Convegno SIN
Regione Calabria

INQUADRAMENTO DELLE MALATTIE NEUROMUSCOLARI

Corrado Angelini
San Camillo IRCCS s.r.l., Venice, Italy

Malattie neuromuscolari: diagnosi

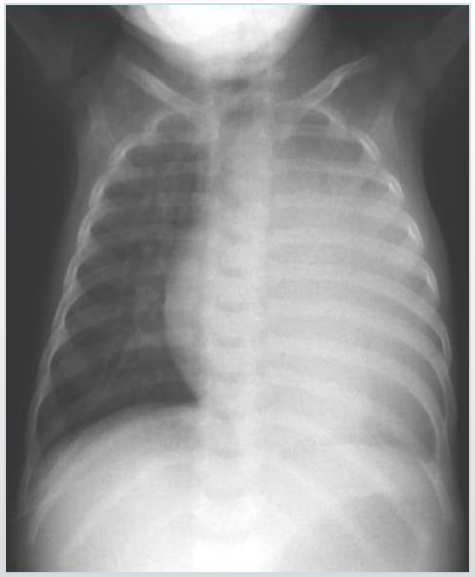
1. **Valutazione clinica neurologica:**

- Marcia anserina o steppante
- deficit di forza muscolare: scala MRC;
- variazione della massa muscolare;
- contratture muscolari;
- Riflessi osteo-tendinei
- Facies
- Piede cavo
- MOE e nervi cranici
- Valutazione neuropsicologica e QoL

2. **Valutazione neurometabolica**

- Esauribilità muscolare e Mialgie
- Insufficienza respiratoria o cardiaca

CASI CLINICI: MALATTIA DI POMPE



- Cardiomegalia
- Cardiomiopatia ipertrofica
- Breve intervallo PR e ampio complesso QRS

1972. La malattia di Pompe è una miopatia metabolica causata da un deficit dell'enzima lisosomiale maltasi acida (alfa 1-4 glucosidasi).

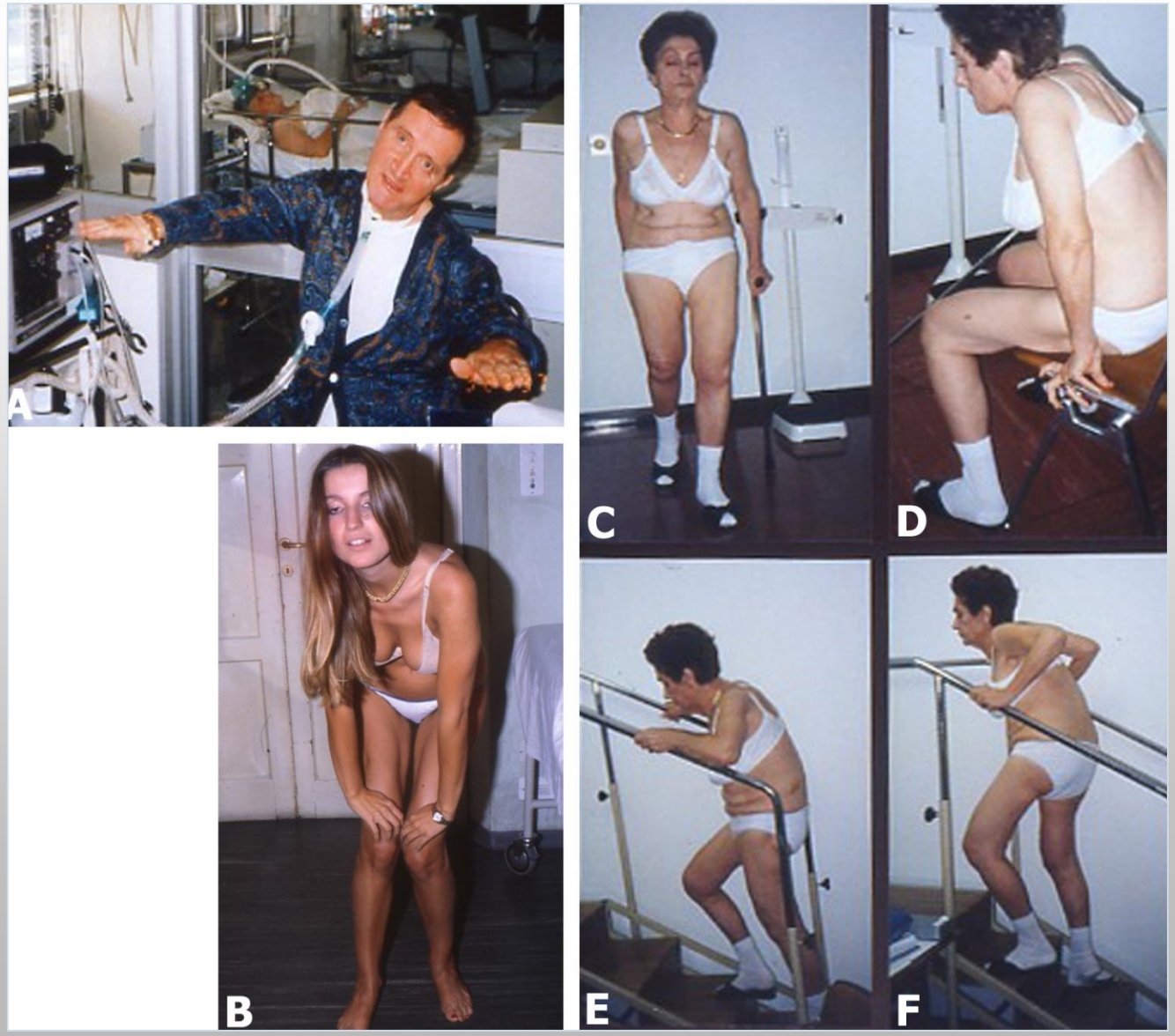
La forma dell'adulto esordisce solitamente dopo i 20 anni di età, più spesso tra i 30 e i 50 anni. L'ipostenia, più prossimale che distale, colpisce prevalentemente i muscoli del dorso e del cingoli pelvico e scapolare.

JUVENILE AMD

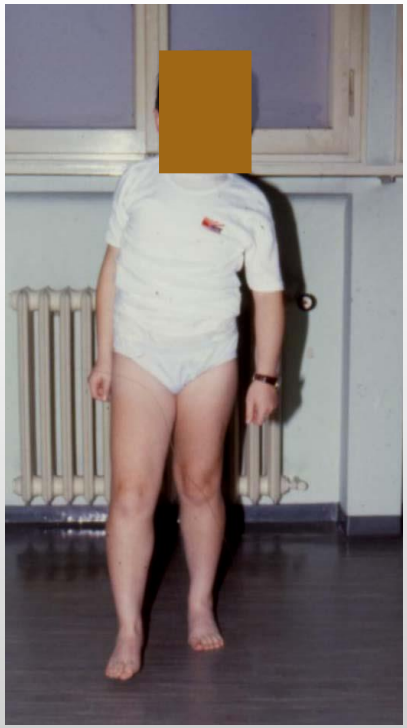
Angelini et al. Clin. Genet. 2007

ADULT AMD

Angelini et al. Basic Appl. Myol. 2004



CASI CLINICI:DISTROFIA DI DUCHENNE

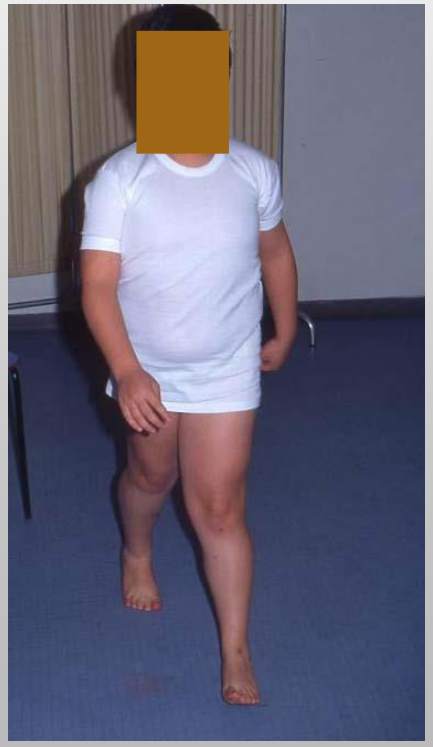


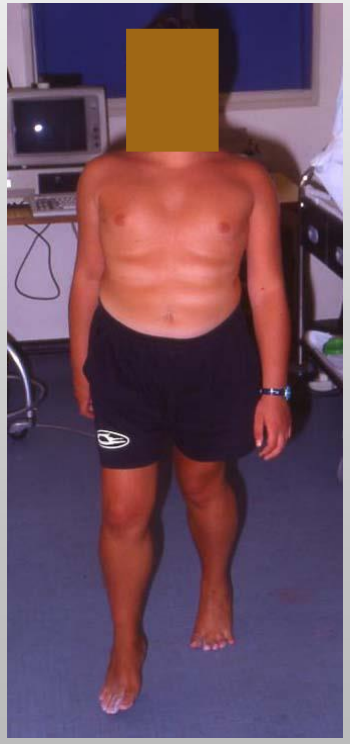
Bambini affetti da DMD osservati durante la deambulazione

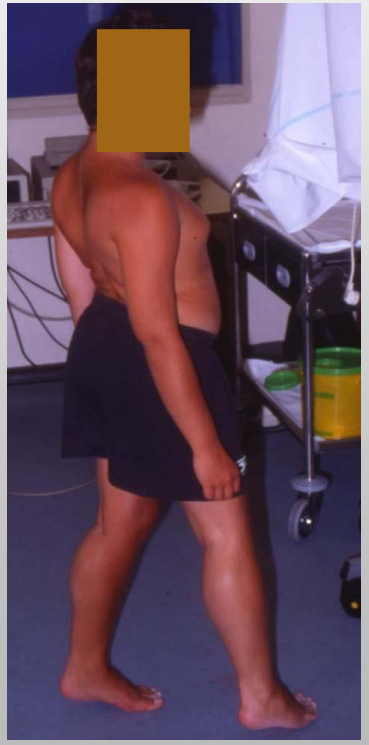
- Perdita della capacità di compiere la manovra di Gowers
- Coinvolgimento cardiaco

NON TRATTATI

TRATTATI CON STEROIDI

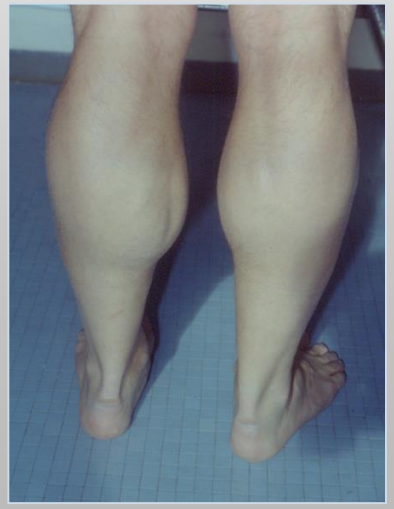


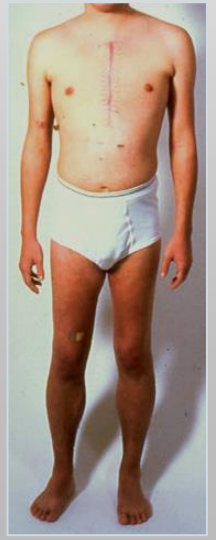


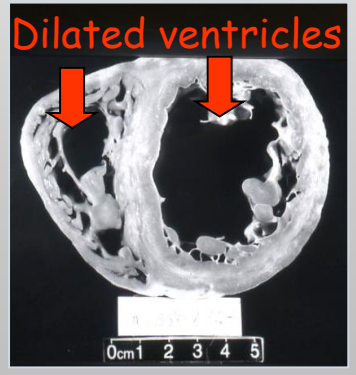


CASI CLINICI: DISTROFIA DI BECKER

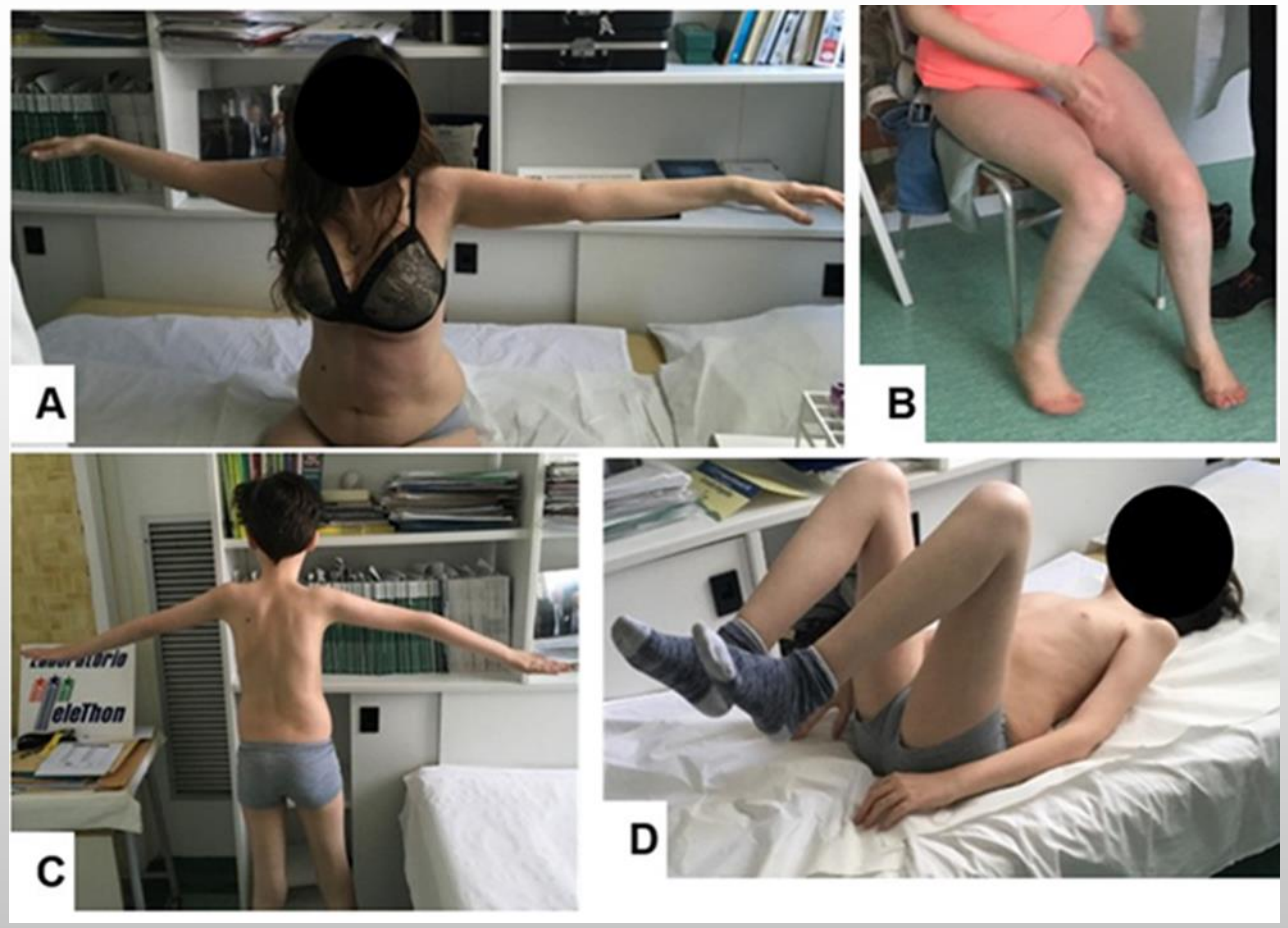
- Inizio della debolezza muscolare prossimale tra i 5-15 anni
- Perdita della deambulazione dopo i 15 anni
- Fenotipo clinico eterogeneo
- Aumento di CK
- Insufficienza respiratoria e coinvolgimento cardiaco: IV-V decade







CASI CLINICI: DISTROFIA MUSCOLARE DEI CINGOLI D2



Famiglia Ungherese affetta da Distrofia dei cingoli D2 (LGMDD2). Madre di 43 anni (A) e figlio di 12 anni (C). Si nota la cifoscoliosi e i piedi piatti nella madre (A,B). Il figlio presenta scapole alate moderate (C) e non riesce ad alzarsi dal pavimento o tenere sollevate le gambe (D).

Angelini et al. 2019

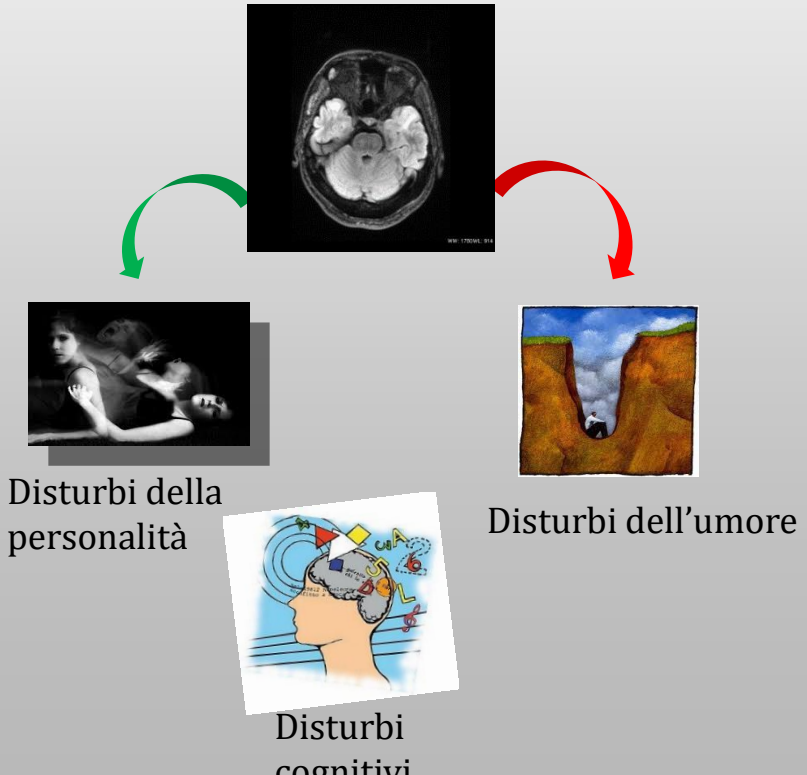
CASI CLINICI: DISTROFIA DI STEINERT (DM1)

Malattia ereditaria a modalità di trasmissione autosomica dominante

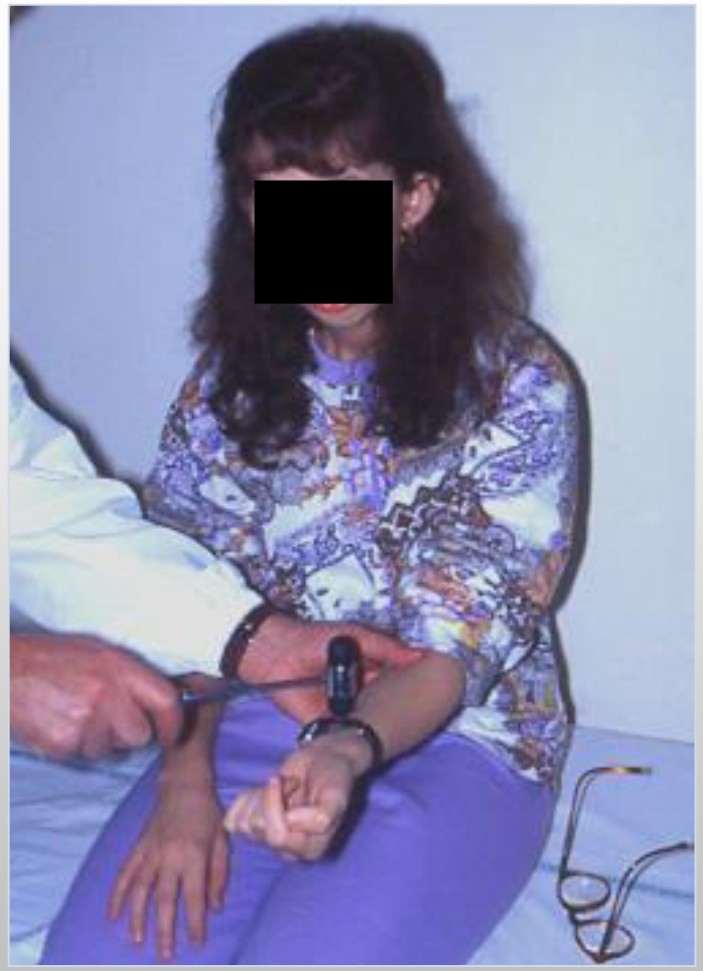
Forma di distrofia muscolare più frequente nell'età adulta (prevalenza:2-14 per 100.000 abitanti; incidenza: 1/8000)

Difetto genico causale: abnorme espansione della tripletta trinucleotidica CTG a livello del cromosoma 19, locus q 13.3.

