SESSIÓ INAUGURAL SCAP
Acadèmia de Ciències Mèdiques
Barcelona, 21 d'octubre de 2021

Myoglobinopathy: the globin disease of striated muscle

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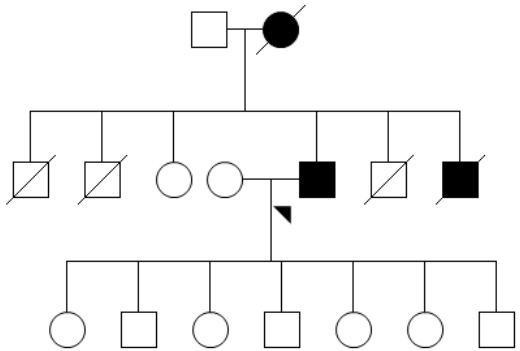
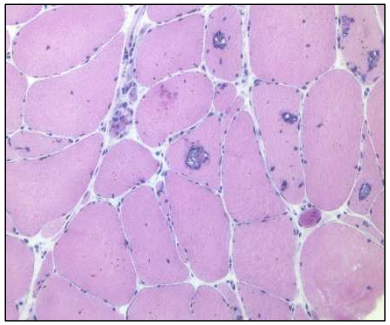
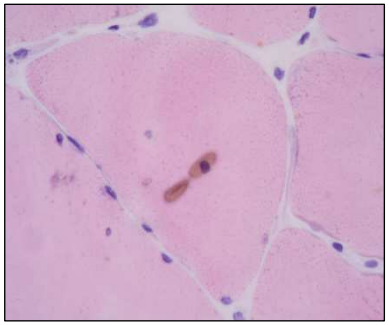
UNIVERSITAT AUTÒNOMA DE BARCELONA

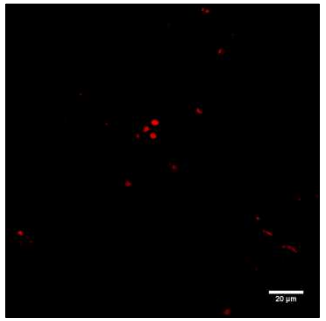
The beginning of a fascinating story

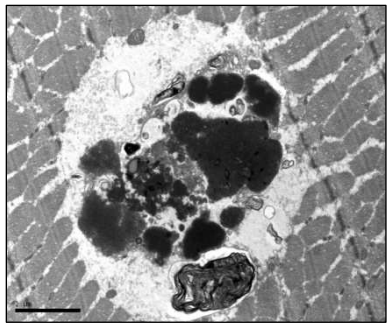


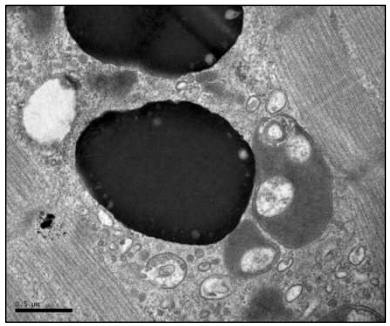
- A 51-year-old man
- Onset at 36 years
- Proximal LL and axial weakness
- Respiratory insufficiency
- Dilated cardiomyopathy





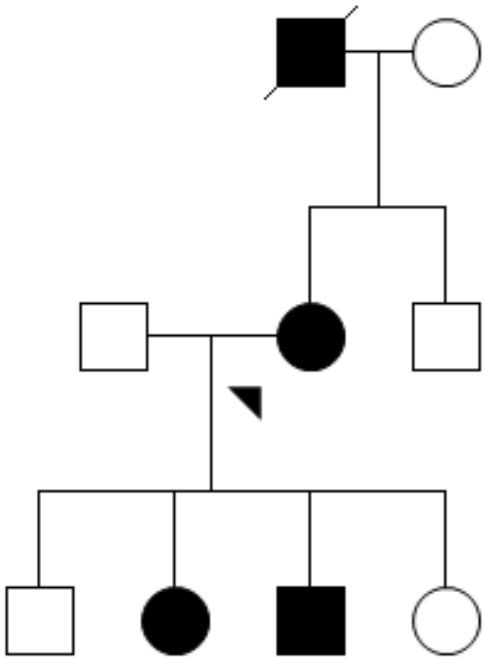


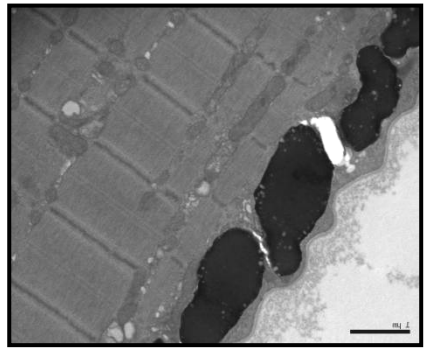




Myopathy with rimmed vacuoles and pigment deposition

9 years later, a second family with exactly the same disease





Journal of the Neurological Sciences, 1980, 47: 171–190

A NEW TYPE OF HEREDITARY DISTAL MYOPATHY WITH CHARACTERISTIC SARCOPLASMIC BODIES AND INTERMEDIATE (SKELETIN) FILAMENTS

LARS EDSTRÖM[1], LARS-ERIC THORNELL[2] and ANDERS ERIKSSON[3]

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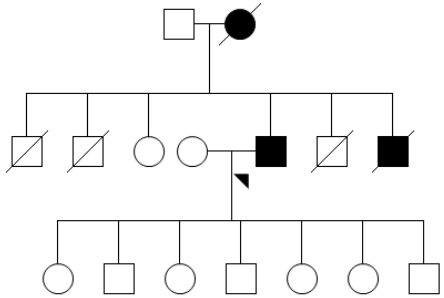
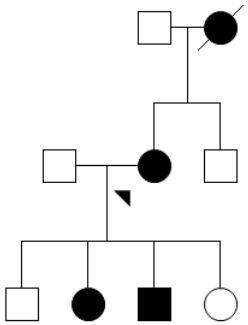
Uncovering the molecular cause of the disease in two Spanish families

Family 1



Family 2



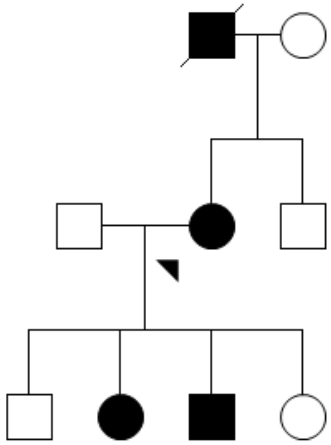
- NGS to sequence a panel of 254 neuromuscular disease genes.
- Two affected individuals from family 1 and one affected and one non-affected from family 2 were exome sequenced, variants with minor allele frequecy >1% filtered out. Heterozygous variants were selected for the analysis according to a dominant pattern of inheritance.
- Genes with a promoter showing more than 1,000-fold enriched expression in skeletal muscle were selected as candidate disease genes. **A resulting list of 82 genes was screened for variants.**

