31st European Congress of Pathology

European Society of Pathology

Integrated morphologic and molecular approach to diagnosis of cardiovascular disease

Cardiomyopathies in neuromuscular disease

C.Giordano

Inherited neuromuscular diseases are a broad spectrum of genetic disorders including dystrophic and non-dystrophic myopathies, mitochondrial myopathies, storage disease, and muscle channelopathies.

Heart is involved in many NMDs, in the form of cardiomyopathy, conduction defects, and/or arrhythmias.

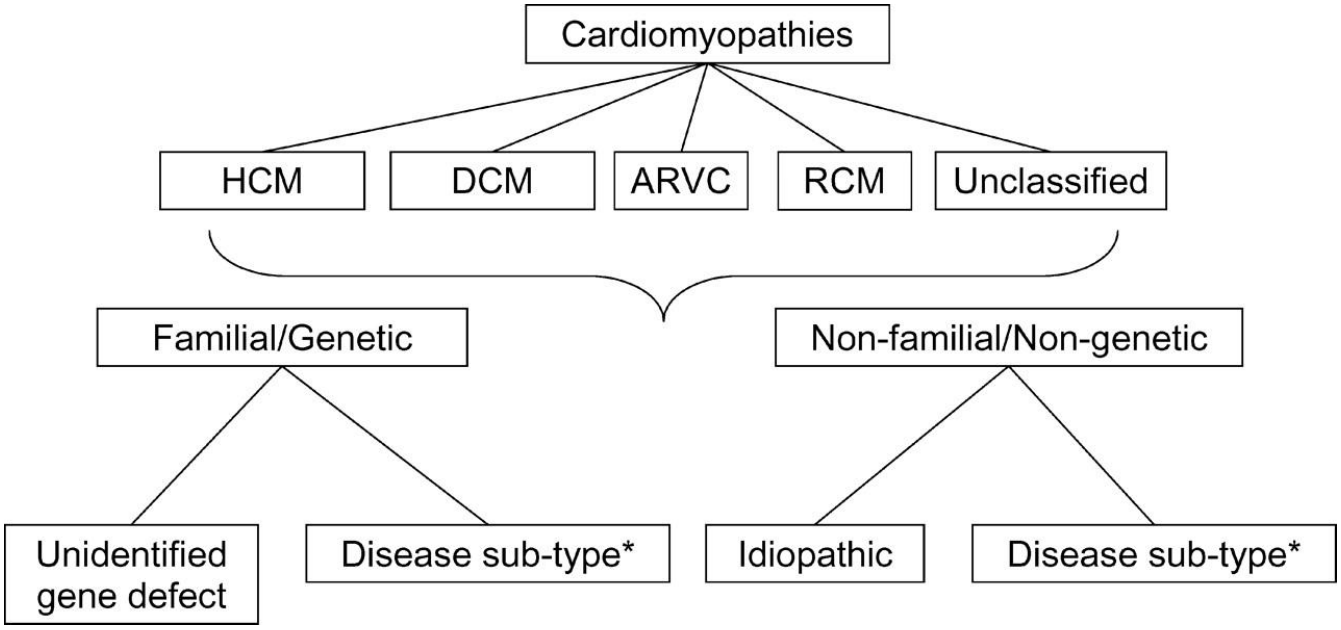
Prevalence, age at onset and severity of cardiac involvement varies significantly according to the specific etiology, with phenotype/genotype correlations continuously evolving.

For many NMDs, cardiac disease represents a major cause of morbidity and mortality

European Heart Journal (2008) **29**, 270–276
doi:10.1093/eurheartj/ehm342

Definition and classification of cardiomyopathy

"A myocardial disorder in which the heart muscle is structurally and functionally abnormal, in the absence of coronary artery disease, hypertension,
valvular disease and congenital heart disease sufficient to cause the observed myocardial abnormality"



In the clinical setting cardiac involvement:

1) **May manifest after the development of skeletal myopathy, when clinical and/or genetic diagnosis of NMDs has been already established (e.g. Duchenne muscular dystrophy)**
2) **May occur concurrently with an undiagnosed skeletal myopathy**
3) **May be the first or predominant manifestation.**

In the clinical setting cardiac involvement:

1) **May manifest after the development of skeletal myopathy, when clinical and/or genetic diagnosis of NMDs has been already established (e.g. Duchenne muscular dystrophy)**

In this circumstance diagnosis takes advantage from cardiac screening programs that are now part of the routine diagnostic workup (e.g. Duchenne muscular dystrophy, DMD)

AHA SCIENTIFIC STATEMENT

Management of Cardiac Involvement Associated With Neuromuscular Diseases

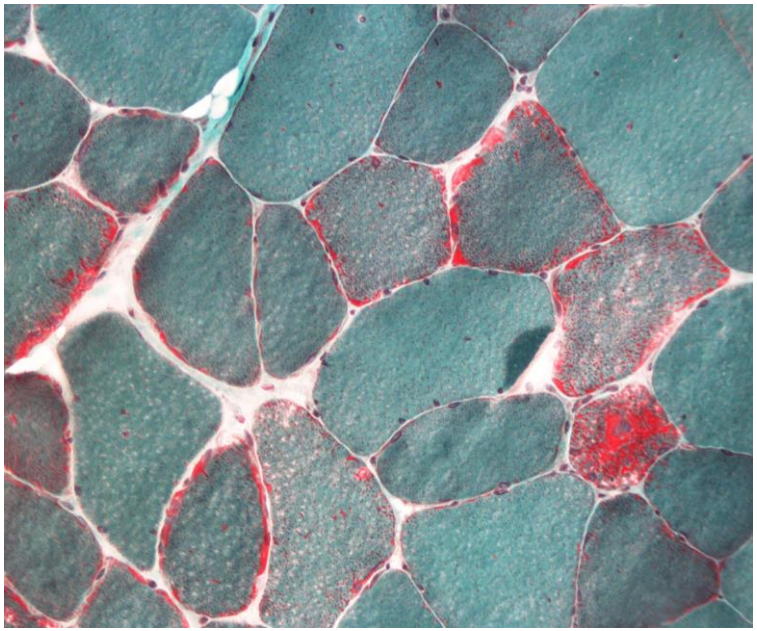
A Scientific Statement From the American Heart Association

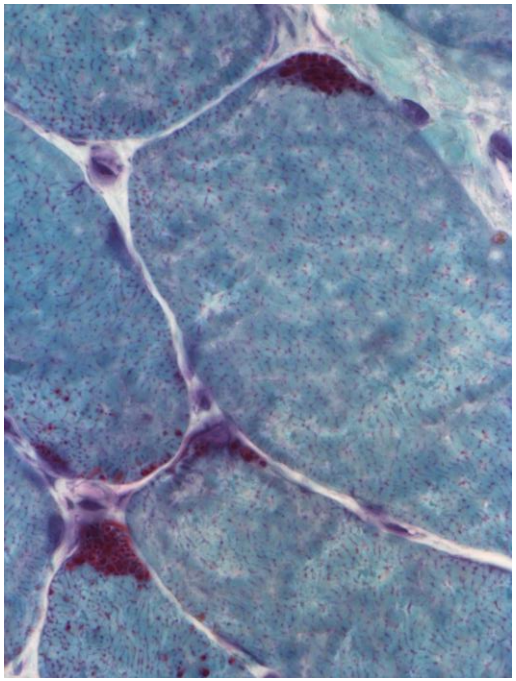
Circulation. 2017;136:e200–e231.

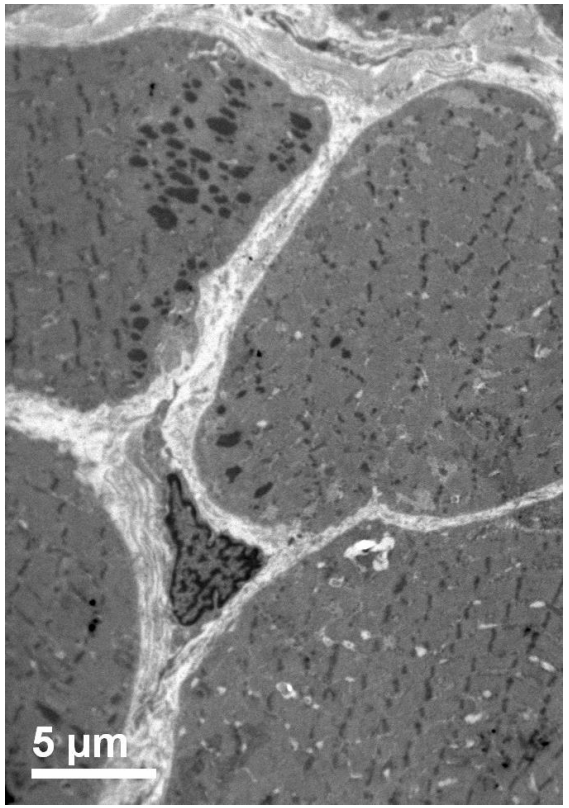
In the clinical setting cardiac involvement:

2) May occur concurrently with an undiagnosed skeletal myopathy

Cardiac phenotypes may guide diagnostic suspicion of a specific cause, muscle biopsy is often part of the diagnostic flowchart and guide rational selection of specialized tests, including, biochemical or genetic analysis







In the clinical setting cardiac involvement:

3) May be the first or predominant manifestation.

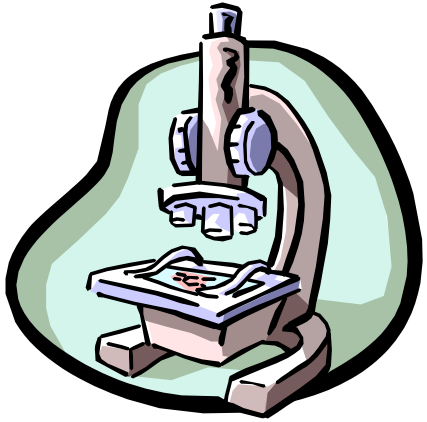
When the clinical presentation is dominated by cardiac symptoms, skeletal muscle involvement may not be obvious. The systematic search for diagnostic “red flags” (pattern of inheritance, markers of muscle damage, specific cardiac phenotype), may provide clues for specific diagnosis.

Diagnostic work-up in cardiomyopathies: bridging the gap between clinical phenotypes and final diagnosis. A position statement from the ESC Working Group on Myocardial and Pericardial Diseases

EUROPEAN SOCIETY OF CARDIOLOGY®

European Heart Journal (2013) **34**, 1448–1458
doi:10.1093/eurheartj/ehs397

Role of Pathologist in the diagnostic flowchart



Pathologist may be involved in the diagnostic flow chart:

- In selected cases, when endomiocardial biopsy is performed (e.g. isolated cardiomyopathy)

- After cardiac transplant and at autopsy (remarkably, cardiomyopathies may be severe enough to cause heart failure or sudden cardiac death).

Role of Pathologist in the diagnostic flowchart

2011 Consensus statement on endomyocardial biopsy from the Association for European Cardiovascular Pathology and the Society for Cardiovascular Pathology

Cardiovasc Pathol. 2012 Jul-Aug;21(4):245-74.

Virchows Arch (2017) 471:691–705
DOI 10.1007/s00428-017-2221-0

CrossMark

ORIGINAL ARTICLE

Guidelines for autopsy investigation of sudden cardiac death: 2017 update from the Association for European Cardiovascular Pathology

- Snap-freeze at least one myocardial fragment for immunostains (dhystrophin), histoenzymatic stains (i.e. cytochrome c oxidase, COX and succinic dehydrogenase, SDH)
- Fix a sample in 4% glutaraldehyde for TEM.

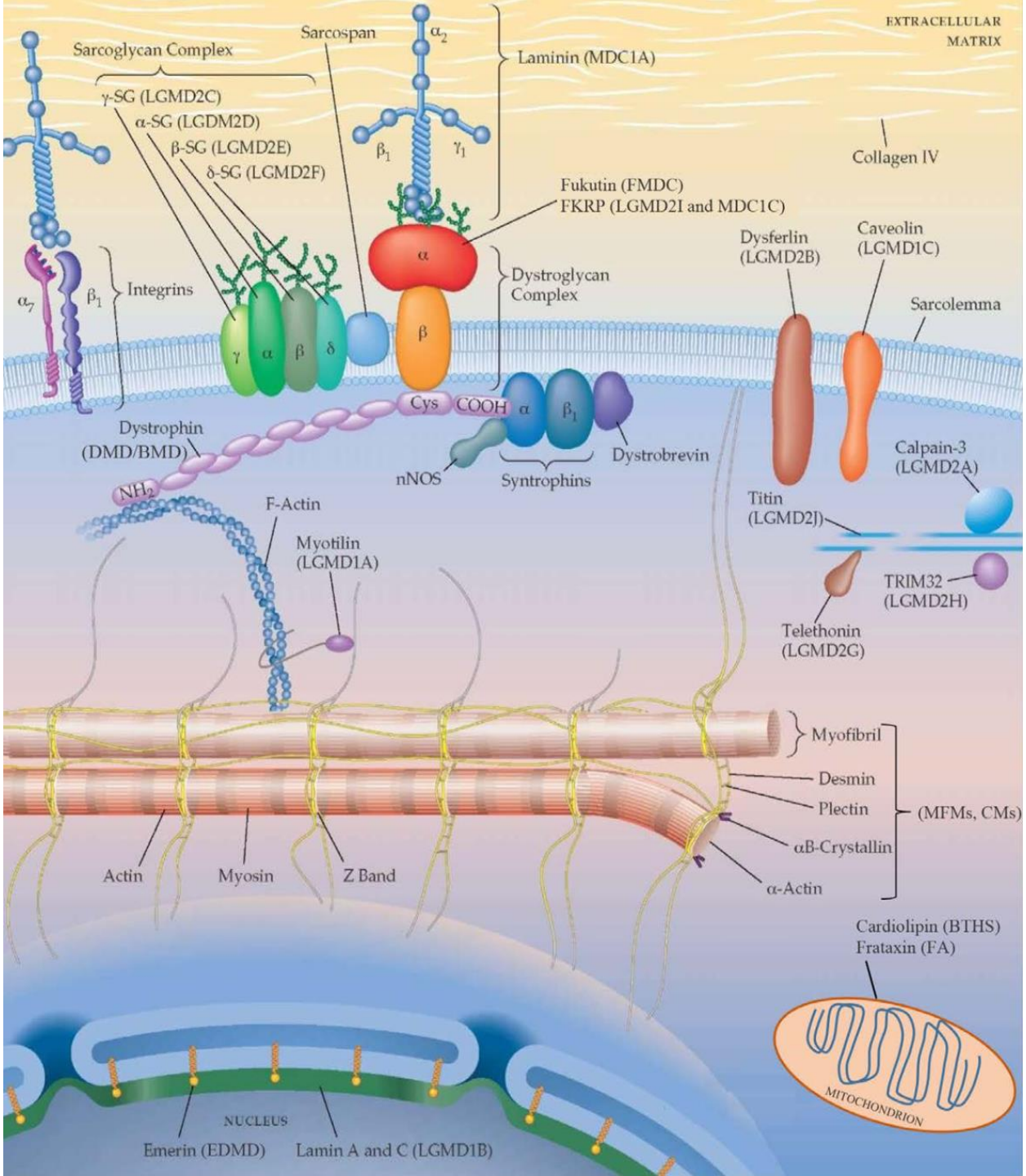
Dilated Cardiomyopathy (DCM)

Left ventricular dilation and left ventricular systolic dysfunction in the absence of abnormal loading conditions or coronary artery disease sufficient to cause global systolic impairment.

Dilated Cardiomyopathy (DCM) in neuromuscular disorders

Condition	Gene Locus	Gene Product	Heritance	Cardiac Features		
				Cardiomyopathy	Arrhythmia	Conduction
X-linked recessive muscular dystrophies						
Duchenne	Xp21	Dystrophin	XLR	Common (DCM)	Common (late)	Rare (late)
Becker	Xp21	Dystrophin	XLR	Common	Common	Rare (late)
Emery-Dreifuss	Xq28	Emerin	XLR	Rare	Common	Common (SD)
Limb-girdle muscular dystrophies						
LGMD1B	1q11–q21	Lamin A and C	AD	Common (DCM)	Common (AT, VT)	Common (SD)
LGMD1C	3p25	Caveolin-3	AD	Rare (DCM)	Not reported	Rare (AVB)
LGMD1E	7q36	DNAJB6 (co-chaperone)	AD	Rare	Rare	Rare
LGMD2B	2p13	Dysferlin	AR	Rare (DCM)	Not reported	Not reported
LGMD2C	13q12	γ-Sarcoglycan	AR	Common (DCM)	Rare	Rare
LGMD2D	17q12–q21	α-Sarcoglycan	AR	Common (DCM)	Rare	Rare
LGMD2E	4q12	β-Sarcoglycan	AR	Common (DCM)	Common	Common
LGMD2F	5q33–q34	δ-Sarcoglycan	AR	Rare	Rare	Rare
LGMD2I	19q13.3	Fukutin-related protein	AR	Common (DCM)	Rare	Rare
Congenital myopathies						
Central core disease	19q13.2	Ryanodine receptor	AD/AR	Rare (DCM)	Not reported	Not reported
Nemaline myopathy	1q21, 2q21–q22, 1q42.13, 19q13.4	α-Tropomyosin, nebulin, skeletal muscle α-actin, troponin T	AR/AD	DCM, HCM	Rare (long QT)	Common (mild)

Feingold B et al. Management of Cardiac Involvement Associated with Neuromuscular Diseases: A Scientific Statement From the American Heart Association. Circulation. 2017;136(13):e200-e231


EXTRACELLULAR MATRIX
Sarcoglycan Complex
Sarcospan
α_2
Laminin (MDC1A)
γ-SG (LGMD2C)
α-SG (LGDM2D)
β-SG (LGMD2E)
δ-SG (LGMD2F)
β_1
γ_1
Collagen IV
Fukutin (FMDC)
FKRP (LGMD2I and MDC1C)
Dysferlin (LGMD2B)
Caveolin (LGMD1C)
α_7
β_1
Integrins
α
β
γ
α
β
δ
Dystroglycan Complex
Sarcolemma
Cys
COOH
α
β_1
Dystrophin (DMD/BMD)
Dystrobrevin
nNOS
Syntrophins
Calpain-3 (LGMD2A)
NH_2
F-Actin
Titin (LGMD2J)
Myotilin (LGMD1A)
TRIM32 (LGMD2H)
Telethonin (LGMD2G)
Myofibril
Desmin
Plectin
αB-Crystallin
α-Actin
(MFMs, CMs)
Actin
Myosin
Z Band
Cardiolipin (BTHS)
Frataxin (FA)
MITOCHONDRION
NUCLEUS
Emerin (EDMD)
Lamin A and C (LGMD1B)

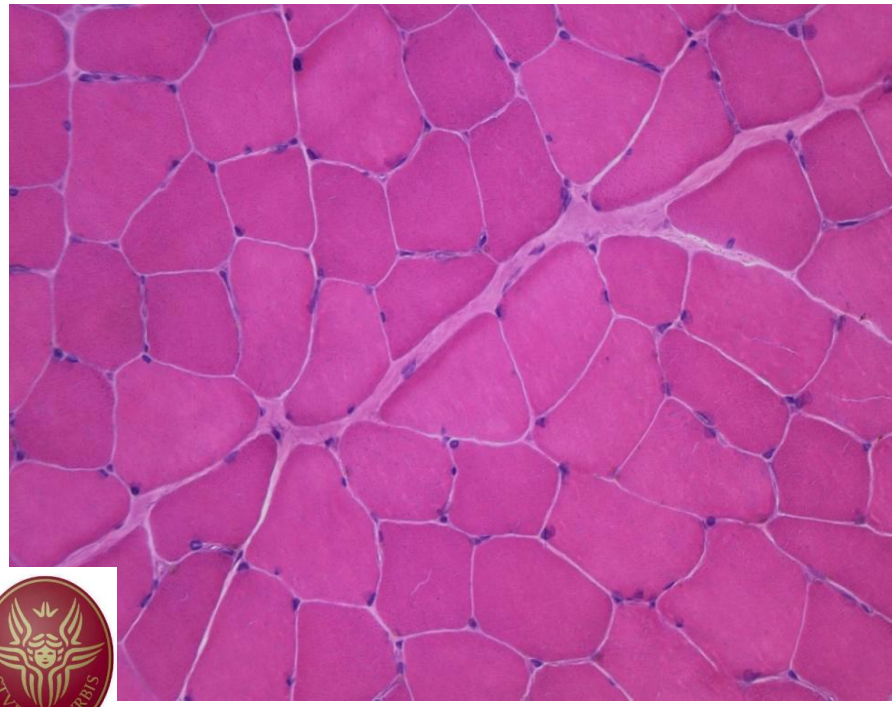
Dystrophinopathy

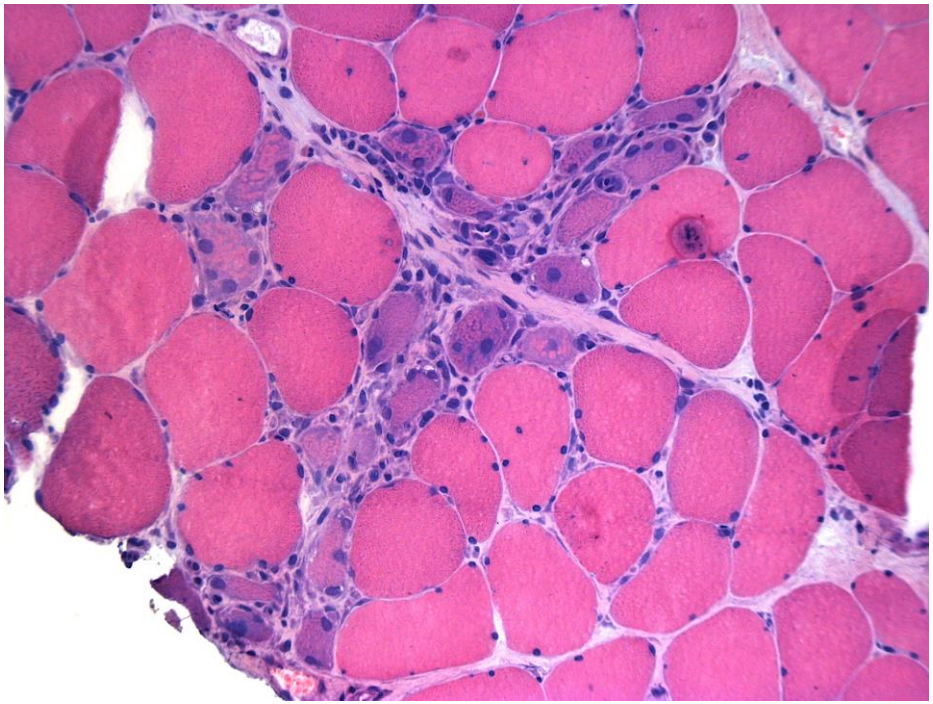
- Duchenne muscular dystrophy (DMD)
- Becker muscular dystrophy (BMD),
- X-linked dilated cardiomyopathy,
- DMD and BMD female carriers

Dystrophinopathy

- DMD: weakness in the early childhood progressing to loss of the ability to walk and respiratory insufficiency by the 2°-3° decade.
- BMD: milder and more variable phenotype

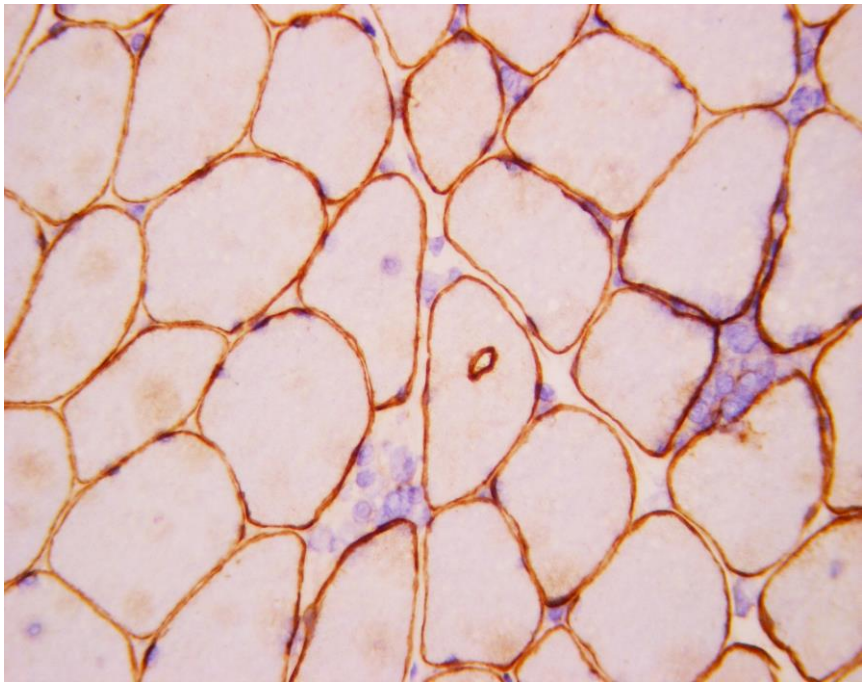
Elevated CK level is a diagnostic sign that should prompt to perform muscle biopsy or genetic testing.

