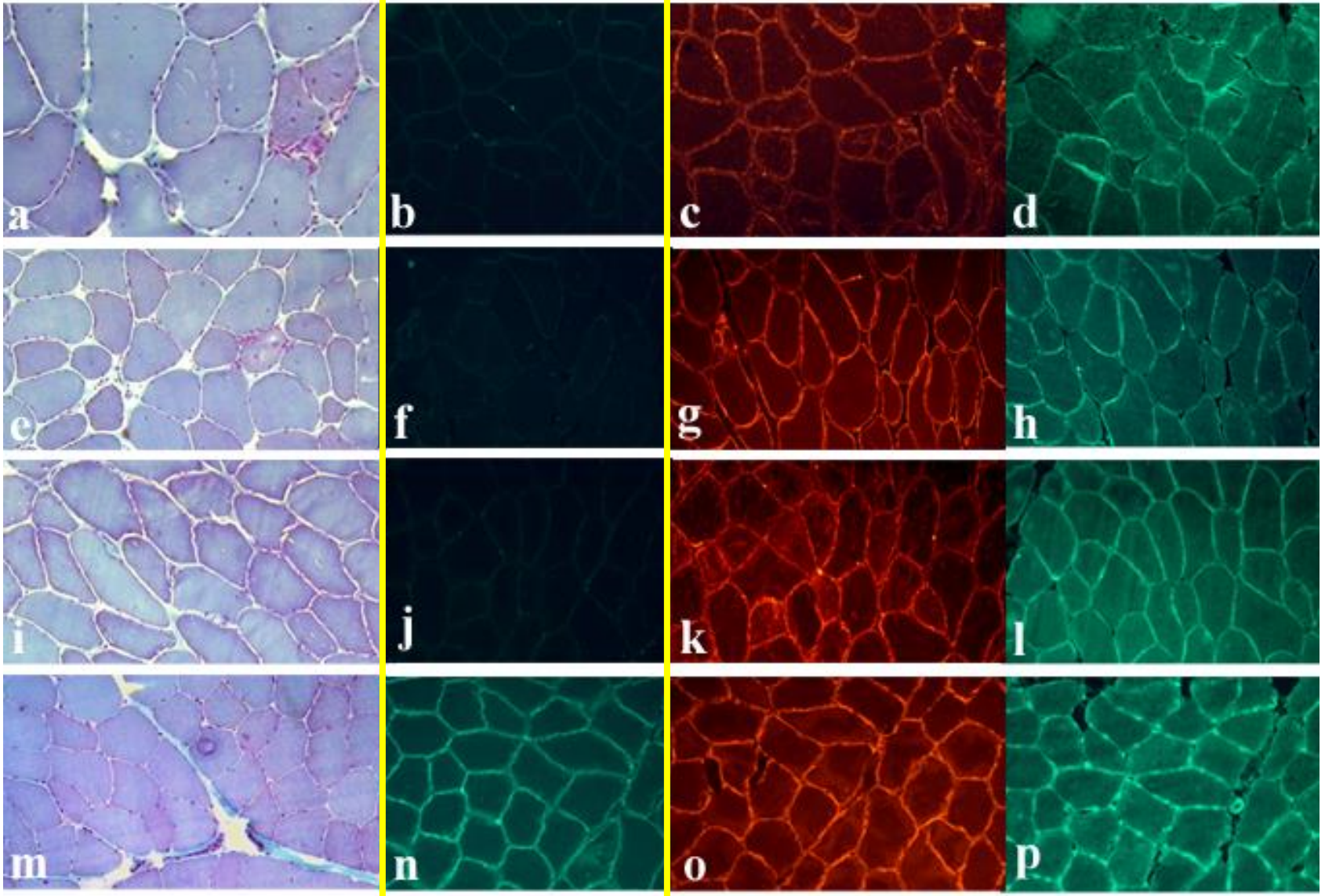
Abstract—*Background:* Caveolin-3 is the muscle-specific protein product of the caveolin gene family and an integral membrane component of caveolae. Mutations in the gene encoding caveolin-3 (*CAV3*) underlie four distinct disorders of skeletal muscle: the autosomal dominant form of limb–girdle muscular dystrophy type 1C (LGMD-1C), rippling muscle disease (RMD), sporadic and familial forms of hyperCKemia, and distal myopathy. *Objective:* To characterize a multigenerational Italian family affected by an autosomal dominant myopathic disorder and to assess the expression of caveolin-3, dystrophin, dystrophin-associated glycoproteins, and neuronal nitric oxide synthase in the myocardium of an affected patient. *Methods:* Clinical analysis involved 15 family members. Skeletal muscle expression of sarcolemmal proteins was evaluated by immunohistochemistry and western blot analysis in three affected individuals. Caveolar structures were analyzed through electron microscopy in muscle biopsies and in one heart biopsy. *Results: CAV3* genetic analysis showed a heterozygous 3-bp microdeletion (328–330del) in affected individuals, resulting in the loss of a phenylalanine (Phe97del) in the transmembrane domain. In the skeletal muscle, the mutation was associated with severe caveolin-3 deficiency and caveolar disorganization, whereas the expression of the other analyzed muscle proteins was unaltered. Remarkably, caveolin-3 was expressed in myocardium at a level corresponding to about 60% of that of control individuals and was correctly localized at the myocardial cell membranes, with preservation of cardiac myofiber caveolar structures. Clinical analysis revealed the concomitant presence in this family of the following phenotypes: RMD, LGMD, and hyperCKemia. *Conclusions:* Intrafamilial phenotypic heterogeneity is associated with caveolin-3 Phe97 microdeletion. The molecular network interacting with caveolin-3 in skeletal muscle and heart may differ.

NEUROLOGY 2003;61:1513–1519

Varibilità clinica intrafamiliare

Differenti fenotipi clinici sono associati con la stessa mutazione di CAV3 nella stessa famiglia