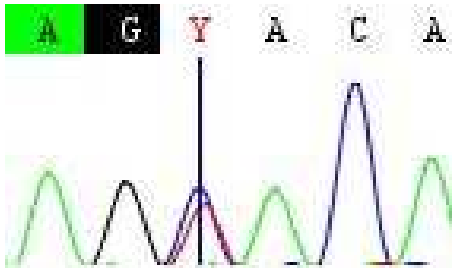
Myoglobinopathy

MB, p.His98Tyr

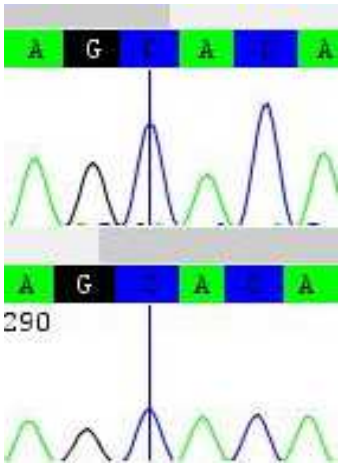
RESULTS

Family 1

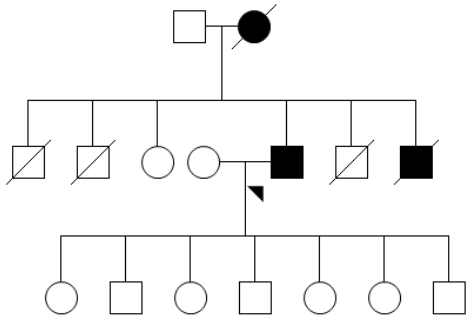


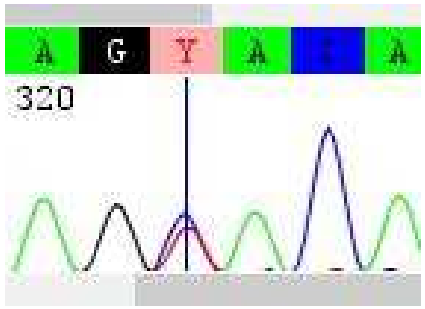


Control



Family 2

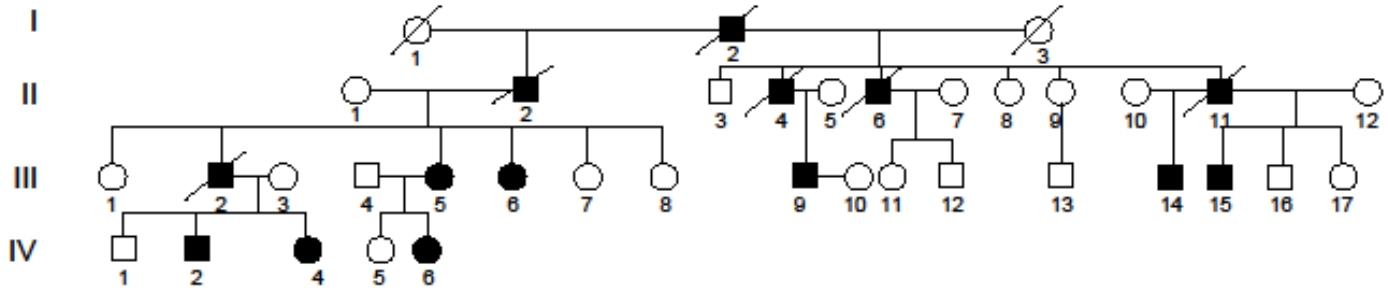




MB p.His98Tyr variant is not present in 1000 genomes, ExAC or GnomAD and involves a well conserved residue among the species, up to zebrafish.

H. sapiens	98	IKPLAQSHATK**H**KIPVKYLEFIS
Mutant	98	IKPLAQSHATK**Y**KIPVKYLEFIS
P. troglodytes	98	IKPLAQSHATK**H**KIPVKYLEFIS
M. mulatta	98	IKPLAQSHATK**H**KIPVKYLEFIS
F. catus	98	IKPLAQSHATK**H**KIPVKYLEFIS
M. musculus	98	IKPLAQSHATK**H**KIPVKYLEFIS
G. gallus	98	IKPLAQSHATK**H**KIPVKYLEFIS
D. rerio	98	IKPLAQSHATK**H**KIPVKYLEFIS

Identification of disease causative gene in the original Swedish family



Markers	III:1	IV:1	IV:2	IV:3	IV:4	III:3	III:4	III:5	IV:5	IV:6	III:6	III:7	III:8	II:3	II:5	III:9	III:10	II:7	III:11	III:12	II:8	III:13	II:11	III:14	II:12	III:15	III:16	III:17
D22S427	1 7	1 5	4 7	1 4	5 7	4 5	1 4	1 6	4 6	1 1	1 6	1 7	1 7	1 1	4 4	4 6	0 0	1 7	4 7	4 7	1 1	1 4	4 6	1 6	4 4	4 6	4 4	4 4
D22S539	2 2	2 2	2 2	2 7	2 2	2 6	2 1	2 2	2 2	1 2	2 2	2 2	2 2	2 4	2 2	2 2	2 2	2 5	2 2	2 2	2 4	2 4	2 2	2 2	2 5	2 5	2 2	2 5
D22S1174	5 4	5 6	4 6	3 5	4 6	6 4	5 4	5 4	5 4	4 2	4 4	5 4	5 4	5 4	5 4	5 4	5 4	5 1	5 1	5 1	5 5	5 5	4 5	1 4	5 5	4 5	5 5	5 5
D22S315	5 4	5 2	5 2	4 4	5 4	2 4	0 0	5 5	0 0	0 0	3 5	5 4	5 4	3 3	4 5	5 5	3 5	3 3	3 3	3 3	3 5	3 5	5 3	4 5	4 4	5 4	3 4	3 4
D22S1154	4 1	2 3	4 3	1 1	4 2	3 2	4 1	4 2	4 2	4 4	4 4	2 1	2 1	1 1	1 1	1 4	1 1	1 3	4 4	4 4	1 4	1 4	4 1	1 4	2 3	4 2	1 2	1 3
D22S531	2 2	2 3	0 0	5 2	2 2	3 2	4 1	2 2	4 2	1 2	2 2	2 2	2 2	2 2	2 2	2 1	2 2	2 3	3 3	2 3	2 3	2 3	2 2	2 3	2 3	2 3	2 2	2 2
D22S689	3 5	3 5	4 5	5 6	4 5	4 5	4 6	3	3 5	4 5	3 5	3 5	3 5	4 4	5 5	5 5	2 4	5 6	5 6	6 6	4 6	4 5	4 5	5 5	1 5	1 5	4 5	4 5
D22S280	2 3	5 5	4 7	5 5	4 7	4 5	2 5	5	5 7	2 7	2 7	2 3	3 5	1 4	4 5	5 7	5 5	5 5	5 7	5 7	1 7	1 6	4 7	4 7	4 5	4 7	4 5	4 4
D22S685	3 5	5 6	2 4	3 3	4 2	6 3	5 5	5	5 4	5 4	5 4	3 5	3 5	1 2	4 6	6 4	1 5	2 5	3 5	3 2	2 3	2 3	1 4	6 4	2 5	2 4	1 5	1 5
D22S424	2 2	1 1	1 2	1 2	2 2	1 2	2 2	1	2 1	2 2	2 2	2 2	2 1	2 2	2 1	3 2	3 1	2 1	1 1	2 1	2 2	2 2	2 2	2 1	2 1	2 1	2 2	2 1
D22S683	1 3	1 6	1 3	5 6	5 3	3 6	4 9	1 5	1 5	4 5	3 5	1 3	1 1	4 5	4 9	4 5	0 0	8 9	8 10	8 10	4 10	4 7	5 5	7 5	2 9	2 5	5 9	5 9
D22S1173	1 3	1 1	1 1	2 0	0 1	1 1	1 1	1 1	1 1	1 1	0 0	1 3	1 3	1 1	2 3	2 1	1 1	1 2	1 2	0 0	1 1	1 1	1 1	2 1	1 3	1 1	1 3	1 3
D22S283	5 9	8 9	8 3	2 7	3 5	3 9	4 5	8 5	8 5	4 5	5 5	5 9	8 9	8 8	2 6	6 5	5 6	1 5	5 5	5 5	5 8	8 8	8 5	5 5	6 10	10 5	6 8	6 8
D22S692	1 1	4 4	2 4	1 4	2 4	2 4	1 2	4 4	4 4	1 4	1 4	1 1	1 4	2 2	1 2	1 4	2 5	1 4	2 4	2 4	2 2	2 2	2 4	2 4	2 4	2 4	2 4	2 4
D22S1177	2 4	3 4	1 4	4 5	4 1	1 3	3 4	3 4	4 4	4 4	4 4	2 4	2 4	4 4	5 3	4 3	4 2	3 4	5 4	4 4	4 4	4 3	4 5	4 3	4 3	6 6	4 3	5 3
D22S445	4 5	1 1	1 5	4 6	5 5	1 5	5 5	1 5	1 5	5 5	0 0	4 5	1 4	5 5	1 5	1 5	1 6	4 5	3 5	5 5	5 5	3 5	5 5	5 5	4 5	5 5	4 5	4 5
D22S423	4 6	3 4	3 4	3 7	3 3	4 3	5 3	4 3	3 4	3 3	3 4	6 4	6 4	1 3	3 7	4 3	3 4	3 4	3 4	3 3	3 3	3 5	1 4	4 4	4 7	4 4	1 7	1 7
D22S276	3 4	4 7	4 7	3 3	7 7	5 7	1 7	4 7	4 7	1 7	3 7	3 4	4 4	3 7	4 4	4 6	4 7	4 4	4 6	1 4	1 7	6 7	3 6	1 6	3 4	3 6	3 4	3 4