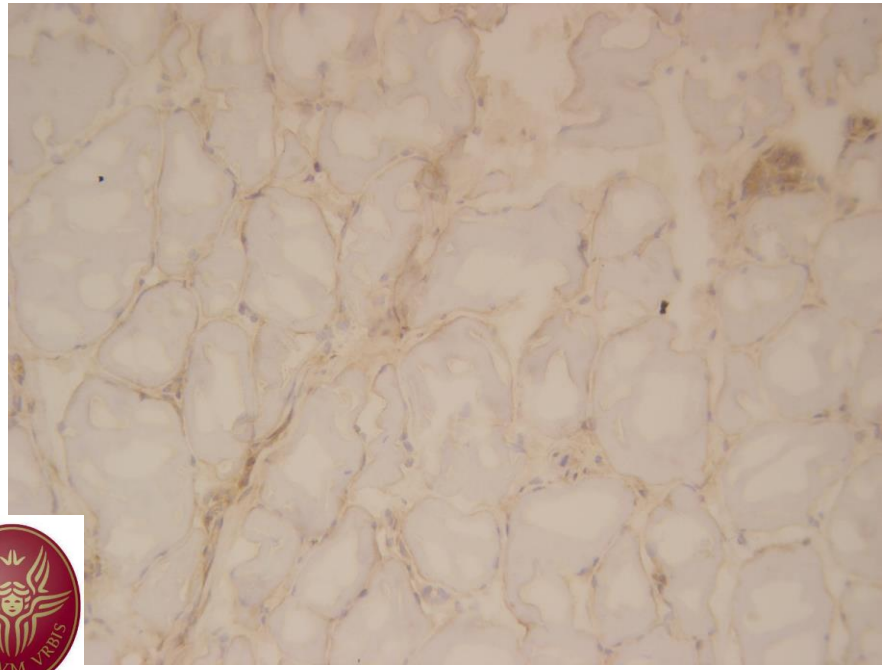


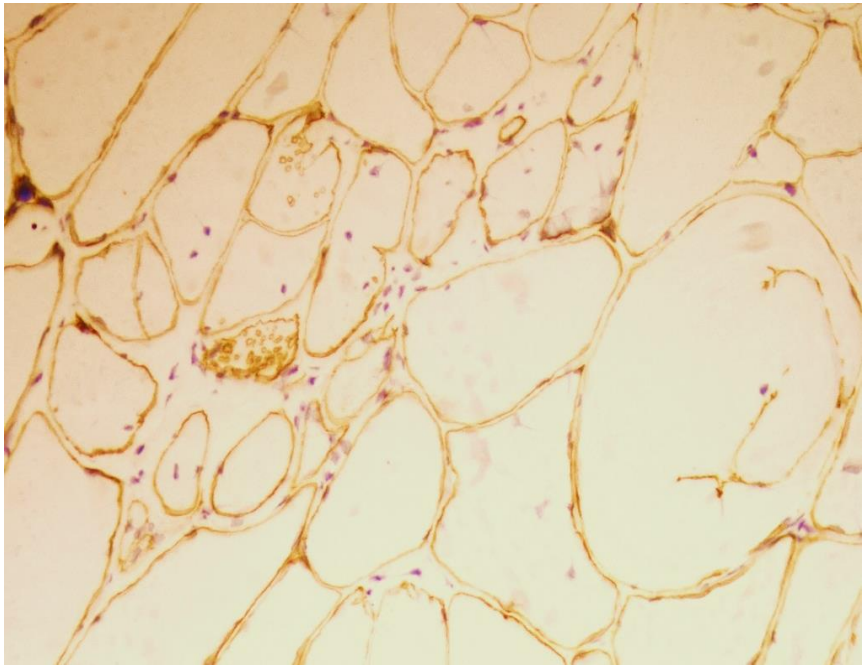


Dystrophinopathy

- Immunostain with antibodies against thew N- and C- domains and rod domains of dystrophin demonstrate the absence of protein in DMD or reduction in BMD.







Dystrophinopathy

Cardiac involvement in DMD always becomes clinically evident after the onset of muscular symptoms with incidence increasing with age :

- 25% at 6 years of age
- 59% by 10 years of age
- more than 90% over 18 years of age

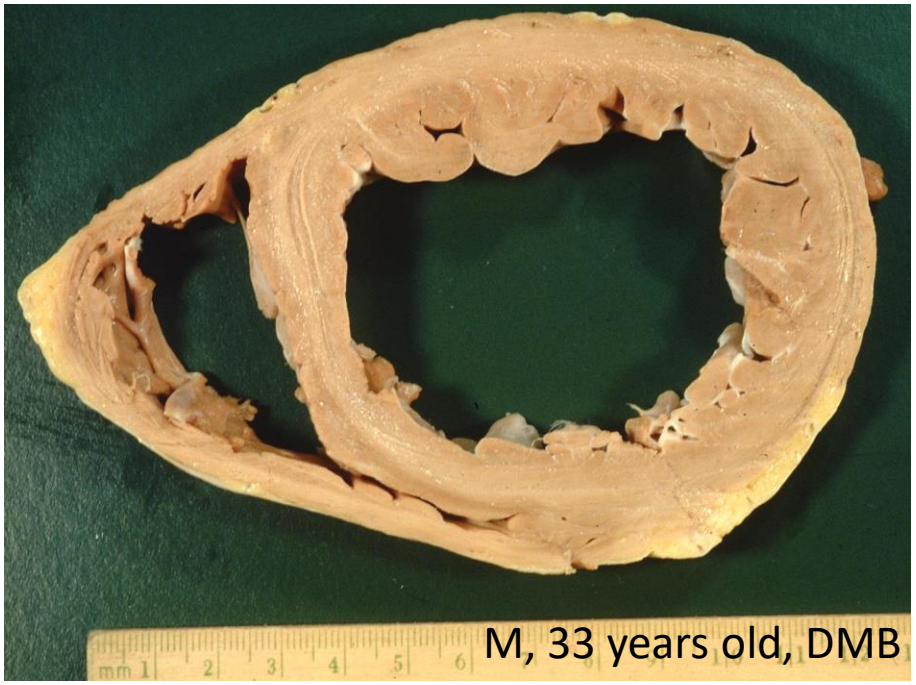
In BMD over 70% of patients develop cardiomyopathy

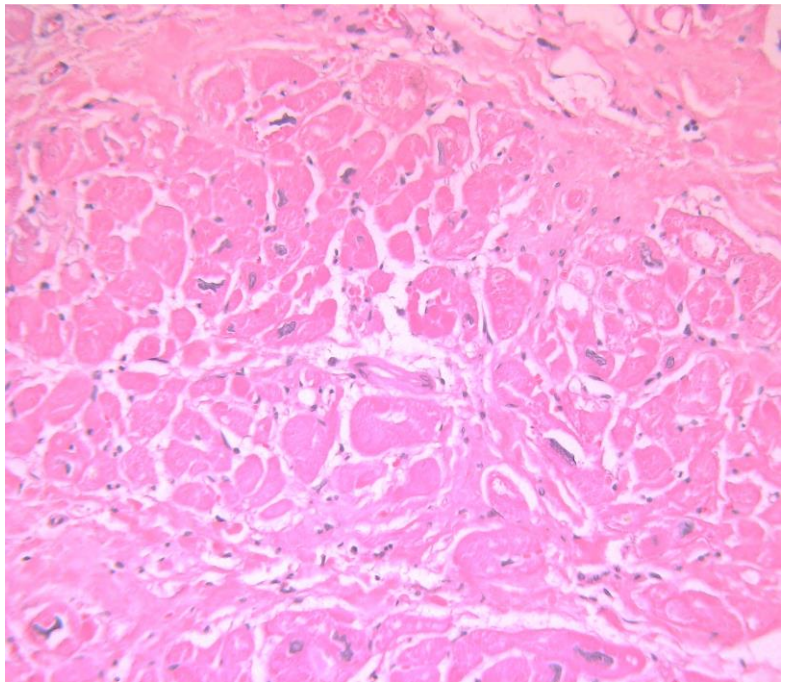
The onset of cardiomyopathy is variable in BMD and is not correlated to skeletal muscle involvement. Cardiomyopathy , often associated with cardiac arrhythmias, may be the predominant manifestation.

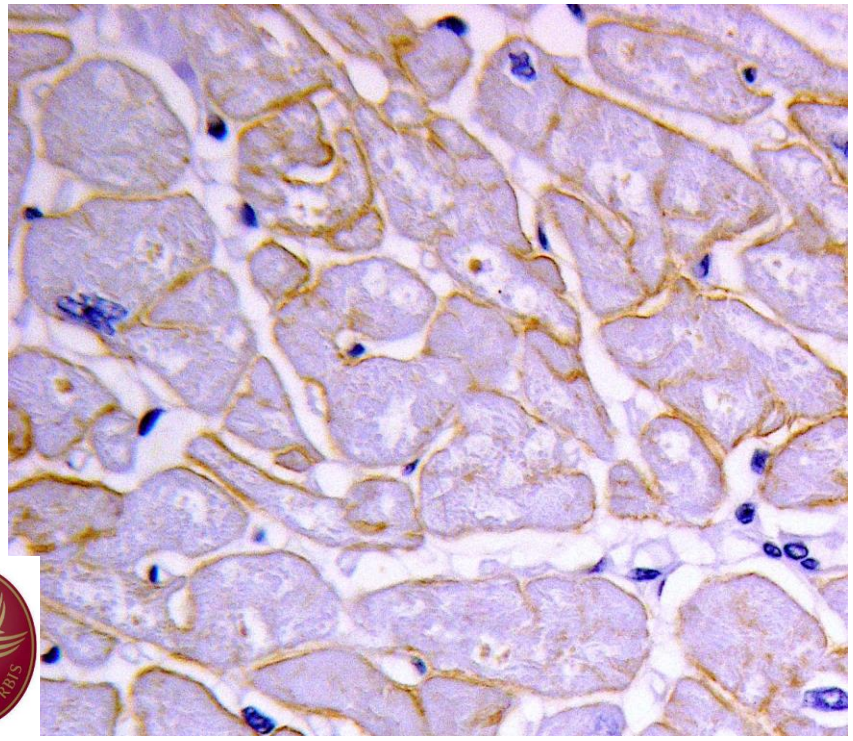
Hyper-CK is common and should be considered a sufficient reason for consultation with a neurologist.

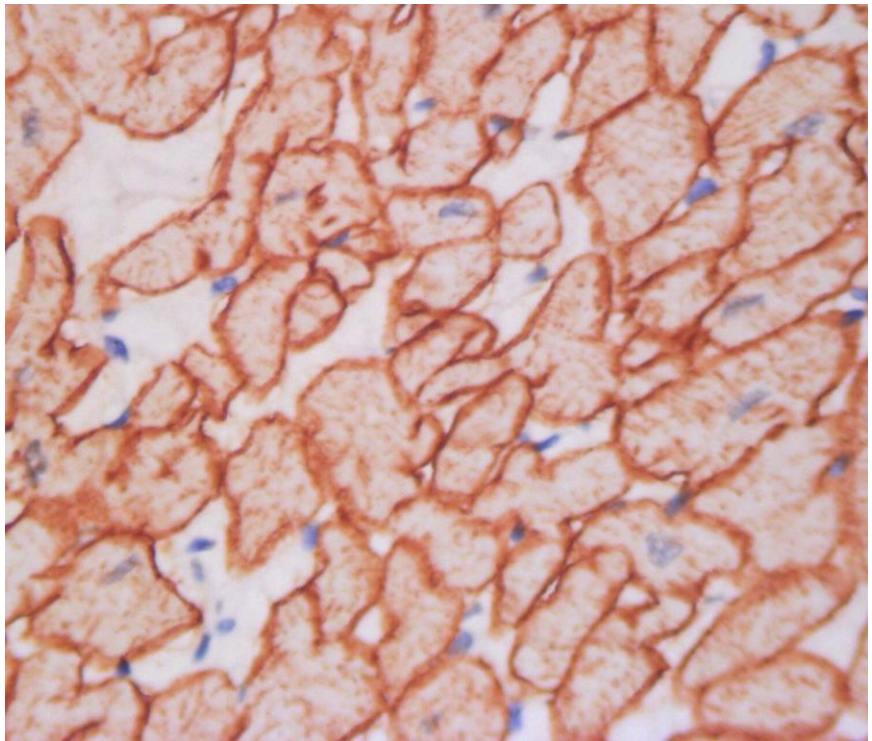
Kamdar F and Garry DJ. Dystrophin-deficient cardiomyopathy.

J Am Coll Cardiol. 2016;67(21):2533-2546


M, 33 years old, DMB





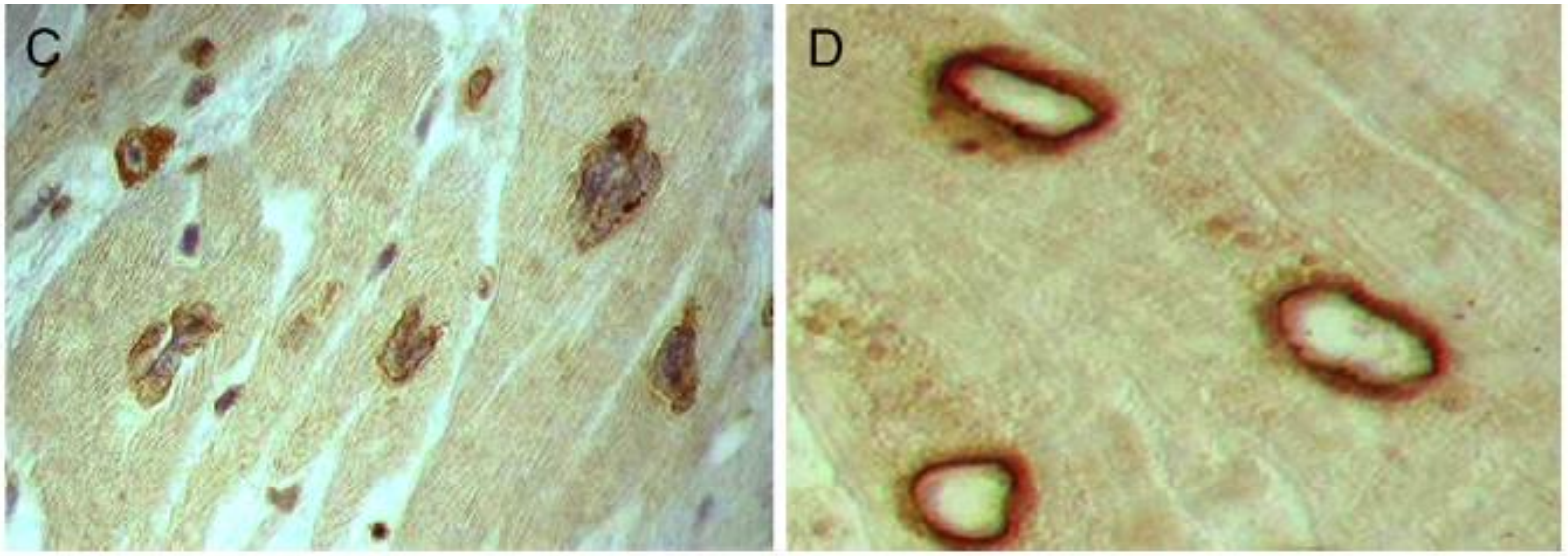


Dystrophinopathy in femal carriers

- The majority of female carriers of DMD and BMD are asymptomatic; however, female carriers can become symptomatic.
- Prevalence of manifesting carriers 20%
- Manifesting carriers can present mild muscle weakness, elevated serum creatinine kinase, and cardiomyopathy.
- X-inactivation is the mechanism where 1 of the 2 X chromosomes in female cells randomly becomes transcriptionally inactive. It is postulated that carriers can become symptomatic on the basis of the extent of random X-inactivation of the normal X chromosome versus the dystrophic X chromosome

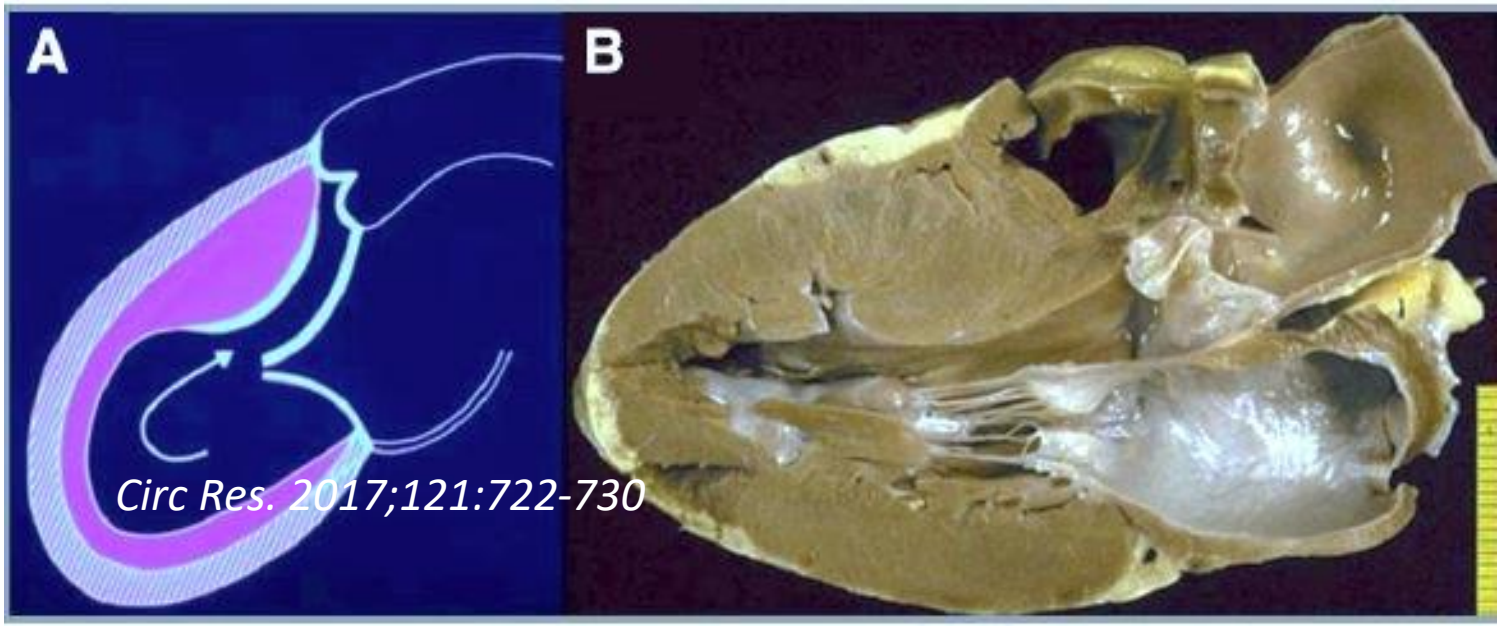
Laminopathy

- ECG abnormalities, conduction system defects and arrhythmias with or without LV enlargement and systolic dysfunction may manifest in subject bearing mutations in lamin A and C protyeins.
- Immunohistochemical staining for lamin A/C may shows discontinuity or loss in perinuclear staining, however data on the use of immunoistochemistry for diagnostic purpose are not available.



2011 Consensus statement on endomyocardial biopsy from the Association for European Cardiovascular Pathology and the Society for Cardiovascular Pathology

Hypertrophic cardiomyopathy (HCM)



Myocardial hypertrophy in the absence of haemodynamic stresses sufficient to account for the degree of hypertrophy

Hypertrophic Cardiomyopathy (HCM) in neuromuscular disorders

Hypertrophic cardiomyopathy is the most frequent cardiac phenotype in metabolic/storage disorders.
Epidemiology and clinical presentation are strictly age-related.

- In the **pediatric population** metabolic/storage disorders account for the vast majority of cardiomyopathy with hypertrophic phenotype.
- In this setting, cardiomyopathy rapidly progresses to heart failure and is often associated with neuromuscular abnormalities.

Hypertrophic Cardiomyopathy (HCM) in neuromuscular disorders

Hypertrophic cardiomyopathy is the most frequent cardiac phenotype in metabolic/storage disorders.

Cardiomyopathies with hypertrophic phenotype sorted by age at presentation

Disease	Protein defect/gene	Clinical diagnostic clues
Infancy		
Mitochondrial disorders	Nuclear genes coding for mt proteins	Mutisystem disease. Severe lactic acidosis.
Disorders of fatty-acid beta-oxidation	Primary carnitine and VLCAD deficiency	Symptoms are often triggered by mild viral illness, physiologic stress, or prolonged exercise
Pompe disease	Lysosomal acid maltase (*α-1,4-glucosidase*)	Autosomal recessive, multiorgan disease, pre-excitation pattern
Noonan/LEOPARD syndromes	Components or regulators of the Ras/MAPK pathway	Congenital heart defects, lentigines, café-au-lait spots

d'Amati G et al. Pathology of endomyocardial biopsy. Diagnostic Hystopathology. 2019; 24(11):433-444

Hypertrophic Cardiomyopathy (HCM) in neuromuscular disorders

Hypertrophic cardiomyopathy is the most frequent cardiac phenotype in metabolic/storage disorders.

Diagnosis is based on the demonstration of a specific biochemical defect and skeletal muscle biopsy is often part of the diagnostic flow-chart.