a
b
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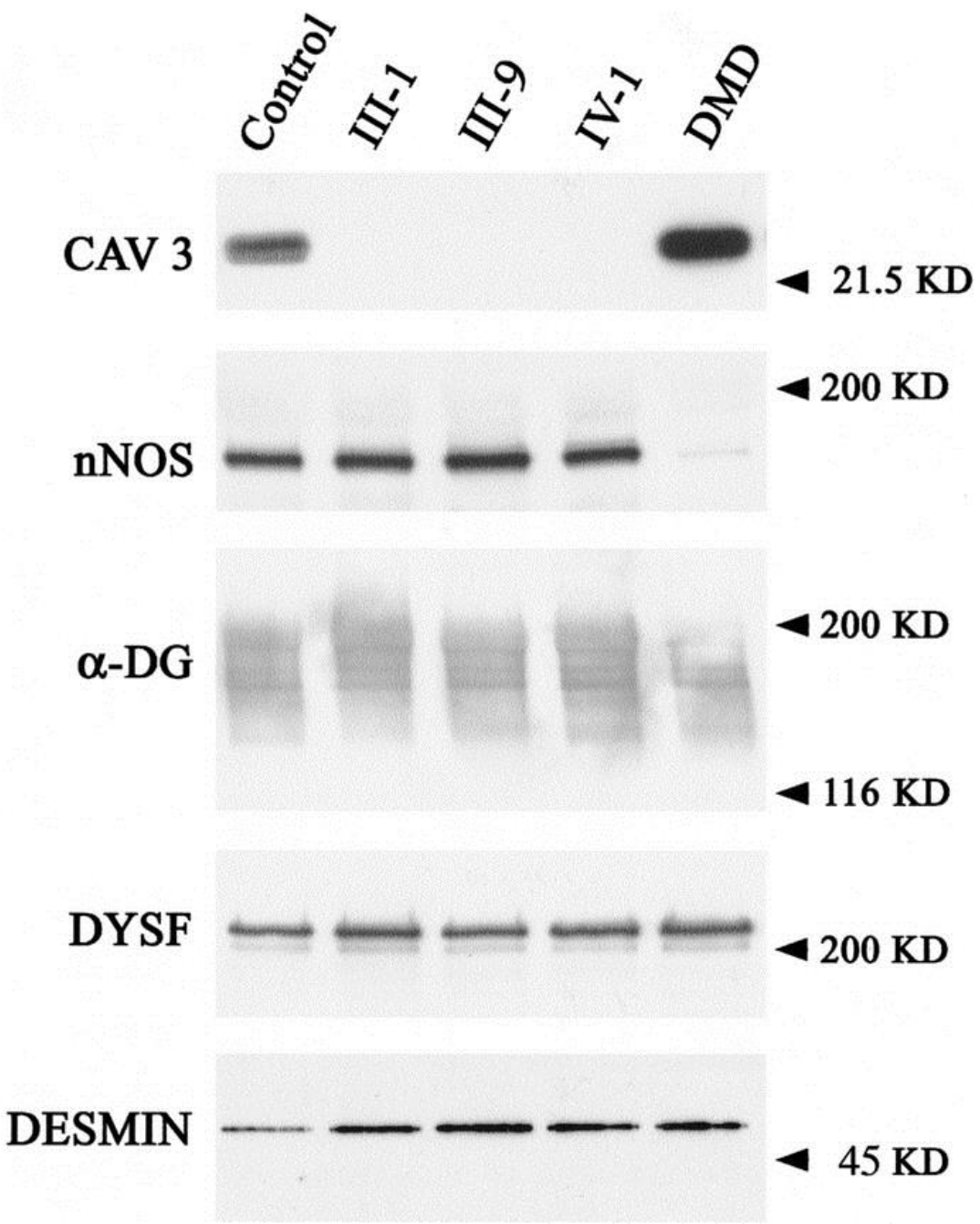