Coinvolgimento muscolare e cerebrale

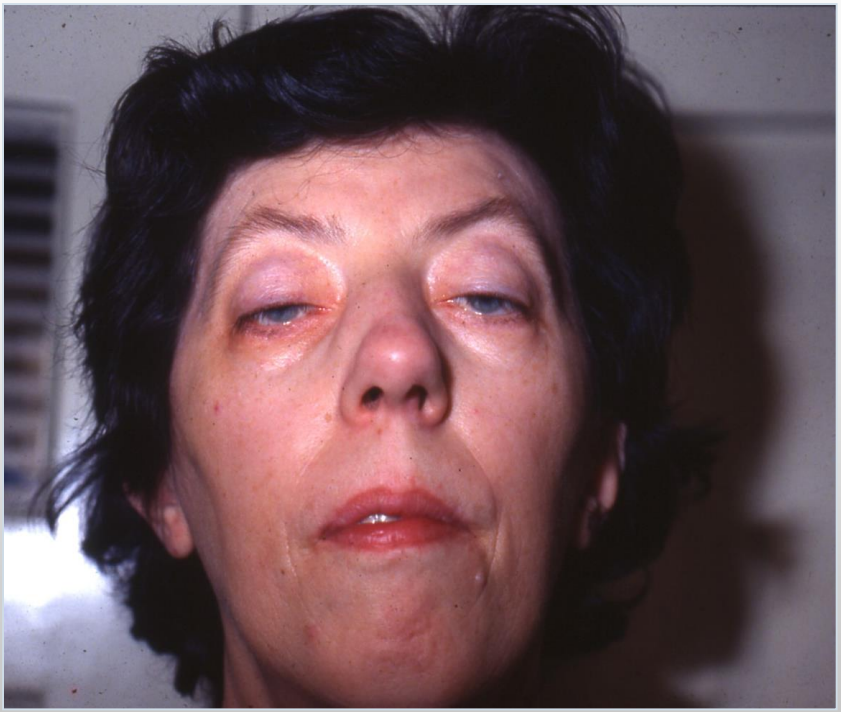


Forma giovanile di DM1



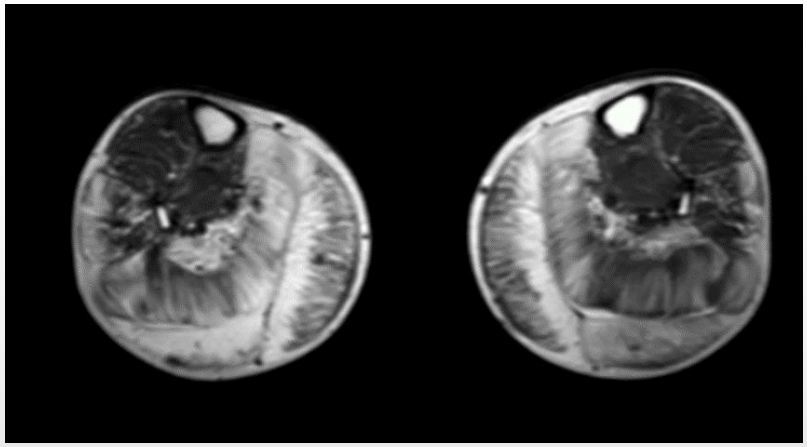
Miotonia meccanica

Forma adulta di DM1



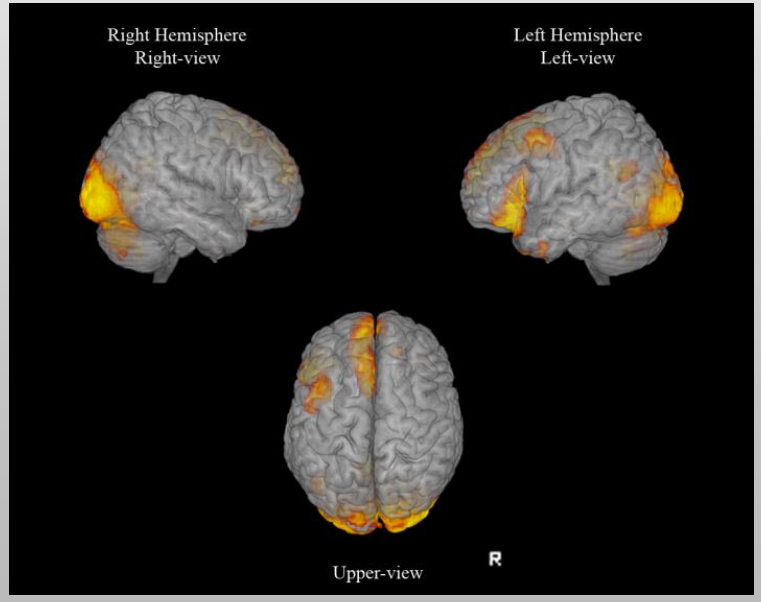
Oftalmoplegia,
facies miopatica

MRI muscolare e cerebrale



MRI muscolare

Alterazioni della massa muscolare e del connettivo analizzate con scala di Mercuri in paziente affetto da BMD



MRI cerebrale

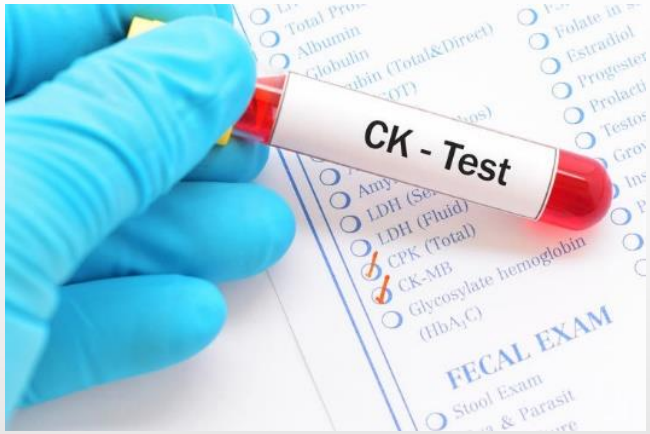
Attivazione aree cerebrali nella functional fMRI in 20 pazienti DM1 anosognosici e non asognosici nel lobo occipitale e temporale (in giallo).

Malattie neuromuscolari: diagnosi

3. Indagini di laboratorio

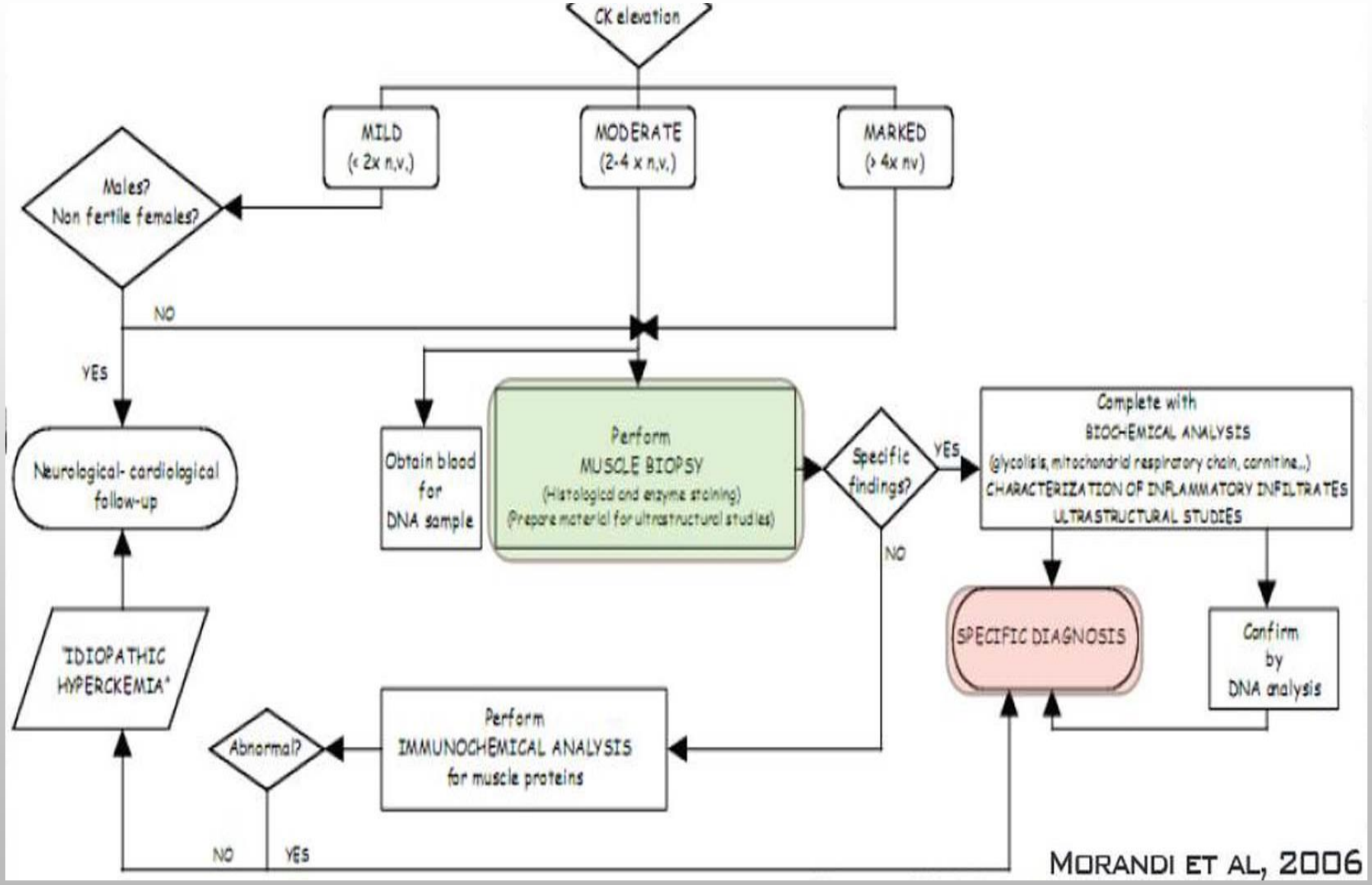
-MRI muscolare e cerebrale

- **CK (creatinchinasi)** e Mioglobina
- **LDH (lattato deidrogenasi)**
- Dosaggio dell'acido lattico
- Profilo Acyl-carnitine con GC/MS
- Anticorpi AchR-Ab o anti-Musk (Stifmen syndrome) e anticorpi per Polimiositi etc.
- Striscio di sangue per vacuoli nei granulociti o nei megacariociti.
- **Dry blood spot (DBS) per malattie metaboliche**
- Colture di fibroblasti
- **Biopsia muscolare**
- **DNA genomico con studio mutazioni con NGS o WES**
- Vitamina D (D3, D4, TSH)

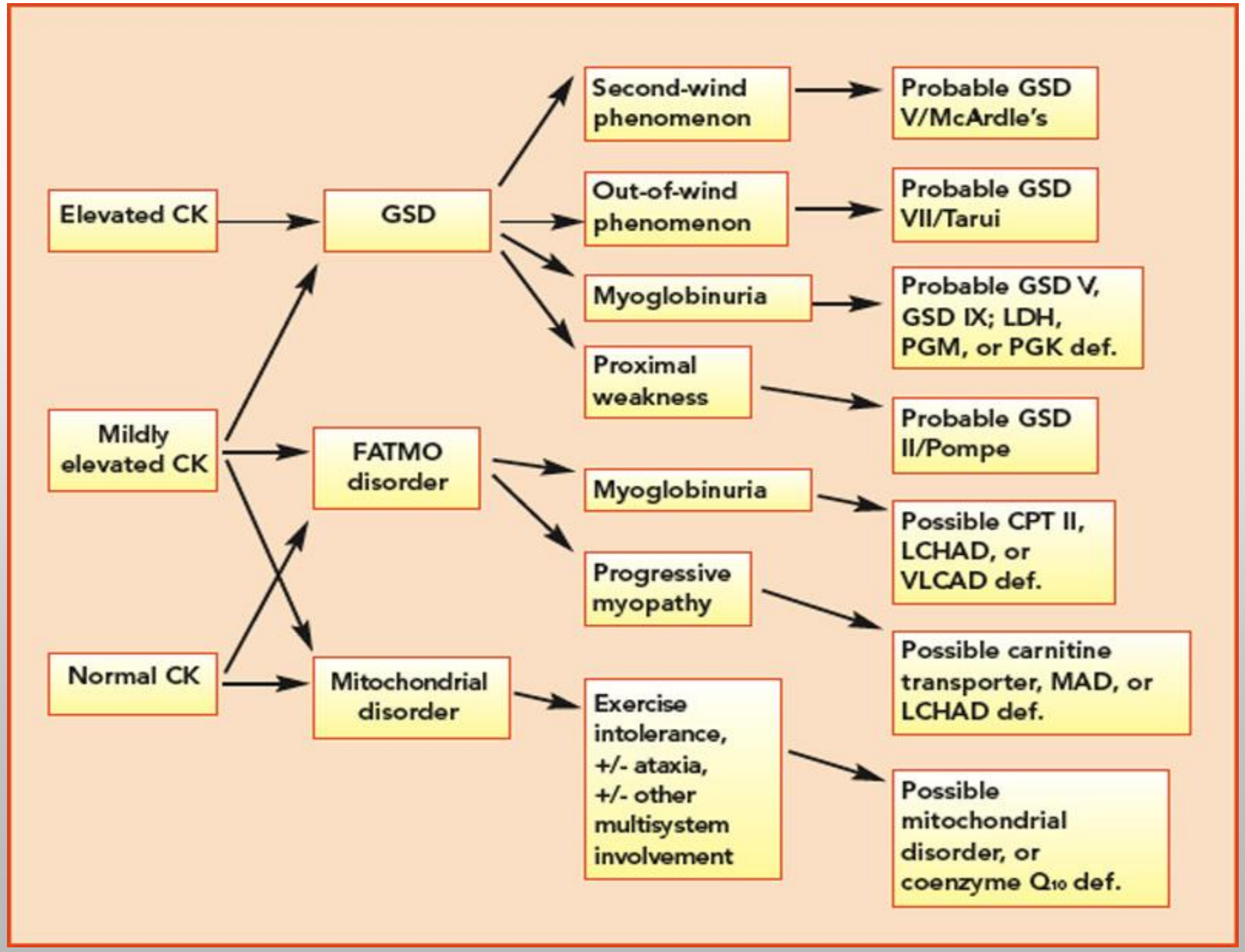


Enzimi sierici e metaboliti

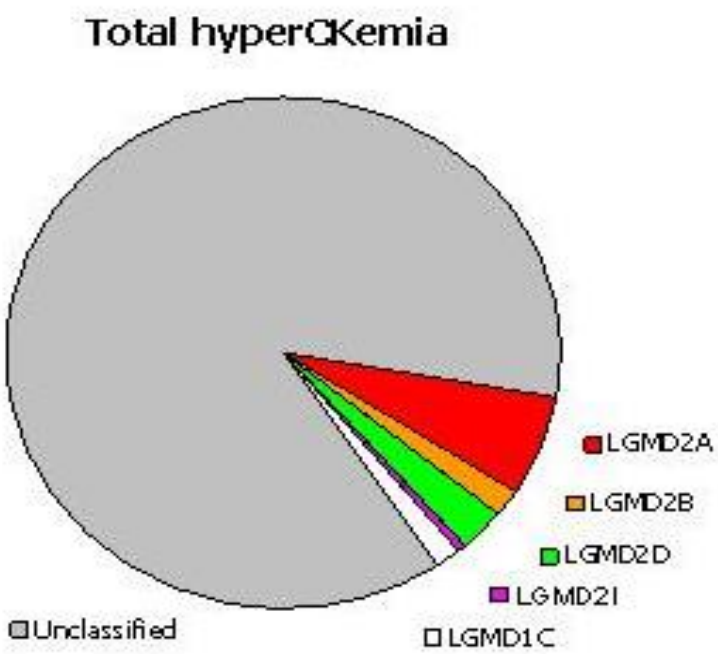
CREATIN CHINASI (CK) NEL SIERO



CREATIN CHINASI (CK) NEL SIERO



MIOPATIE GENETICHE CON IPERCKEMIA

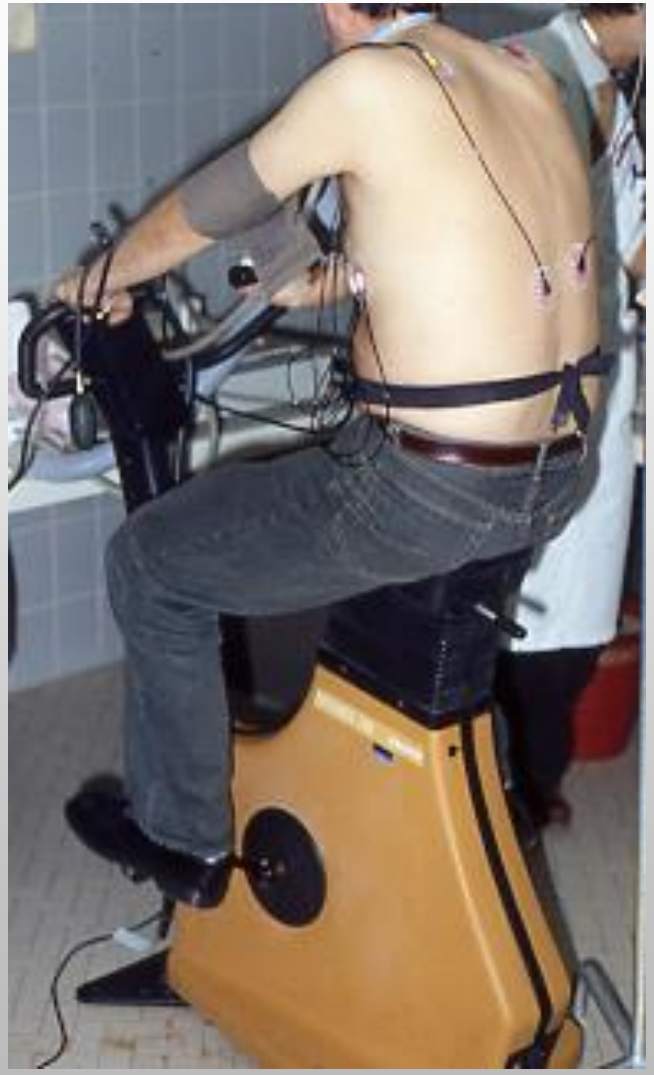


Adult onset glycogenosis type II
Caveolinopathy (Caveolin-3)
Calpainopathy (Calpain-3)
Desminopathy
Dysferlinopathy (LGMD and Miyoshi)
Fukutin-related protein (FKRP) LGMD 2I
Dystrophinopathy (also female carriers)
Sarcoglycanopathy
Myotonic dystrophy type 2