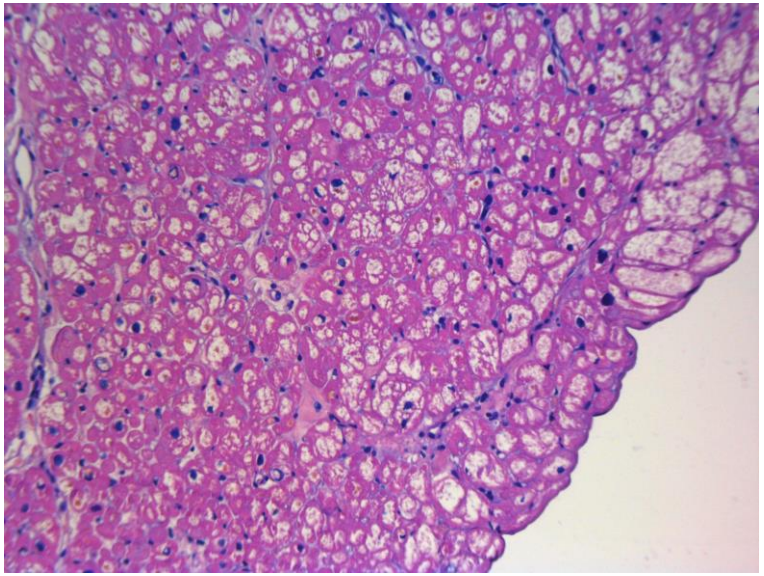
Hypertrophic Cardiomyopathy (HCM) in neuromuscular disorders

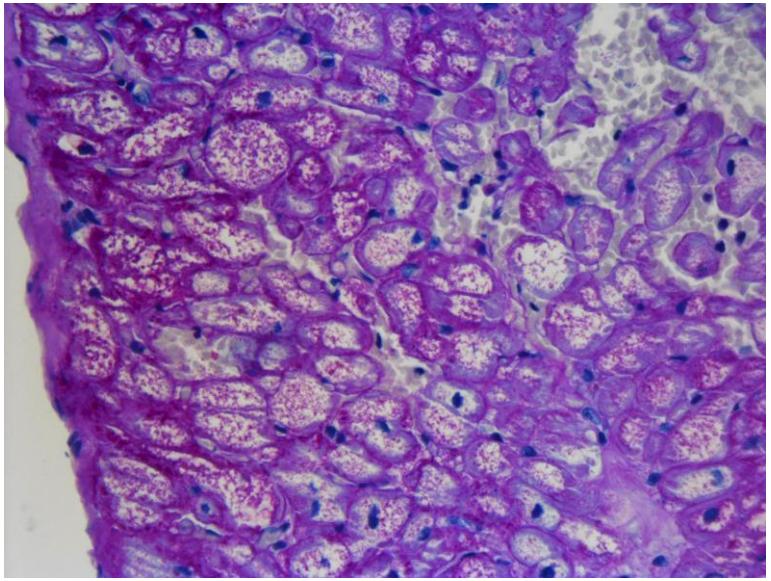
- In **adult patients**, unexplained cardiac hypertrophy is mostly related to mutations in genes encoding sarcomere-associated proteins.
- Massive hypertrophy may be the only manifestation of a metabolic disorder (phenocopy of sarcomeric HCM).

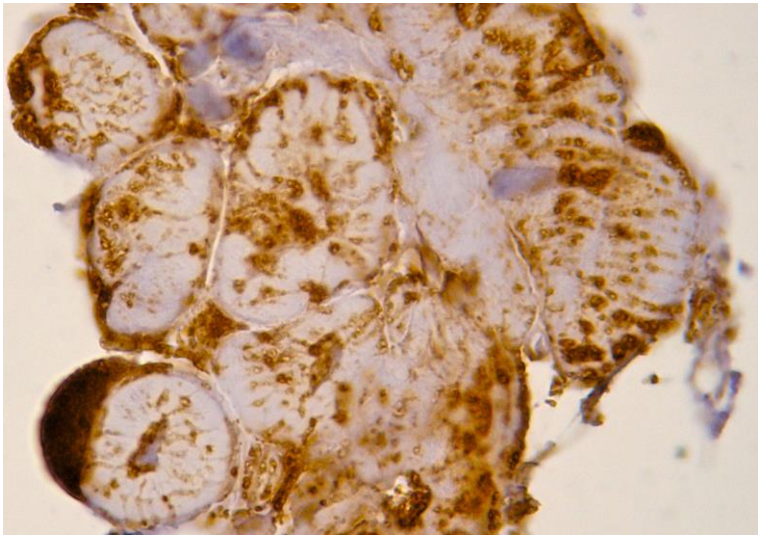
- Clue for differential diagnosis with sarcomeric HCM are:
  - Pattern of inheritance
  - Symmetric pattern of hypertrophy
  - Conduction abnormalities (WPW)
  - Subclinical myopathy (CK)

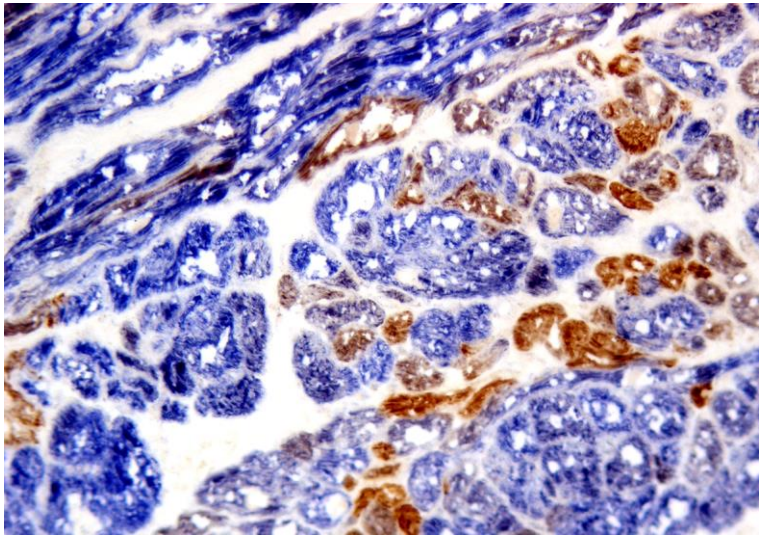
Endomiocardial biopsy may help in diagnosis.

Hypertrophic Cardiomyopathy (HCM) in neuromuscular disorders



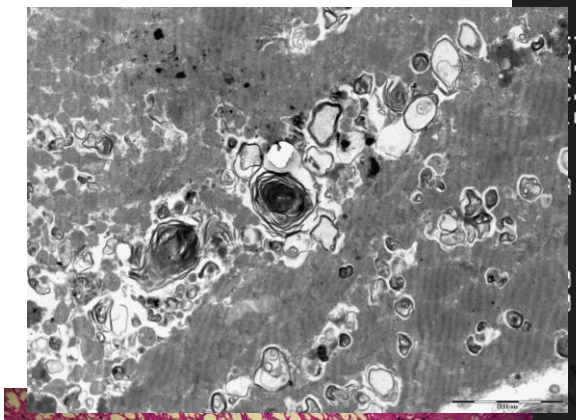


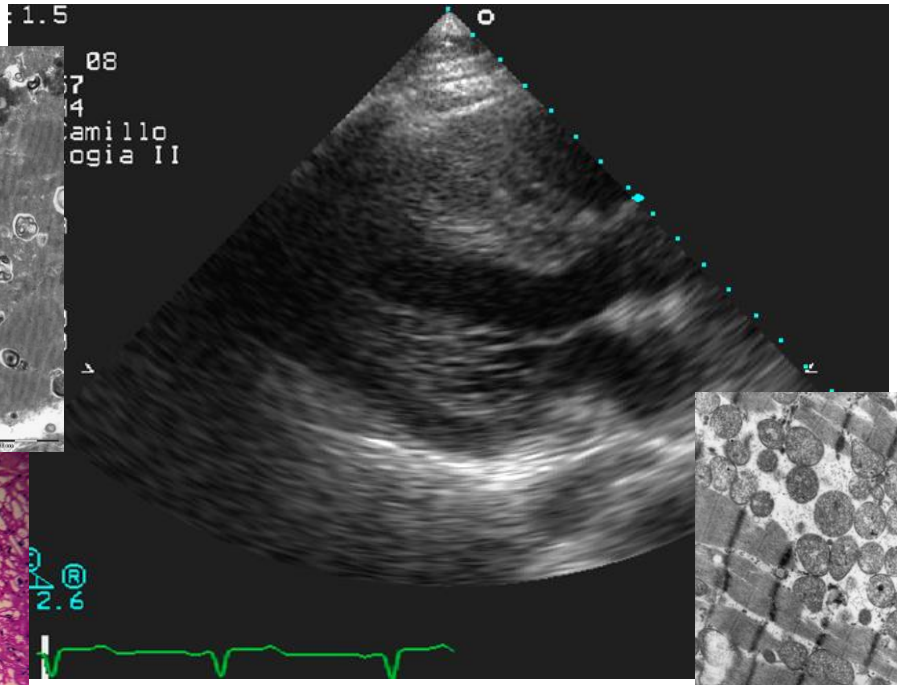


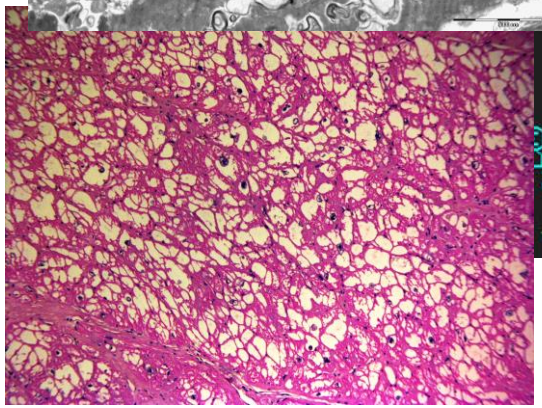


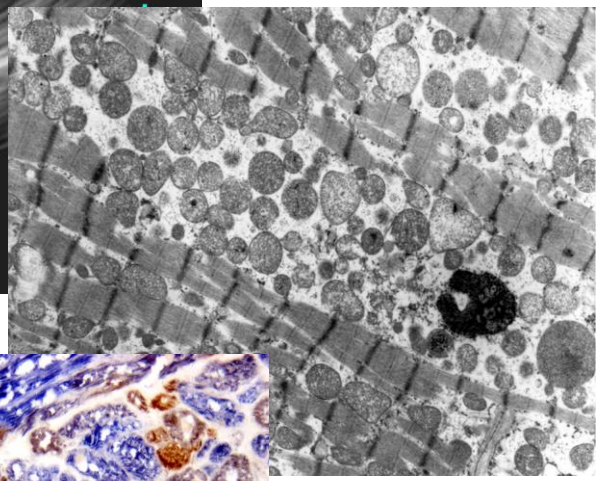
Endomiocardial biopsy may help in diagnosis.

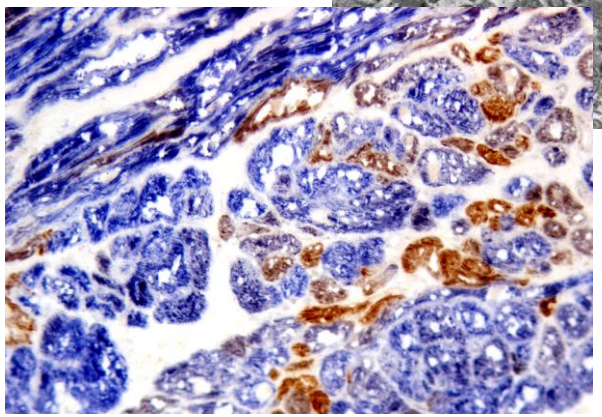
Phenocopy of sarcomeric HCM









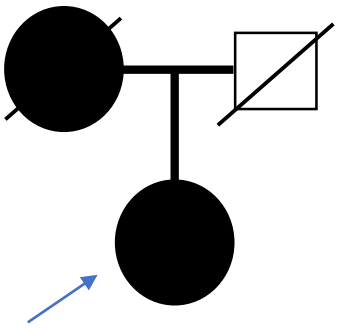


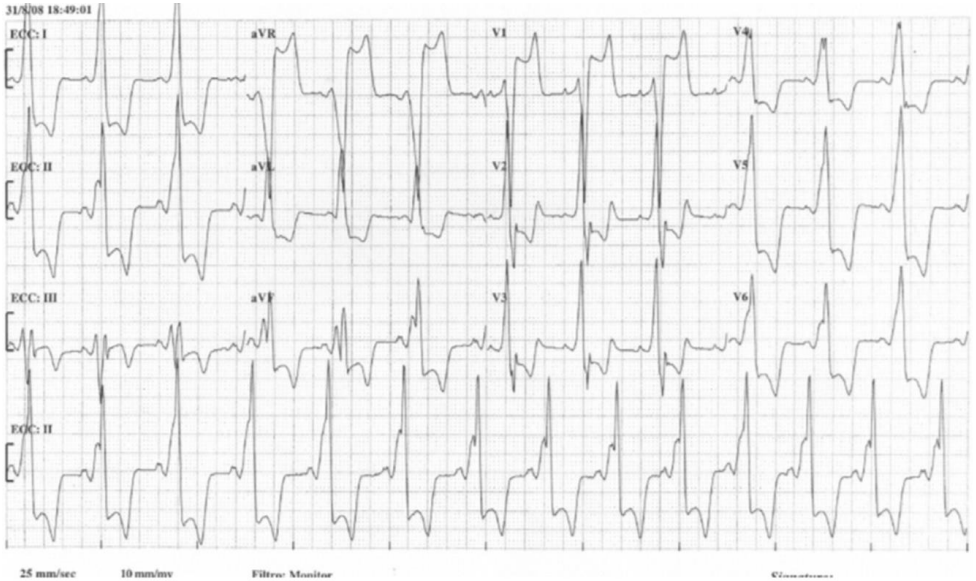
Storage disease
PRKAG2 mutations
Danon disease
Glycogenosis
Fabry disease

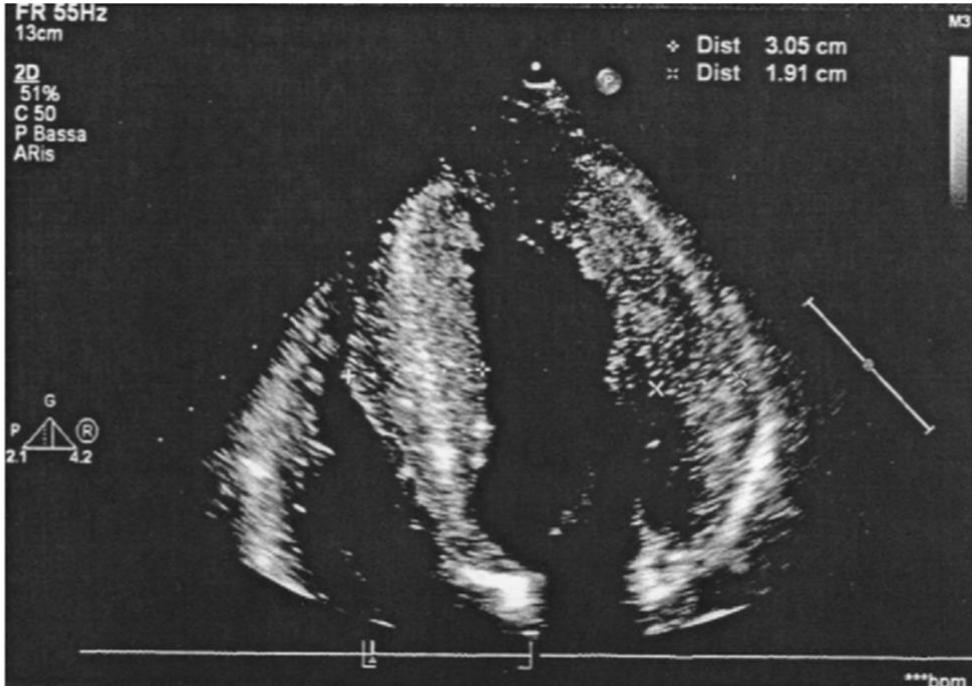
Mitochondrial cardiomyopathies

Female, 21-years old,
mild mental retardation.
Dyspnea, mild muscular weakness.
Pre-excitation pattern
Mild LV dilation

Family history positive for DCM







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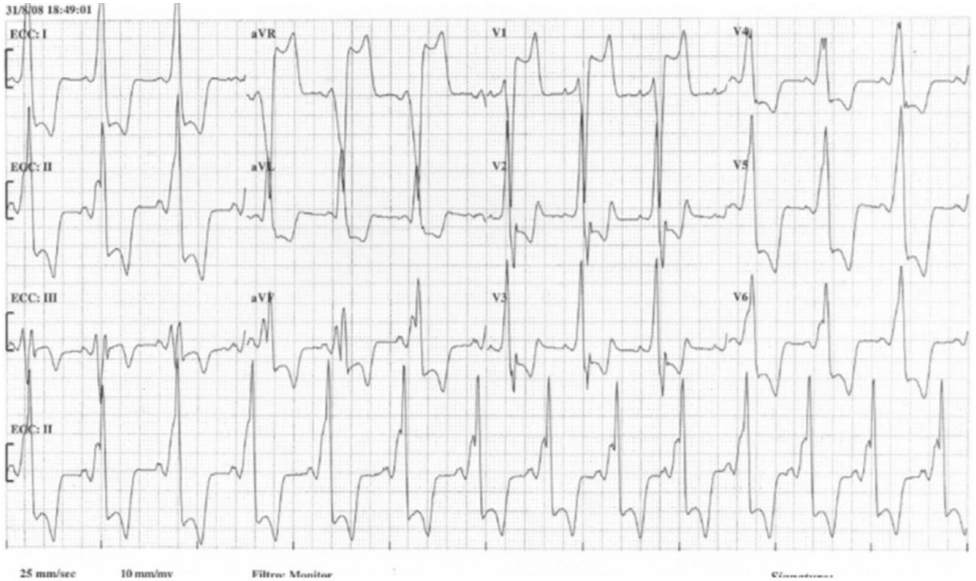

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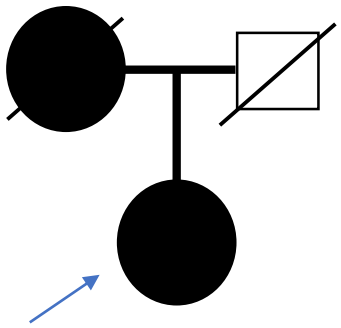

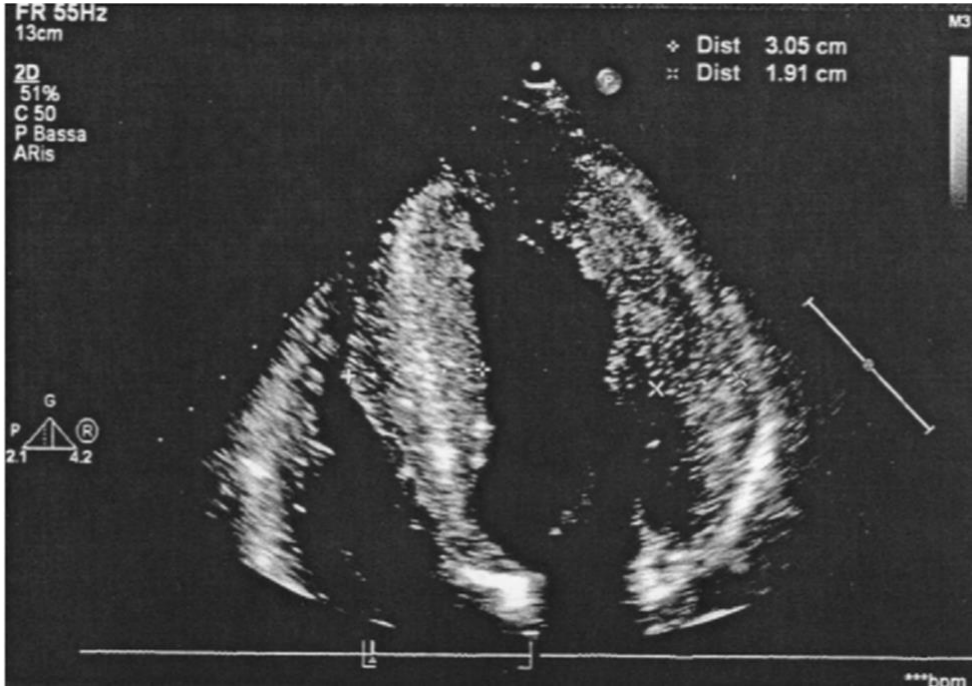

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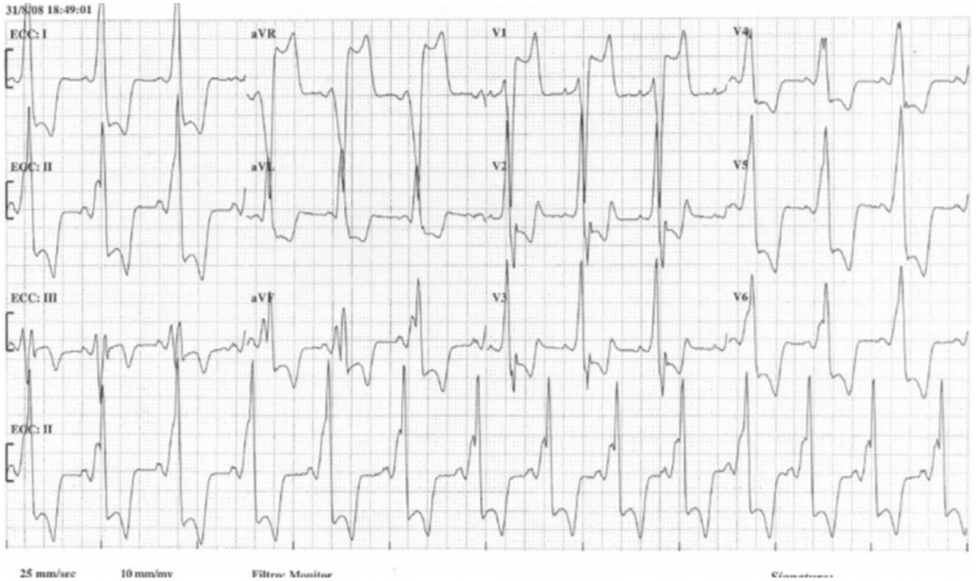
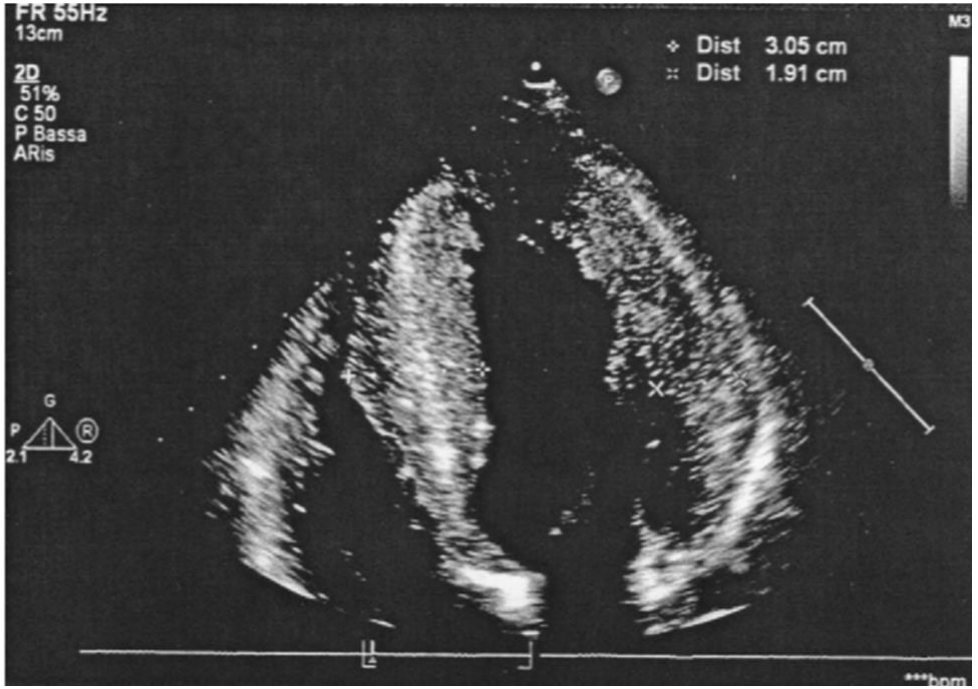
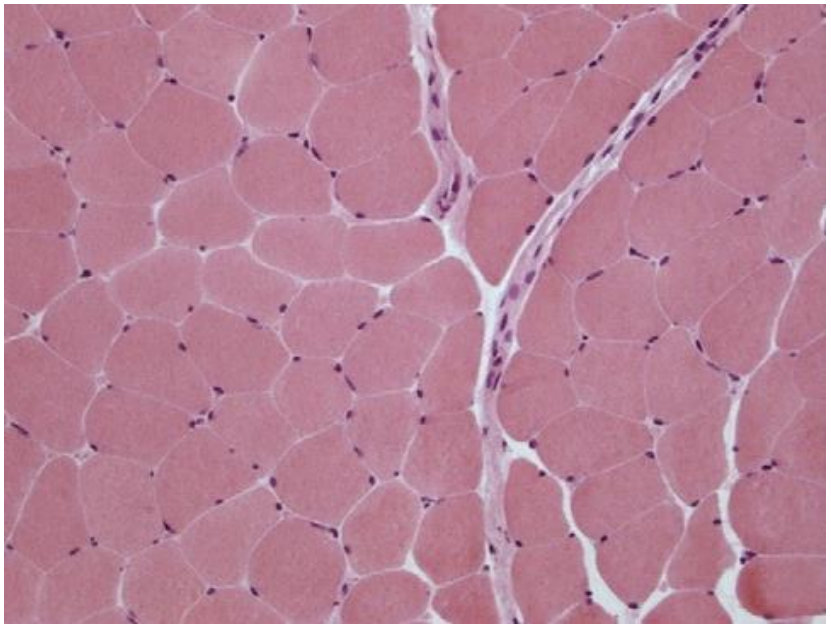