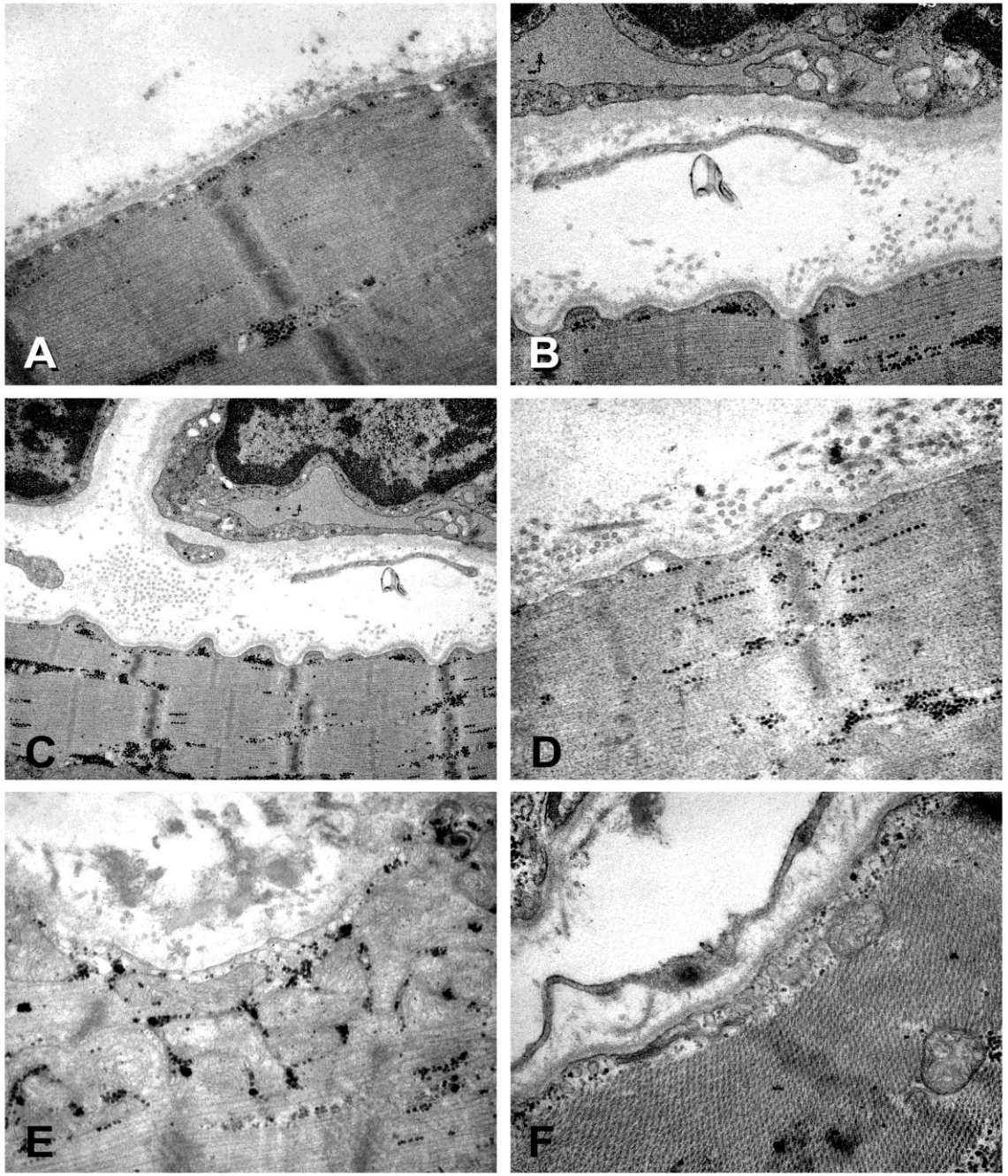
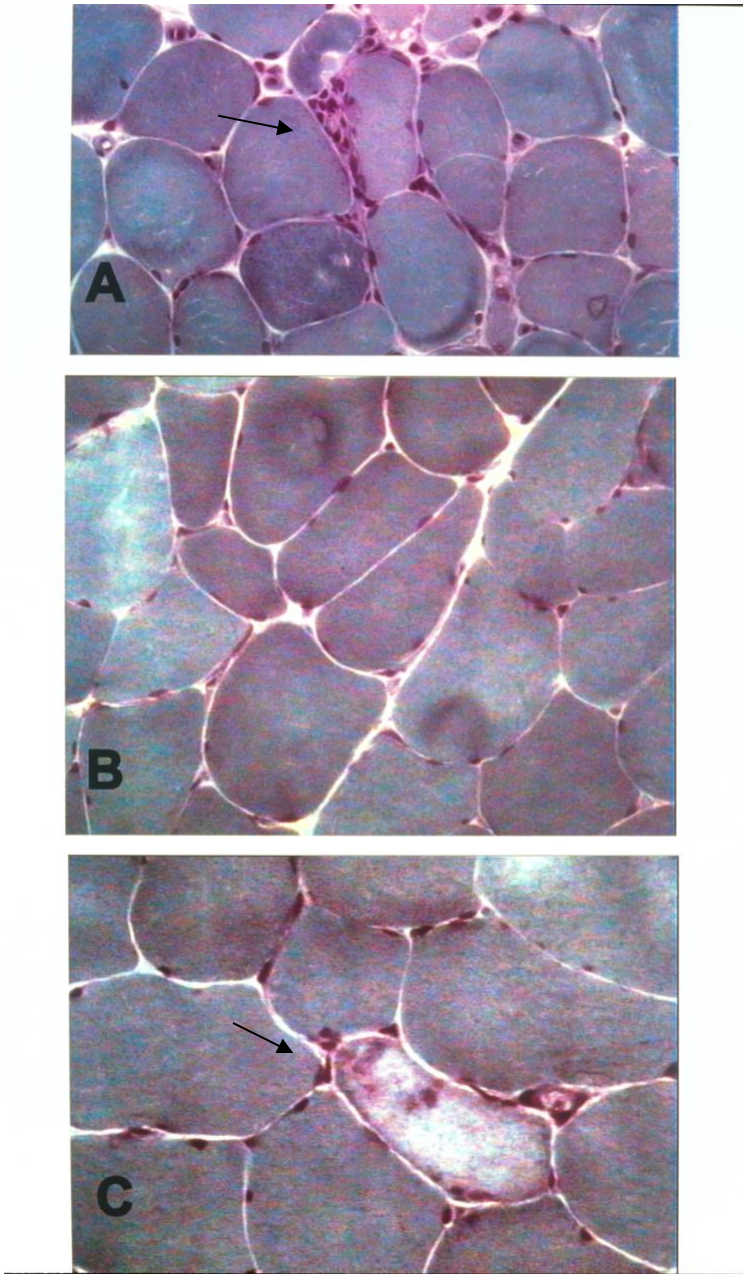
p