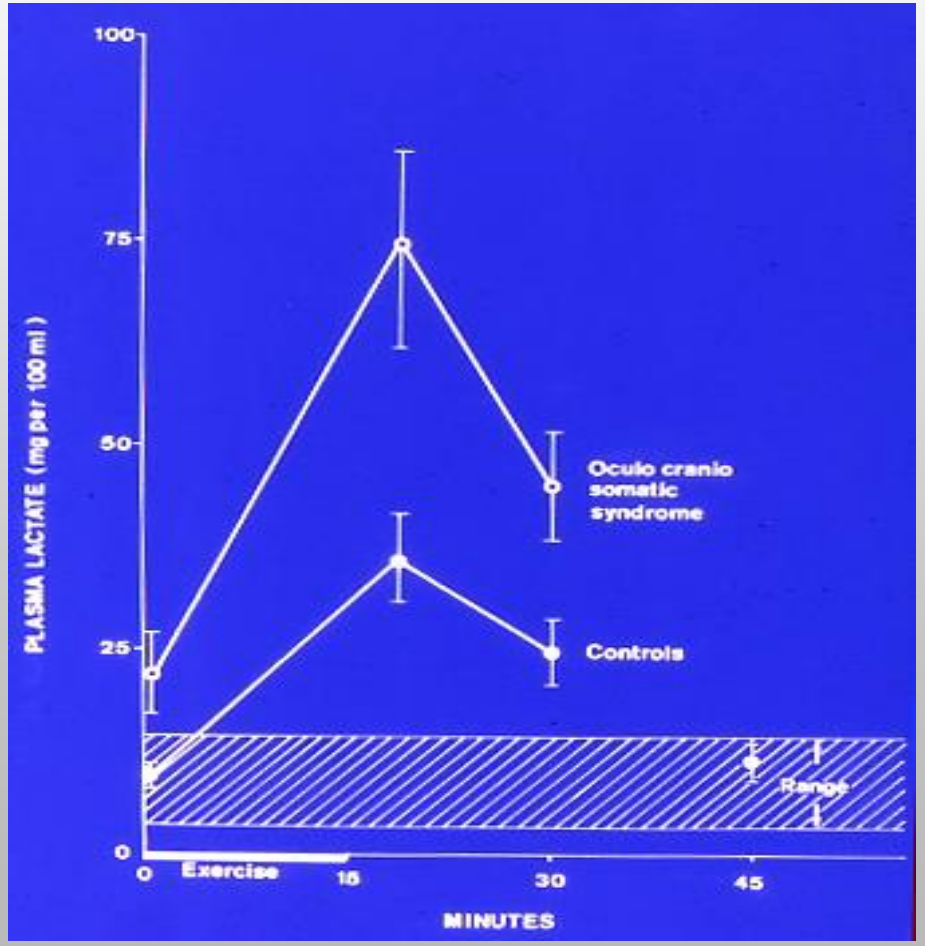
Fanin et al. 2009

Kyriakides et al. 2010

LATTATO DOSAGGIO

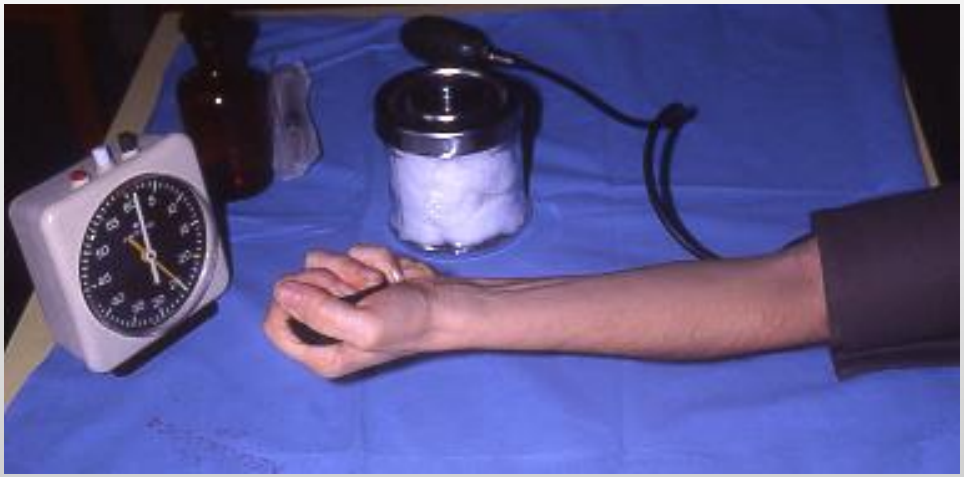


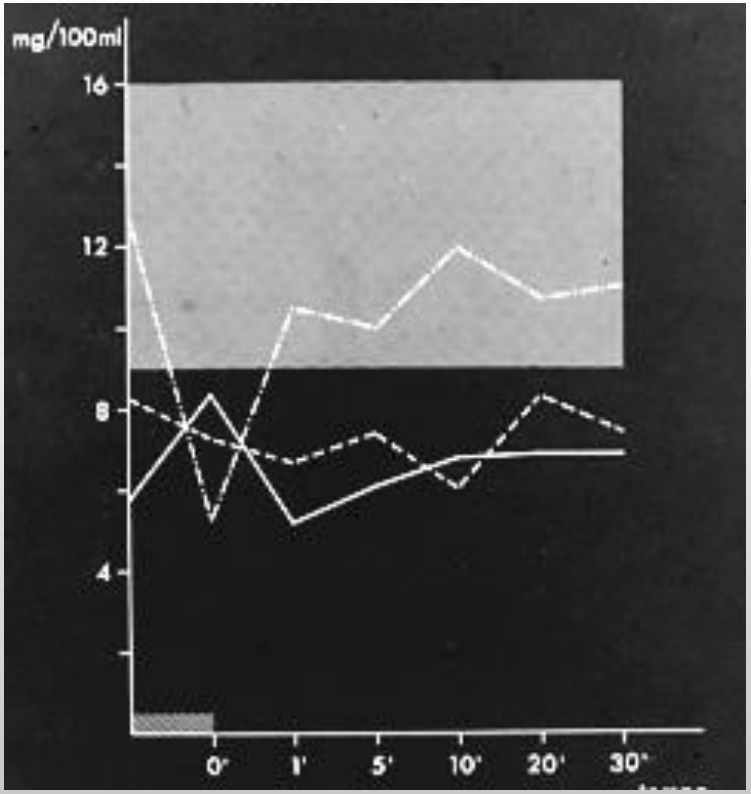
Test aerobico
(difetto nella catena respiratoria)



LATTATO DOSAGGIO

Test anaerobico
(difetto degli enzimi glicolitici)

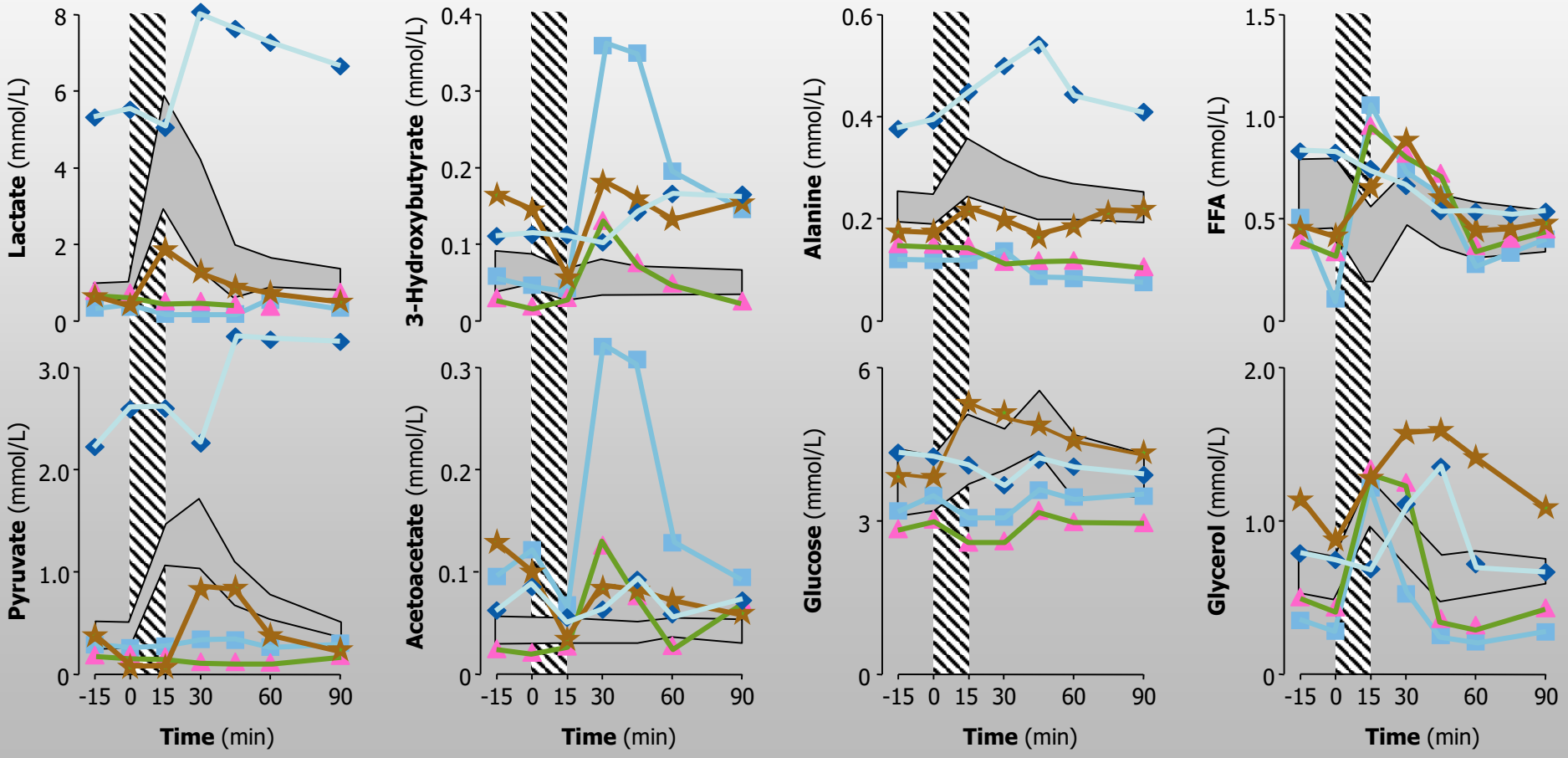




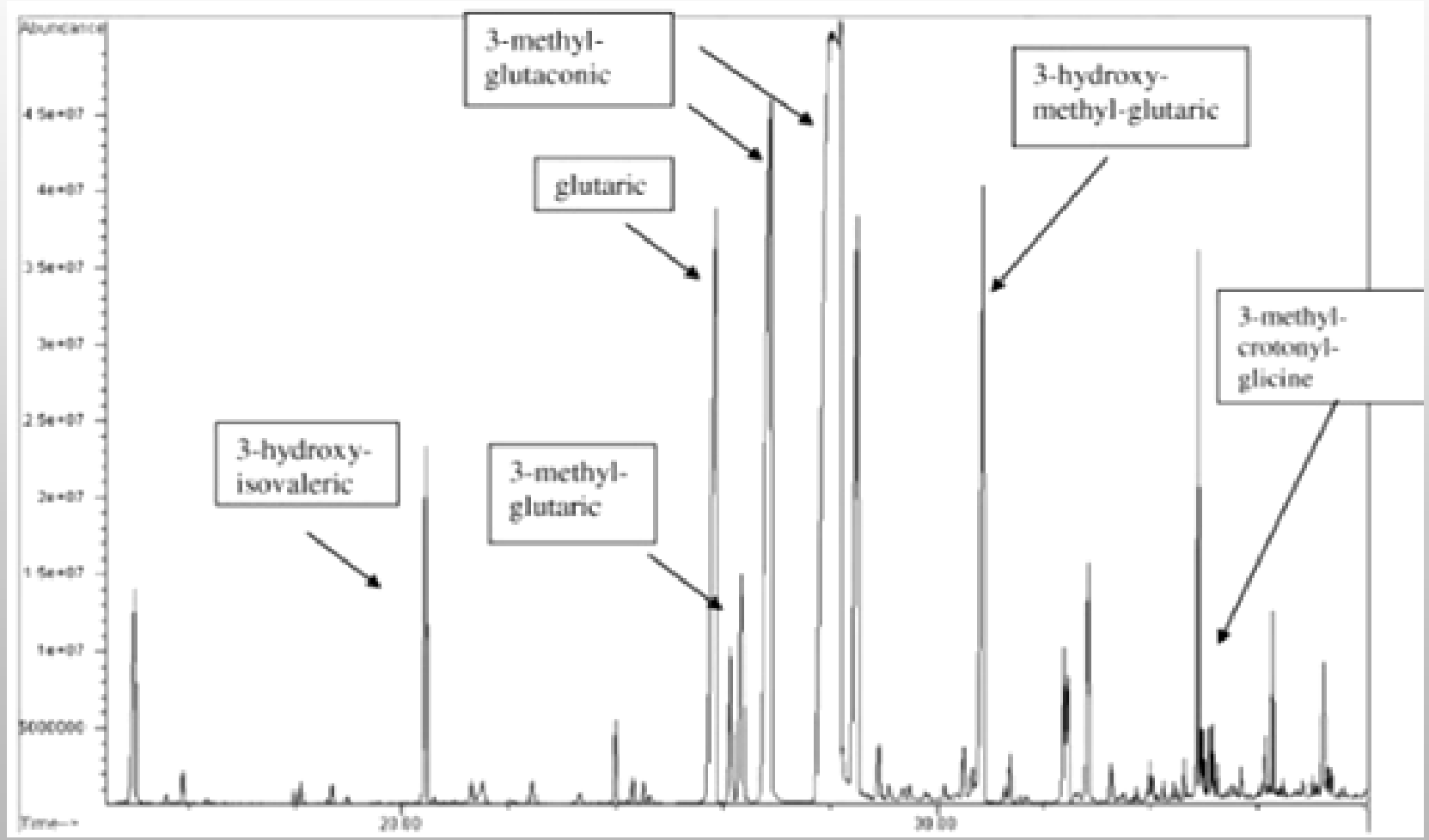
Risultati anormali (curva flat) in deficit di miofosforilasi, PFK, PGAM, PGK; risultati normali in difetti di maltasi acida ed enzimi deramificanti.

METABOLITI NELLA GLICOGENOSI

Lactate, pyruvate, alanine, glucose, FFA (free fatty acids), and ketone bodies in type I, type II, and type V glycogenoses during and after 15 minutes of exercise. Shaded area: mean values of controls.



PROFILO DEGLI ACIDI ORGANICI MEDIANTE SPETTROSCOPIA DI MASSA (CG/MS) IN ACIDURIA GLUTARICA



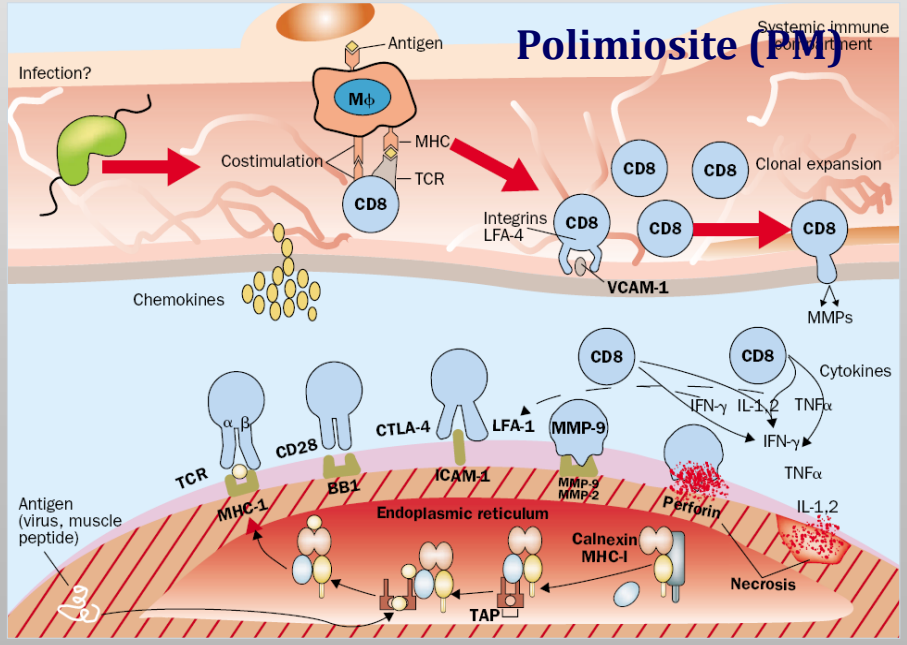
Anticorpi circolanti

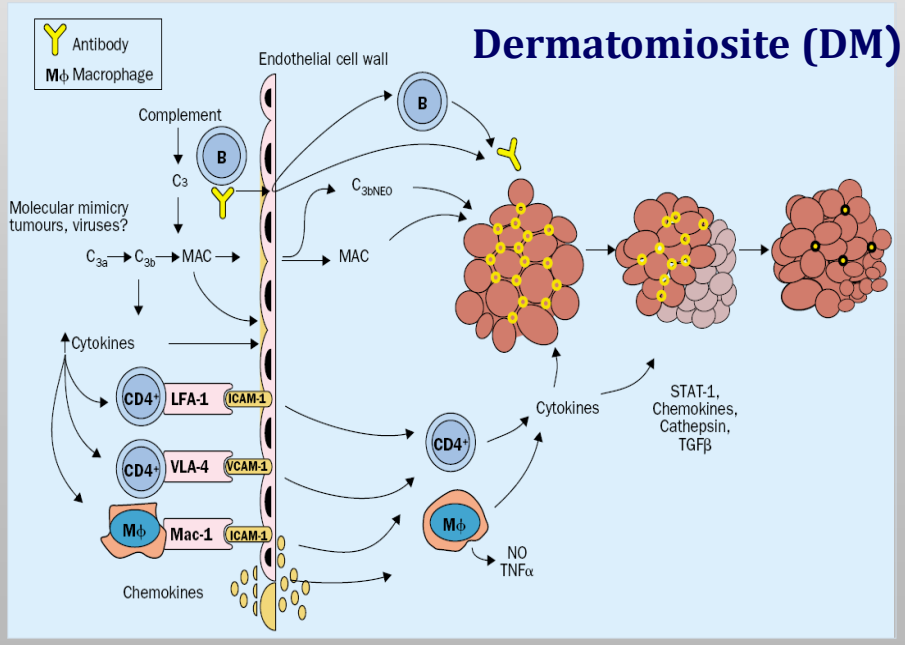
Gli anticorpi circolanti sono utili per diagnosticare la Miosite, IBM, o malattie trattabili che interessano la giunzione neuromuscolari (es. Miasthenia Gravis e la sindrome di Lambert-Eaton).

AUTOANTICORPI SPECIFICI PER LA MIOSITE

- anti-Jo-1, anti-Mi-2, anti-SRP sono presenti nel 30-40% della DM e PM senza distinzione

- anti-Mi-2b è associato con la presenza di papule di Gottron
- diagnosi di IBM è difficile quando anti-Jo-1 è presente





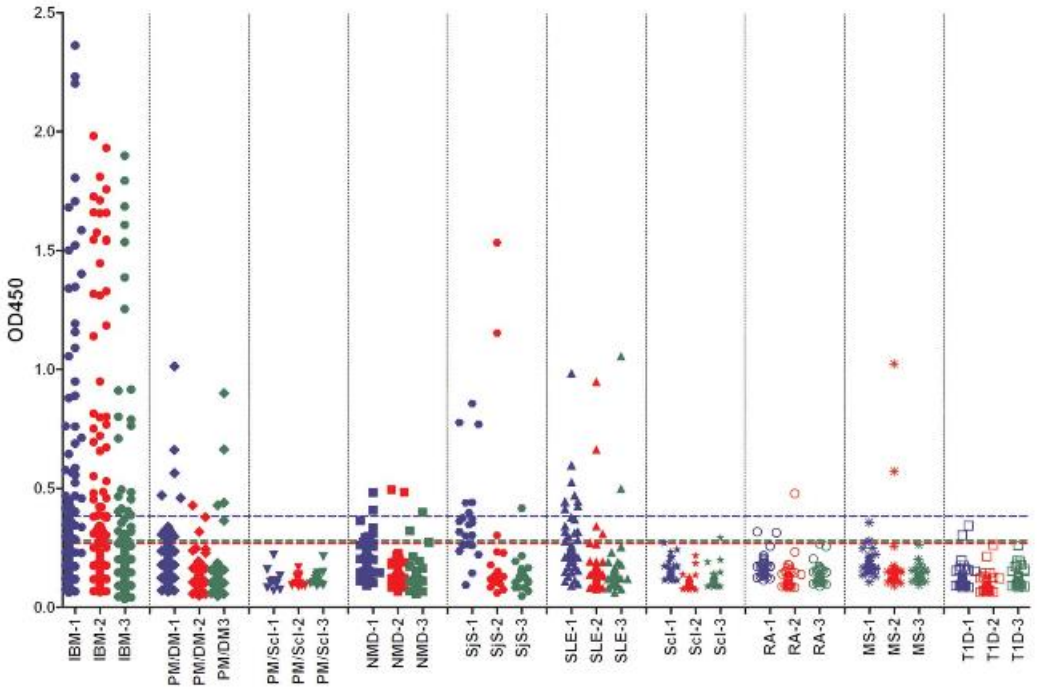
AUTOANTICORPI SPECIFICI NELLA MIOSITE A CORPI INCLUSI (IBM)

- anti-cN-1A sono presenti nel 37% di IBM

Table 1 Sensitivity and specificity of anti-cN-1A autoantibodies

Sera	Number	Anti-cN-1A reactivity* n	Anti-cN-1A reactivity* Per cent
Inclusion body myositis	238	88	37
Polymyositis/dermatomyositis	185	8	4
Polymyositis/scleroderma overlap	12	0	0
Neuromuscular diseases	93	4	4
Sjögren's syndrome	22	8	36
Systemic lupus erythematosus	44	9	20
Scleroderma	44	1	2
Rheumatoid arthritis	44	1	2
Multiple sclerosis	40	2	5
Type 1 diabetes	40	0	0
Disease controls†	*458*	*16*	*3*

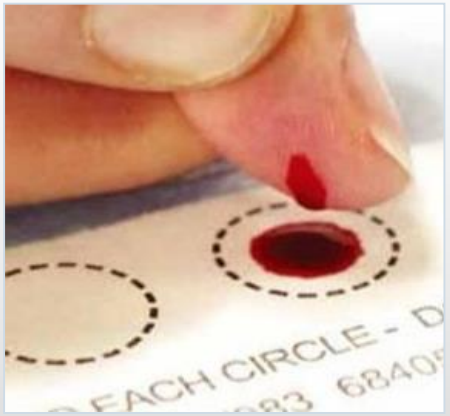
*Reactivity with at least one of the three cN-1A peptides higher than cut-off.
†Disease controls: total of all disease control groups except IBM, SLE and SjS.
cN-1A, cytosolic 5′-nucleotidase 1A; IBM, inclusion body myositis; SjS, Sjögren's syndrome; SLE, systemic lupus erythematosus.