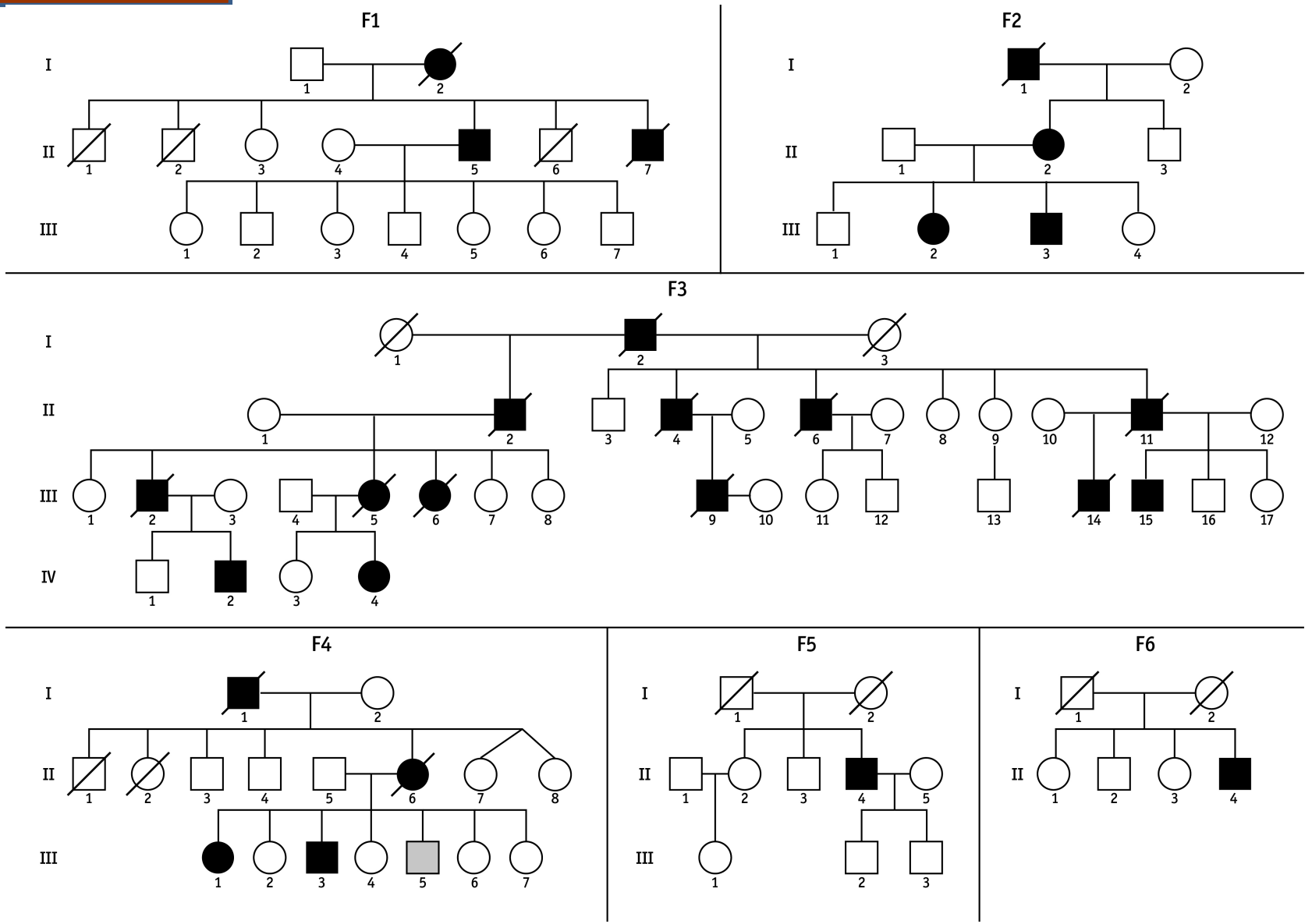
Linkage chrom 22

Identification of disease causative gene in the Swedish SBM family

- Six affected and two unaffected family members were selected for target sequencing of the entire linkage region.

- The *MB* p.His98Tyr variant was present in all 6 affected and none of the two unaffected patients.

- Subsequent analysis of the whole family showed that *MB* p.His98Tyr variant was present in all available affected patients and in none of the healthy subjects.

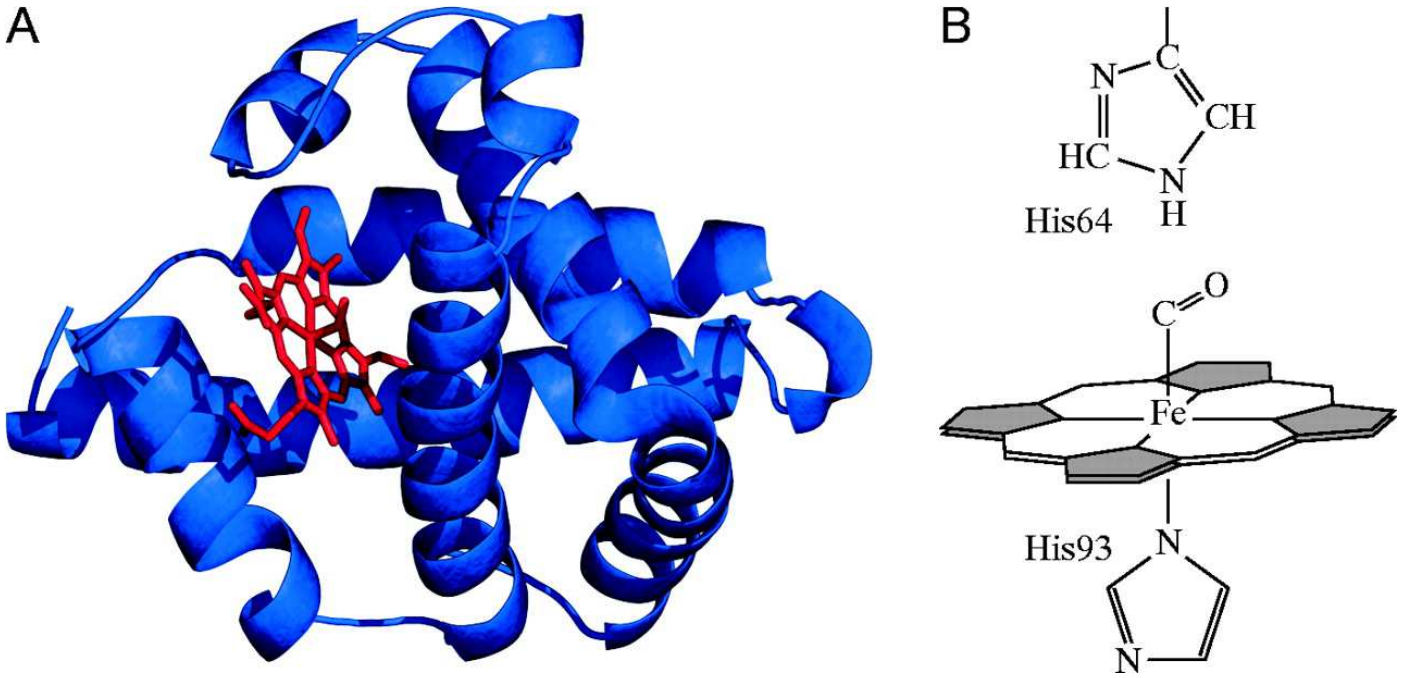


6 unrelated families carrying the same *MB* p.His98Tyr variant

Myoglobin

- Small, cytoplasmic globular hemoprotein highly expressed in cardiac and oxidative myofibers.
- The pigment that gives muscle its red color.
- Myoglobin binds O_2 , facilitates O_2 release from red cells to mitochondria during periods of increase metabolic activity.
- Serves as a reservoir of O_2 during hypoxic and anoxic conditions.
- Implicated in the control of redox pathways in skeletal and cardiac muscle.
- Myoglobin is encoded by *MB,* chrom 22q12.3, three exons, 10.4 kb DNA, 154 aa.
- No primary myoglobin disease has been identified to date.

MYOGLOBIN STRUCTURE



Eight α-helices assigned the letters A to H that wrap around a central pocket containing a heme group, a porphyrin ring that contains a central bounded iron atom that is normally in the ferrous oxidation state. The heme-binding domain is responsible for the reversible binding to various ligands including oxygen, carbon monoxide and nitric oxide.