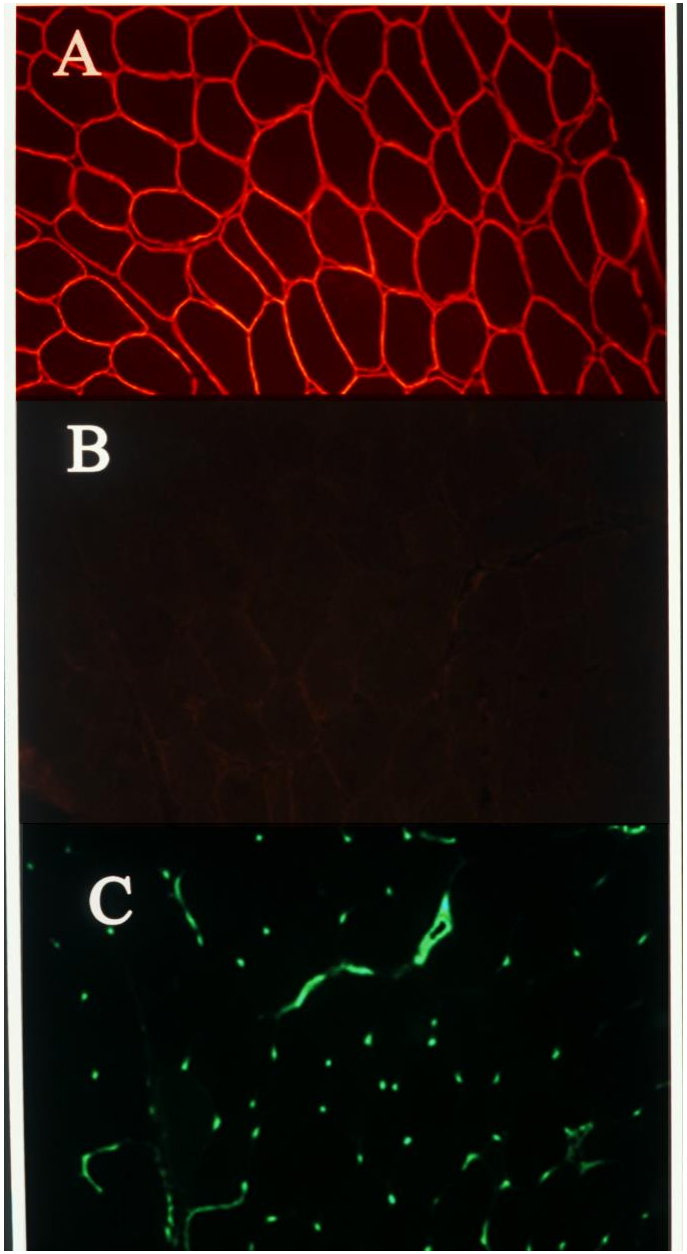

Control
III-1
III-9
IV-1
DMD
CAV 3
21.5 KD
nNOS
200 KD
α-DG
200 KD
116 KD
DYSF
200 KD
DESMIN
45 KD


A
B
C
D
E
F

Disferlinopatie LGMD 2B

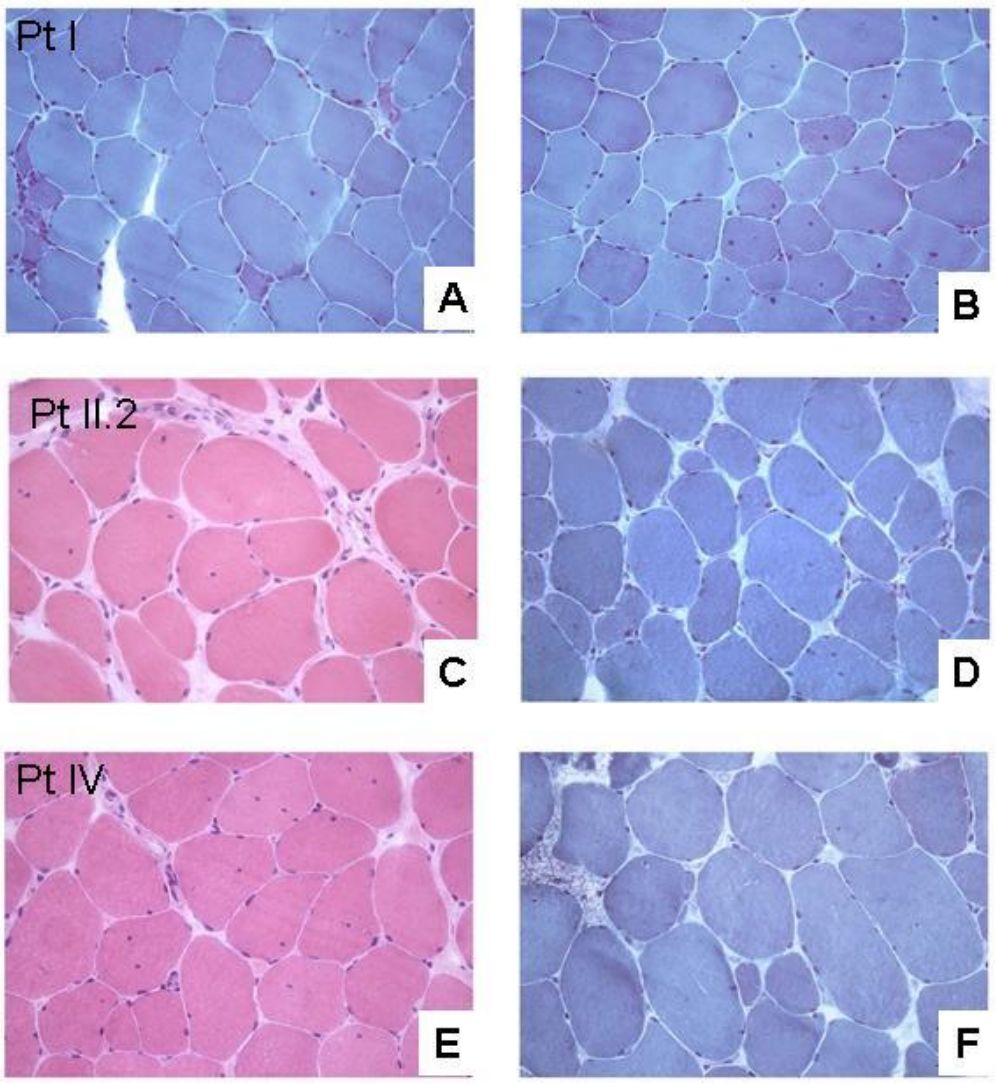
- Ereditarietà autosomica recessiva.
- Ipostenia distale oltre che prossimale
- CPK molto elevate
- Quadro bioptico con componente infiammatoria
- DD con LGMD2L (mutazioni gene Anoctamina 5)


A
B
C


A
B
C

Anoctamina-5

- Famiglia di geni di cui fanno parte 10 anoctamine. Quattro di queste anoctamine sono canali cloro calcio dipendenti.
- Ruolo di anoctamina 5 sconosciuto.
- Mutazioni dominanti nel gene ANO5 sono associate a gnatodisplasia (rara malattia scheletrica).
- La più comune mutazione riscontrata è la c.191dupA nell'esone 10 descritta sia in omozigosi sia in eterozogosi composta.
- Maschi affetti in modo molto più severo


Pt I
A
B
Pt II.2
C
D
Pt IV
E
F

Malattie Muscolari - Nosografia

Geneticamente determinate (AD, AR, X-linked, Matrilineari) / Acquisite

Distrofie: Distrofinopatie di Duchenne e Becker, congenite, dei cingoli, distrofia Facio Scapolo Omerale , Emery-Dreifuss.

Miopatie congenite / Miopatie miofibrillari.

Miopatie metaboliche: Miopatie mitocondriali, da accumulo lipidico o glucidico, miopatie lisosomiali.

Canalopatie: Malattia di Steinert (DM1, DM2)

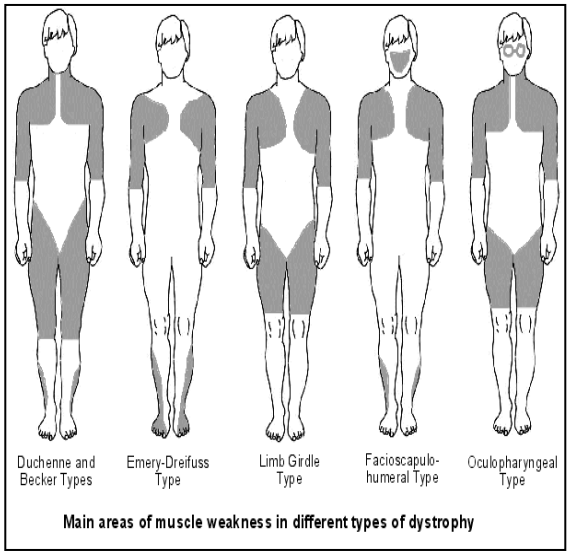
Infiammatorie: Polimiositi, dermatomiositi, miopatie a corpi inclusi

Iatrogene, tossiche.

Alterazioni della giunzione neuromuscolare: miastenia, Eaton Lambert.

FSHD

Contrazione sequenza D4-Z4
Cromosoma 4



Main areas of muscle weakness in different types of dystrophy

- Terza più comune forma di distrofia muscolare
- Ereditarietà autosomica dominante ma ……
- Prevalenza 1:15000 - 1:20000
- Caratteristiche cliniche: precoce coinvolgimento della muscolatura facciale, progressiva ipostenia e atrofia della muscolatura scapolare e omerale, ipostenia muscolare selettiva ma…..
- Variabilità clinica tra i membri della stessa famiglia
- Esordio seconda - terza decade con ipostenia facciale o cingolo scapolare
- Progressione lenta
- Aspettativa di vita generalmente normale