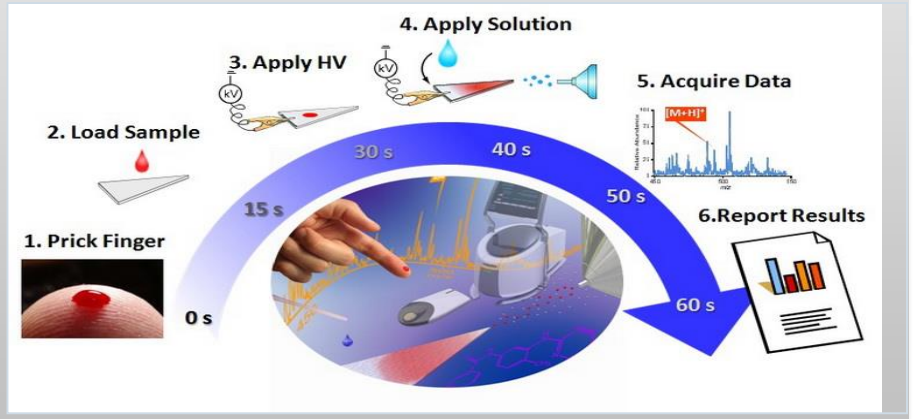


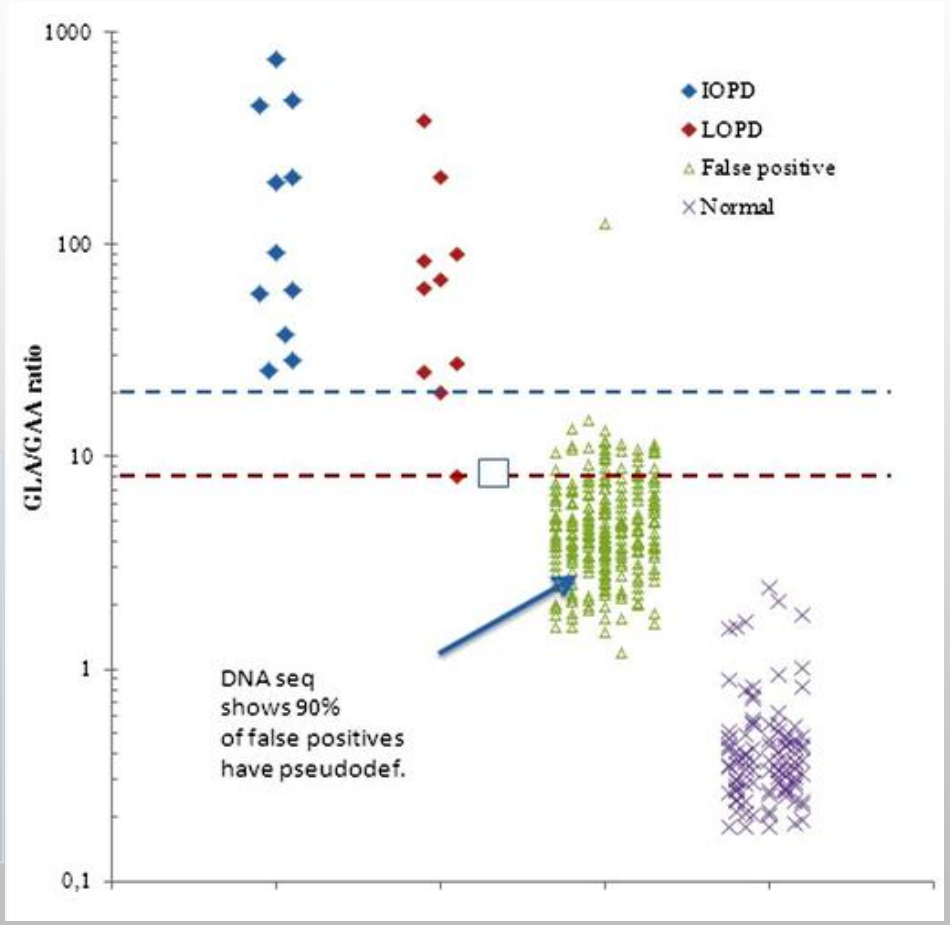
Herbert MK, *et al*. *Ann Rheum Dis* 2016;**75**:696–701. doi:10.1136/annrheumdis-2014-206691

Dry blood spot

DRIED BLOOD SPOT: MS ANALISI DELLA MALTASI ACIDA (a-glucosidase acid, GAA)



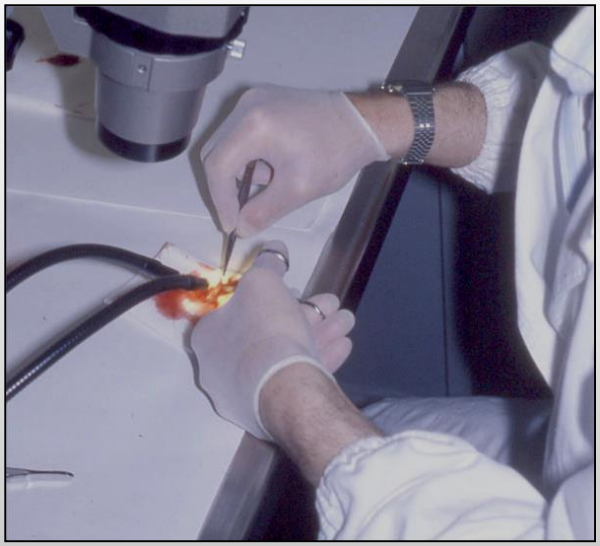


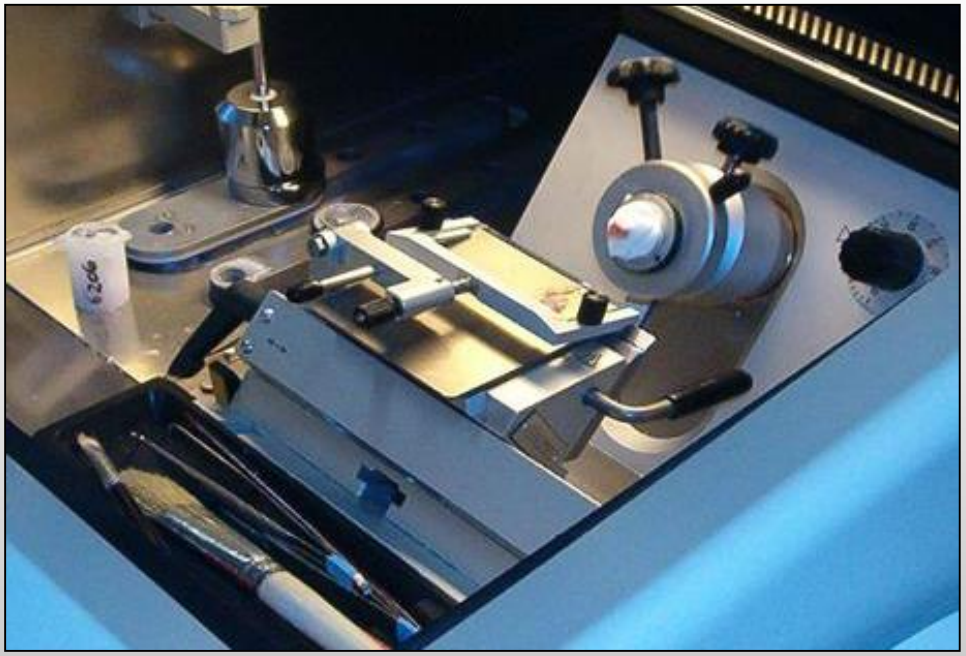


La MS per attività GAA distingue le forme di glicogenosi di tipo 2 a insorgenza infantile (IOPD) e ad esordio tardivo (LOPD) dalle pseudodeficienze.