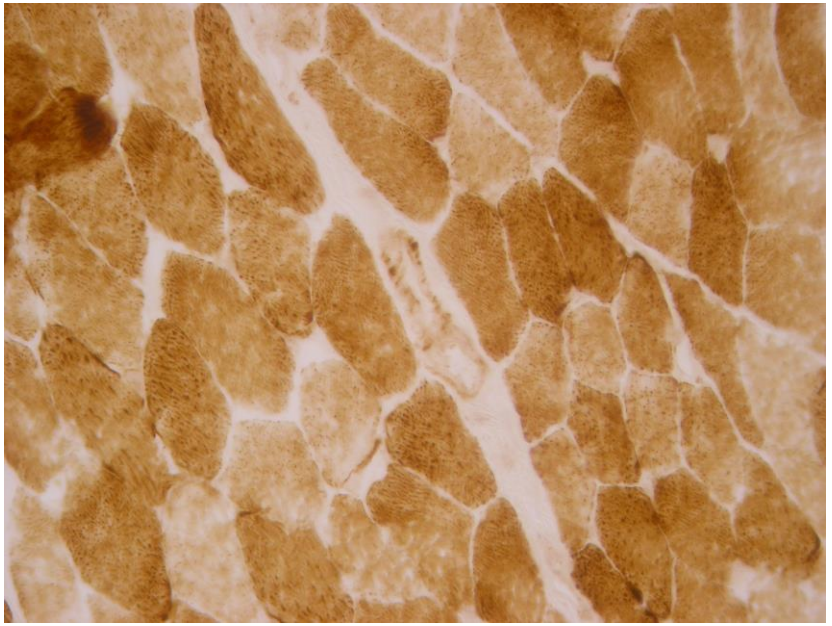
Family history positive for DCM

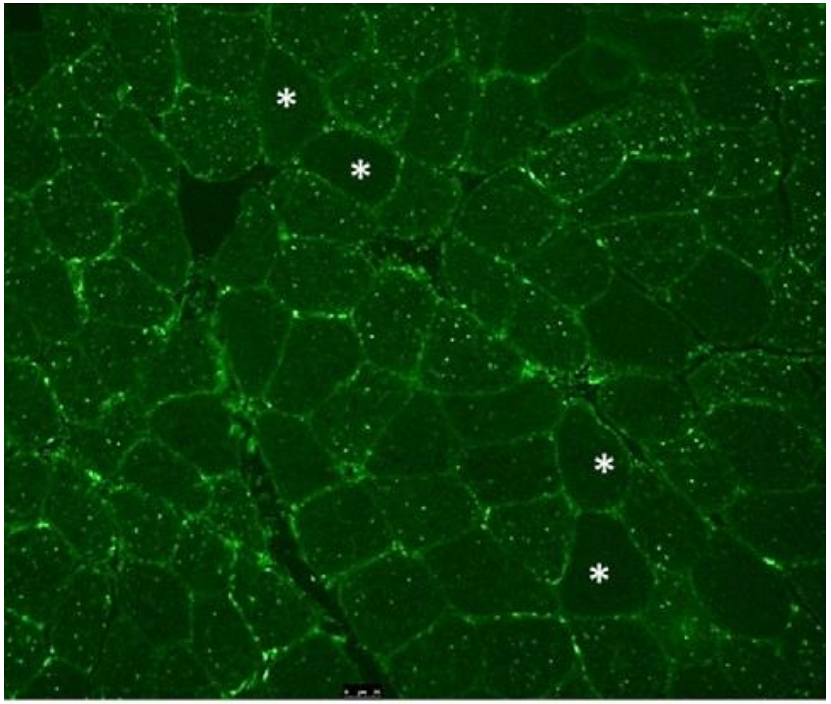
Danon disease

X-linked disorder caused by the primary deficiency of lysosome-associated membrane protein 2 (LAMP2), which coats the inner surface of the lysosomal membrane and is supposed to act as a receptor for proteins to be imported and degraded within lysosomes

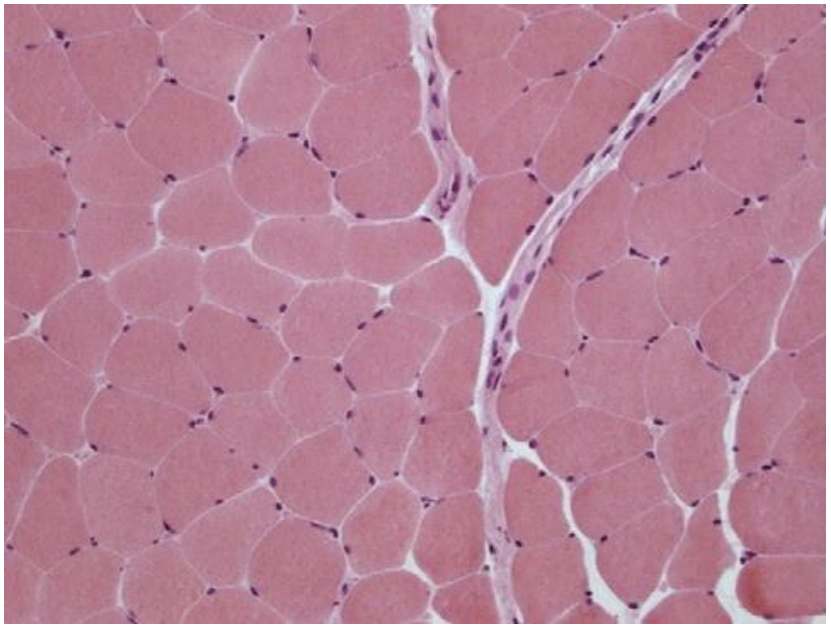


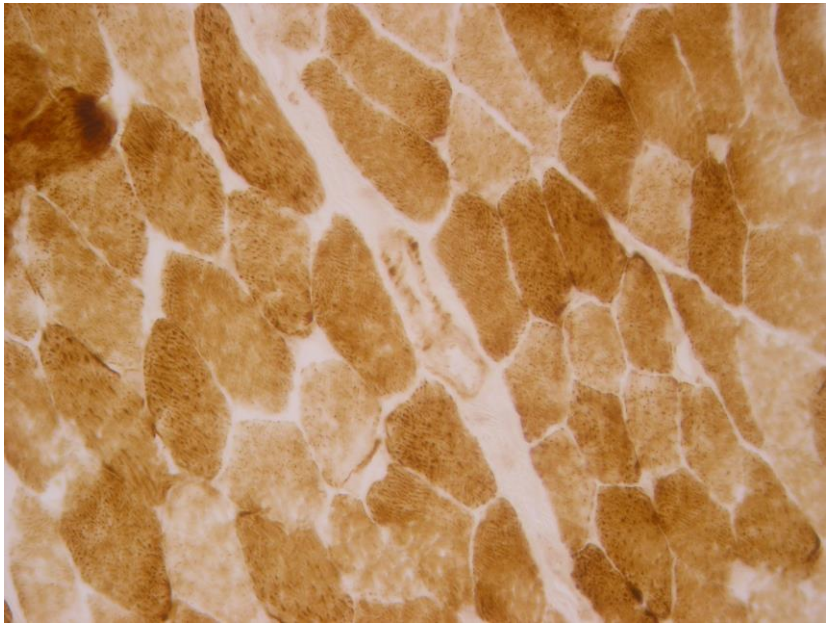


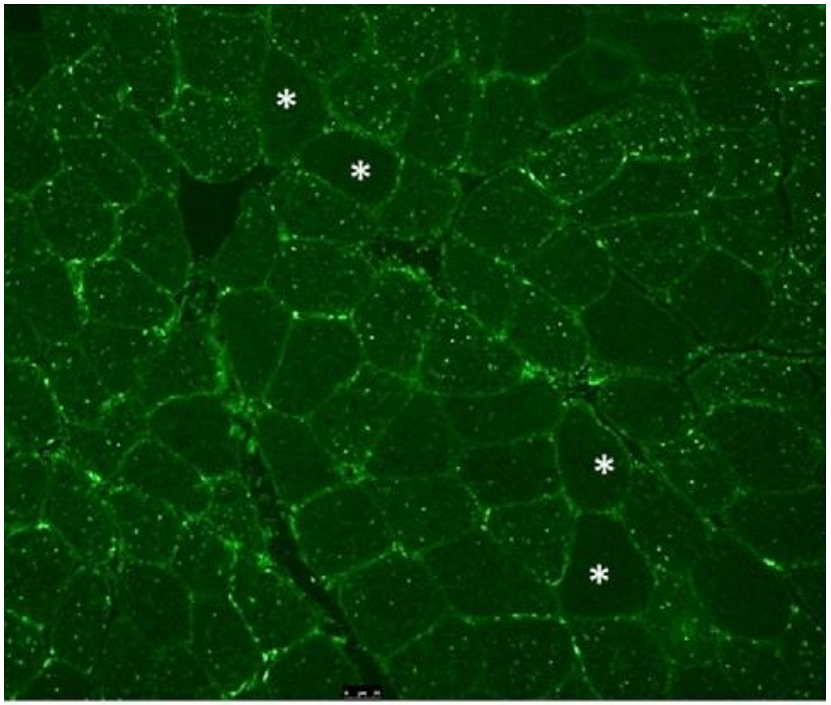




Rare LAMP2 negative fibers at immunofluorescence consistent with a status of manifesting carrier of Danon disease.

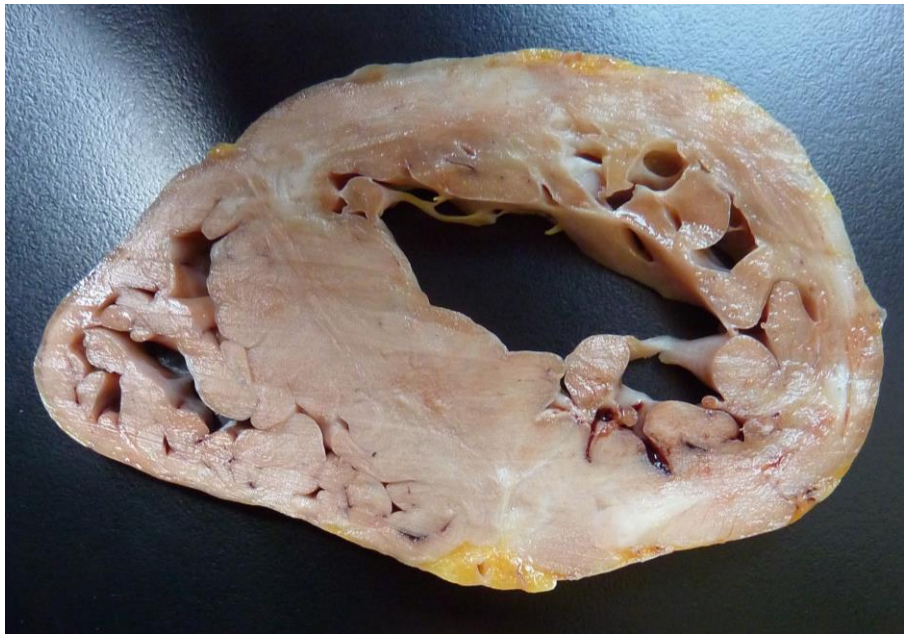


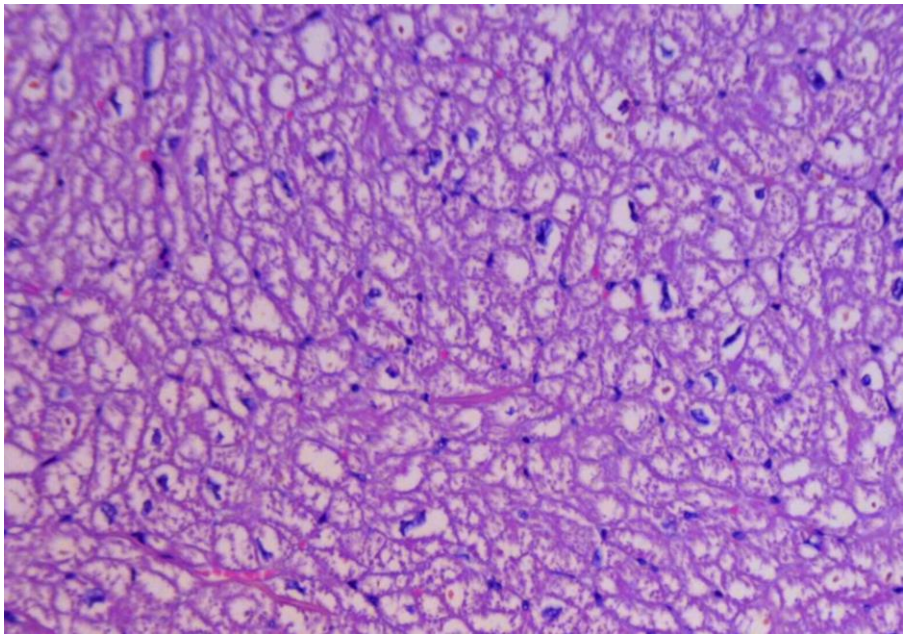


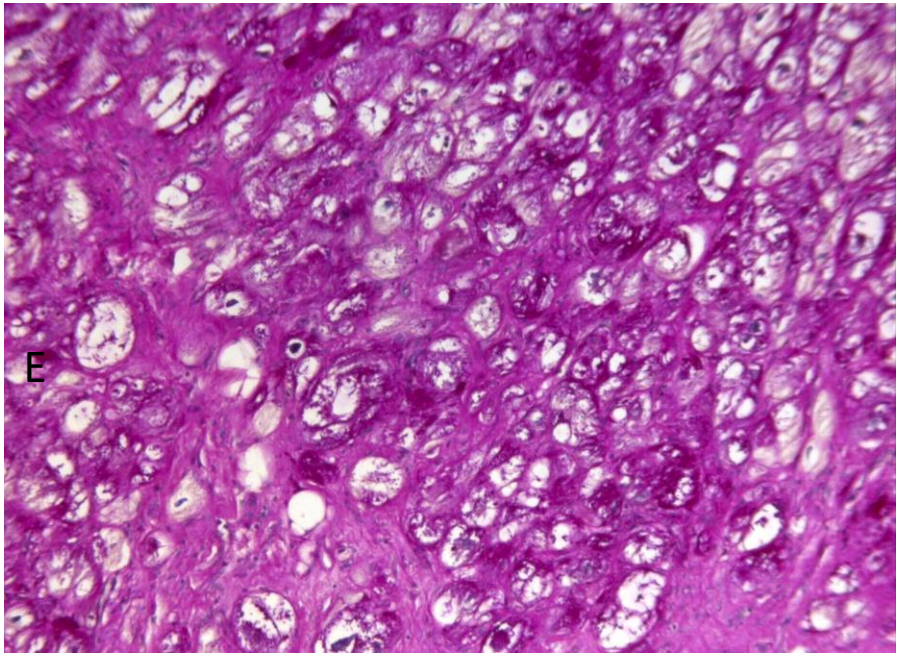


Rare LAMP2 negative fibers at immunofluorescence consistent with a status of manifesting carrier of Danon disease.

One year later the patient experienced severe heart failure and underwent cardiac transplantation.