MYOGLOBIN STRUCTURE

662 NATURE March 8, 1958 Vol. 181

A THREE-DIMENSIONAL MODEL OF THE MYOGLOBIN MOLECULE OBTAINED BY X-RAY ANALYSIS

By Drs. J. C. KENDREW, G. BODO, H. M. DINTZIS, R. G. PARRISH and H. WYCKOFF
Medical Research Council Unit for Molecular Biology, Cavendish Laboratory, Cambridge
AND
D. C. PHILLIPS
Davy Faraday Laboratory, The Royal Institution, London

John C. Kendrew
Nobel Lecture

Nobel Lecture, December 11, 1962

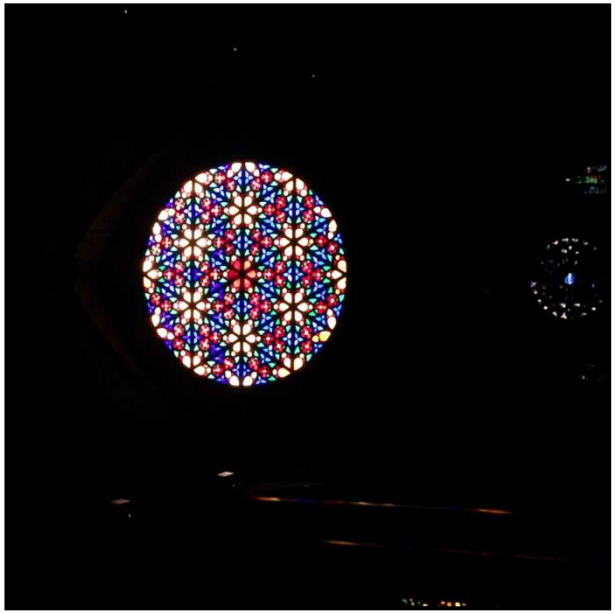
Myoglobin and the Structure of Proteins

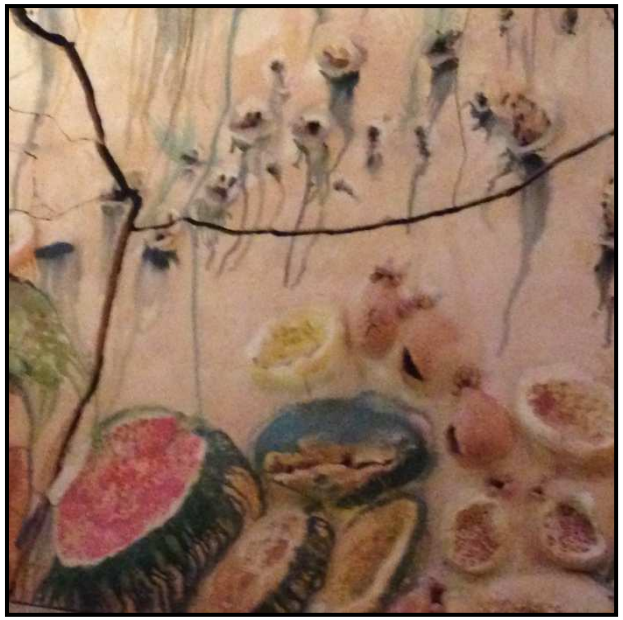
Read the Nobel Lecture
Pdf 730 kB

TO DESCRIBE THE CLINICAL PHENOTYPE OF MYOGLOBINOPATHY……

Son Espases
hospital universitari

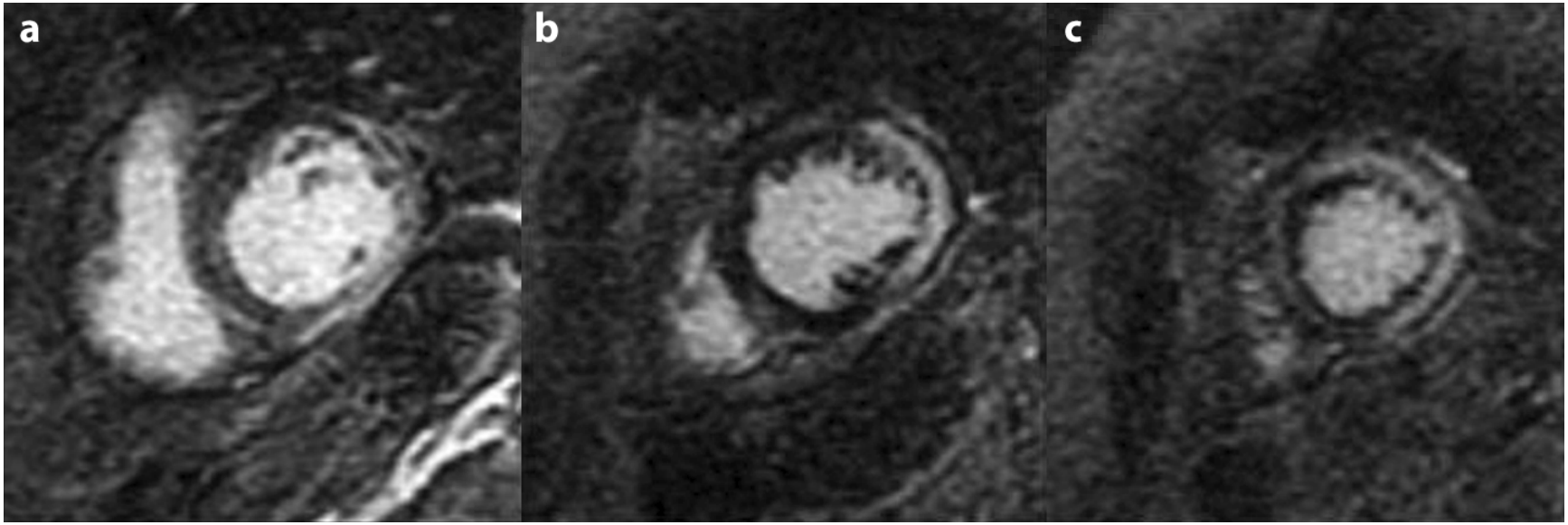
Taking advantage from the trip to Mallorca