Istopatologia muscolare

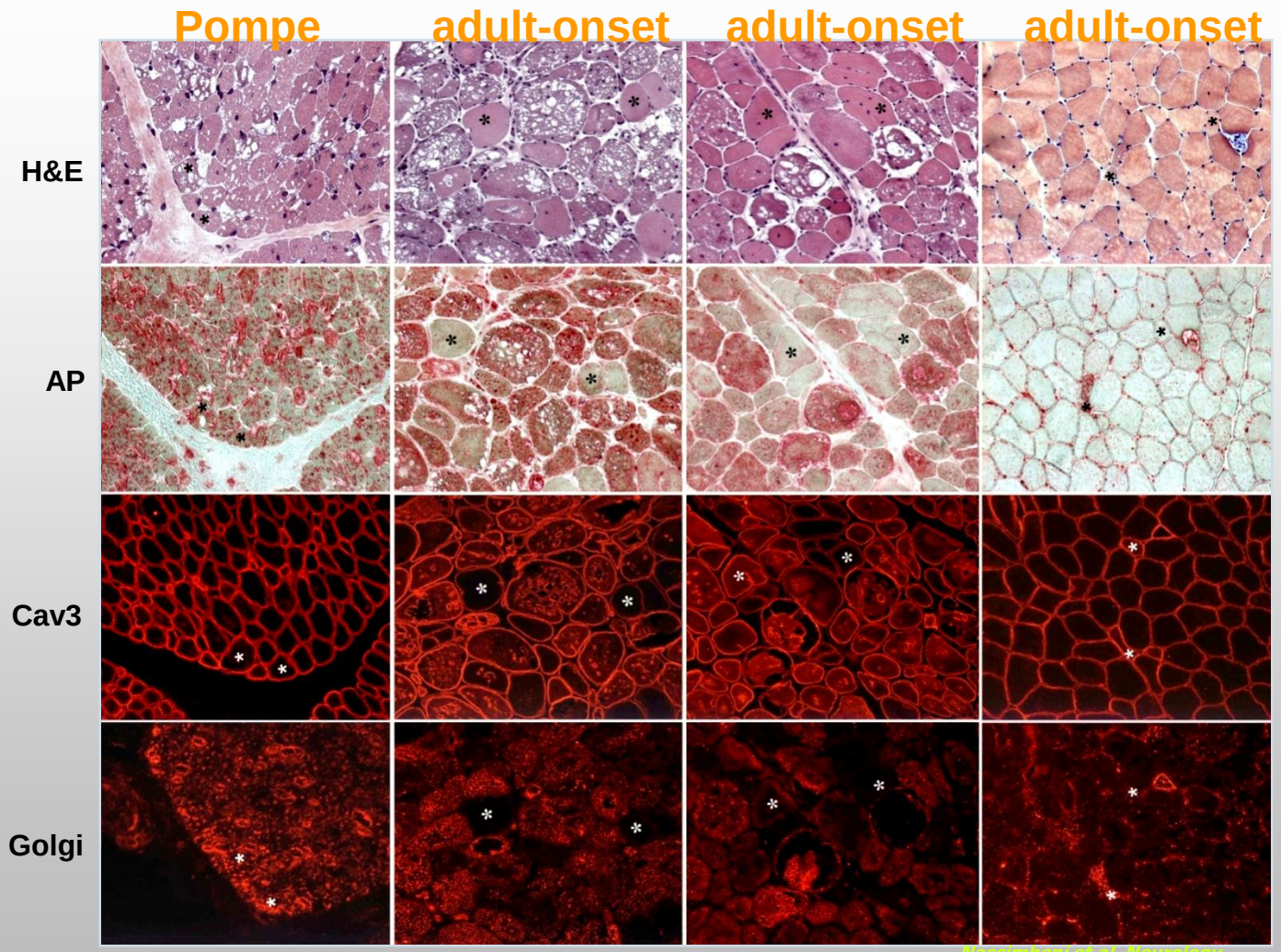
PROCESSAMENTO E CRIOSEZIONAMENTO DI CAMPIONI DI BIOPSIA MUSCOLARE





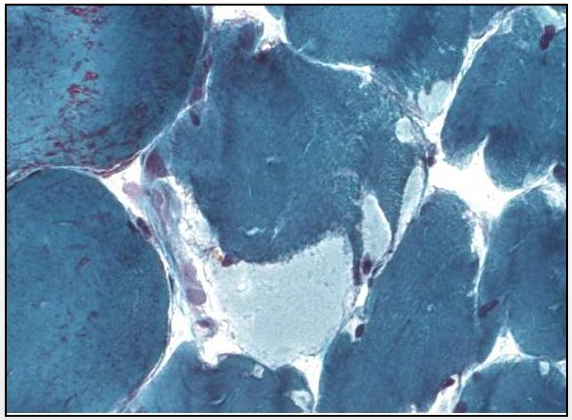
Esame al microscopio

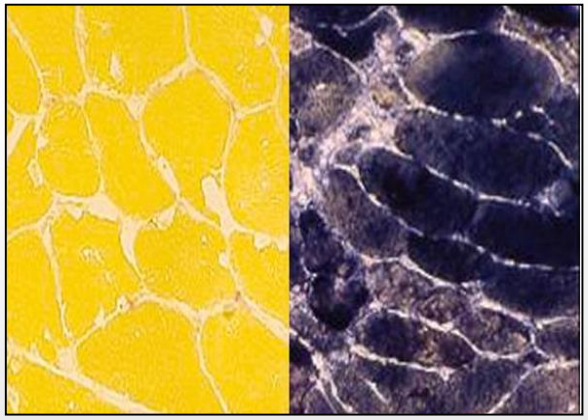
MALATTIA DI POMPE INFANTILE E ADULTA



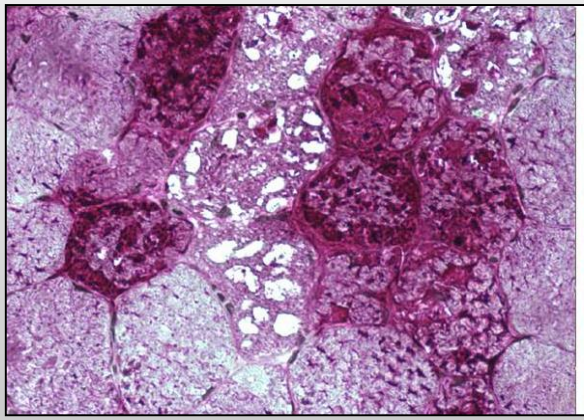
Nascimbeni et al. Neurology 2008

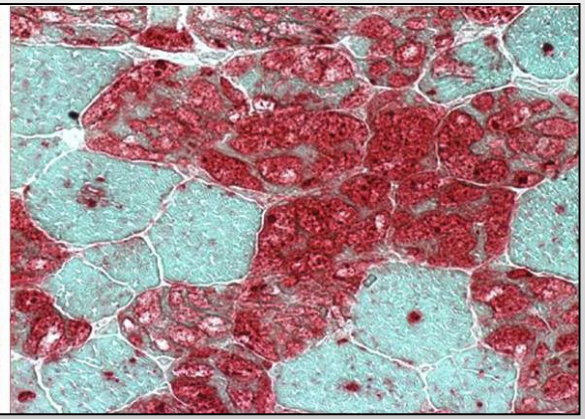
DISORDINI LISOSOMIALI E GLICOGENOSI



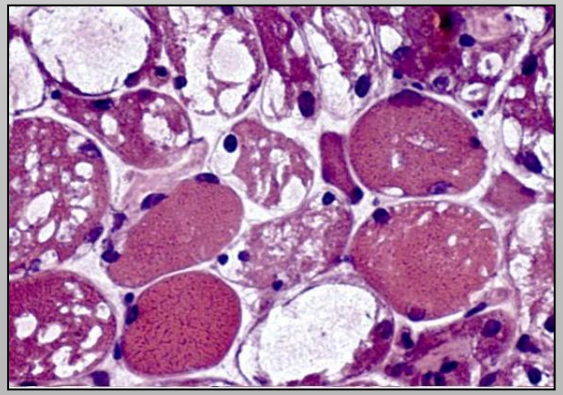


Glicogenosi di tipo V
(McArdle):
Difetto fosforilasi



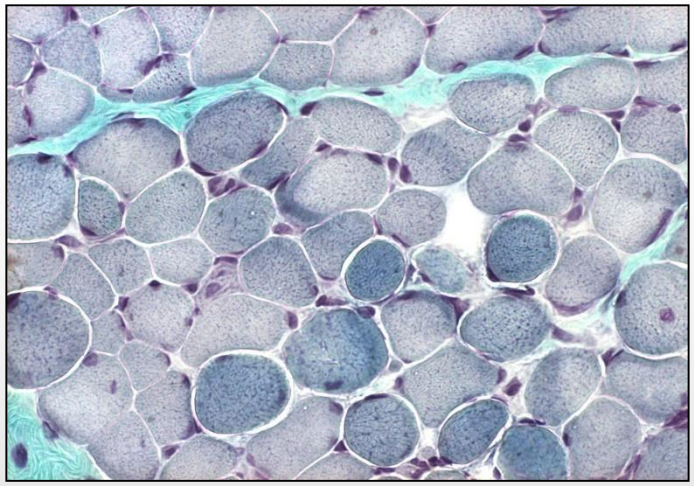


Glicogenosi di tipo II:
difetto maltasi acida

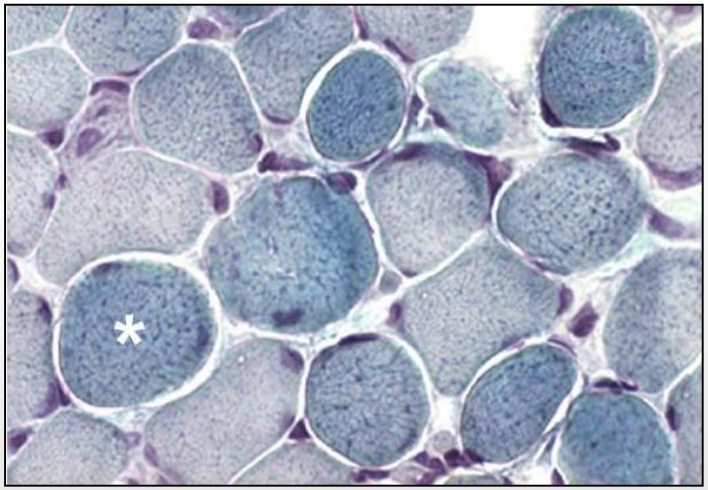


Malattia di Danon:
difetto LAMP-2 lisosomiale

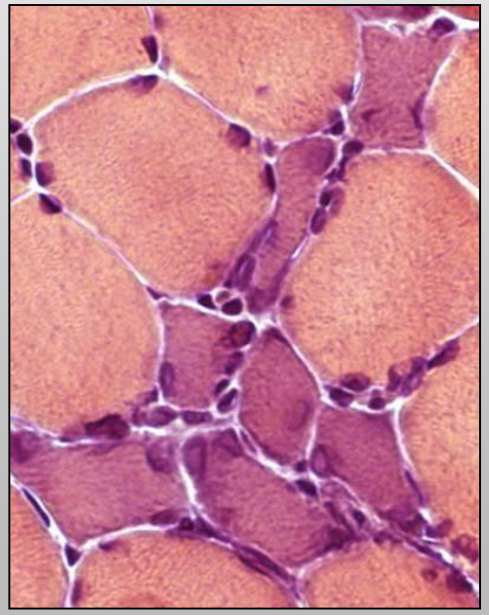
DISTROFIA MUSCOLARE



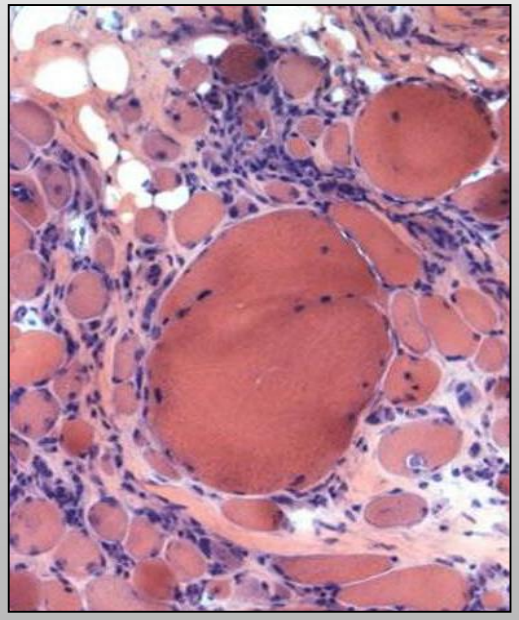
Variabilità nella forma delle fibre



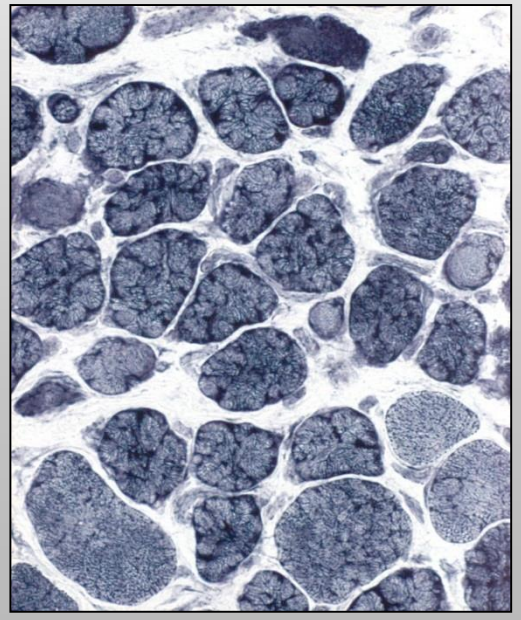
Fibre opache



Fibre rigeneranti

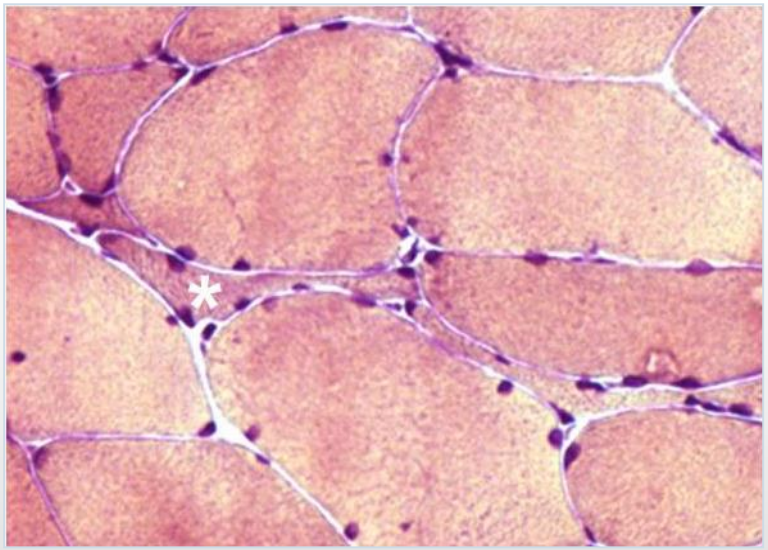


Inclusione citoplasmatica

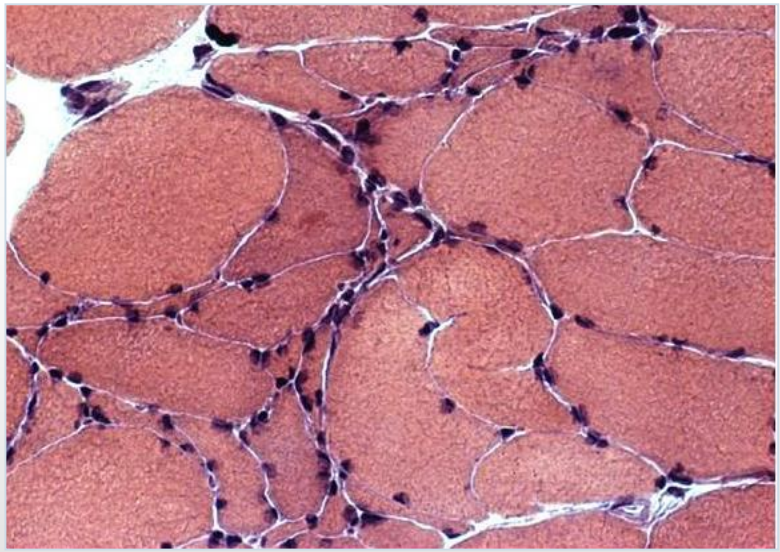


Fibre lobulate

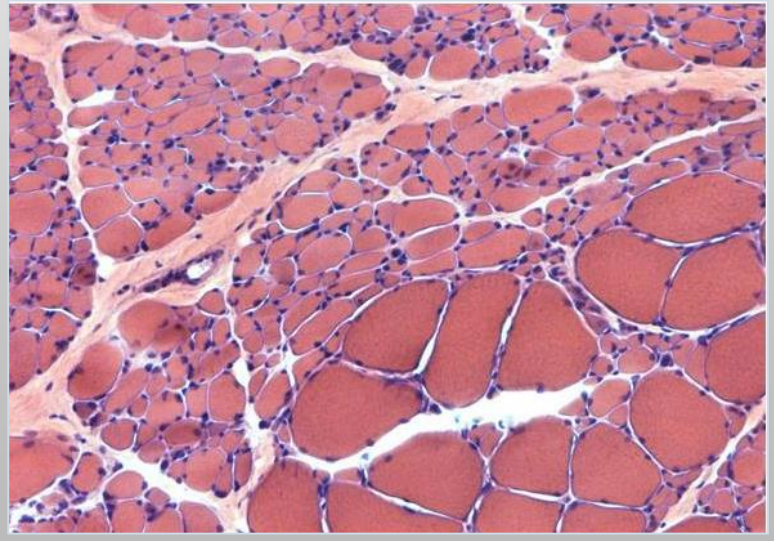
ATROFIA NEUROGENA



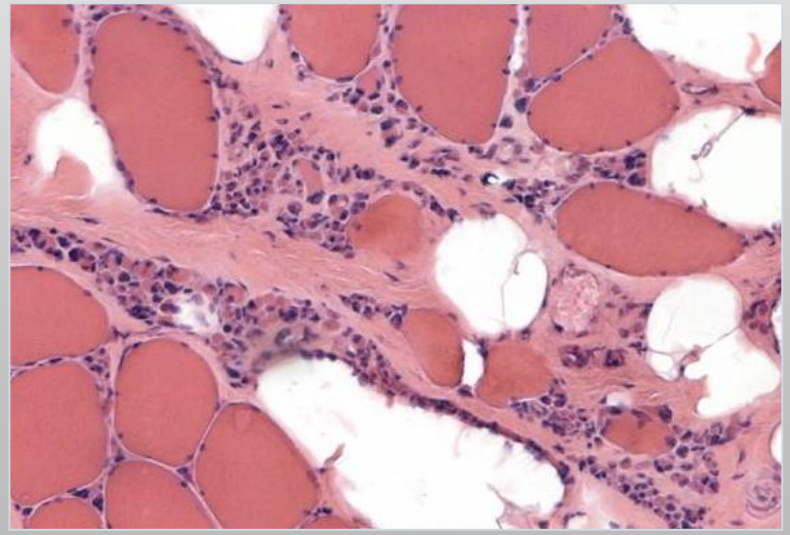
SLA



SLA

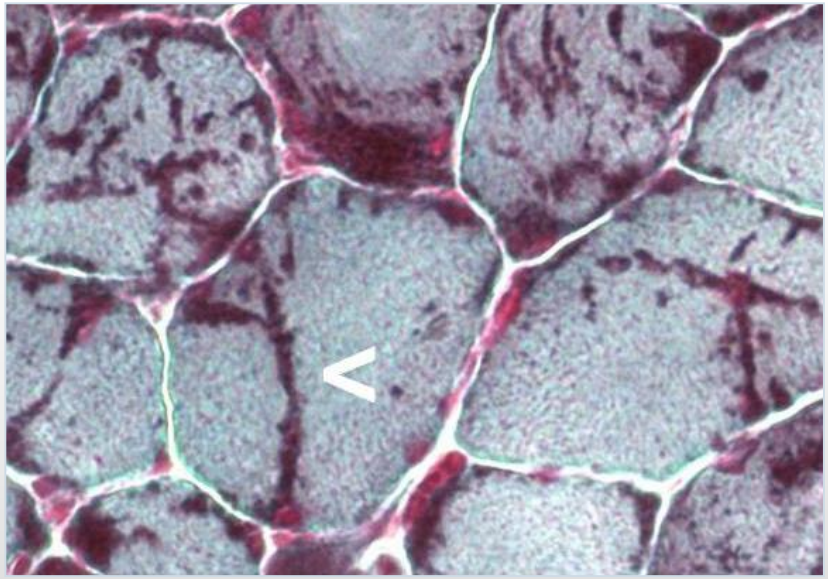


SMA-II

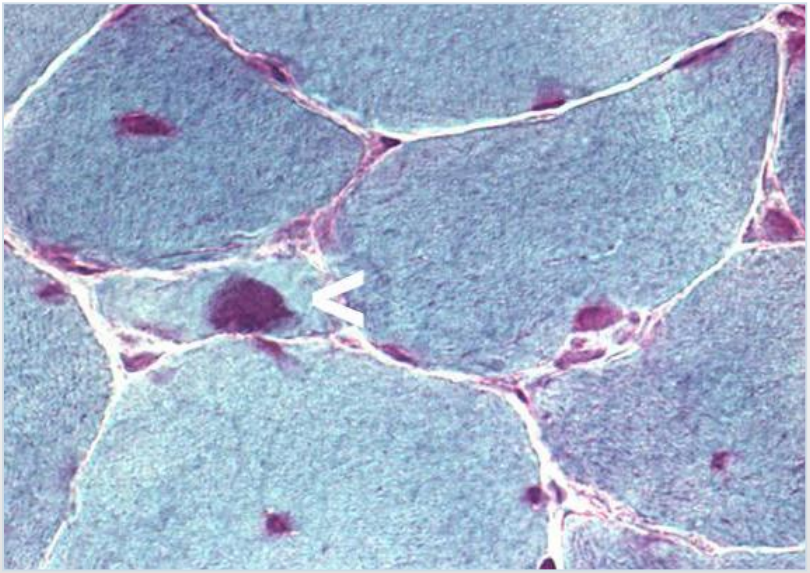


SMA-III

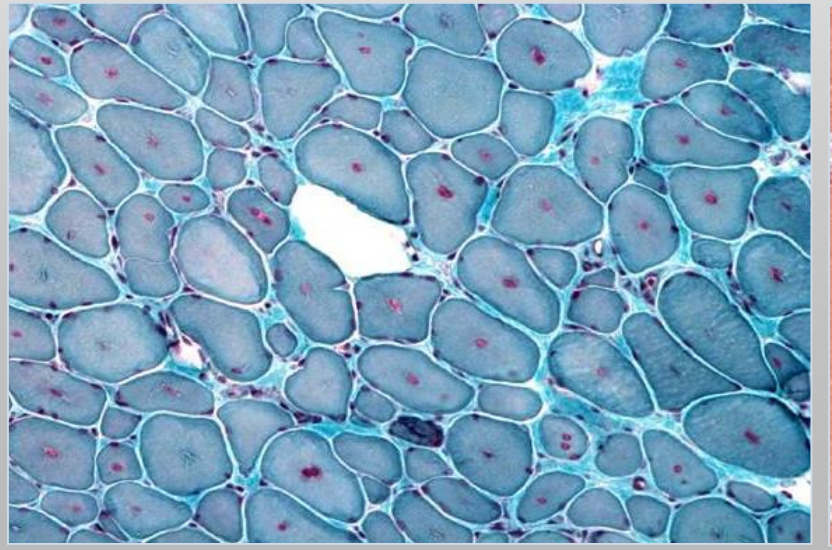
MIOPATIE CONGENITE



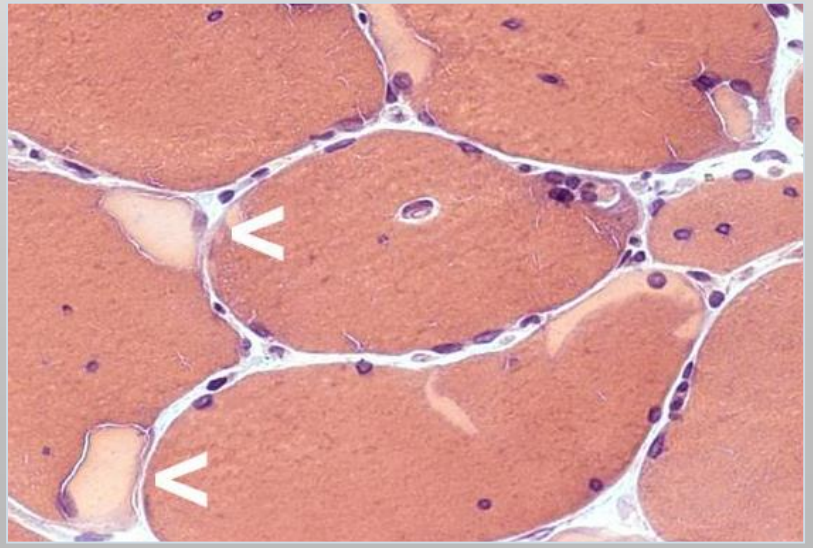
Corpi nemalinici



Corpi citoplasmatici

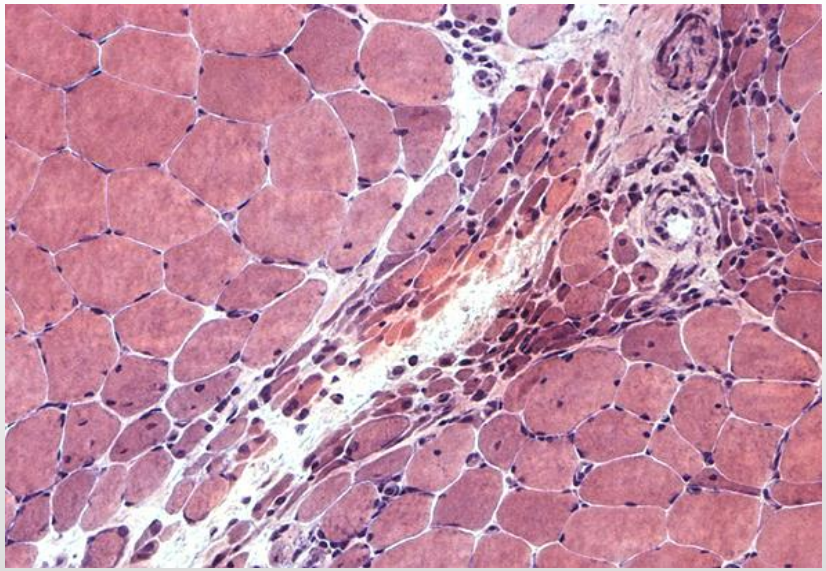


Centronucleari

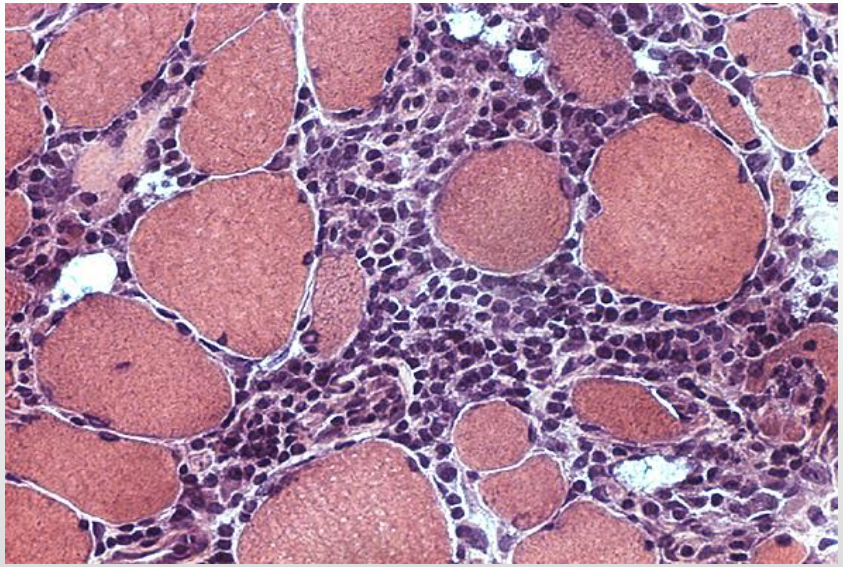


Corpi ialini

MIOPATIE INFIAMMATORIE



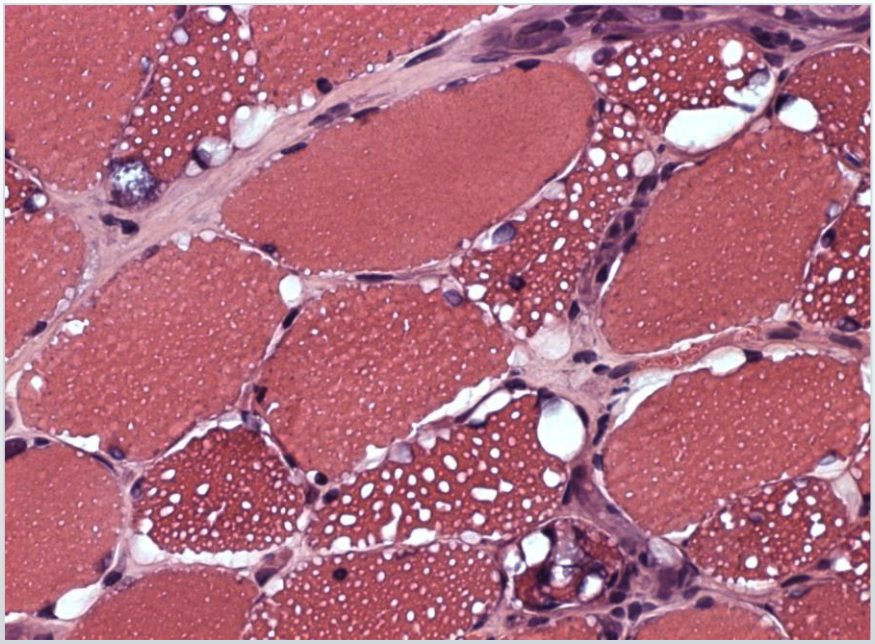
Dermatomiosite

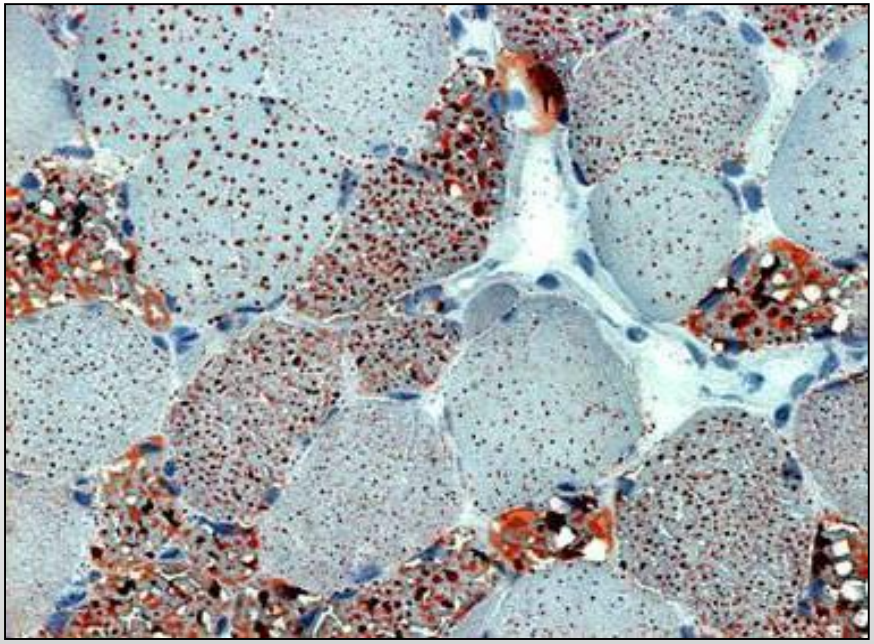


Polimiosite

- Cellule infiammatorie composte da linfociti, plasmacellule, cellule eosinofiliche
- Necrosi e fagocitosi delle fibre muscolari
- Fibre rigeneranti con citoplasma basofilo, nuclei vescicolari con nucleoli prominenti
- Ipotrofia delle fibre muscolari
- Atrofia delle fibre perifascicolari