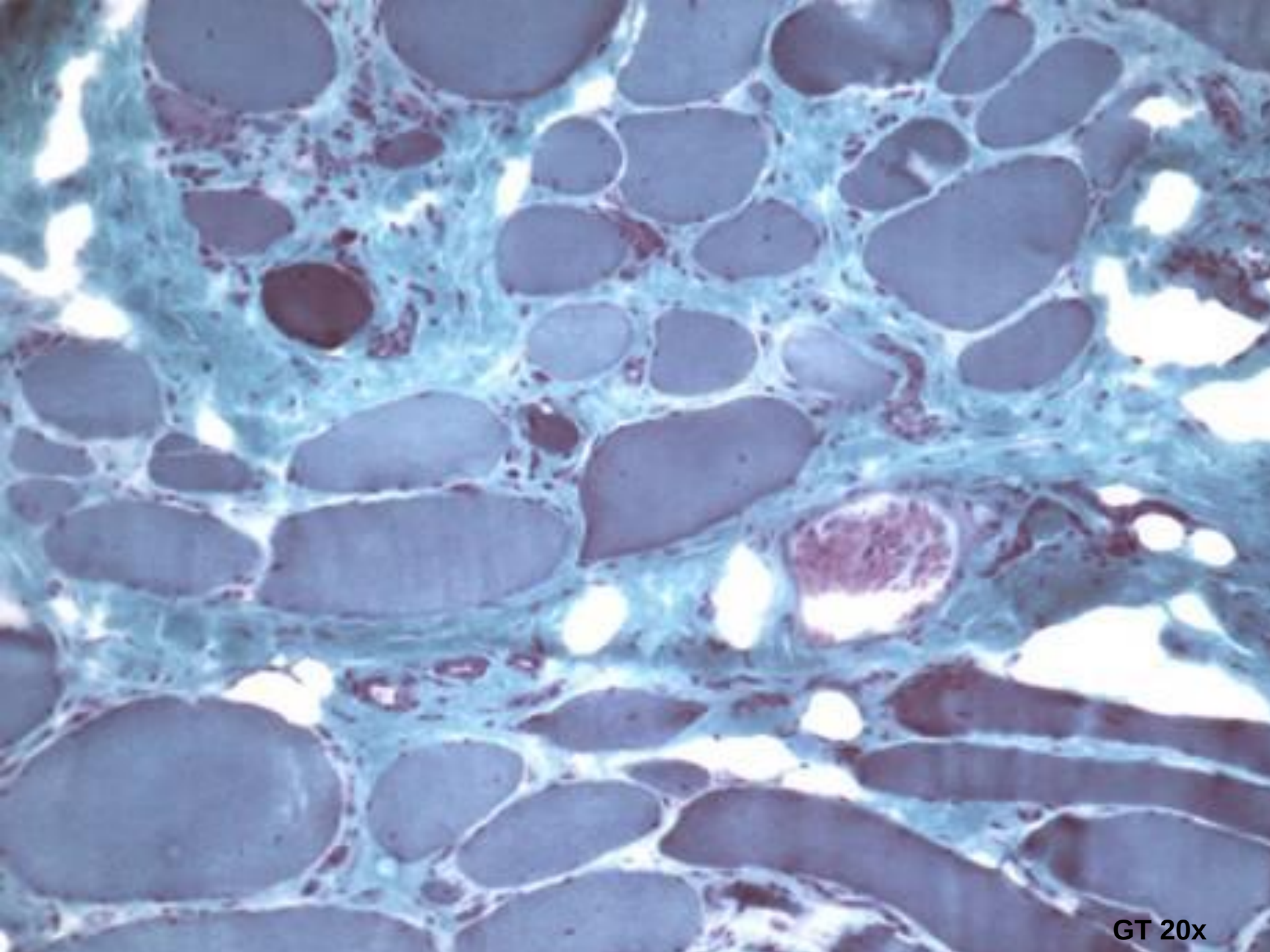
FSHD ad esordio infantile

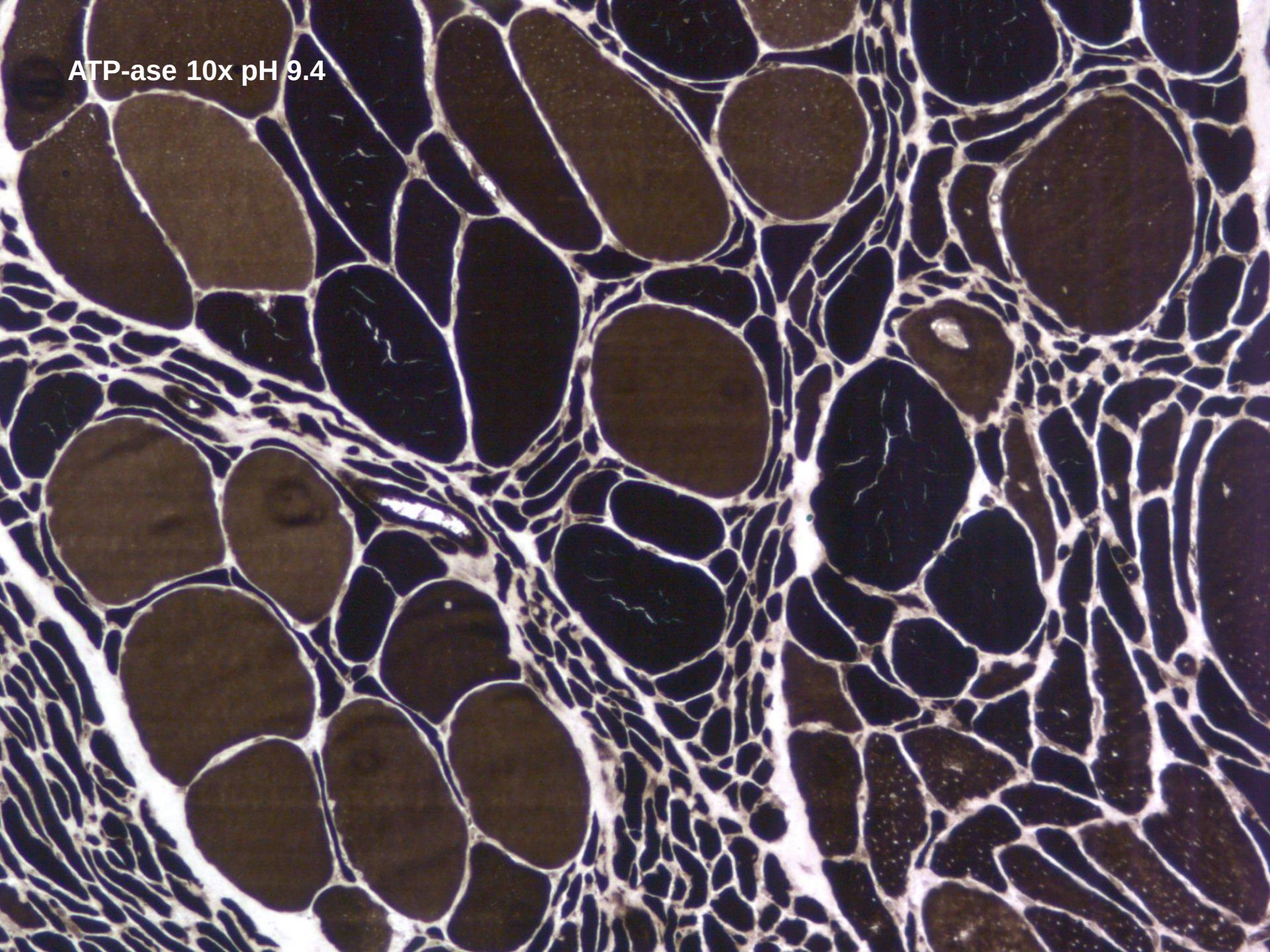
- Relativamente rara (circa 4% dei pazienti)
- Frammento D4-Z4 generalmente molto corto (< 13Kb)
- Fenotipo più severo
- Severa ipostenia facciale dall'infanzia
- Più frequente presenza di complicanze extramuscolari (soprattutto anomalie retiniche e ipoacusia neurosensoriale)
- Possibile ritardo mentale ed epilessia
- Coinvolgimento cardiaco (aritmie) poco frequente