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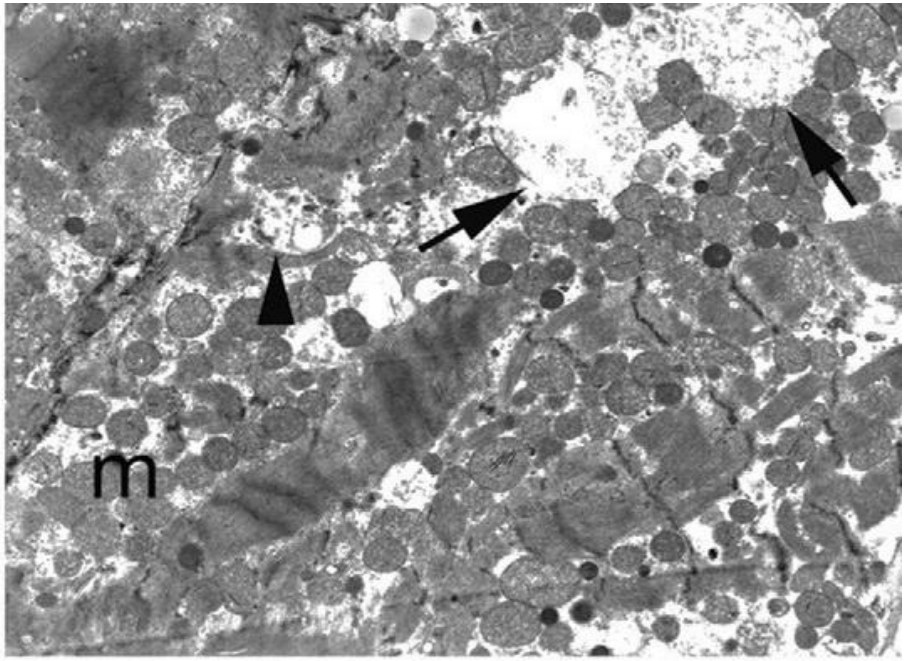
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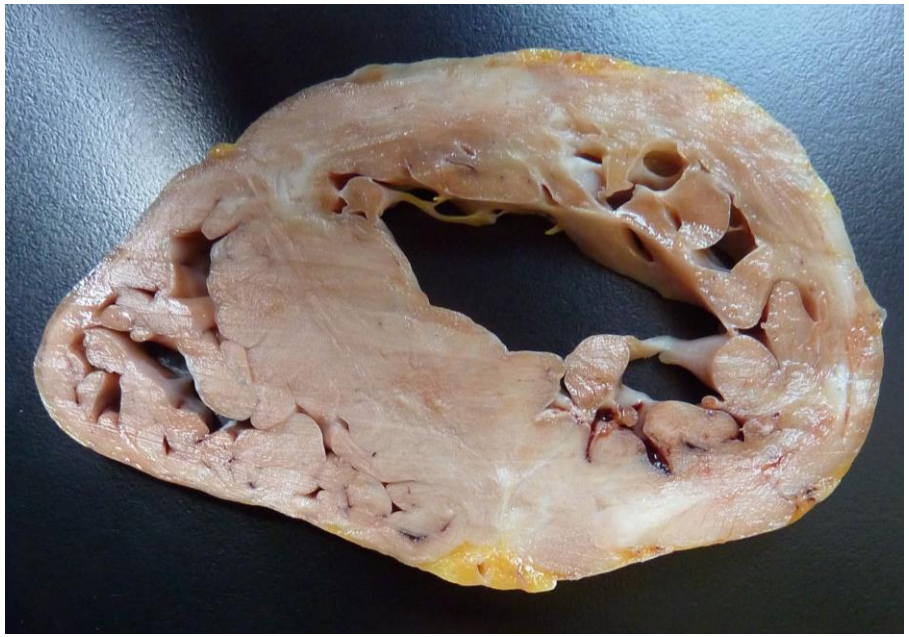
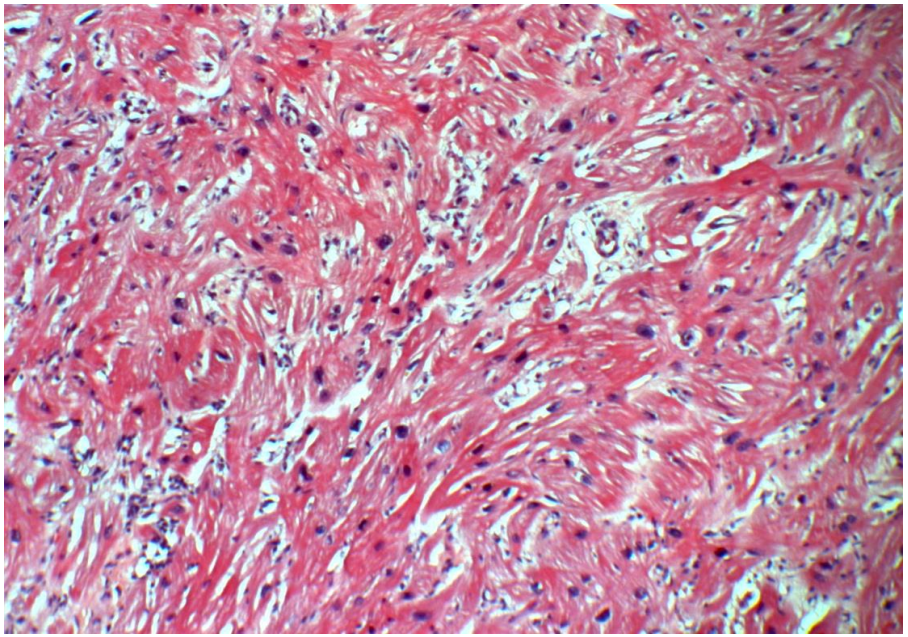
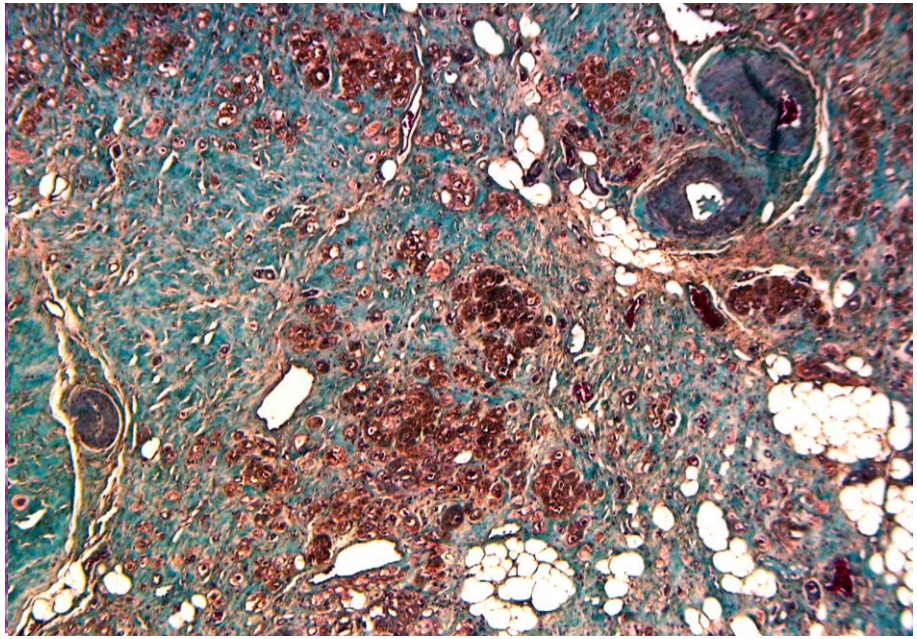
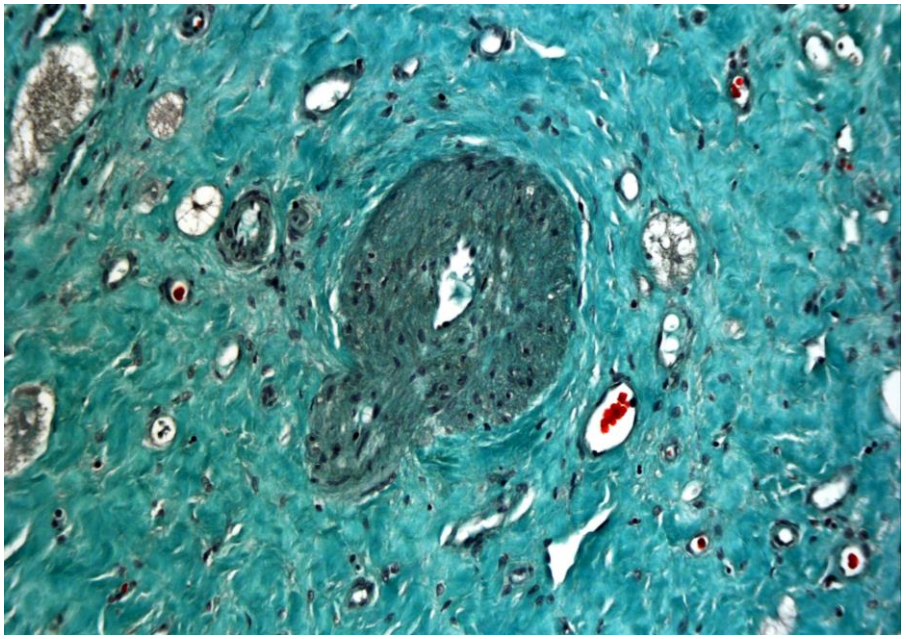
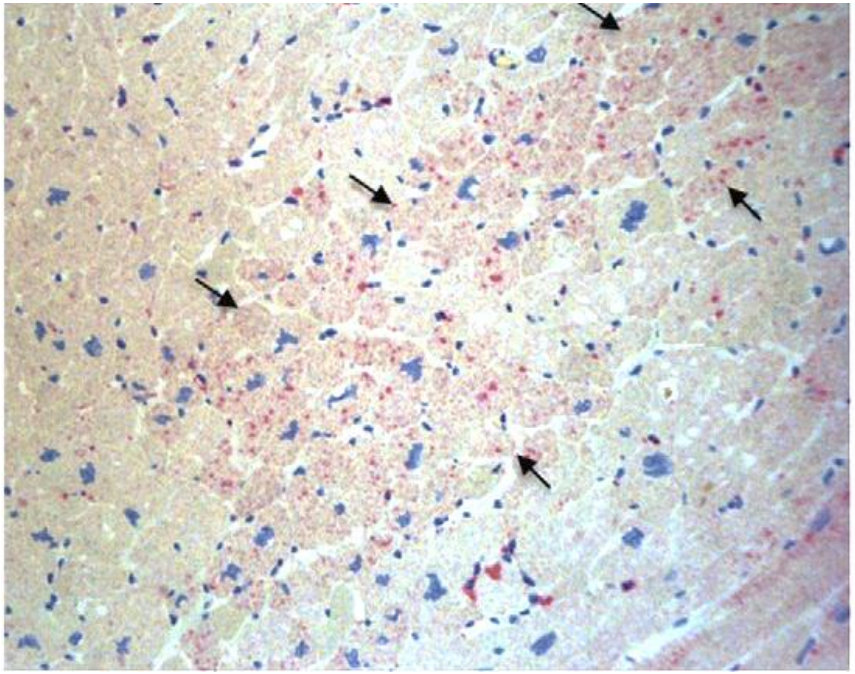
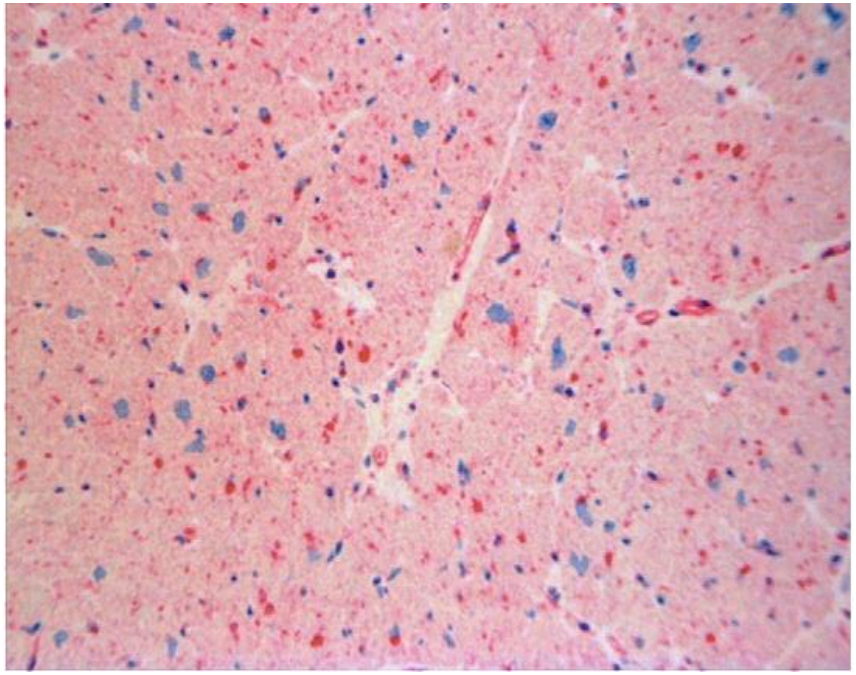






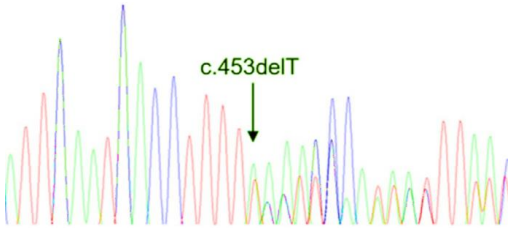






Paternal allele (WT) of the X chromosomes was inactivated in the left ventricle (56% of methylation) and in the septum (61% of methylation).

Next generation sequence analysis with a panel including LAMP+ 91 genes involved in CM disclose a a truncating mutation in LAMP2 (chrX::c.453delT, p. F151fs)



A novel *LAMP2* mutation associated with severe cardiac hypertrophy and microvascular remodeling in a female with Danon disease: a case report and literature review☆

Bottillo et al. Cardiovasc Pathol. 2016;25(5):423-31.

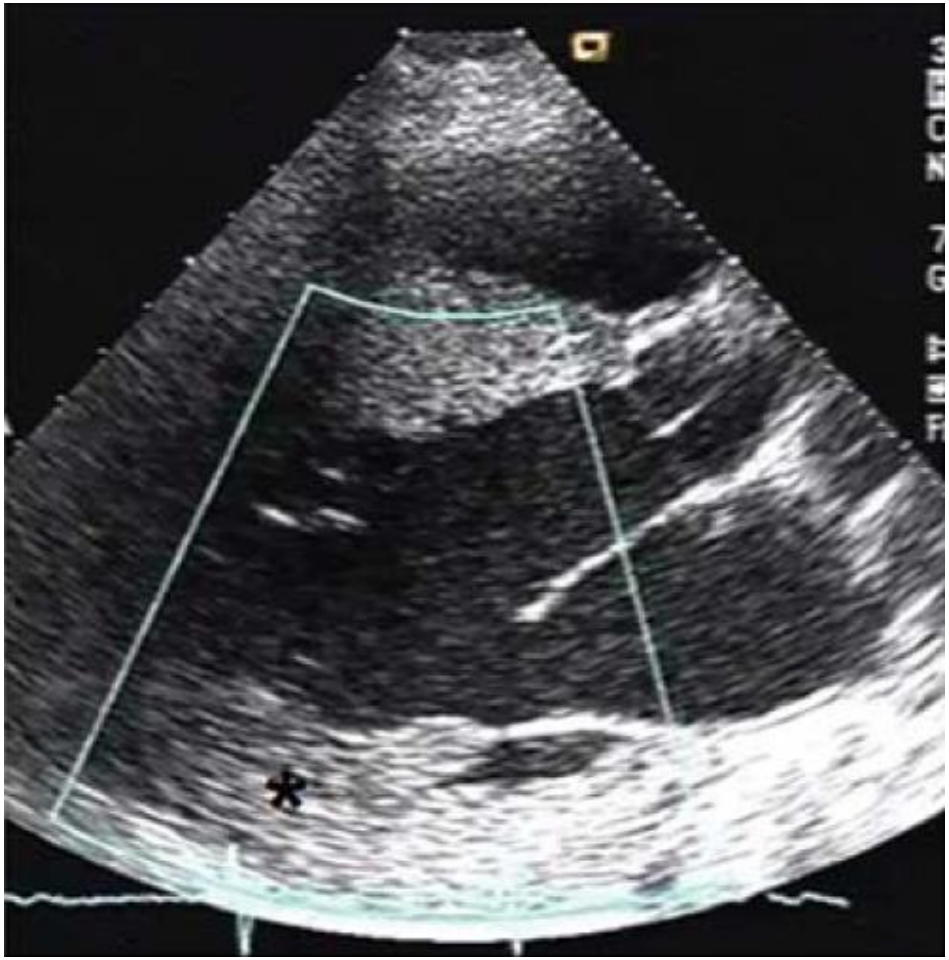
Danon disease

- Heart disease is the dominant clinical feature in both males and females, and it is often associated with conduction disease. Remarkably, the ECG pattern of WPW is observed in about 68% of men and 27% of women
- The onset of cardiac symptoms is usually 10 years later in affected women as compared to males and life expectancy is about 25 years longer.

Early onset cardiomyopathy in females with Danon disease

Carola Hedberg Oldfors [a,*], Gyöngyvér Máthé [b], Kate Thomson [c], Mar Tulinius [d], Kristjan Karason [e], Ingegerd Östman-Smith [f], Anders Oldfors [a]

Male, 16 years old,
Symmetric LV hypertrophy
Sensorineural hearing loss from the age of 2
CK (324 IU/L)



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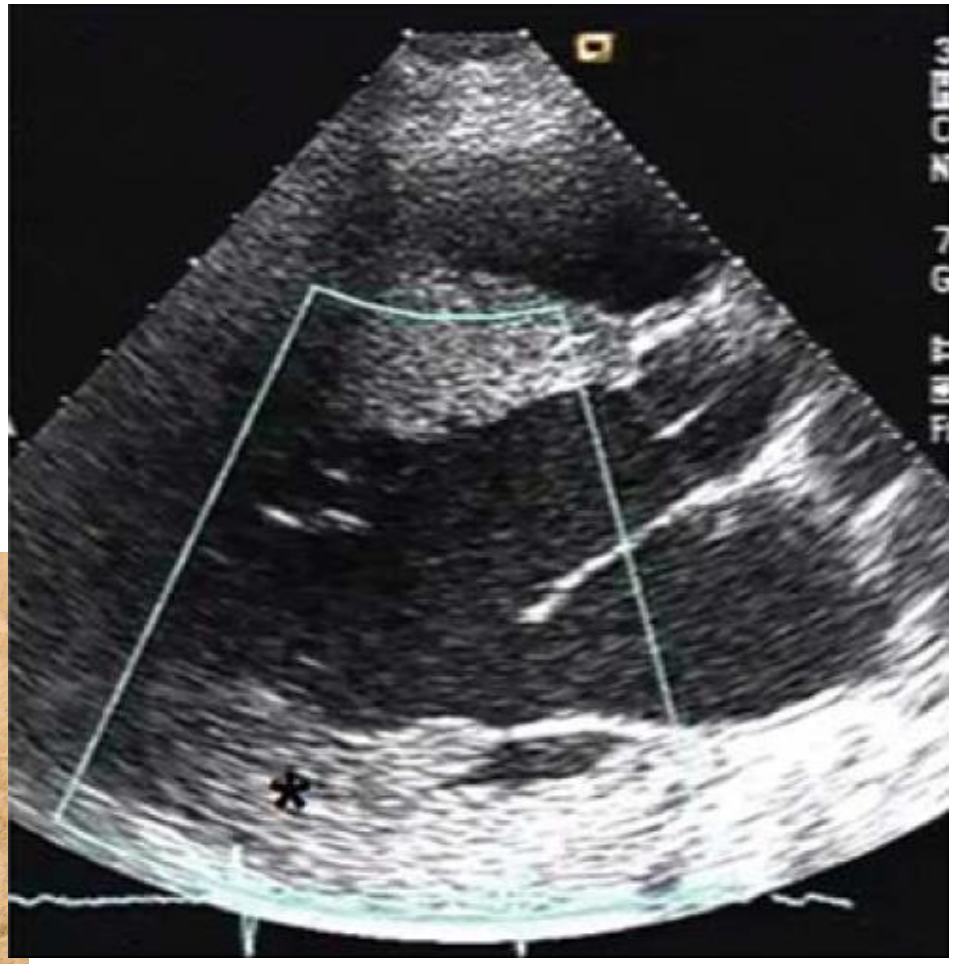

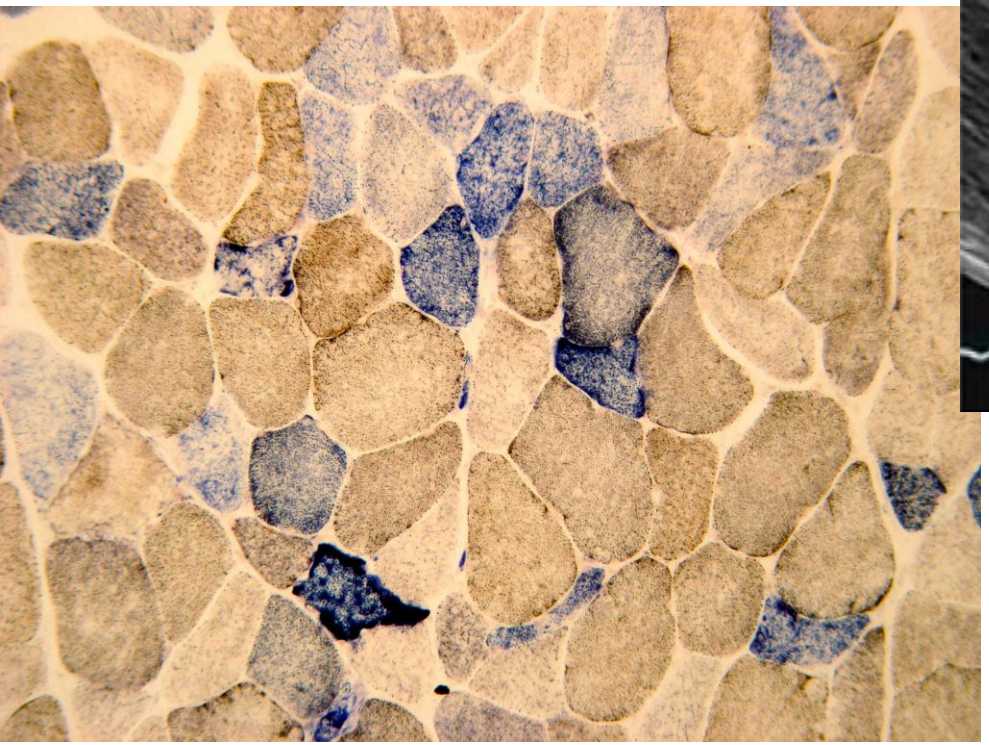

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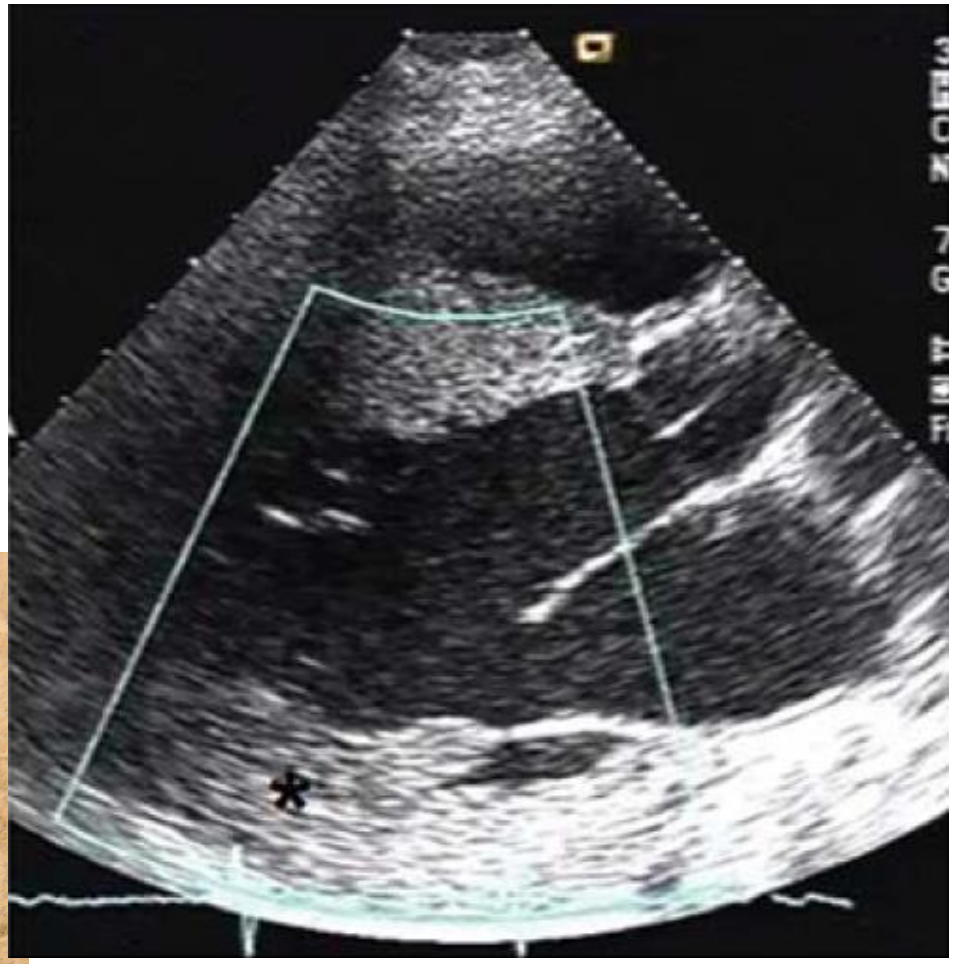
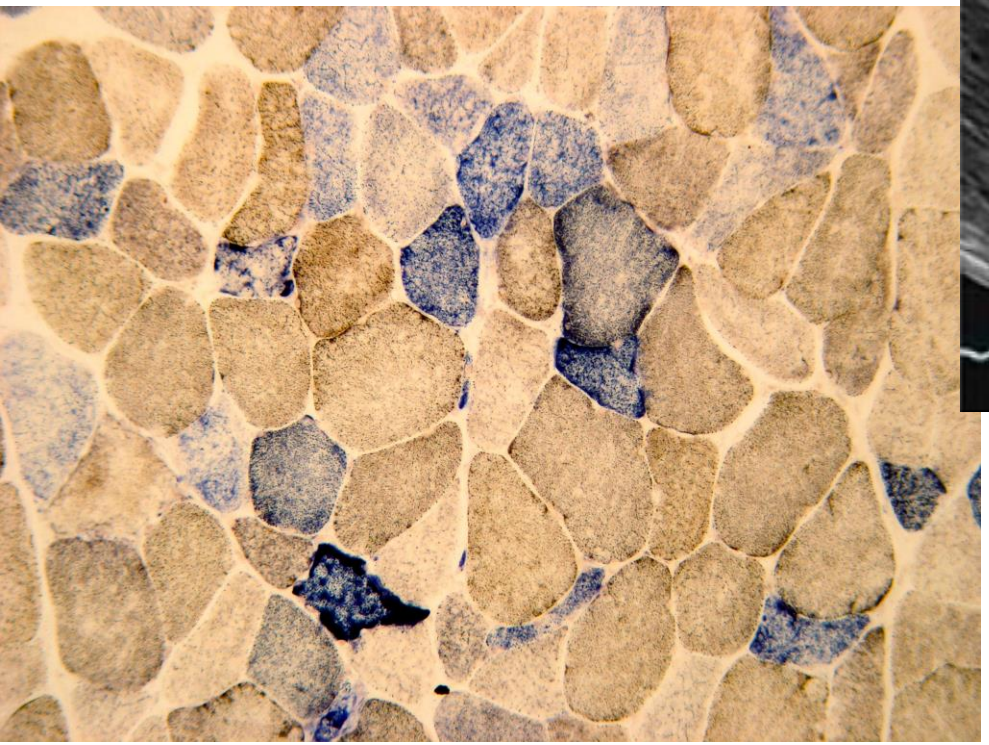
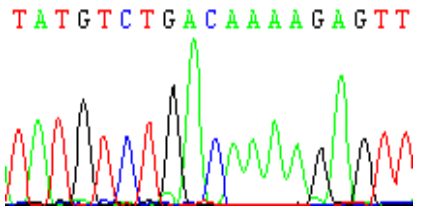
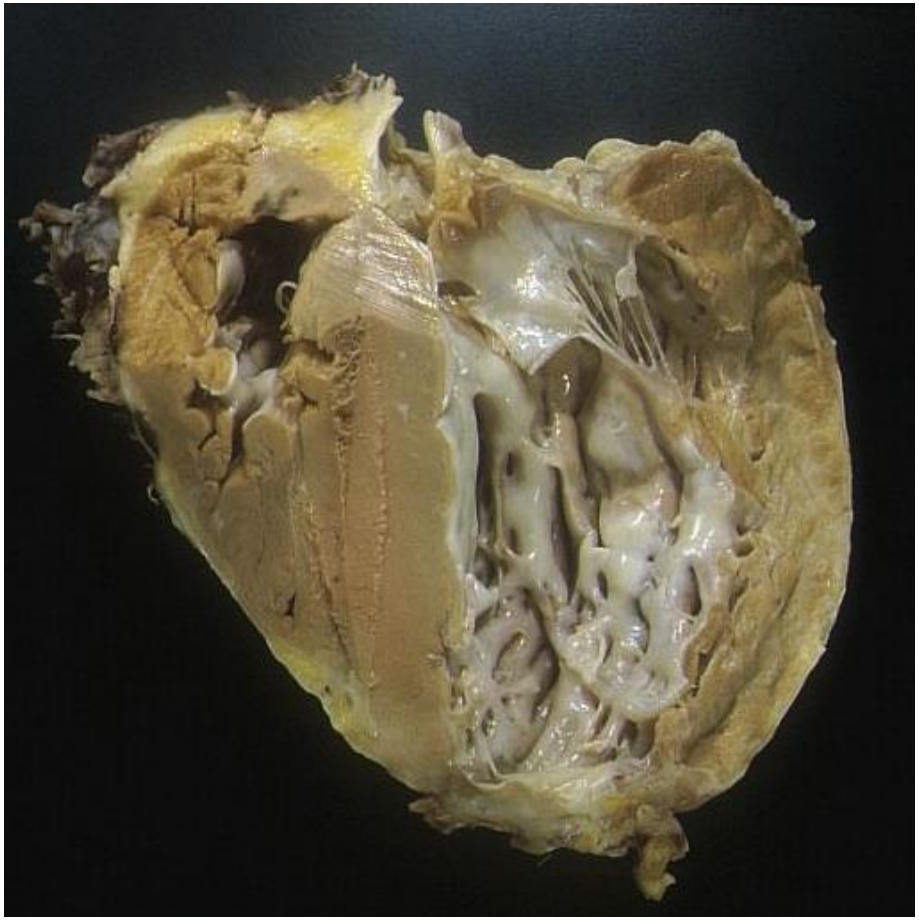
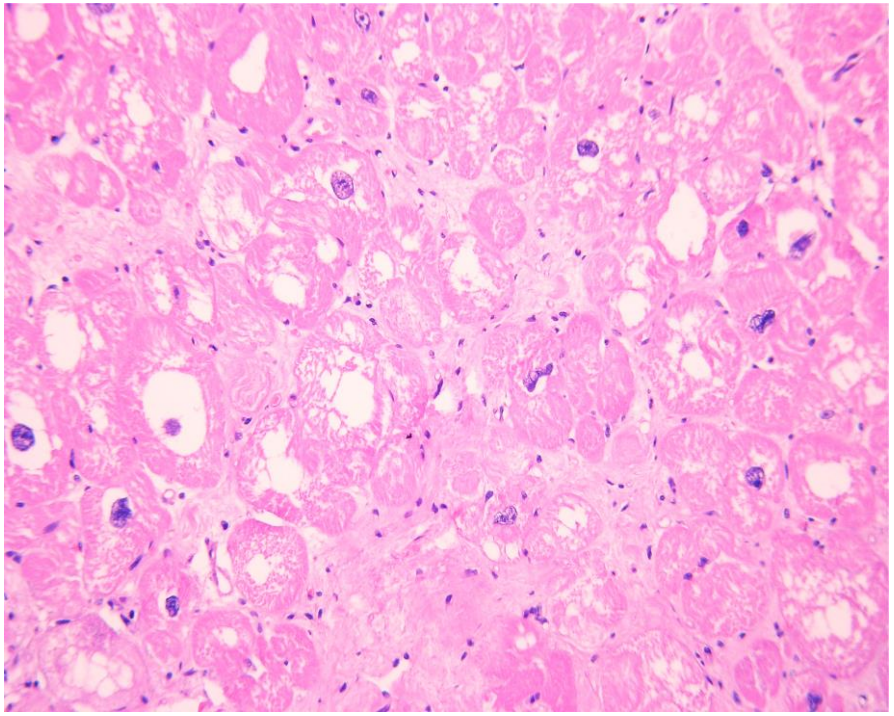