MIOPATIE DA ACCUMULO DI LIPIDI (LSM)

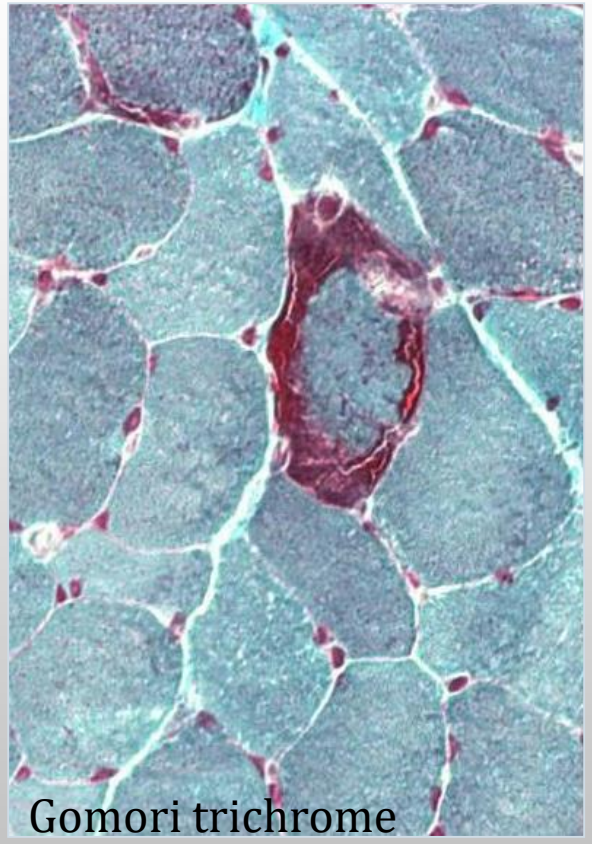




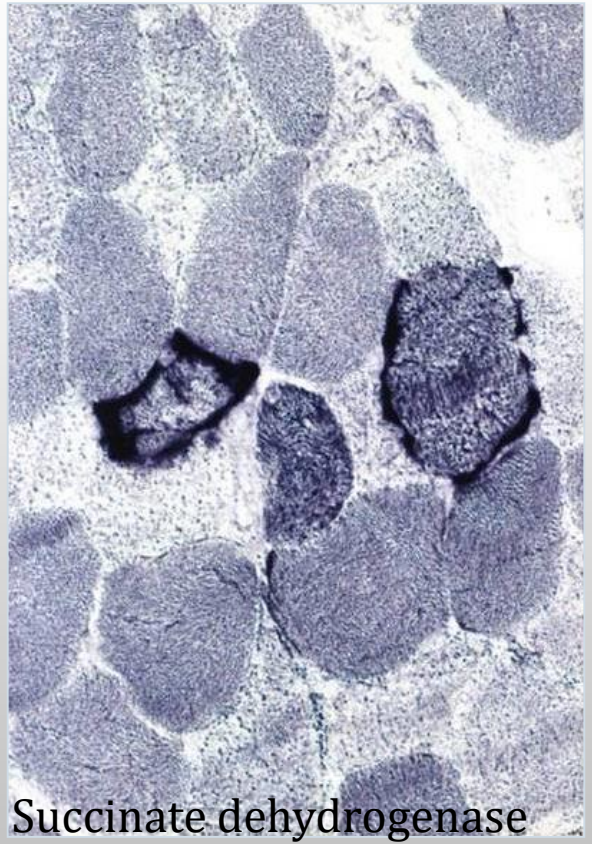
Possibili difetti molecolari:

- *OCTN2*
- *ETFDH*
- *CGI58*
- *PNPLA2*

MIOPATIE MITOCONDRIALI



Gomori trichrome



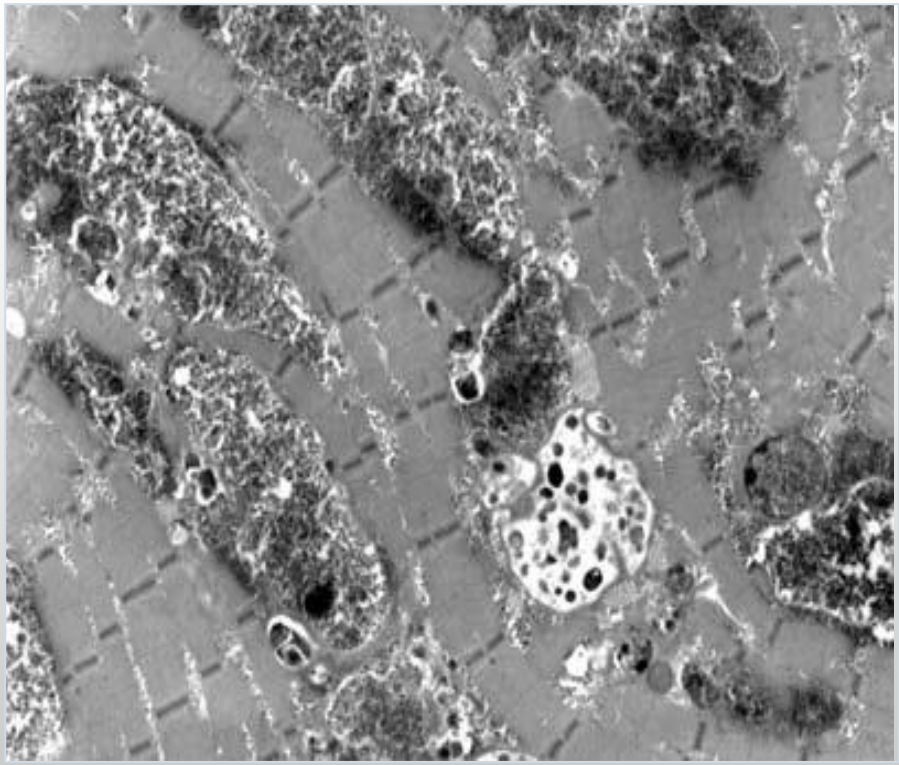
Succinate dehydrogenase

Cytochrome oxidase

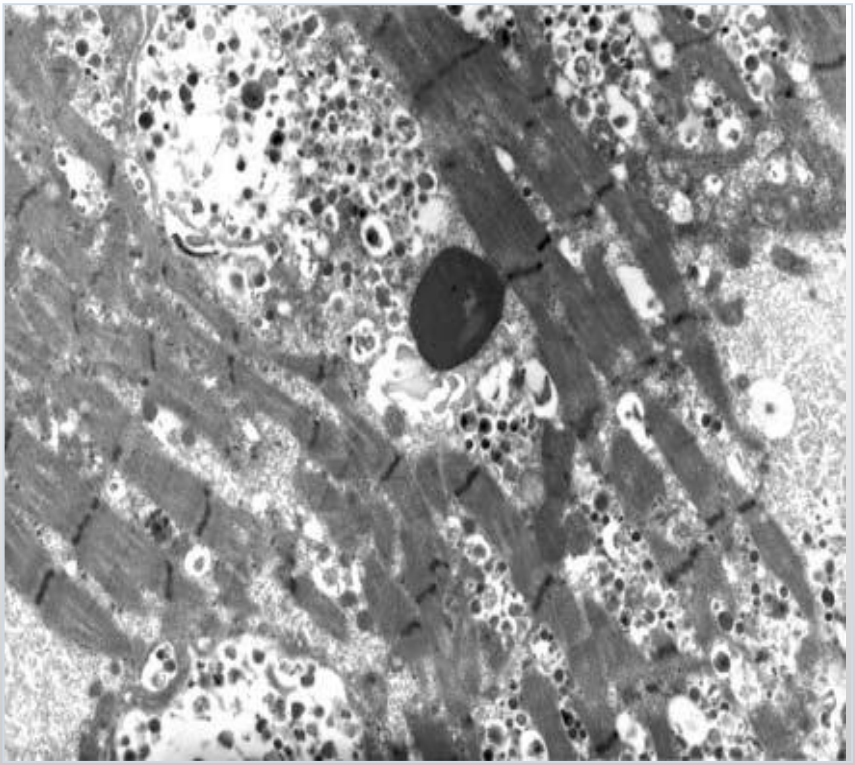
Ragged-red fibers

COX deficiency

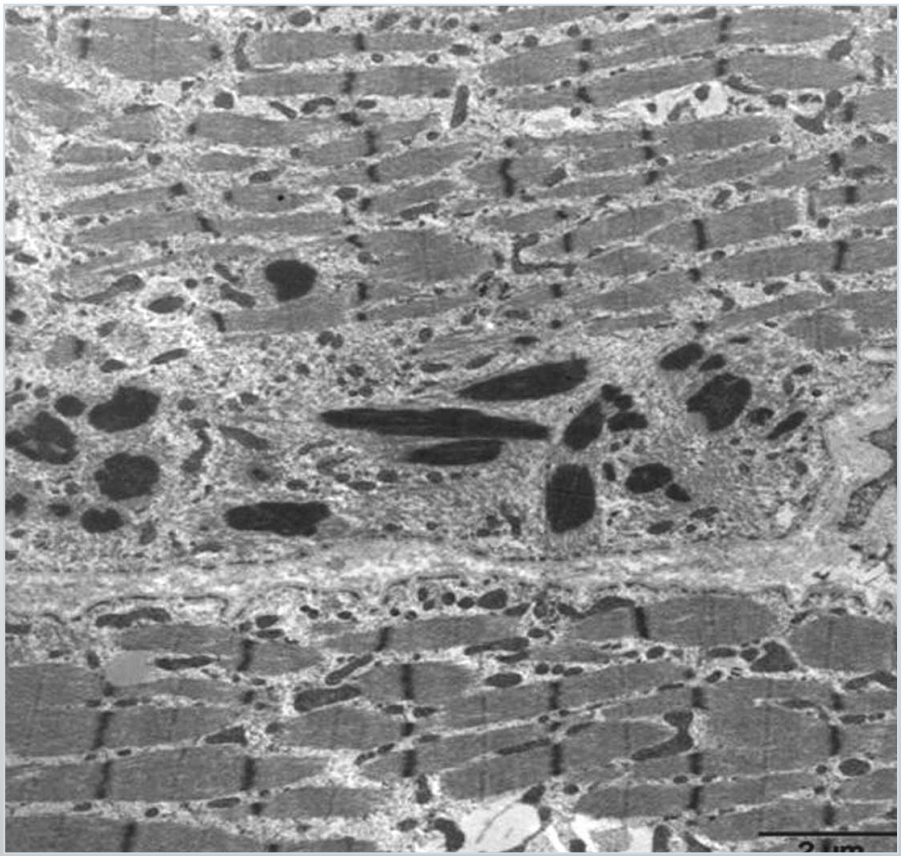
Microscopia elettronica



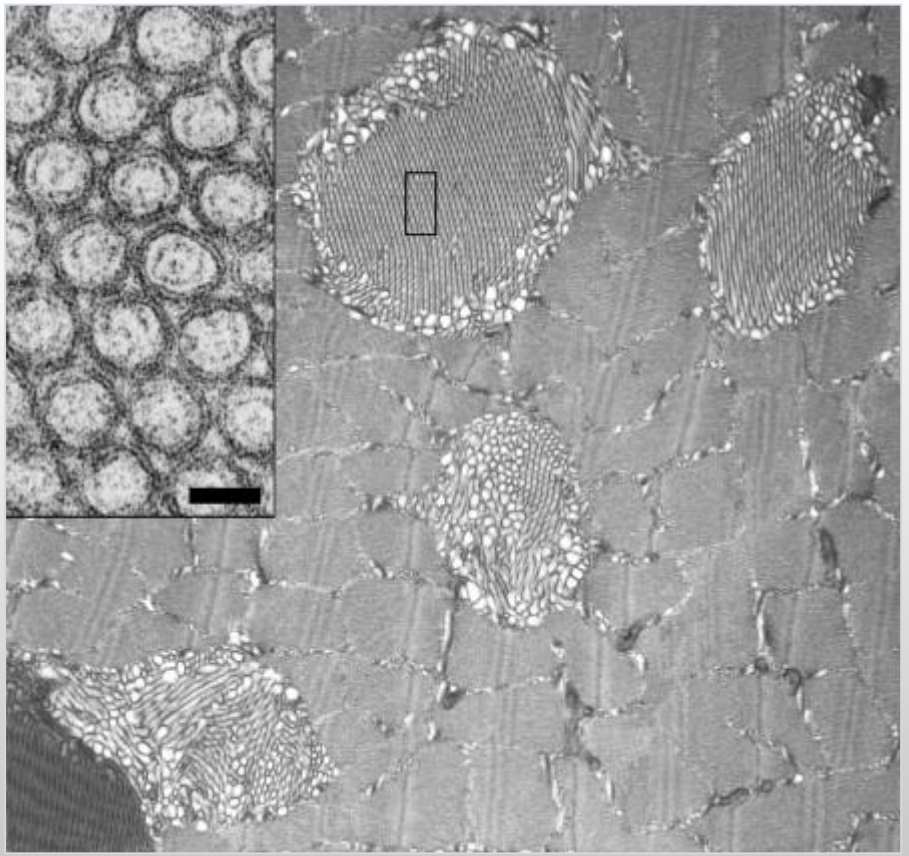
GSDII: vacuoli contenti aggregati di particelle di glicogeno



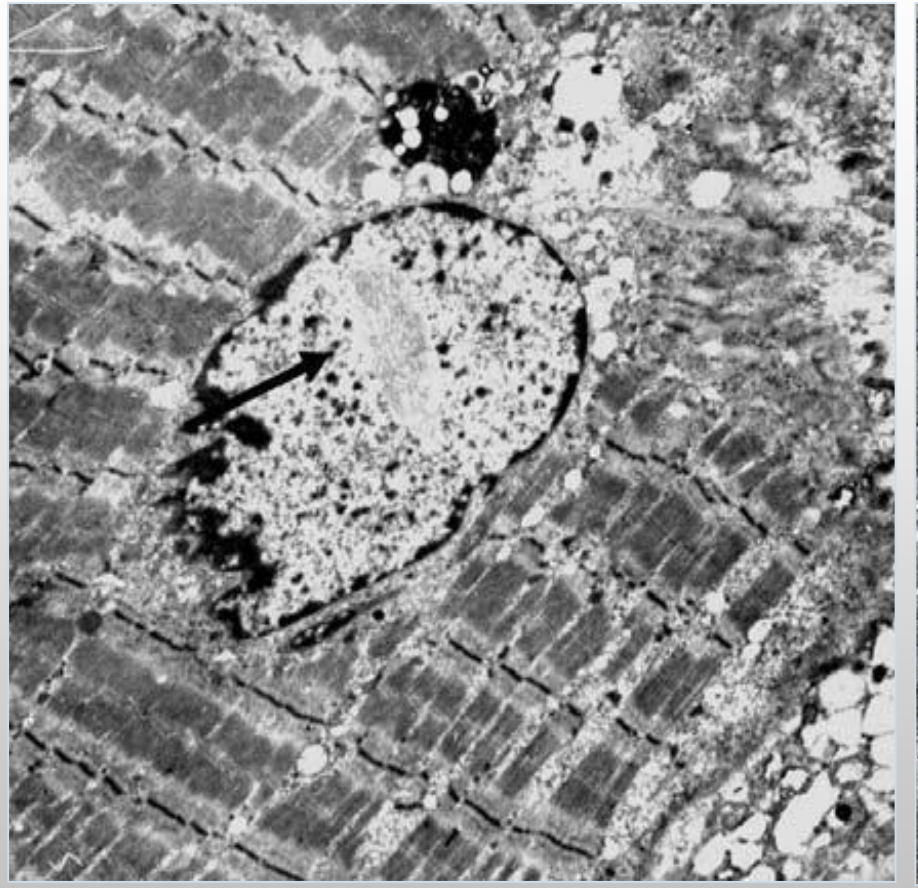
MALATTIA DI DANON: vacuoli autofagici



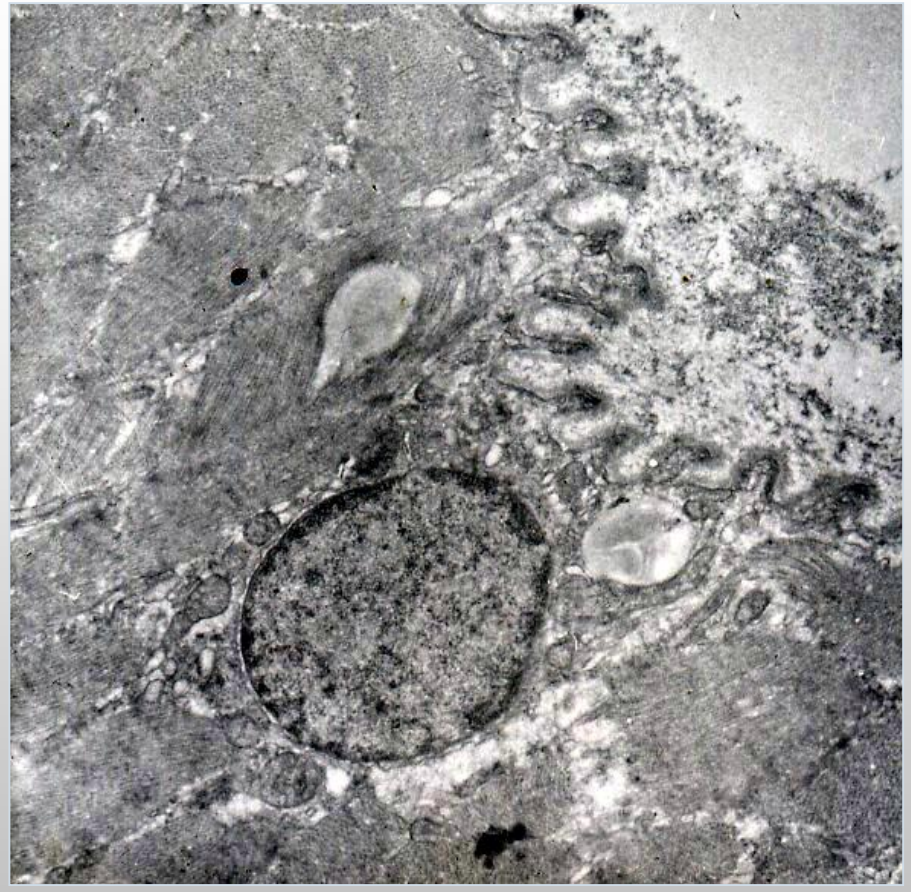
Miopatia congenita nemalinica: corpi nemalinici a forma di bastoncini



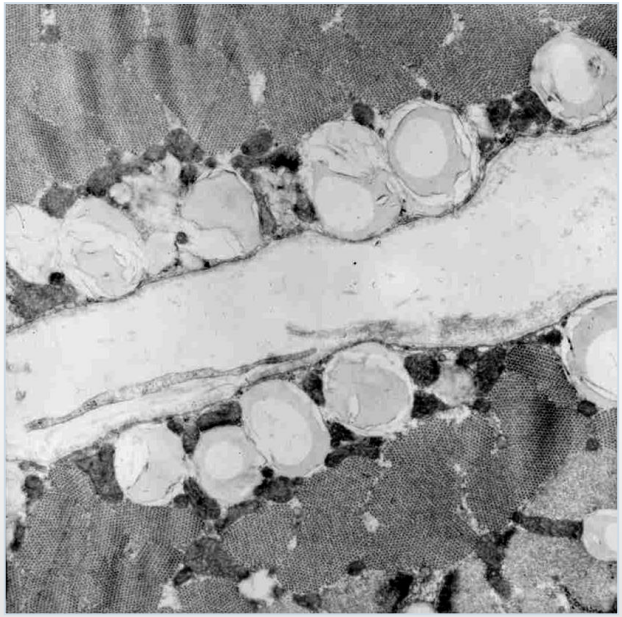
Miopatia congenita con aggregati tubulari