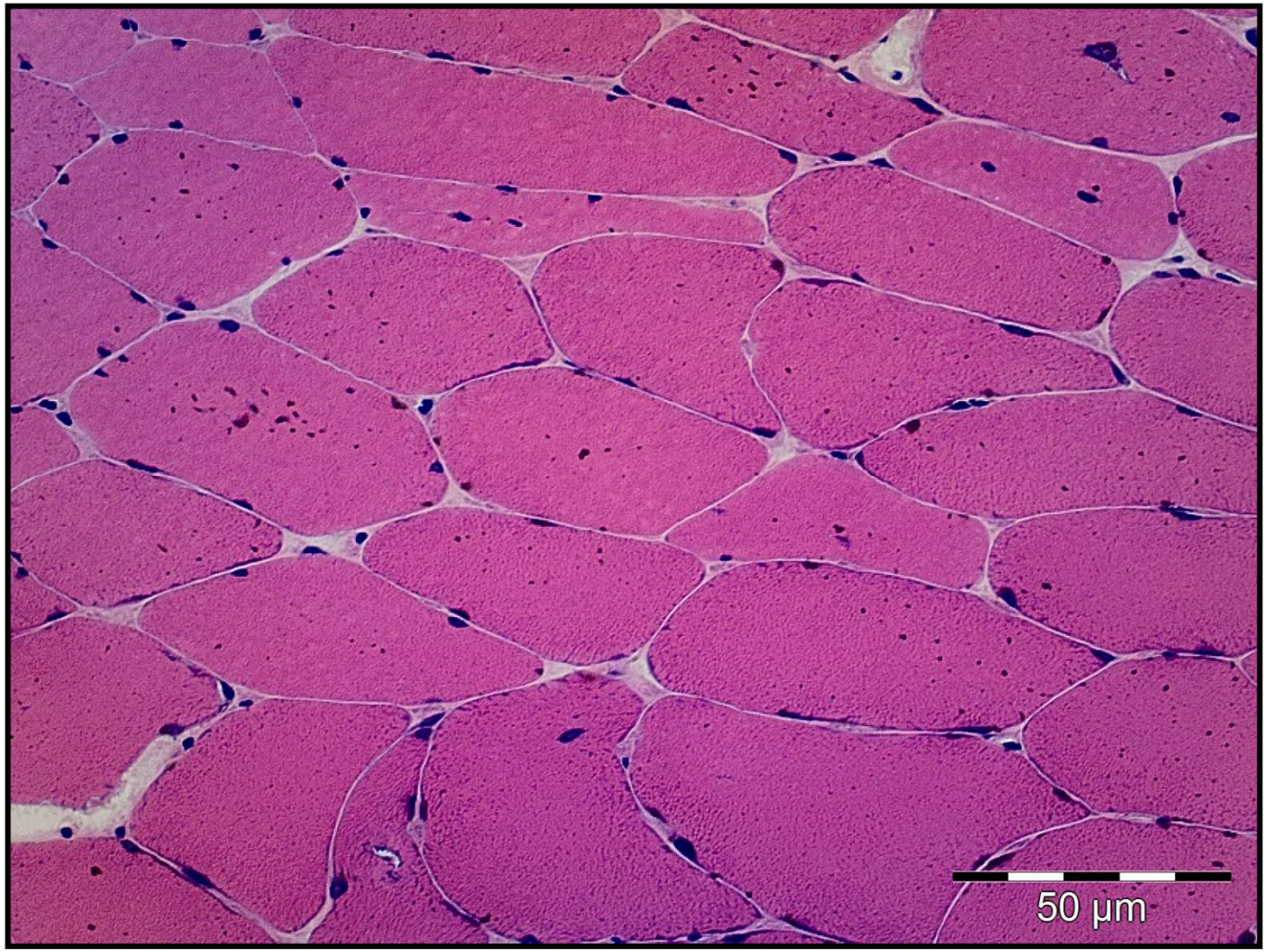
MYOGLOBINOPATHY: CLINICAL PHENOTYPE

	Family 1	Family 2	Family 3	Family 4	Family 5	Family 6
Inheritance pattern	AD	AD	AD	AD	UK	UK
Myoglobin mutation	H98Y	H98Y	H98Y	H98Y	H98Y	H98Y
Country of origin	Spain	Spain	Sweden	France	France	Netherlands
Number of studied patients	2	1	6	3	1	1
Mean age of onset (range)	37.5 (36-39)	38	44.5 (39-49)	46 (44-48)	40	33
Initial symptoms	Proximal LL and axial weakness	Proximal LL and axial weakness	Proximal LL and axial weakness (4) Distal hand weakness (2)	Proximal LL and axial weakness	Proximal LL weakness	Proximal LL and axial weakness
Symptoms at advanced disease						
Distribution of weakness	Proximal and axial>distal 4 EE	Proximal and axial>distal 4 EE	Proximal and axial>distal 4 EE	Proximal and distal 4 EE >axial	Proximal and axial > distal 4 EE	Proximal and axial > distal 4 EE
Involvement of hand muscles	Yes	Yes	Yes	Yes	Yes	No
Facial weakness	No	No	No	No	No	No
Muscle atrophy	Yes	Yes	Yes	Yes	No	Ýes
Dysphagia	2/2	0/1	2/4	0/1	0/2	0/1
Respiratory insufficiency	2/2	1/1	2/6	0/1	2/2	1/1
Cardiac involvement*	2/2	1/1	1/6	ECG normal	1/1	0/1
Clinical outcome						
Mean age at wheelchair dependency (15-20 ado)	54	65	56 (4/6 patients in wheelchair, 2 ambulant)	Not known	56	47
Age at death (mean, range) (25-30 ado)	60.5 (54-67)	-	64 (58-71)	72	-	-

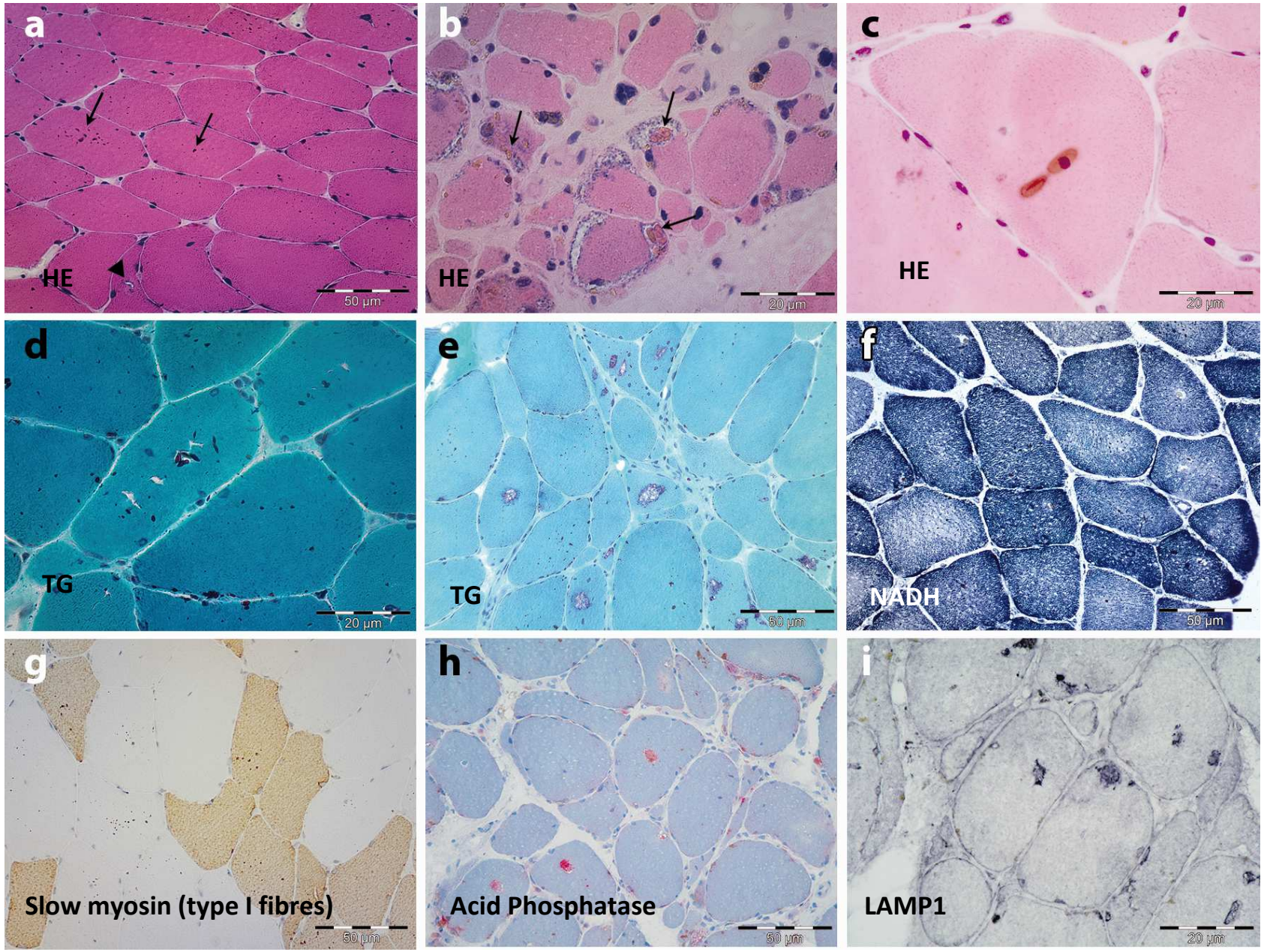


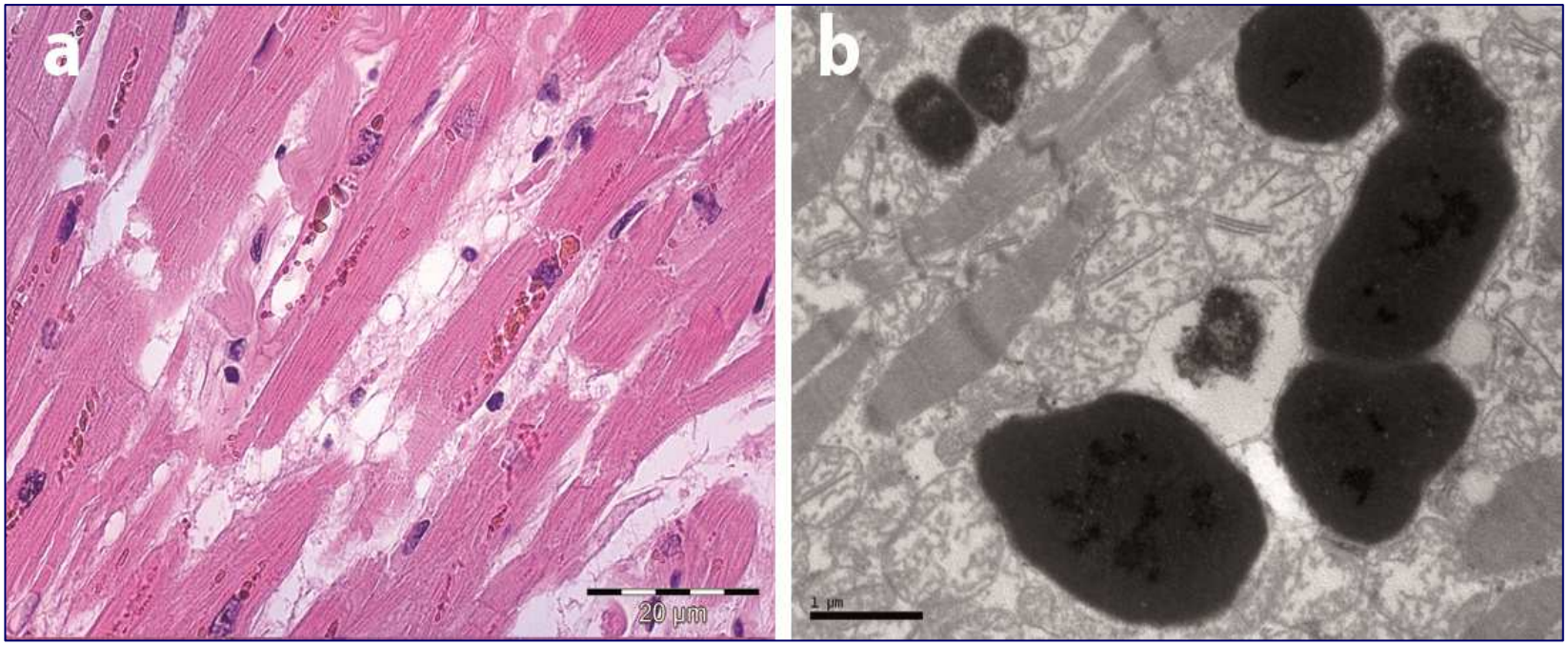
Cardiac MRI from patient F2, II: 2. Short axis images after gadolinium administration showing late transmural enhancement in basal and mid inferolateral segments (a, b) and in all of them at the apical level (c), indicative of fibrosis.