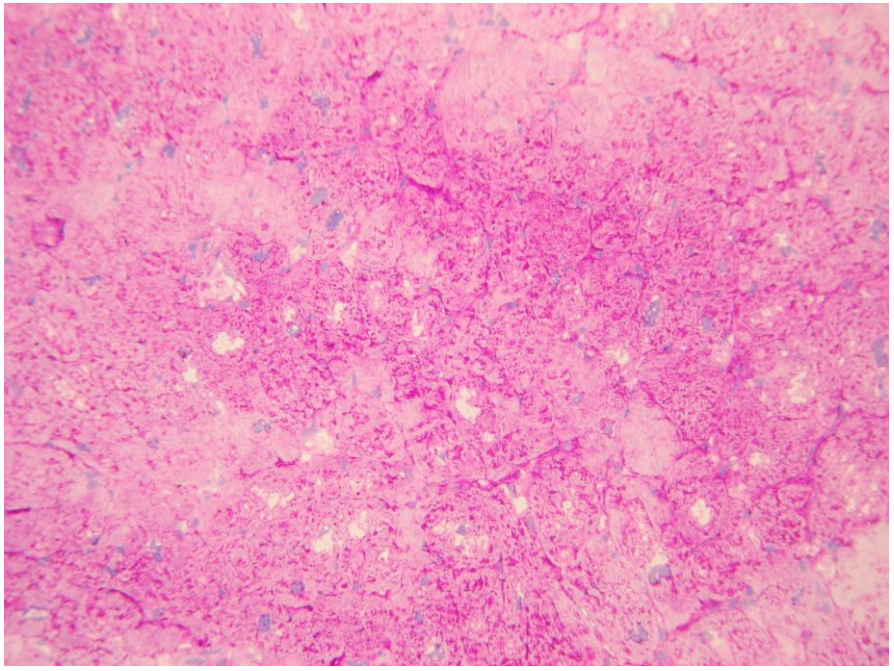


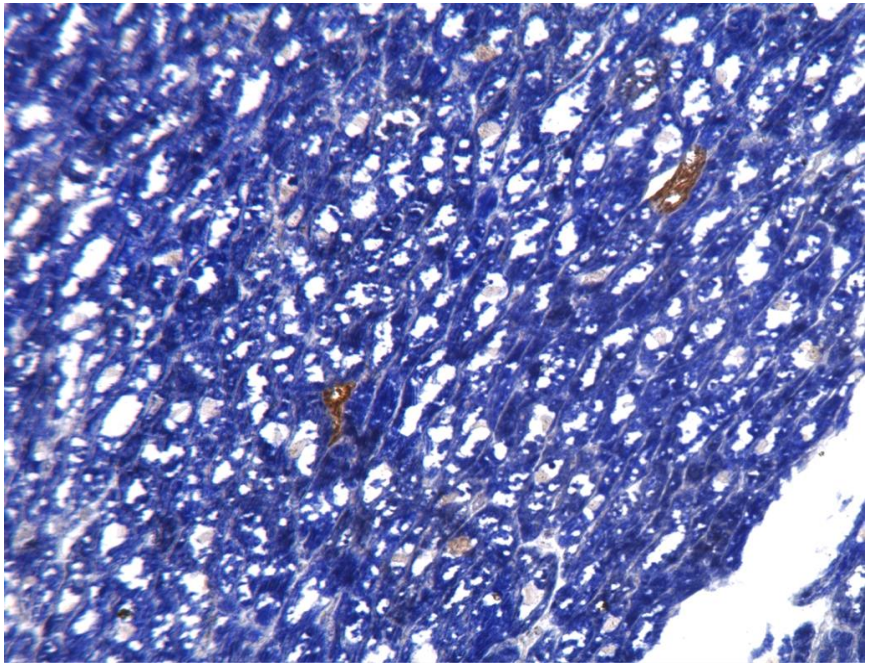
4277T>C,mt-tRNA ^{Ile}

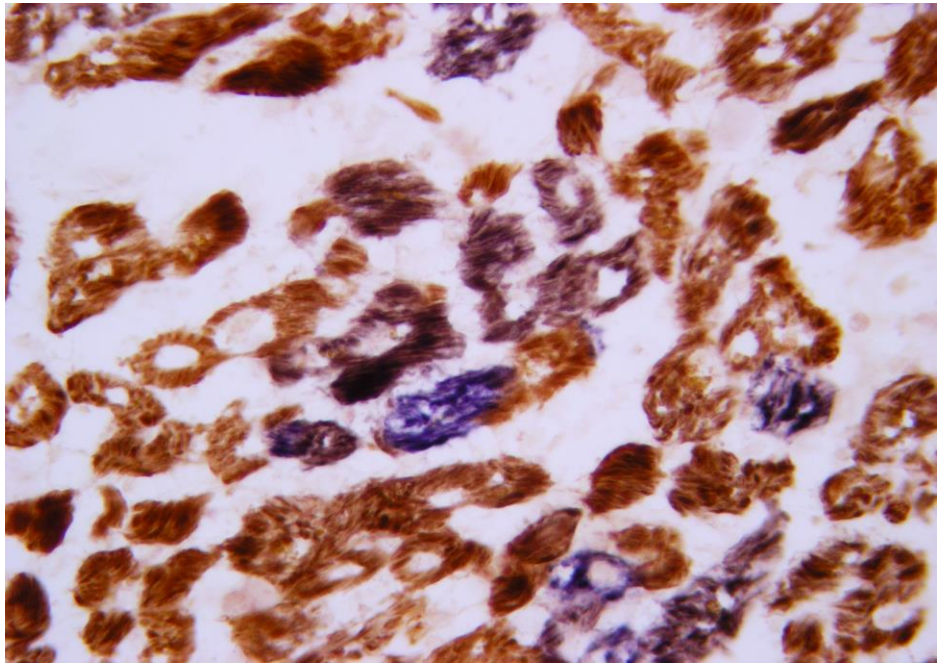


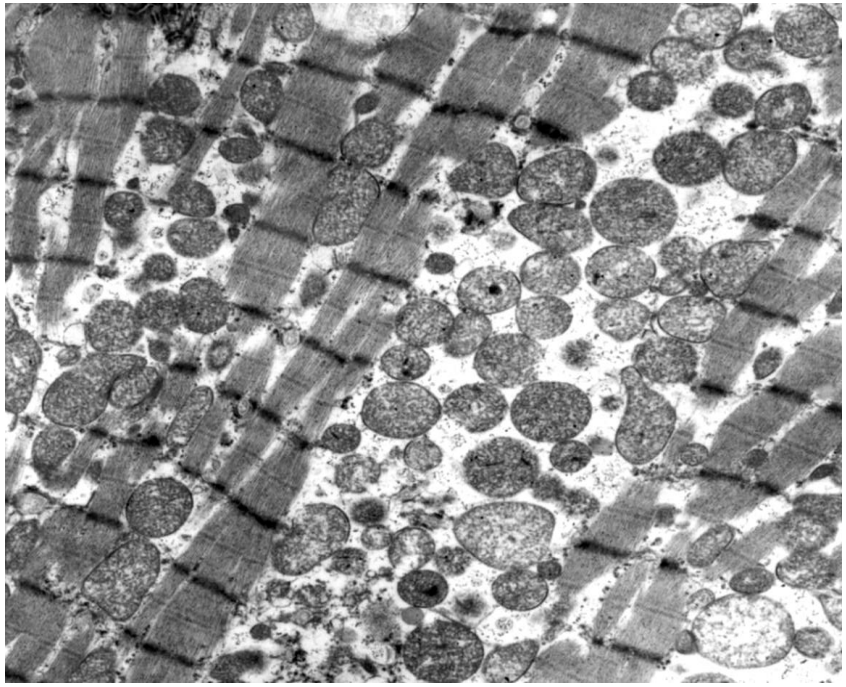


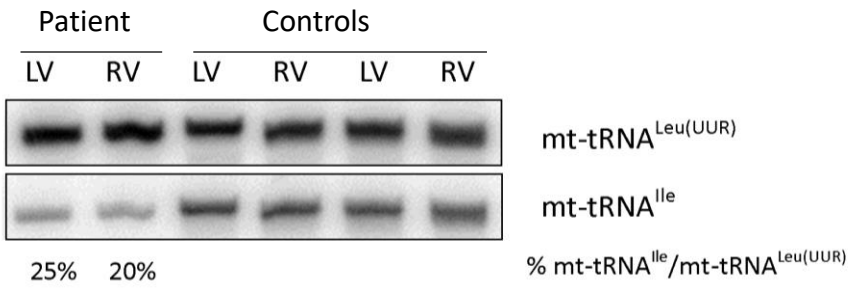












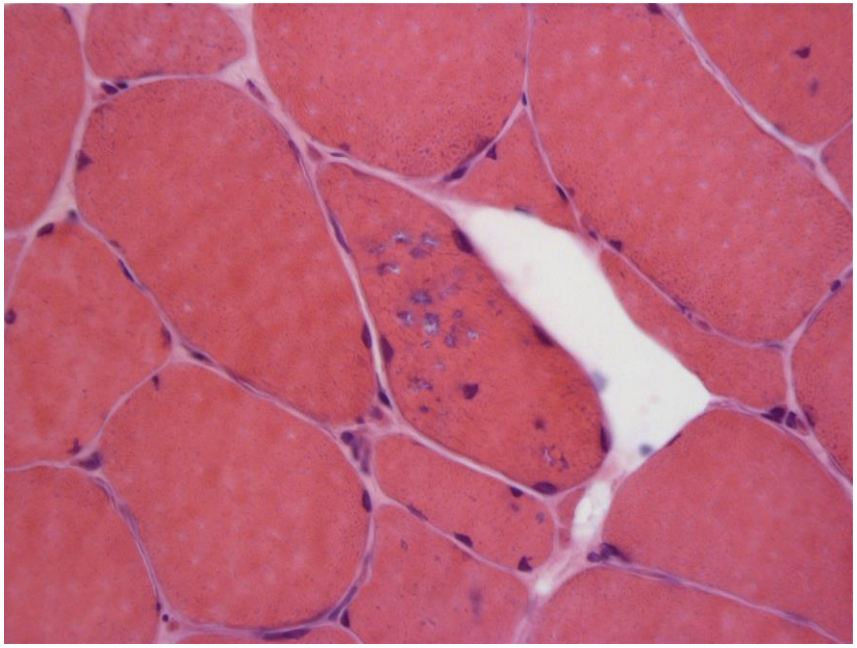
Cardiomyopathies due to homoplasmic mitochondrial tRNA mutations: morphologic and molecular features. Giordano C et al. Hum Pathol. 2013 Jul;44(7):1262-70

Restrictive cardiomyopathy (RCM)

Reduced diastolic relaxation of either the left or both ventricles, with impeded diastolic filling, increased ventricular filling pressure, and normal (or near normal) systolic function.

Myofibrillar myopathies

- Foci of myofibril disruption that begins at the sarcomeric Z-disk associated with abnormal accumulation of myofibrillar degradation products.



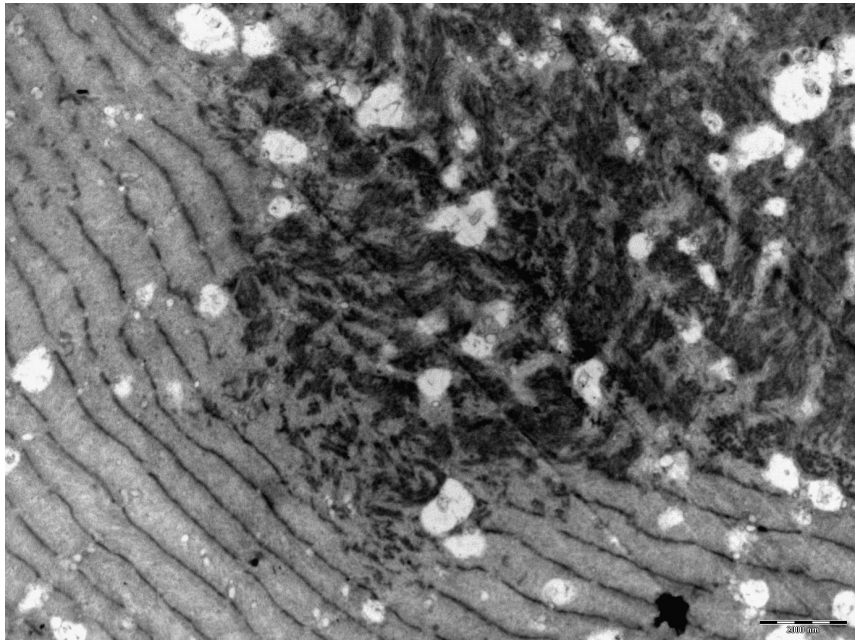


Table 1. Myofibrillar myopathies: clinical presentation. Abbreviations: CB = conduction block; CK = creatine kinase; DCM = dilative cardiomyopathy; RSS = rigid spine syndrome; SPS = scapuloperoneal syndrome; EBS = epidermolysis bullosa simplex.

Disease	Muscle weakness	Heart	Respiratory system	CK levels	Extramuscular
Desminopathy	Distal > proximal, SPS	DCM, CB	Insufficiency	*n* – 5*x*	
aBCopathy	Proximal > distal	DCM,CB		*n* – 7*x*	Cataracts
Filaminopathy	Proximal > distal	DCM, CB	Insufficiency	*n* – 8*x*	Neuropathy
Myotilinopathy	Distal > proximal	DCM	Insufficiency	*n* – 5*x*	Neuropathy, contractures
BAG3opathy	Proximal	DCM	Insufficiency	*n* – 15*x*	RSS, scoliosis, contractures neuropathy
FHL1opathy	Distal = proximal, hypertrophy, SPS	CB	Insufficiency	*n* – 10*x*	RSS, scoliosis contractures
ZASPopathy	Distal > proximal hand muscle atrophy	DCM, CB		*n* – 6*x*	Neuropathy
Plectinopathy	Distal > proximal			*n* – 5*x*	EBS, nail dystrophy

Myofibrillar Myopathies: A Clinical and Myopathological Guide

Rolf Schröder, MD[1]; Benedikt Schoser, MD[2]

Brain Pathology **19** (2009) 483–492

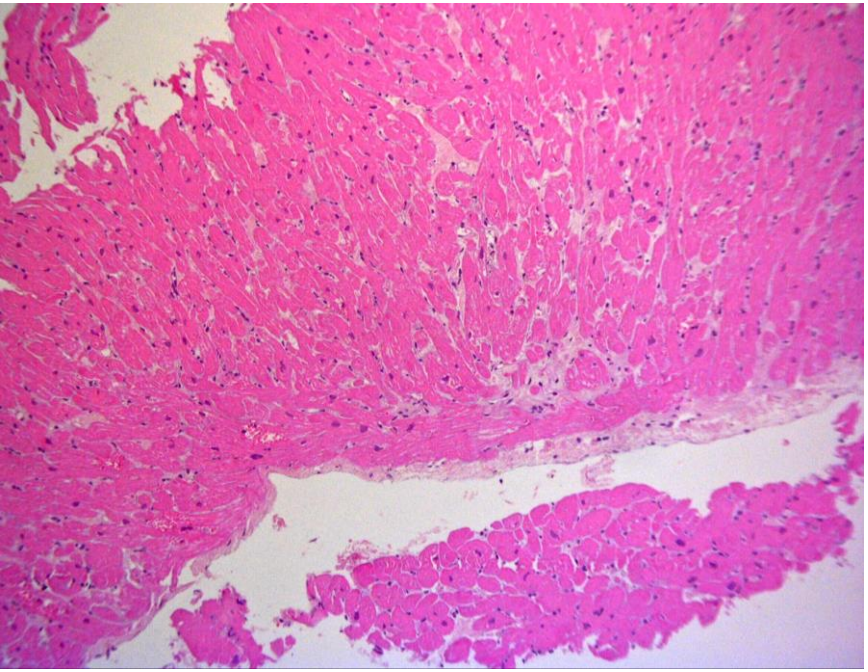
- Clinically, develops later in life (7-77 yeras of age) with slowly progressive muscle weakness, from distal to proximal lower extremities.

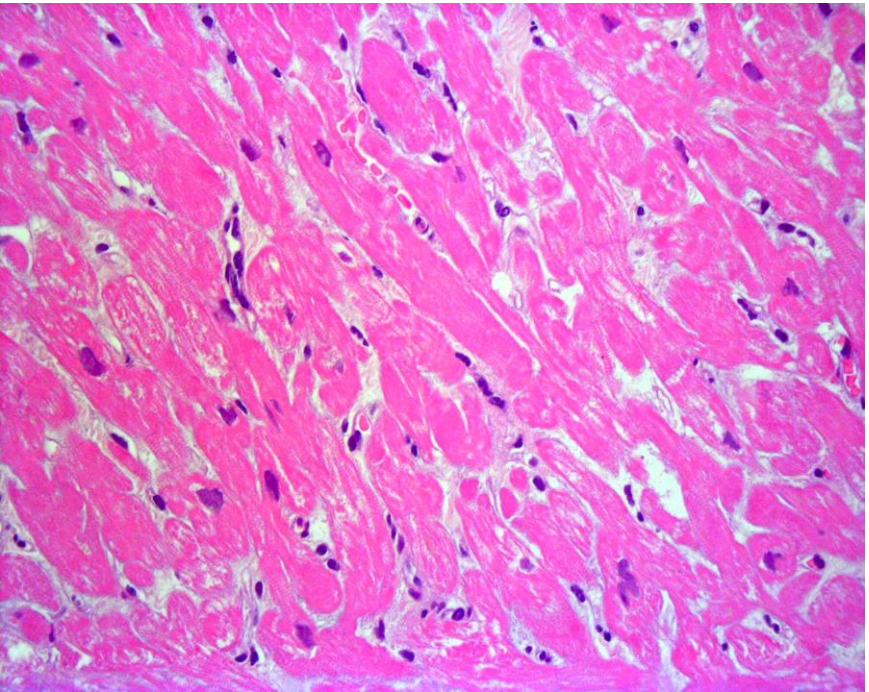
- Peripheral neuropathy and cardiomyopathy are associated features in 15% to 30% of patients.

- Cardiac involvement is more prevalent in mutations that cause childhood-onset MFM.