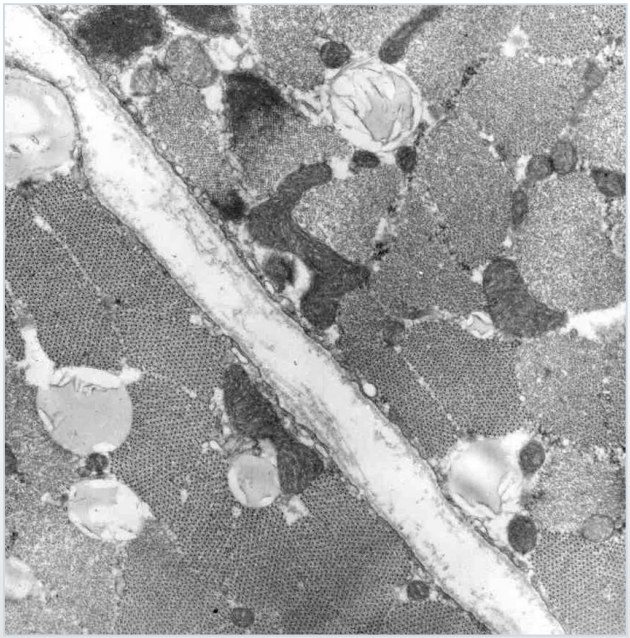


IBM: nucleo contenente inclusioni filamentose

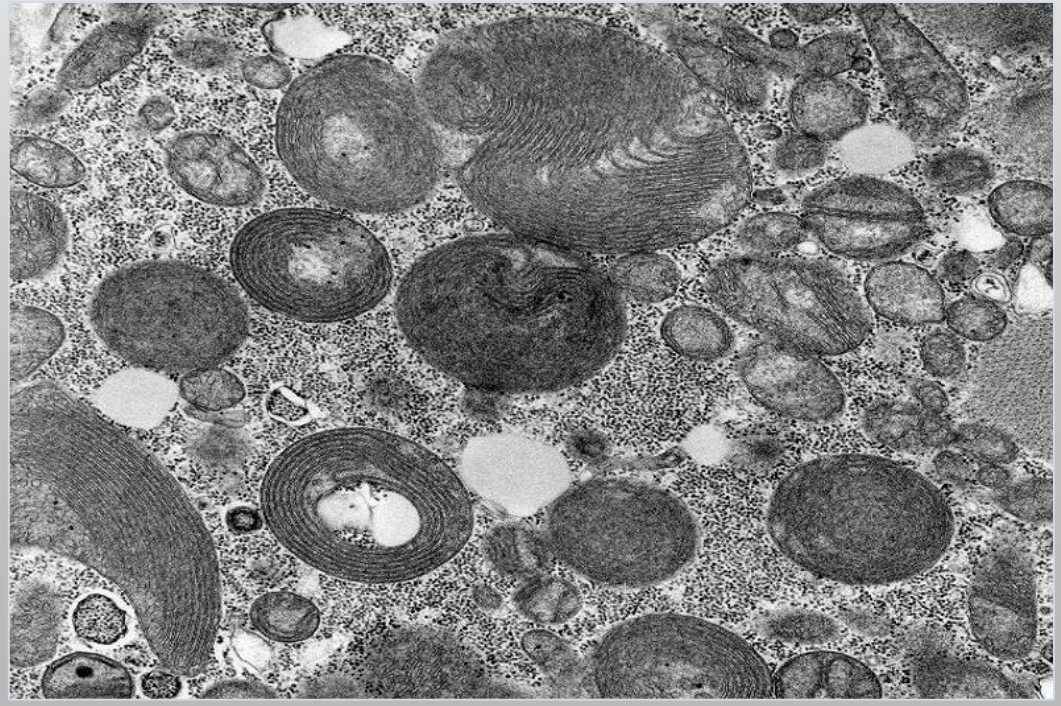


Polimiosite: macrofagi





Neutral lipid storage disease (NLSD): Goccioline lipidiche subsarcolemmali

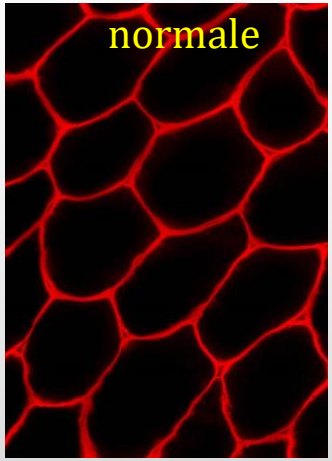


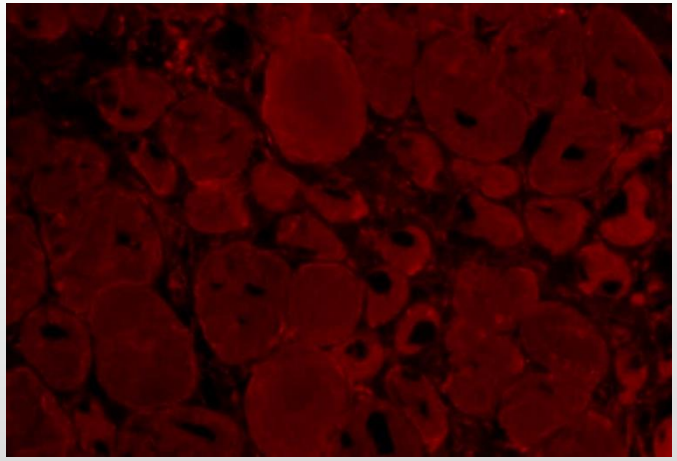
Miopatia mitocondriale: creste mitocondriali anormali

Immunoistochimica muscolare

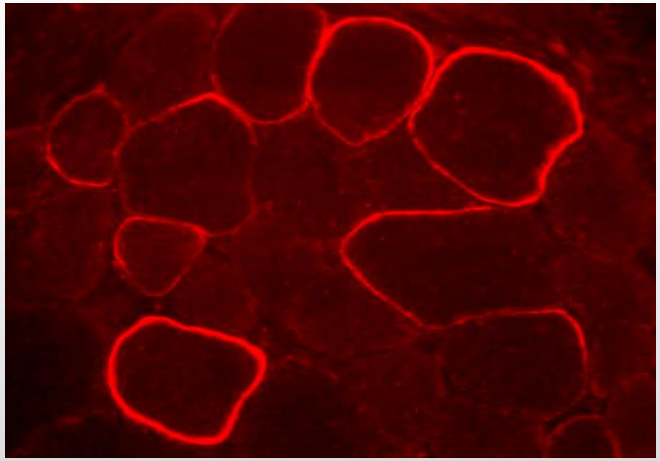
DISTROFIA DI DUCHENNE (DMD), CARRIERS

IHC DISTROFINA

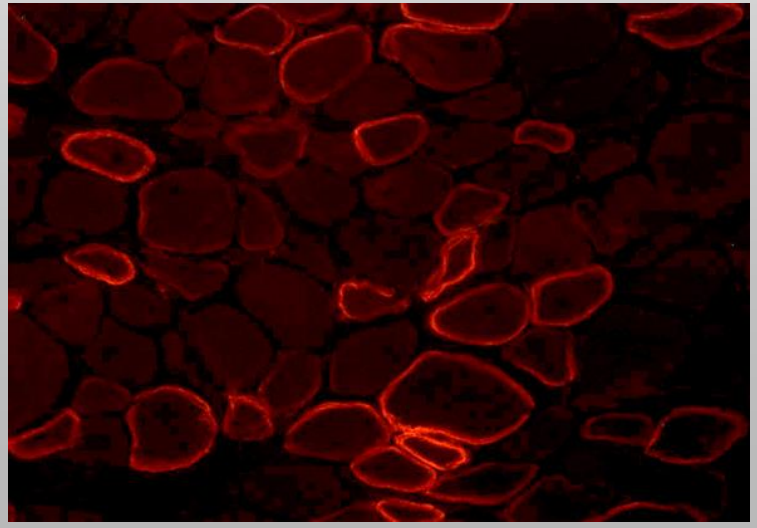




DMD: reazione assente

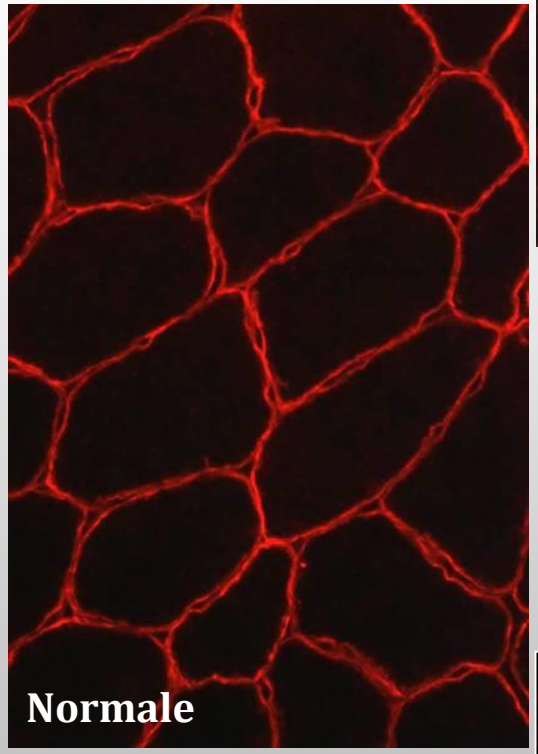


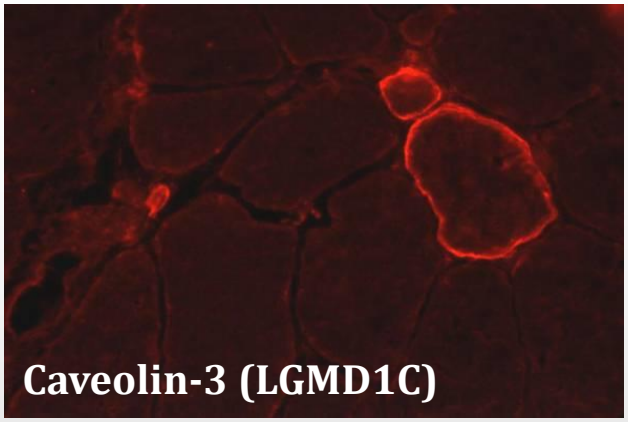
DMD: fibre “revertanti”

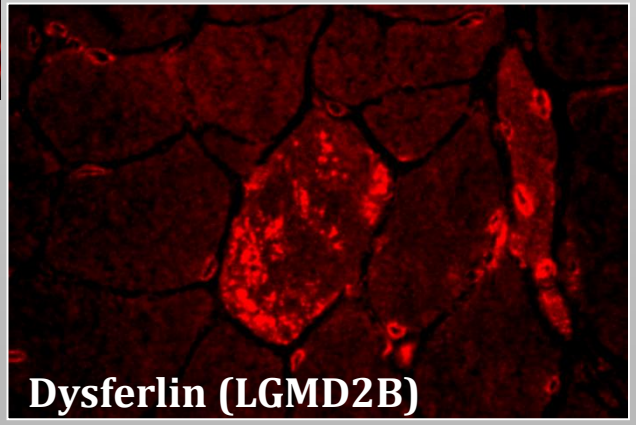


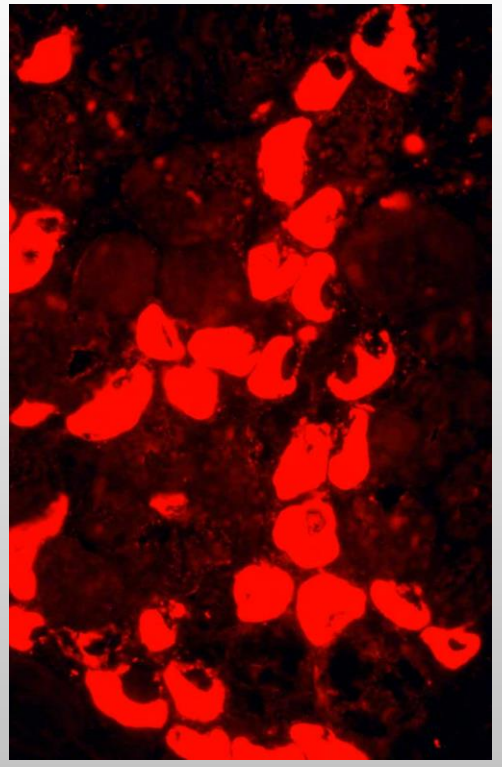
Fibre positive e negative alla distrofina (pattern a mosaico) in carrier DMD

DISTROFIA MUSCOLARE DEI CINGOLI



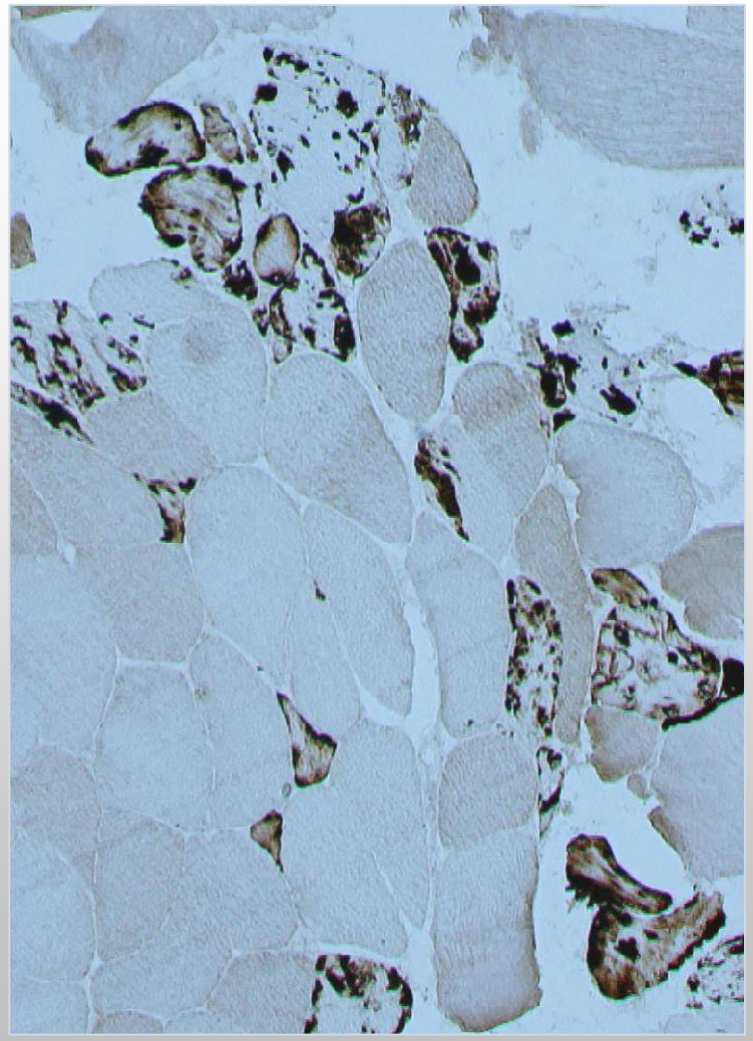




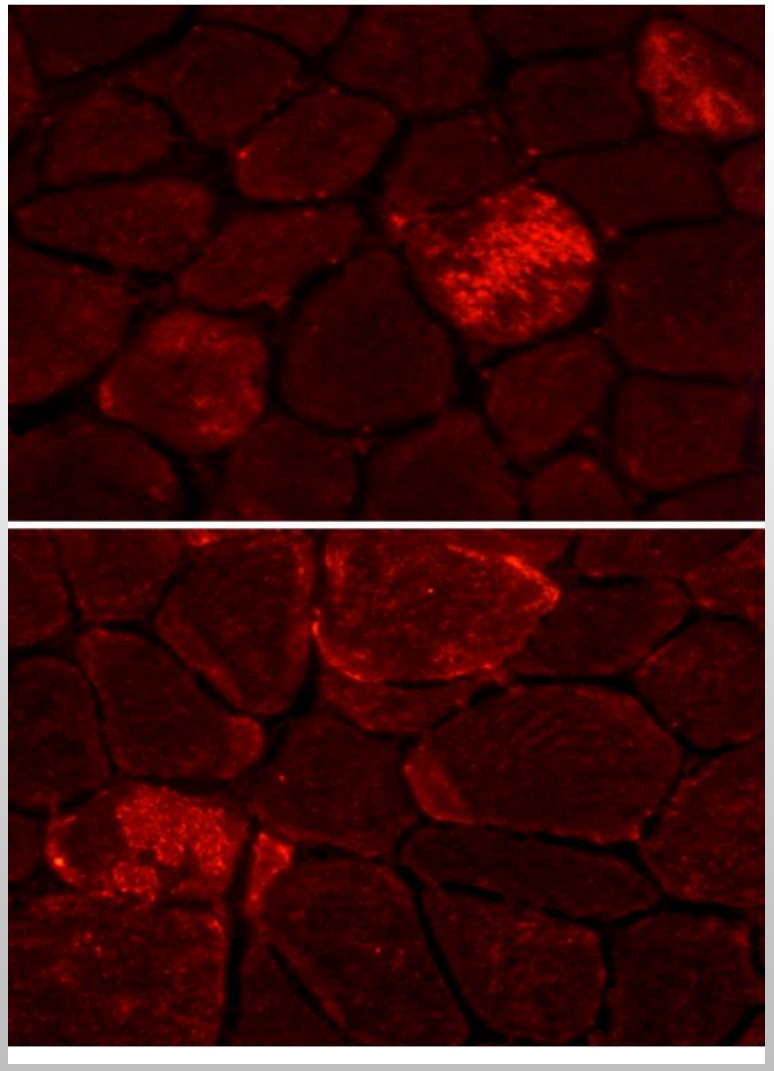


Miosina fetale in fibre rigeneranti

MIOPATIE CON AGGREGATI PROTEICI



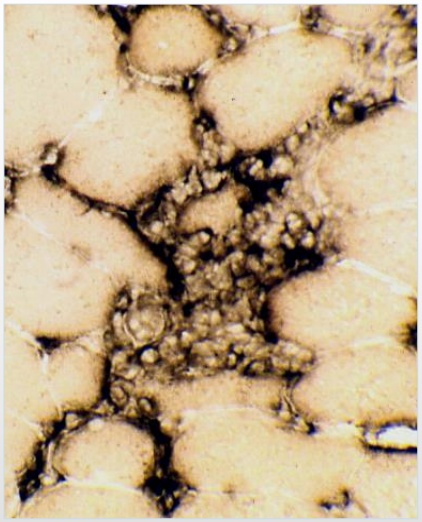
Accumulo di miotilina
in LGMD1A



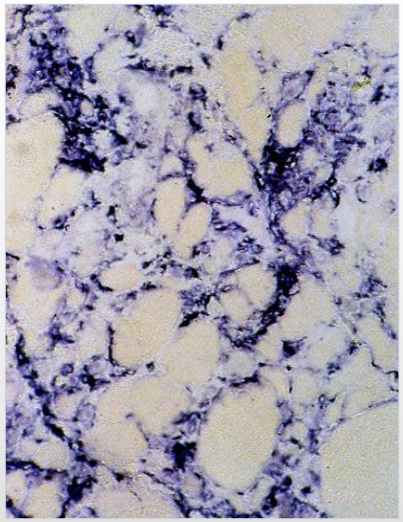
Accumulo di Desmina
in miopatia miofibrillare