SARCOPLASMIC BODIES : PATHOLOGICAL HALLMARK OF MYOGLOBINOPATHY



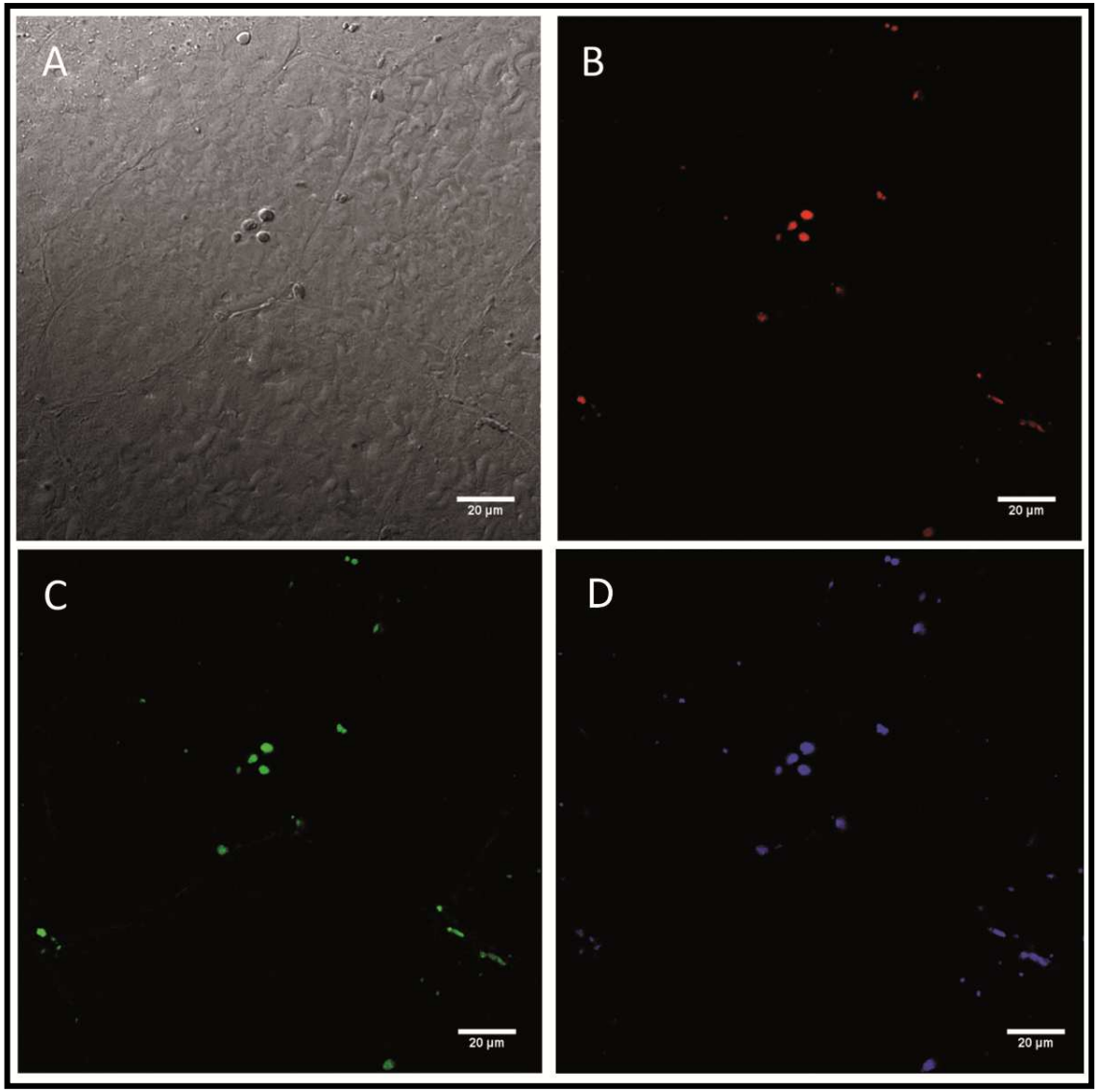
Myoglobinopathy



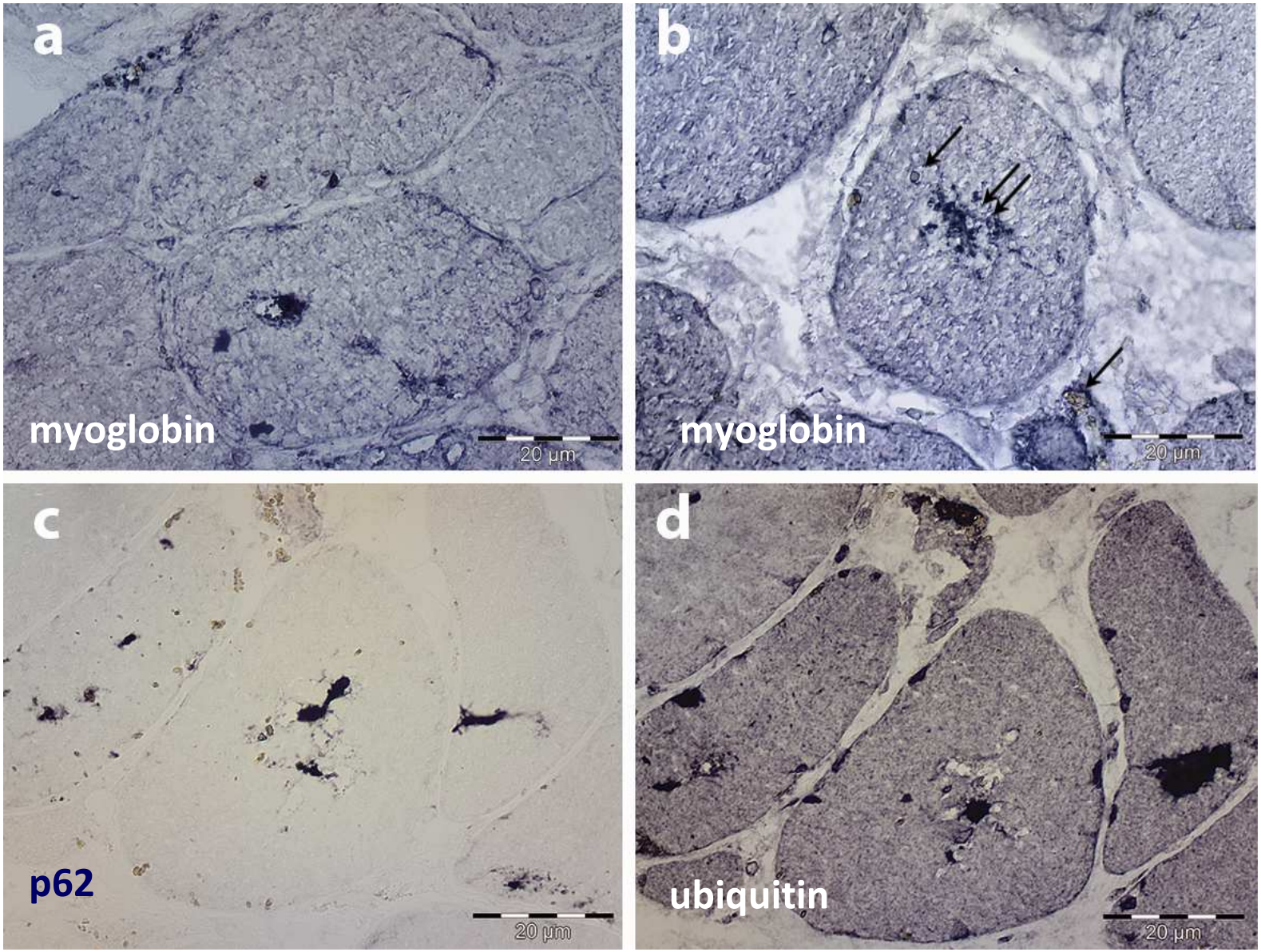


Same inclusions in cardiac muscle

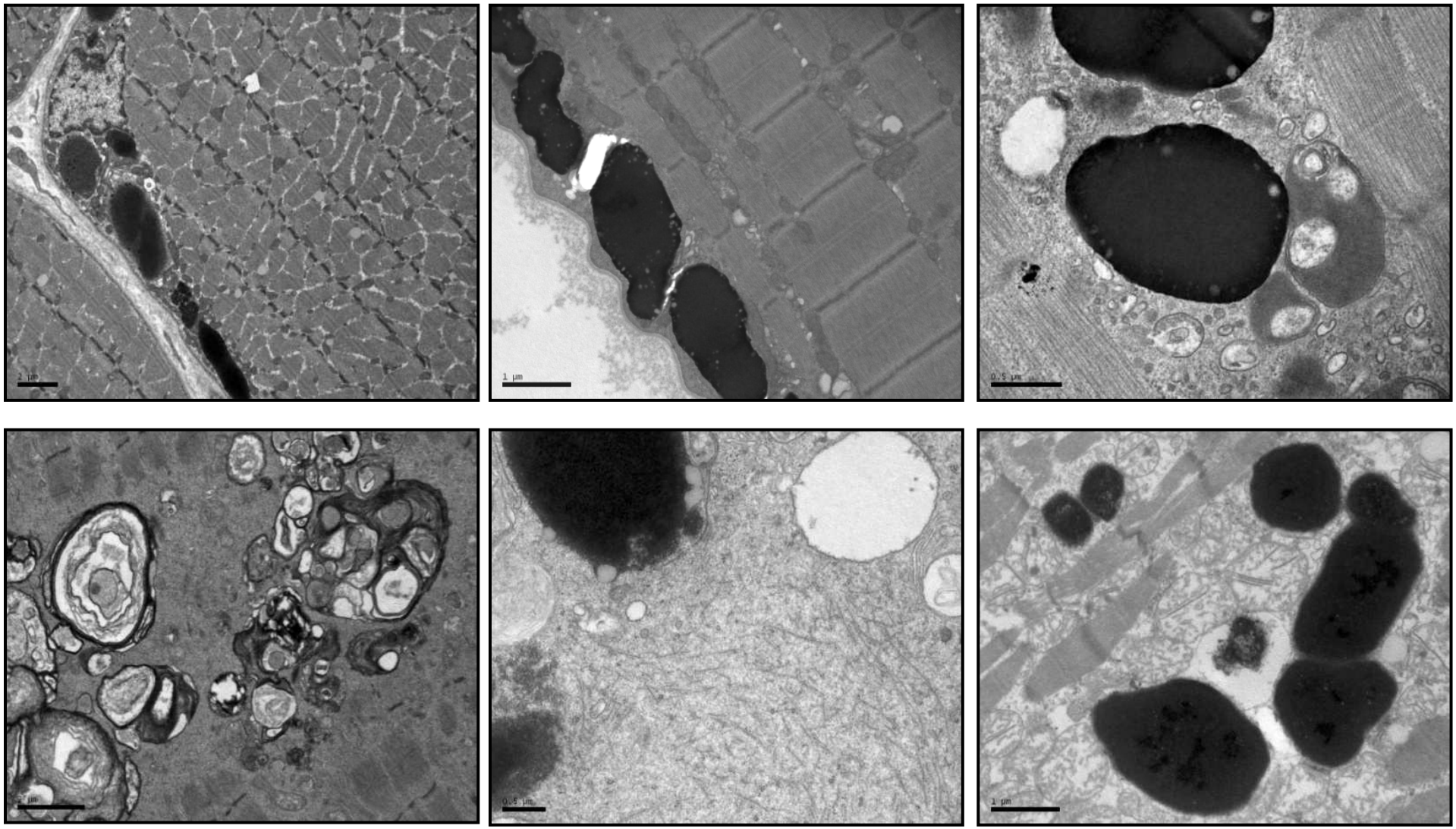
UNSTAINED SECTIONS CONFOCAL MICROSCOPY



Sarcoplasmic bodies exhibit autofluorescence with a wide range of visible laser excitation lines


a
myoglobin