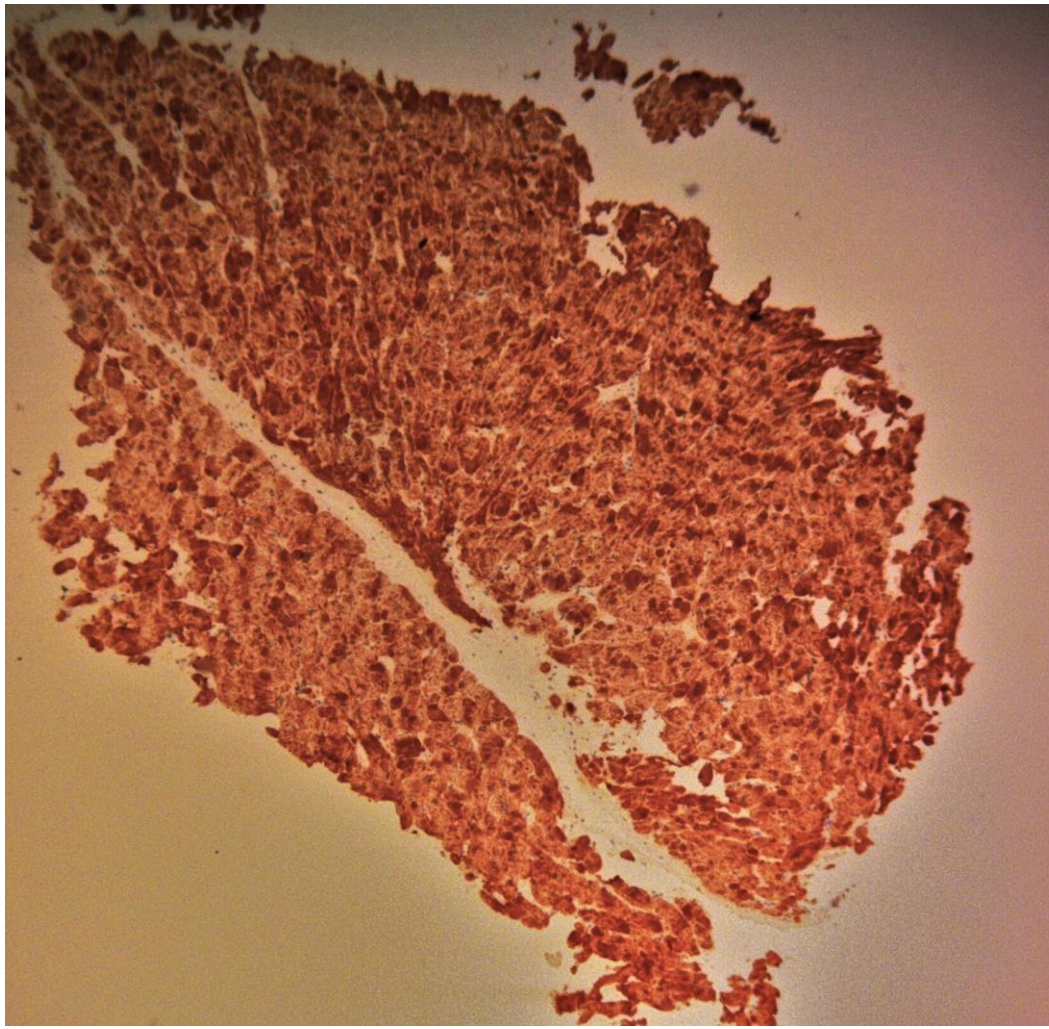
- Although the RCM can be the first clinical manifestation of the disease, the skeletal muscle is always structurally affected, even in the absence of clinically overt myopathy

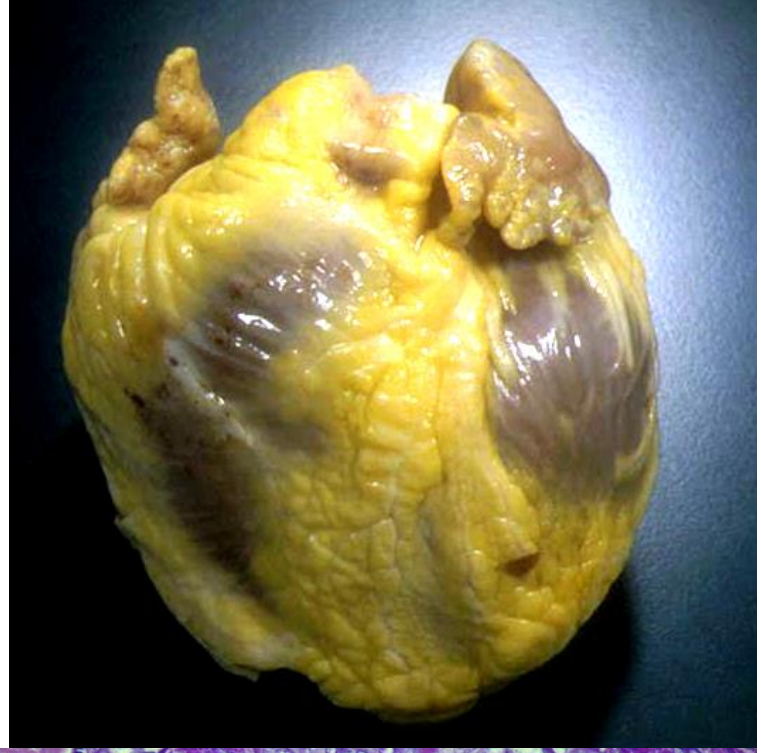
Associated features include sinus node dysfunction, atrioventricular block, supraventricular and ventricular tachycardias, heart failure, and sudden death.

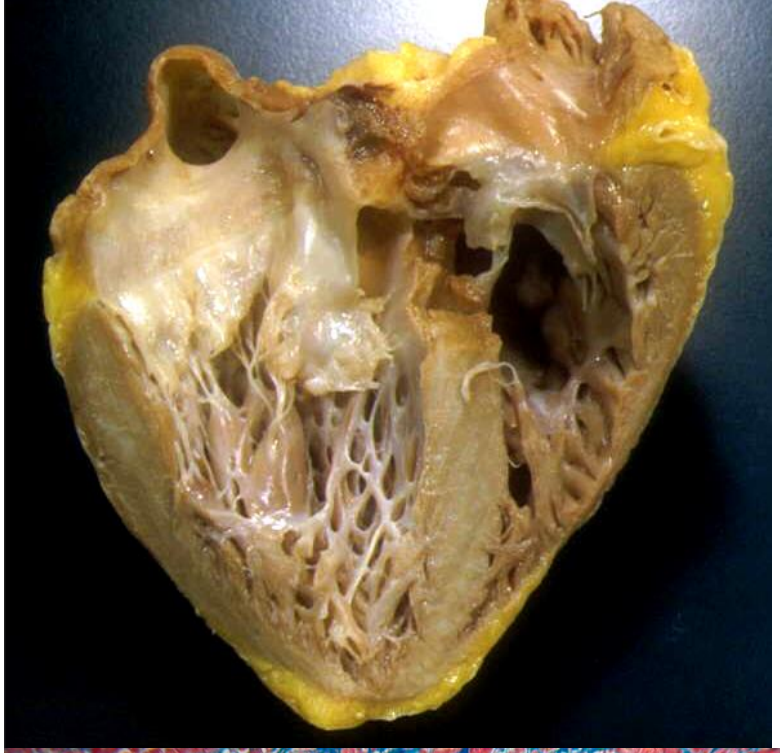


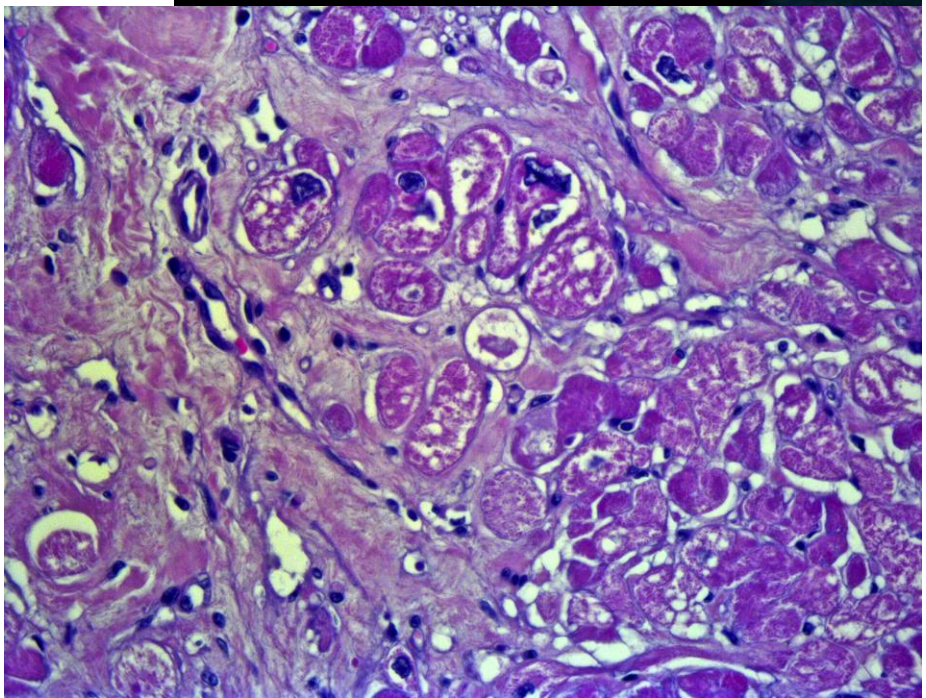


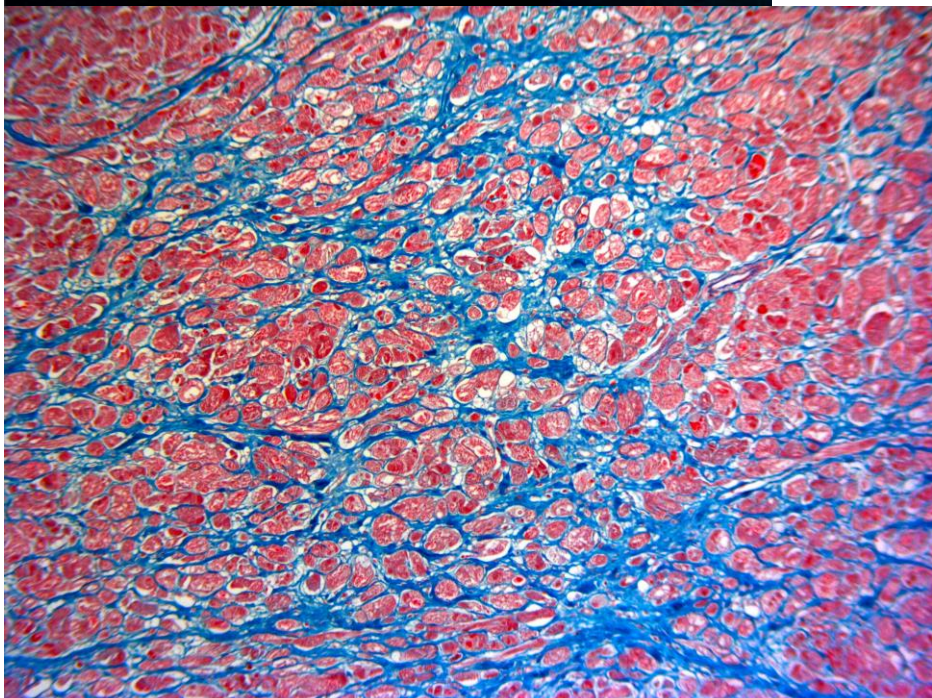
Male, 22 years-old, family history negative for myopathy or cardiomyopathy,
CMP with restrictive pattern and AVB

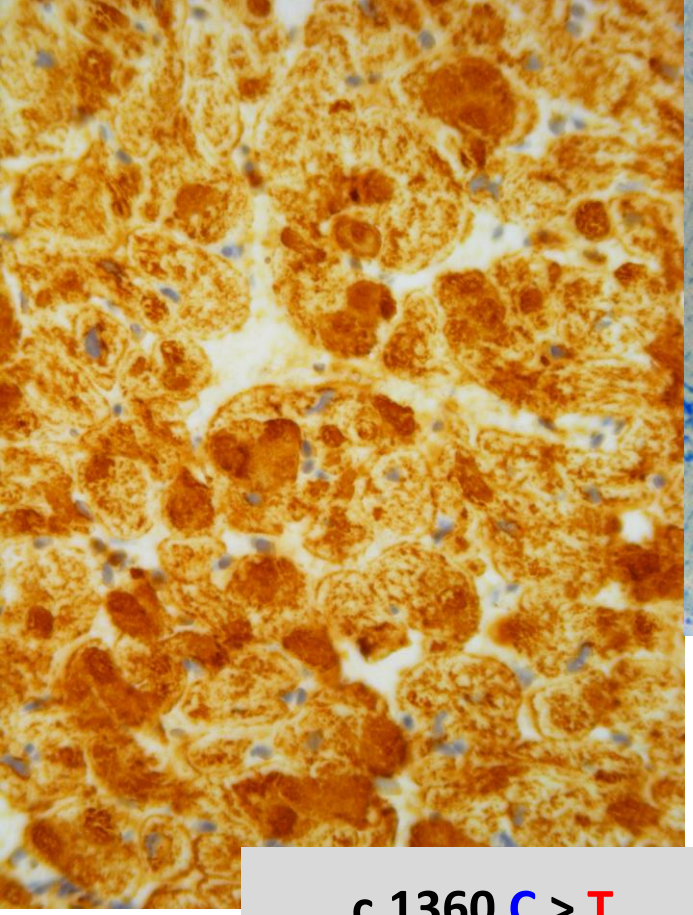


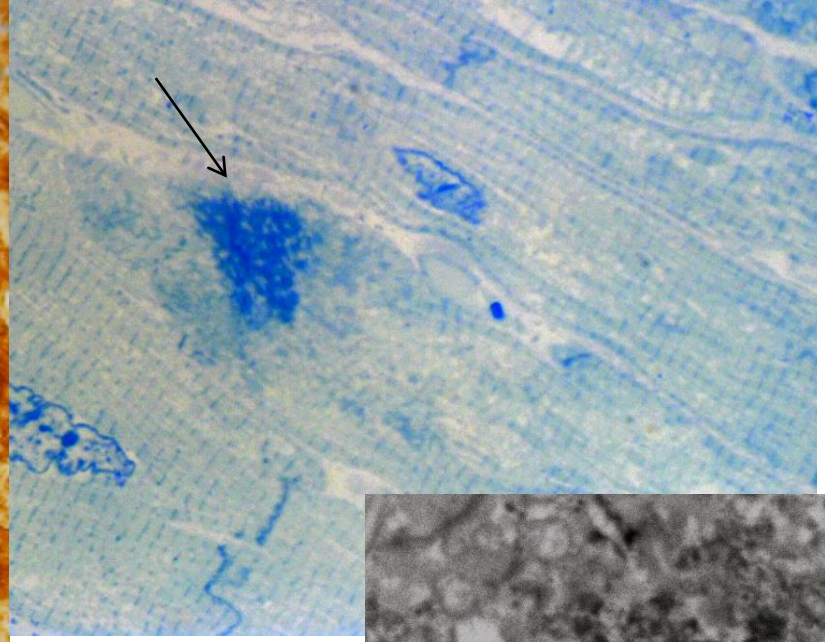


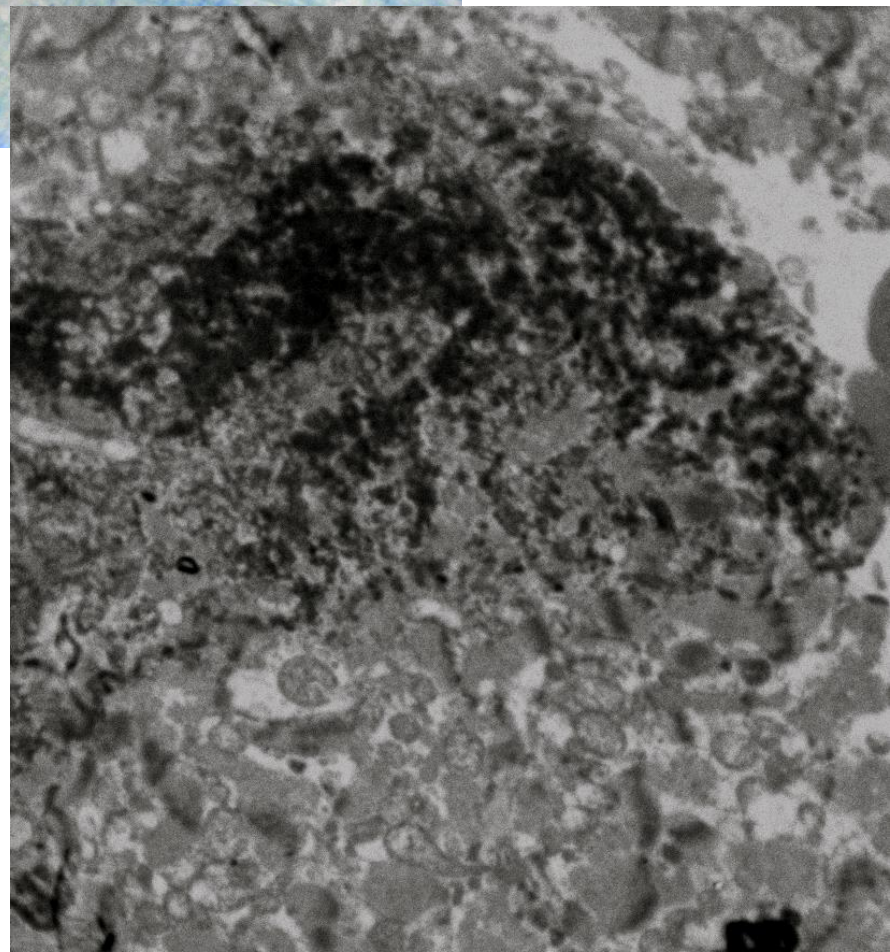












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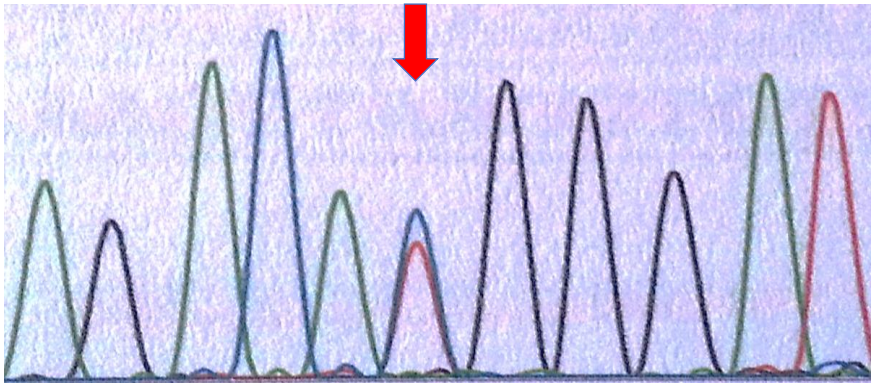
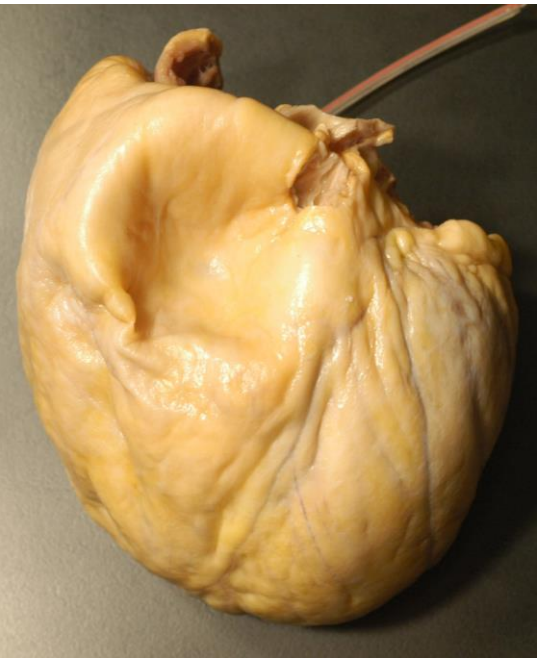
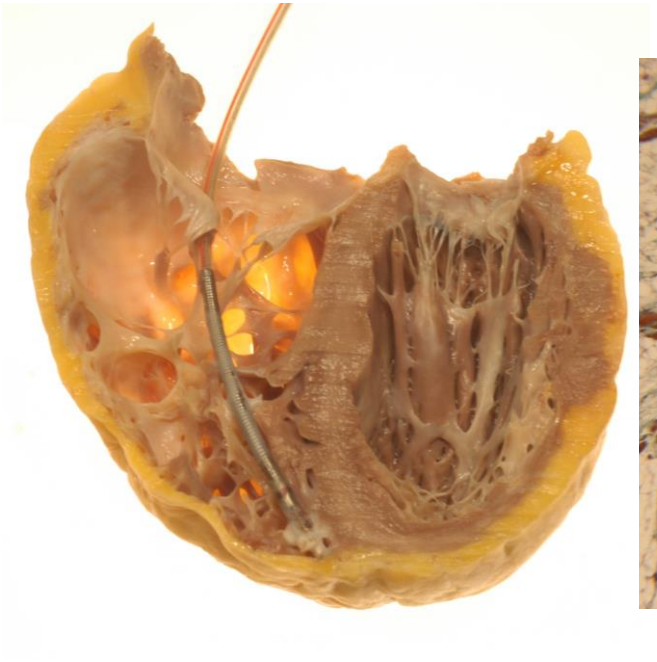
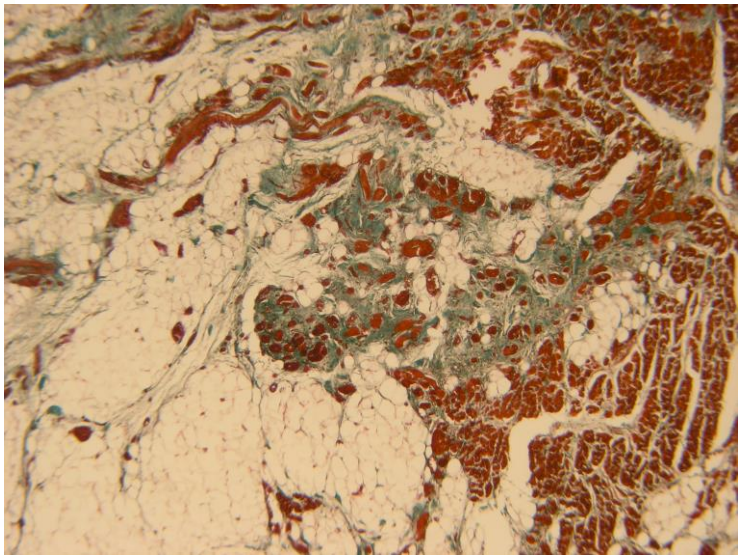


Arrhythmogenic Cardiomyopathy







Family of diseases that feature structural myocardial abnormalities identified by macro- and microscopic pathological examination besides cardiac imaging and ventricular arrhythmia.