REAZIONI INFIAMMATORIE

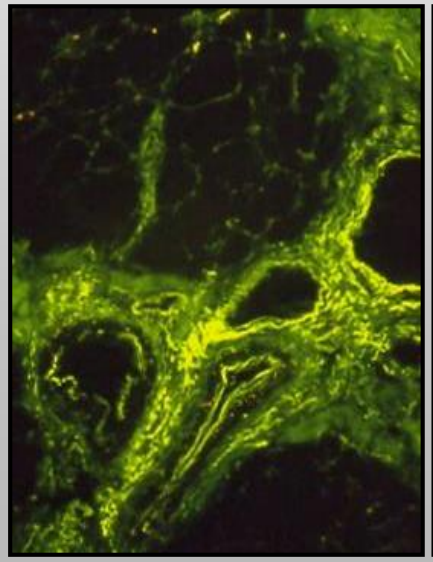
Polimiosite



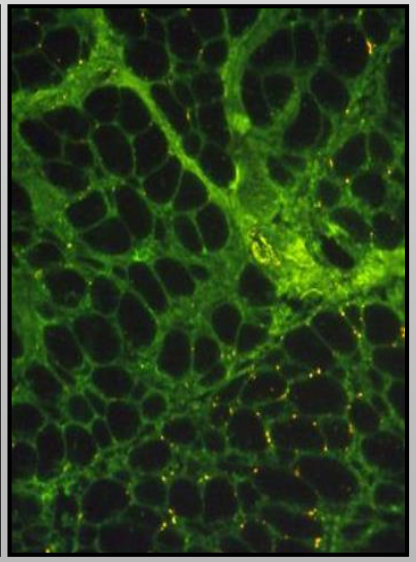
CD8 suppressor
T lymphocytes



Macrophages

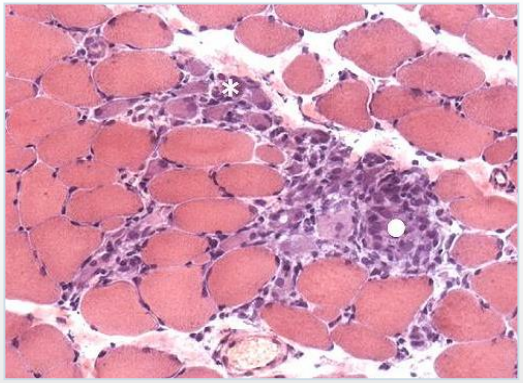


IgG

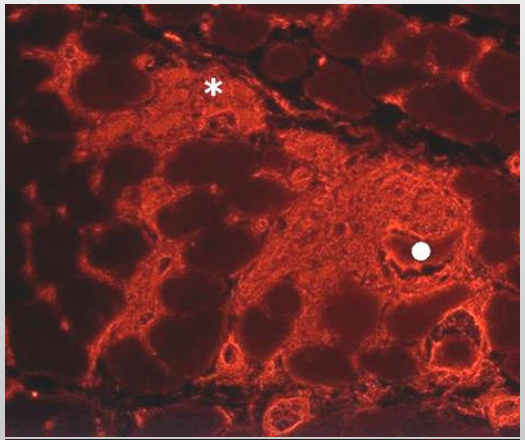


C3 complement

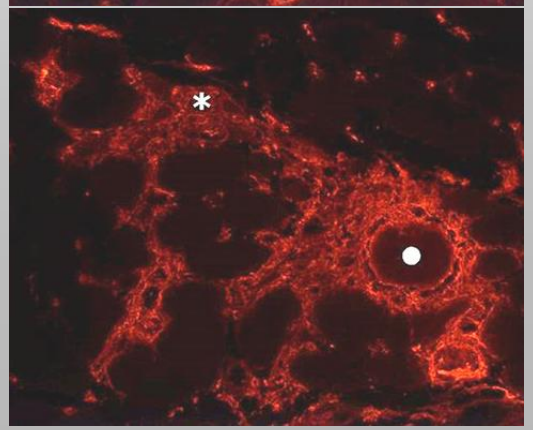
Distrofia muscolare dei cingoli 2A



H&E



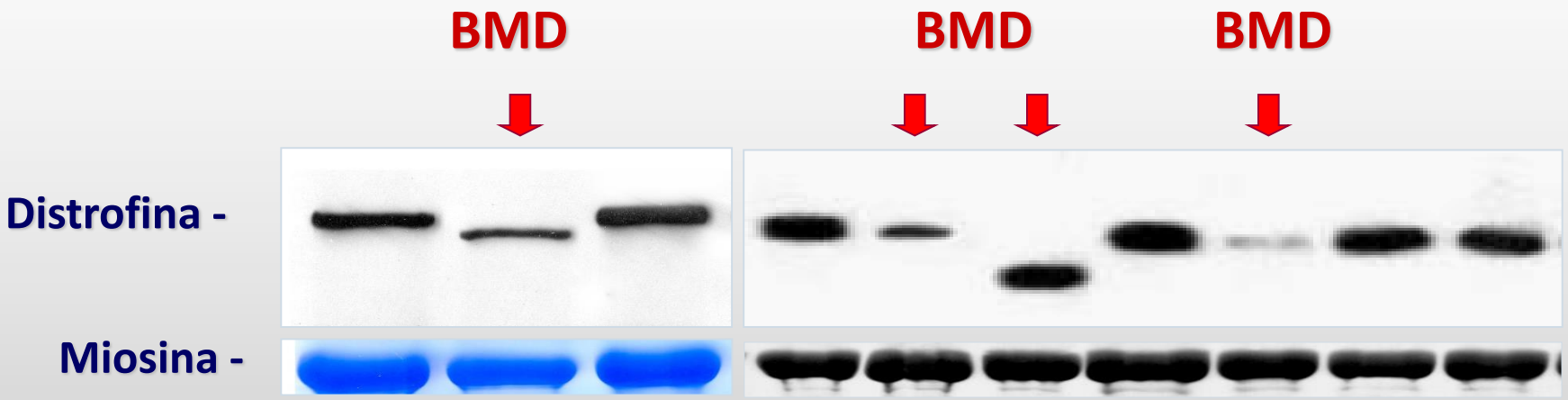
MHC class I



Macrophages

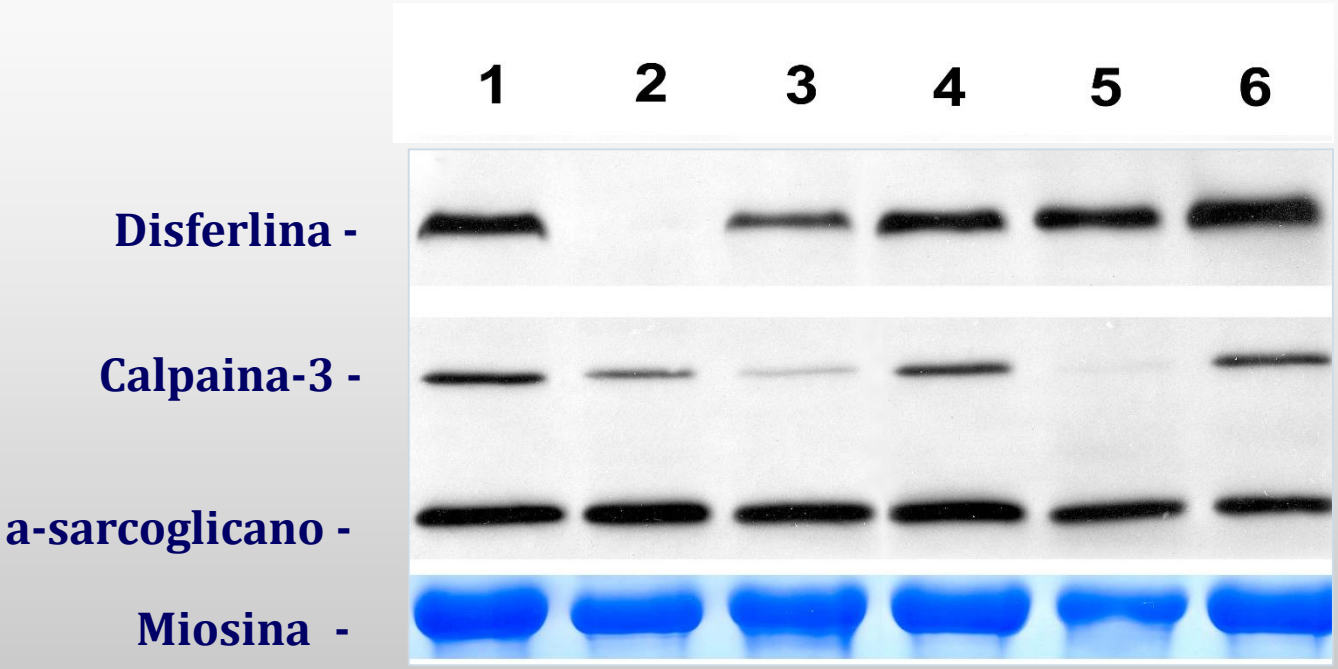
Immunoblotting muscolare

IMMUNOBLOTTING DELLA PROTEINA DISTROFINA IN PAZIENTI AFFETTI DA BMD



Nei casi di BMD la banda della proteina distrofina appare di un peso molecolare differente e/o con quantità ridotte

Immunoblotting di proteine che causano la distrofia dei cingoli (LGMD)



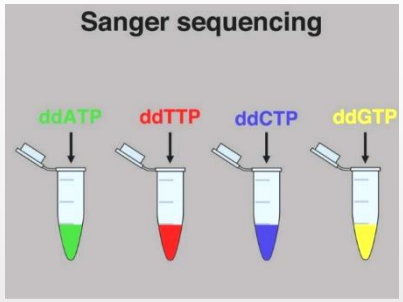
Lane 2: difetto Disferlina (LGMD2B)
Lane 3, 5: difetto Calpaina-3 (LGMD2A)

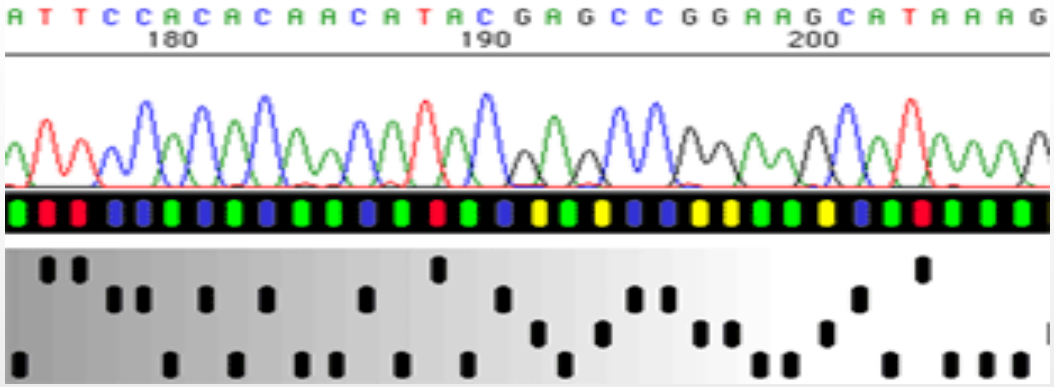
Analisi del DNA
(Sanger sequencing, NSG)

ANALISI MOLECOLARE SU DNA

- **Distrofie miotoniche (DM1, DM2):** espansione nucleotidica delle triplette
- **Distrofia Facio-scapolo-omerale:** contrazione del frammento D4Z4
- **Distrofie Duchenne/Becker e donne carriers:** delezione e duplicazione degli esoni e mutazioni puntiformi nel gene distrofina (MLPA, Sanger sequencing, NGS)
- **Distrofie muscolari dei cingoli:** Sanger sequencing, NGS
- NGS per geni conosciuti
- Exome sequencing per geni sconosciuti

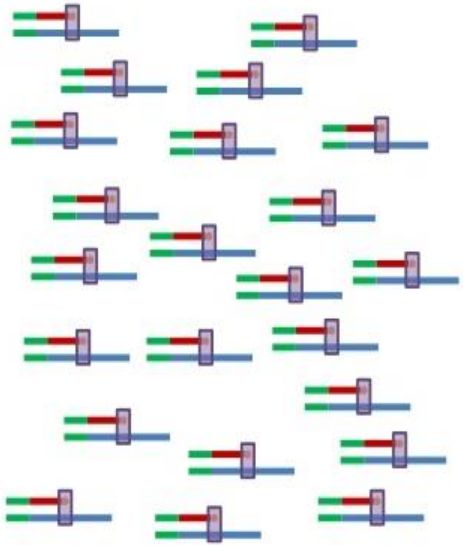
SANGER SEQUENCING vs NGS





What is "next-generation" sequencing?

Massively **Parallel:**



-- first-generation sequencers: –

Sanger sequencer: 384 samples per single batch

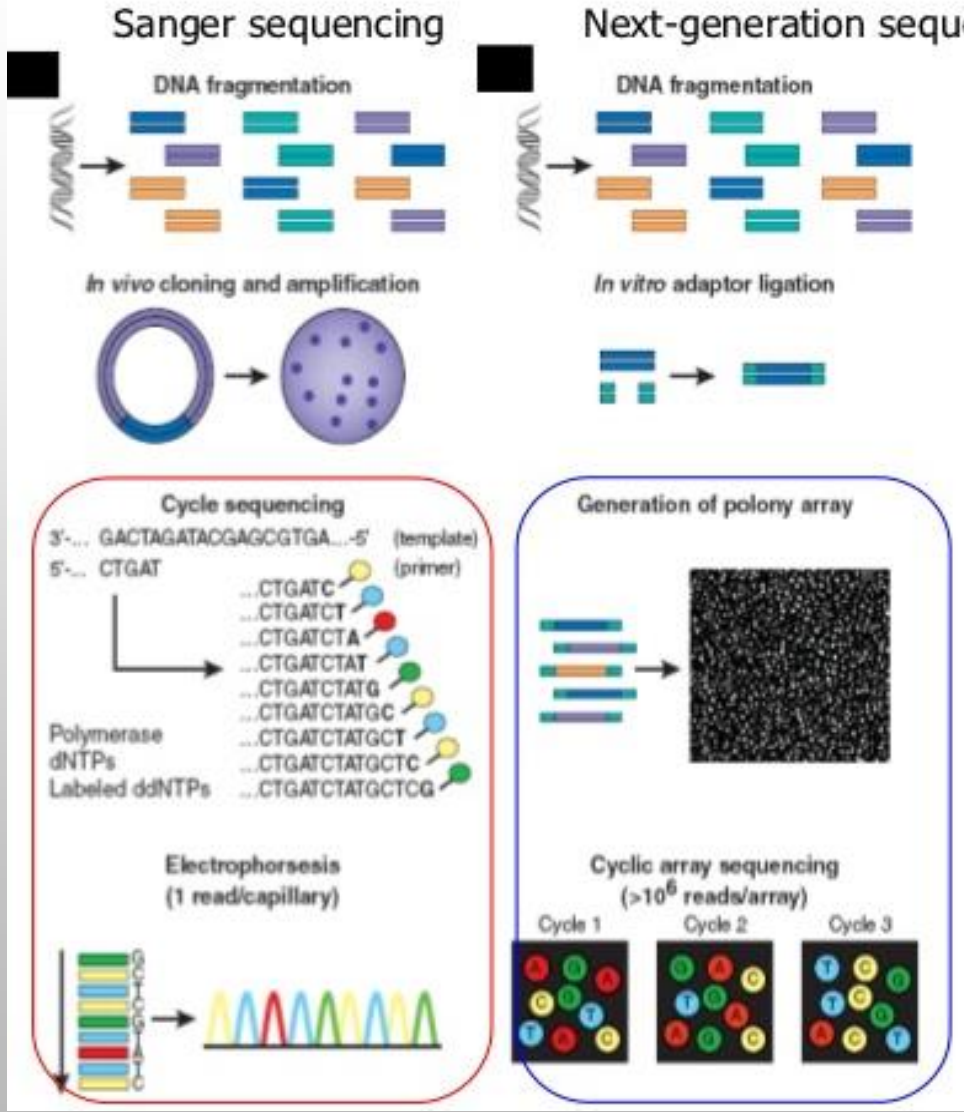
-- next-generation sequencers: --

Illumina, SOLiD sequencer: billions per single batch, ~3 million fold increase in throughput!

Next-Generation Sequencing

- Sequencing is a method for determining the exact order of nucleotides in a given DNA or RNA sequence.
- Next-generation sequencing (NGS) platforms perform massively parallel sequencing, during which millions of DNA fragments are sequenced in unison.
  - Rapid (sequence an entire genome in less than one day)
  - Low cost in comparison to traditional techniques (Sanger sequencing)
- NGS platforms include:
  - Ion Torrent PGM™ (LifeTechnologies, Carlsbad, CA)
  - MiSeq™ (Illumina, San Diego, CA)
  - Several others not discussed

SANGER SEQUENCING vs NGS



Advantages:

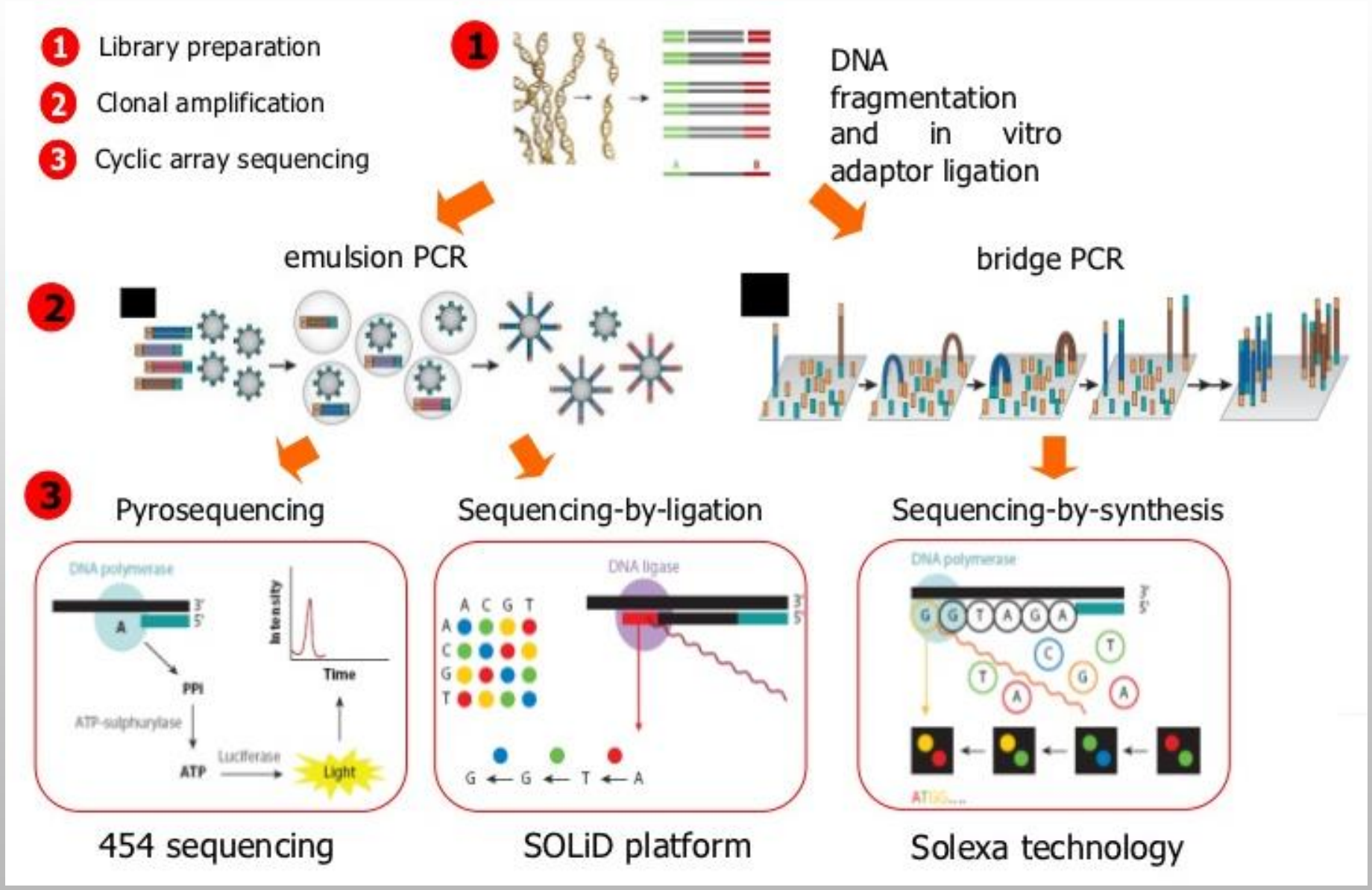
- Construction of a sequencing library → clonal amplification to generate sequencing features

✓ No in vivo cloning, transformation, colony picking...

- Array-based sequencing

✓ Higher degree of parallelism than capillary-based sequencing

NEXT GENERATION SEQUENCING



CONCLUSIONI

PREVENZIONE: I test genetici sono oggi molto avanzati; NGS richiede un'attenta interpretazione dei risultati da parte di un bioinformatico.

DIAGNOSI: L'analisi delle proteine muscolari è molto utile nelle distrofie dei cingoli.

ESAMI IMMUNOLOGICI: Richiedono varie tecniche e gruppi di anticorpi.

TERAPIA: La biochimica muscolare è utile nelle malattie metaboliche (da accumulo di lipidi, glicogenosi e disturbi mitocondriali) che rispondono a diete mirate o supplementazione con carnitina e riboflavina.

Le miopatie infiammatorie vanno trattate secondo protocolli ottimizzati.

La malattia di Pompe infantile e late-onset sono suscettibili ad ERT (rimpiazzo di alglucosidasi alfa ricombinante)