20 µm
b
myoglobin
20 µm
c
p62
20 µm
d
ubiquitin
20 µm

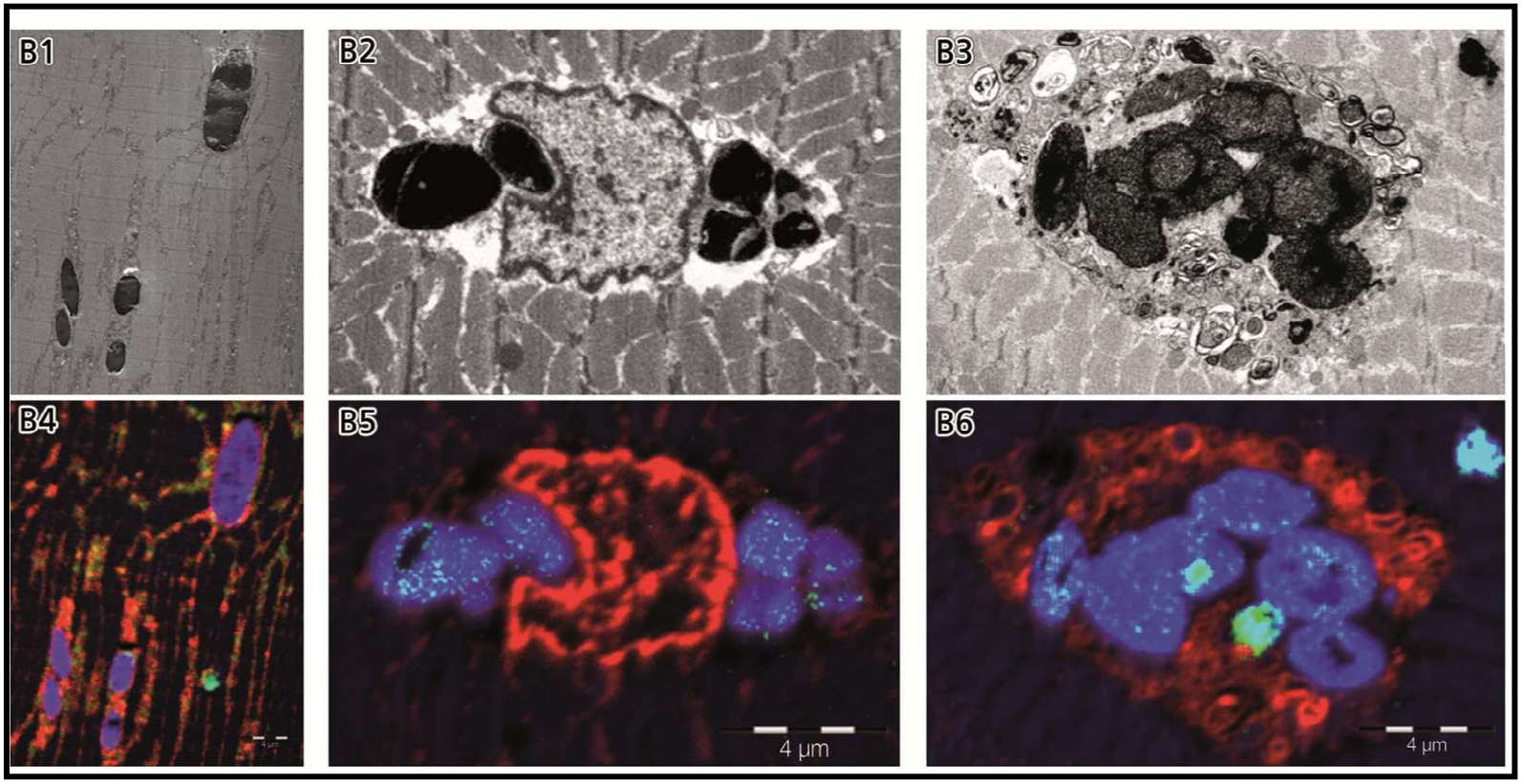
Myoglobinopathy



Characterization of the sarcoplasmic bodies

- Nanoscale Secondary Ion Mass Spectrometry-NanoSIMS
- Fourier transform infrared (µFTIR) microscopy
- Molecular modelling (*in sillico*)
- Electrochemical measurements

Nanoscale Secondary Ion Mass Spectrometry-NanoSIMS



Sulfur (^{32}S)