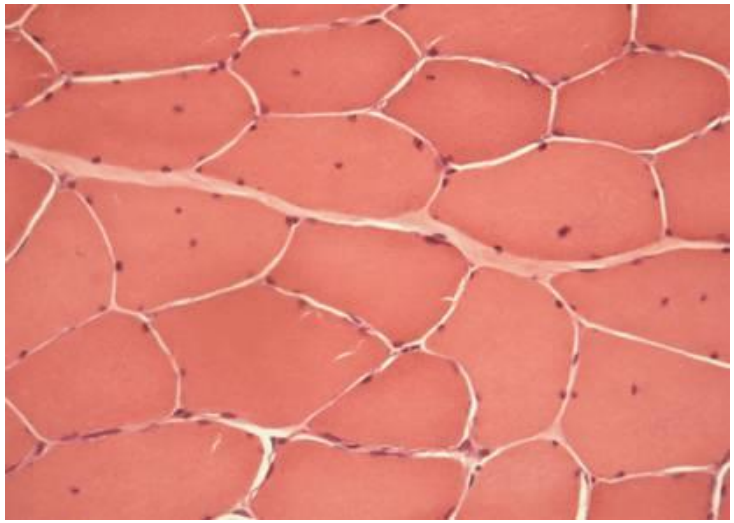
Biopsia muscolare nella FSHD

Presentazione estremamente variabile:

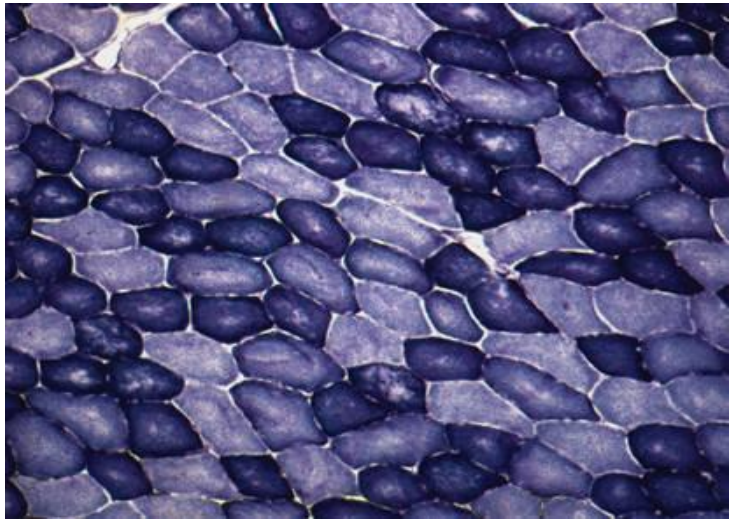
- Pattern distrofico
- Pattern neurogeno
- Lievi alterazioni miopatiche
- Pattern infiammatorio


GT 20x

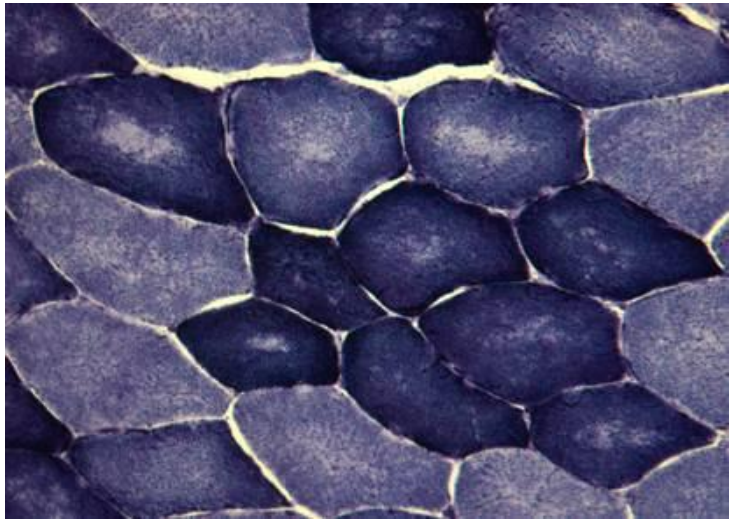

ATP-ase 10x pH 9.4



H&E25x



NADH 10x



NADH 25x

Malattie Muscolari - Nosografia

Geneticamente determinate (AD, AR, X-linked, Matrilineari) / Acquisite

Distrofie: Distrofinopatie di Duchenne e Becker, congenite, dei cingoli, distrofia Facio Scapolo Omerale.

Miopatie congenite / Miopatie miofibrillari.

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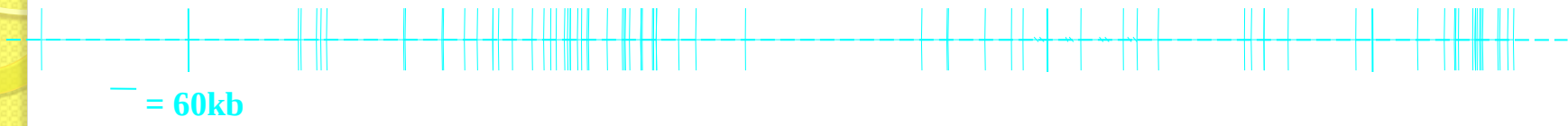
Iatrogene, tossiche.

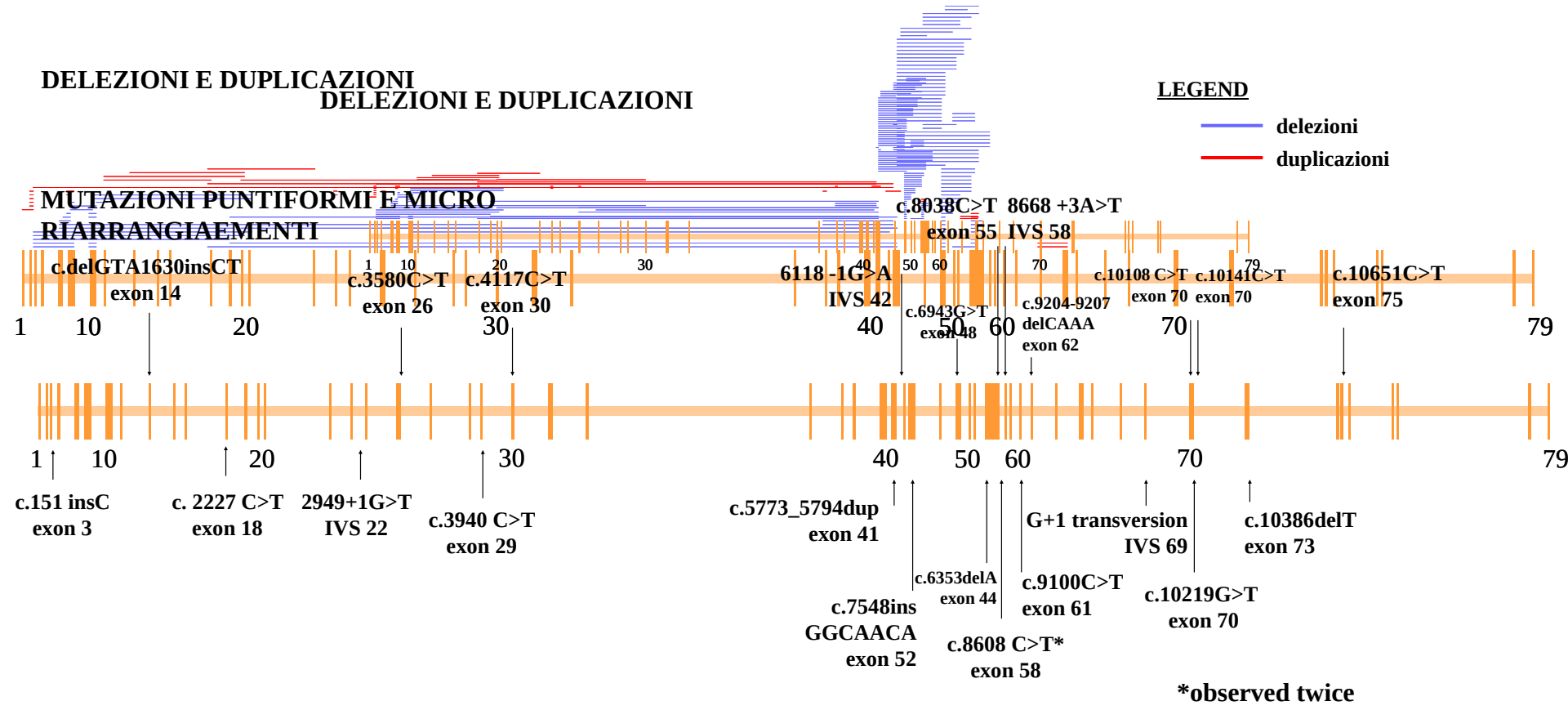
Alterazioni della giunzione neuromuscolare: miastenia, Eaton Lambert.

Analisi Genetica

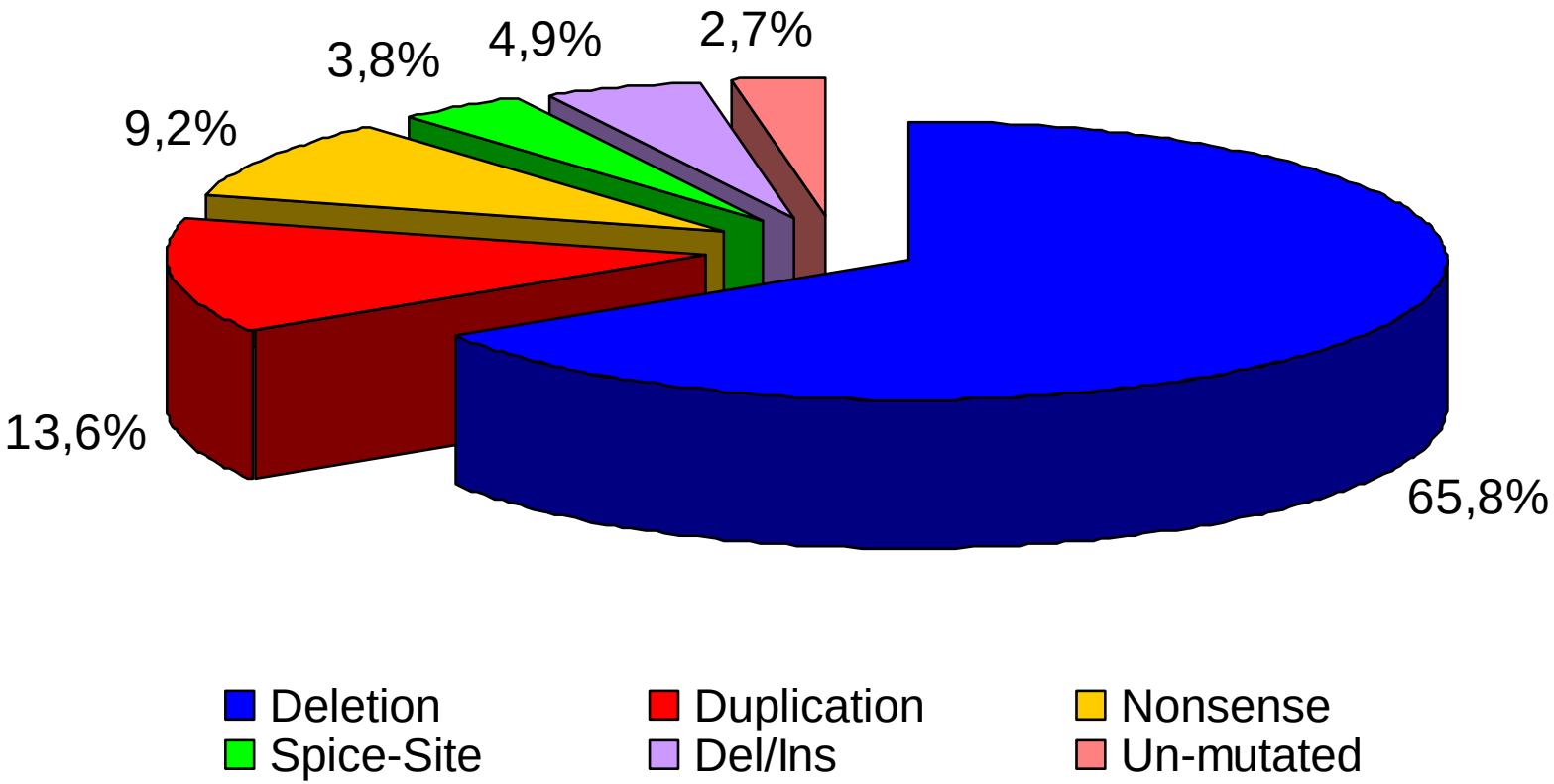


1. **Multiplex PCR per delezioni**
2. **Identificazione Delezioni/Duplicazioni (multiplex ligation-dependent probe amplification, MLPA; Multiplex-Amplifiable Probe Hybridisation, MAPH) CGH array)**
3. **Sequenza genica per identificare mutazioni puntiformi**
4. **Analisi cDNA**