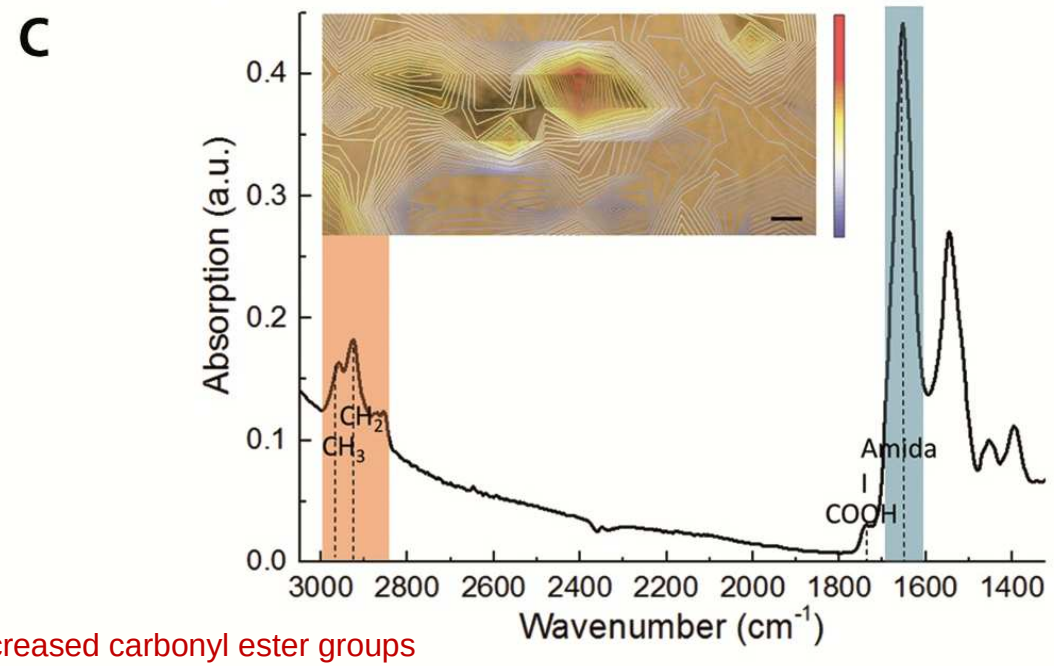
Phosphorus (^{31}P)

Iron (^{56}Fe)

Fourier transform infrared (µFTIR) microscopy

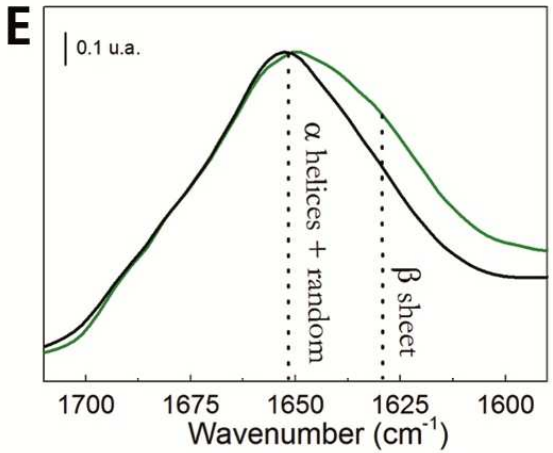
ALBA

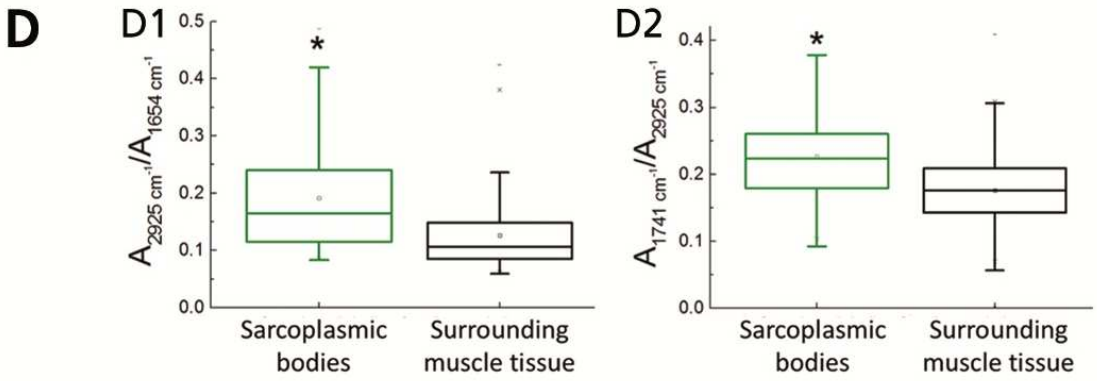
Fourier transform infrared (µFTIR) microscopy



Increased carbonyl ester groups

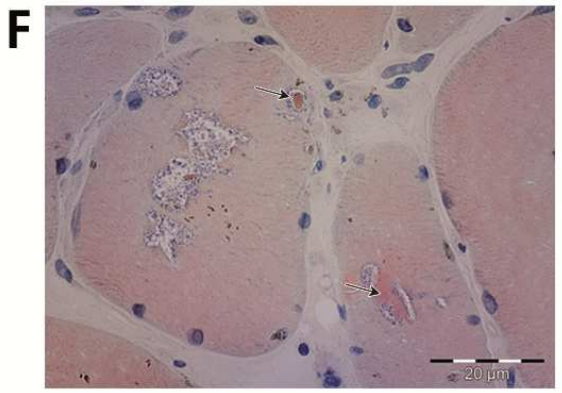
Spectrum of the amida region





Lipid/protein ratio

Lipid oxidation



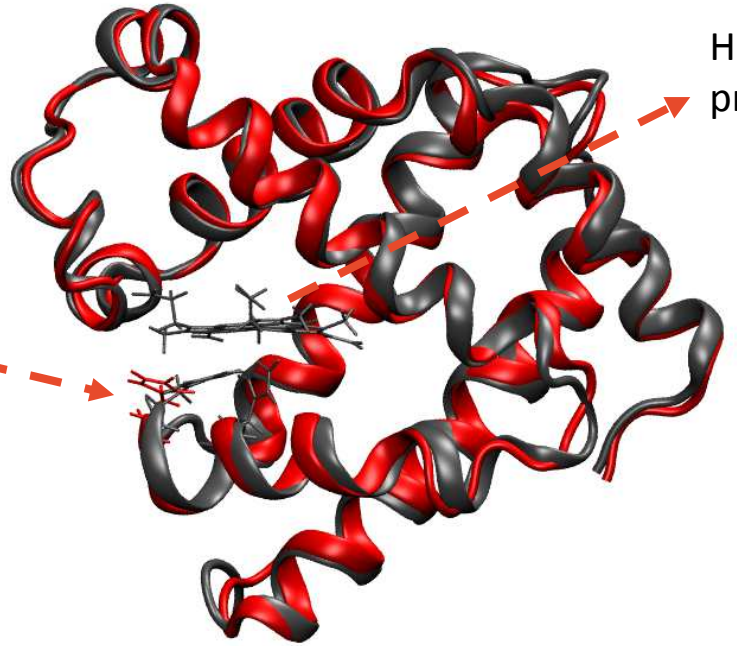
Congo red

Effects of mutation on protein structure - Molecular modelling

Homology modelling

The crystal structure of WT myoglobin is known (red)

The 3D structure of the p.H98Y mutant was calculated *in silico* (grey)



Heme group: Fe atom in a protoporphyrin IX ring

His 98 (Tyr987 in the mutant)

Molecular dynamics

Simulation of the dynamic behavior over time

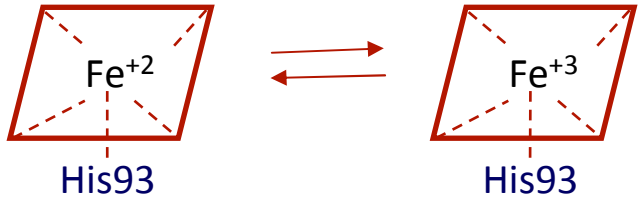
The global fold and dynamics of the WT Mb are CONSERVED in the H98Y mutant

BUT

The heme is slightly more accessible to the water molecules in H98Y and consequenlty the redox potential decreases

Possible downfalls of E° shift upon mutation

Redox potential E° impacts on the equilibrium between Red and Ox Mb in solution



Reduced
Can bind O_2

Oxidized (also known as MetMb)
Does not bind O_2

More negative E° values means that Ox form (inactive towards O_2) is more favored than Red form

The E° of H98Y is approx 50 mV more negative than E° of WT

In patients with H98Y mutation it might be more difficult to maintain Mb in its Reduced form

Altered Mb functionality with respect to O_2 binding/storage
Initiate lipid oxidation cascade reactions

• We have identified a *MB*, p.His98Tyr heterozygous mutation in 6 unrelated European families suffering from an adult-onset myopathy, with highly characteristic inclusions in skeletal and cardiac muscles.

• This represents the first myoglobinopathy reported so far.

• Myoglobinopathy is characterized by AD, adult onset myopathy, initially involving proximal LL and axial muscles. Involvement of cardiac and respiratory muscles occurs at advanced stages of the disease.

• Sarcoplasmic bodies, the morphological hallmark of this disease, correspond to oxidized lipids and missfolded proteins.

•The *MB* p.His98Tyr mutation alters the Mb redox potential towards more negative values, indicating that the His98Tyr substitution stabilizes the oxidized form which is unable to bind and store O2. As a consequence, this presumably initiates protein and lipid oxidation cascade reactions.

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Myoglobinopathy is an adult-onset autosomal dominant myopathy with characteristic sarcoplasmic inclusions

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This work is dedicated to the memory of my brother
Josep Maria Olivé ("Tato"), Neurologist
1953-2015