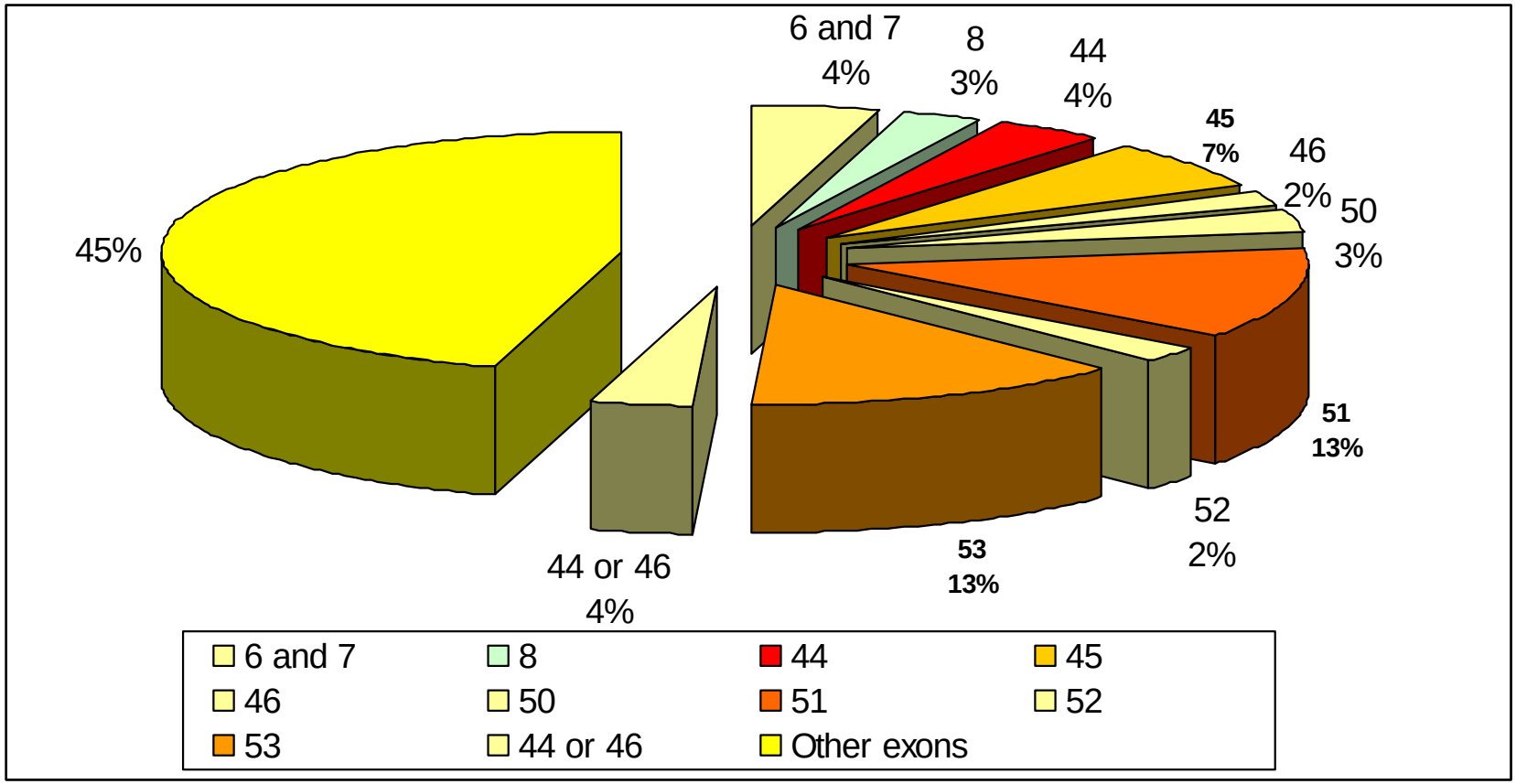
Distribuzione delle mutazioni lungo il gene DYS nella popolazione DMD



Ataluren – Selezione dei pazienti



Exon skipping: 204 pazienti DMD



NEUROMUSCULAR UNIT

Responsible: Moggio M, MD

Sciacco M, MD, PhD
Colombo I, MD
Peverelli L, MD
Villa L, MD

Fagiolari G, Bs
Ciscato P, BS
Napoli L, PhD
Ripolone M, PhD
Violano R, Bs
Tironi R, Bs

Valentini P, Dr. Administration

NEUROLOGICAL UNIT

Responsible: Bresolin N, MD

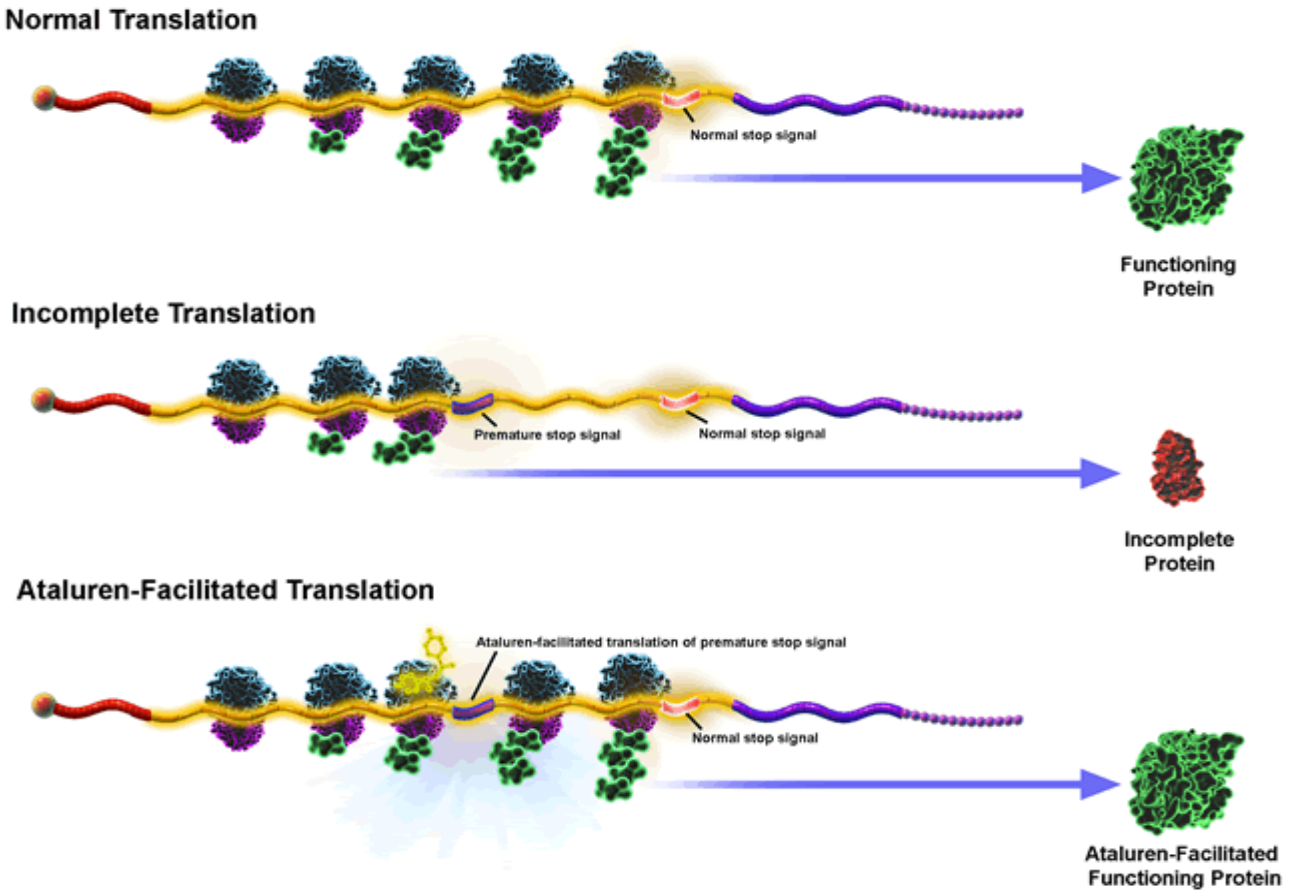
Genetics and Biochemisty

Responsible: Comi GP, MD

Ronchi D, PhD
Bordoni A, BS
Fortunato F, BS
Magri F, MD
Govoni A,MD

Fondazione IRCCS Ca' Granda
Ospedale Maggiore Policlinico-Università degli Studi di Milano

Read through di codoni di stop prematuri - PTC124 (Ataluren)



Exon skipping