ESC European Society of Cardiology

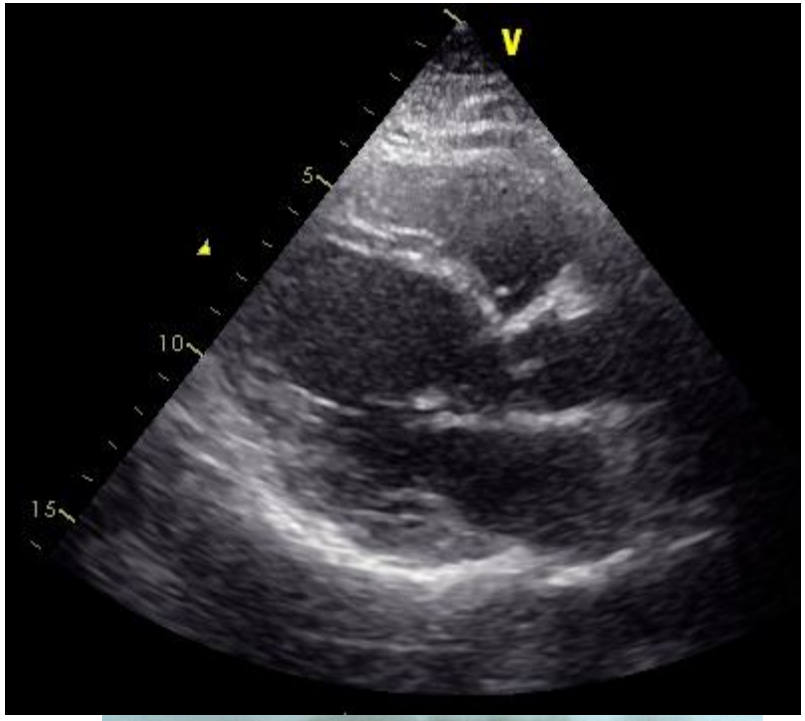
European Journal of Heart Failure (2019)
doi:10.1002/ejhf.1534

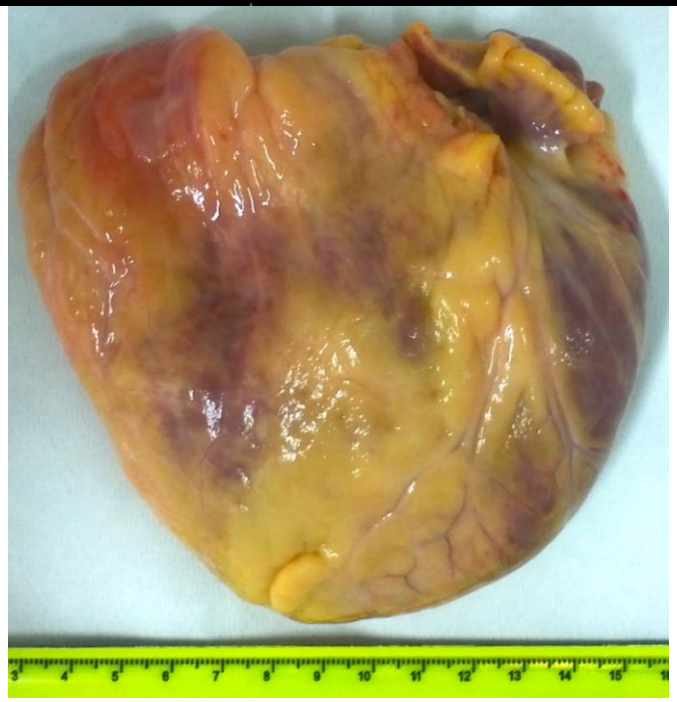
CONSENSUS DOCUMENT

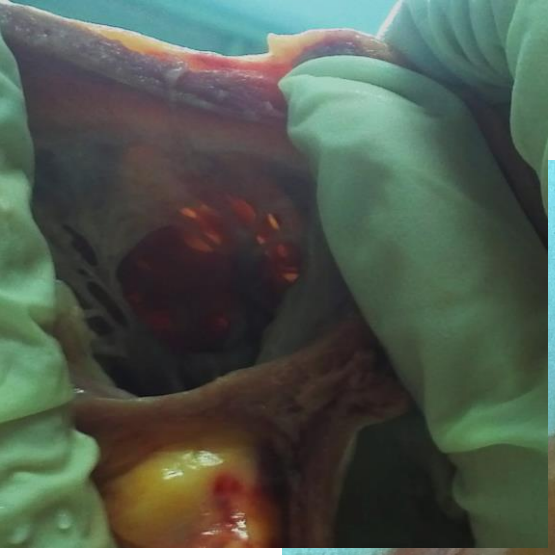
Definition and treatment of arrhythmogenic cardiomyopathy: an updated expert panel report

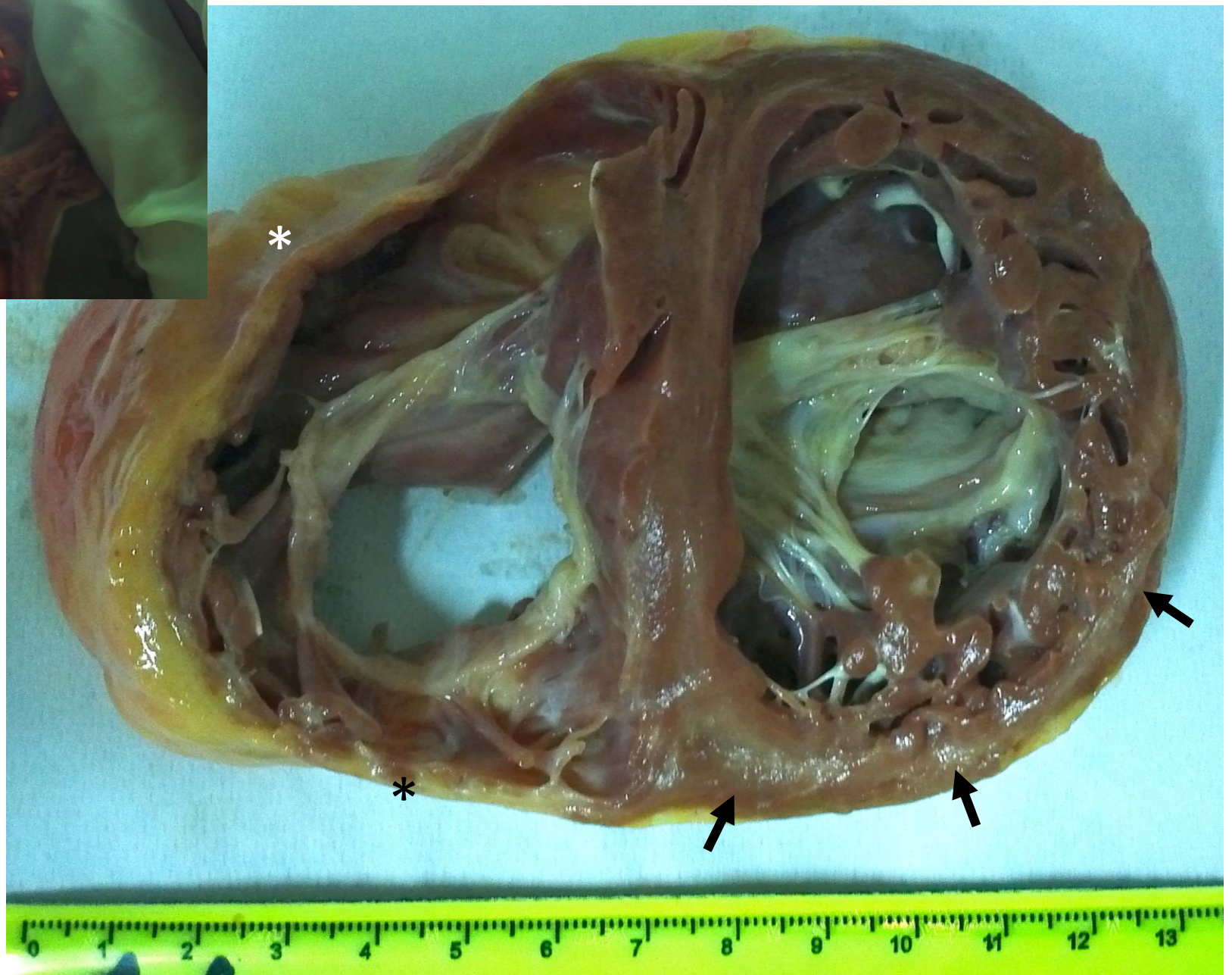
Explanted heart, 23 years old female

- ECG: sinus rhythm in absence of repolarization and depolarization abnormalities.
- **Mild form of ataxia** diagnosed in childhood. Genetic screening for Friedreich and spinocerebellar ataxia (SCA) genes had been done with negative results.
- **Serum lactate level slightly increased** (3,70mM; control<2.2 mM).





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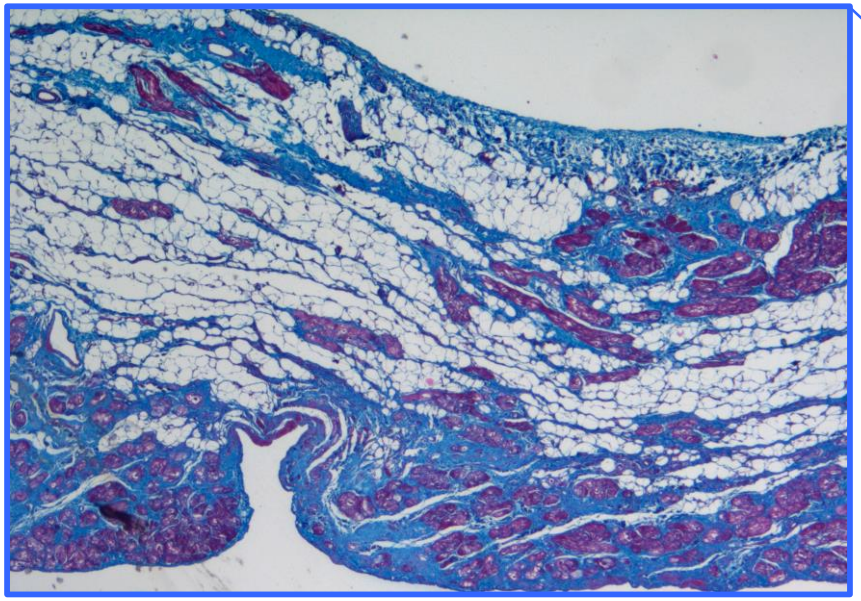
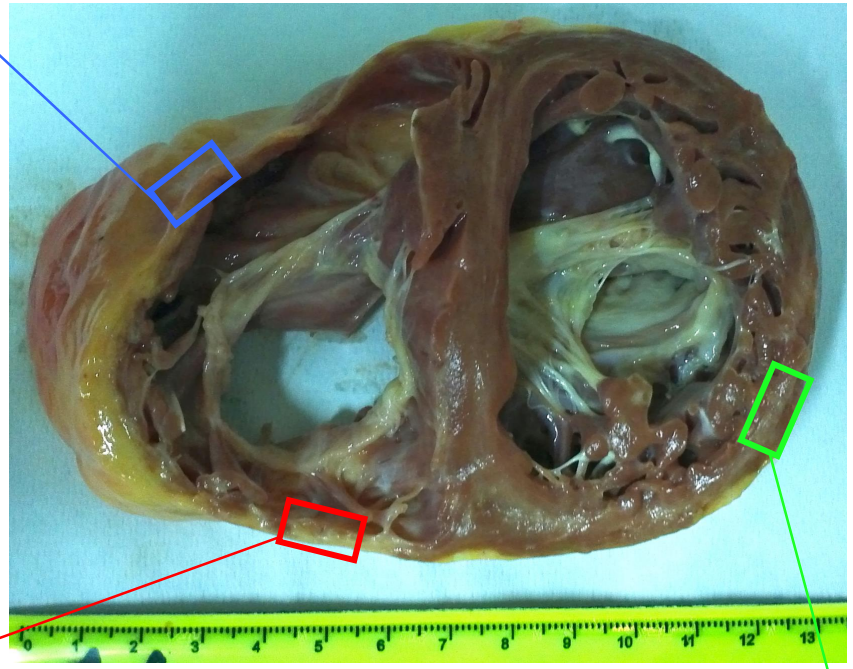
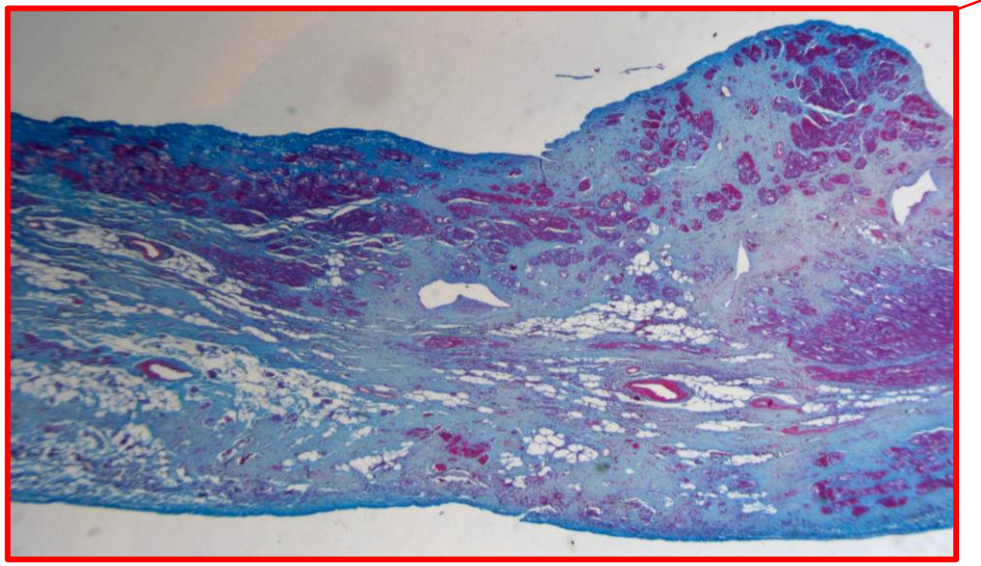
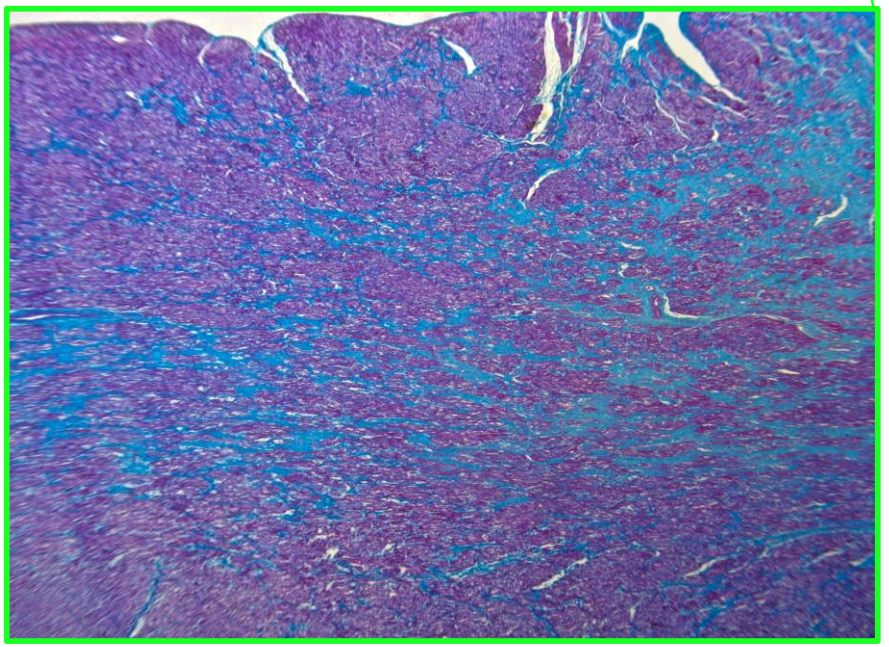
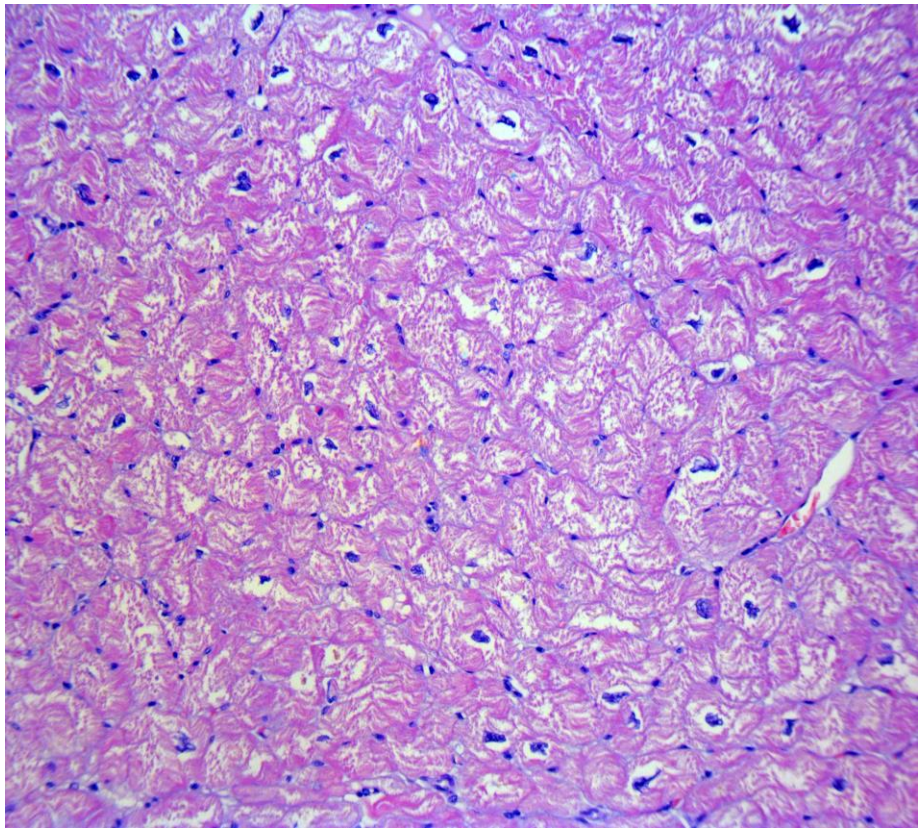
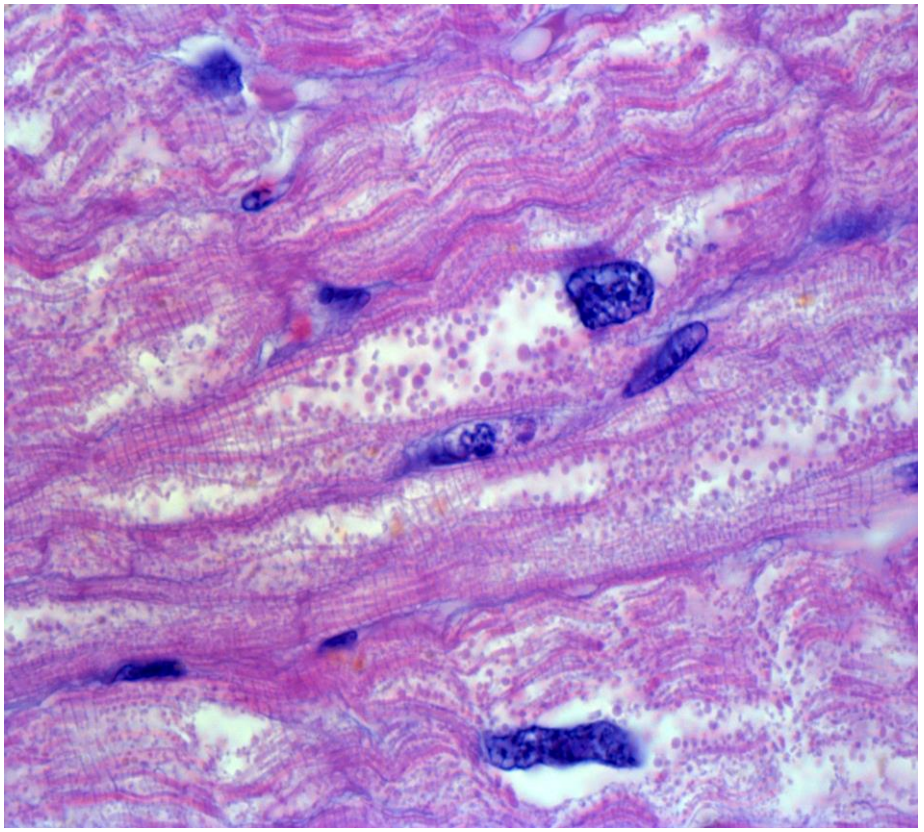


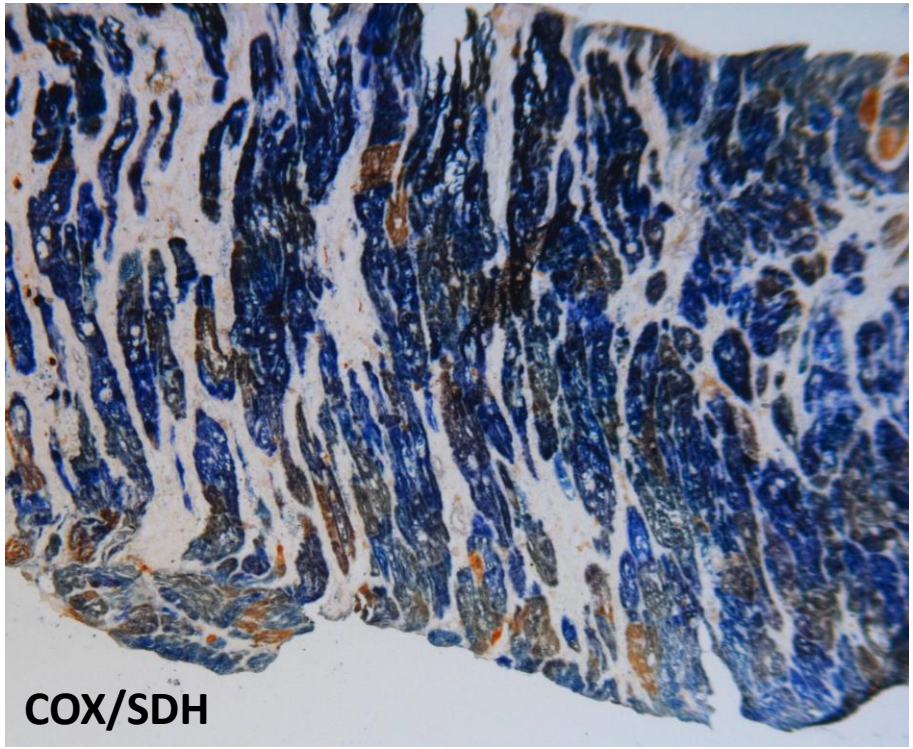


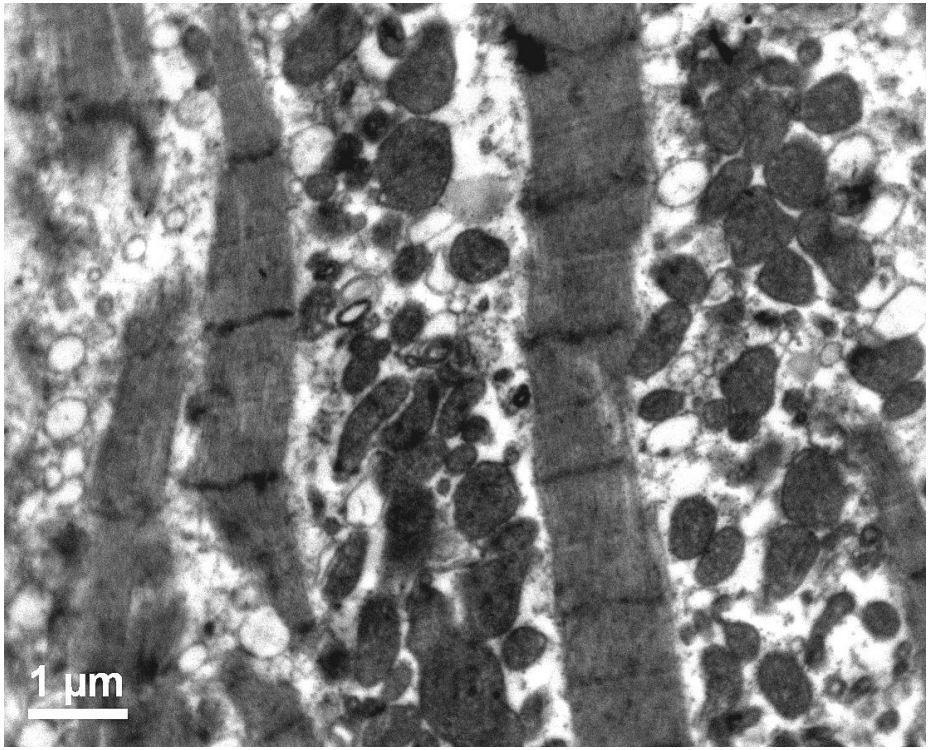




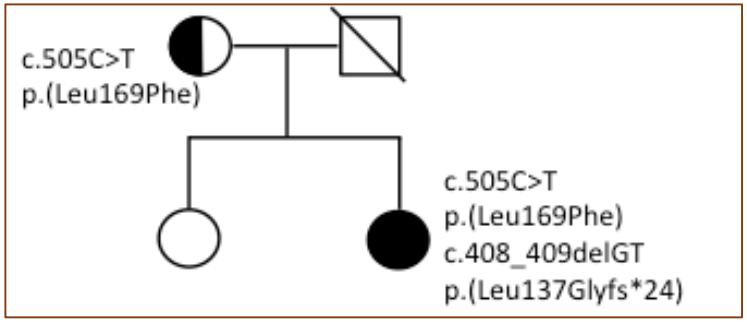




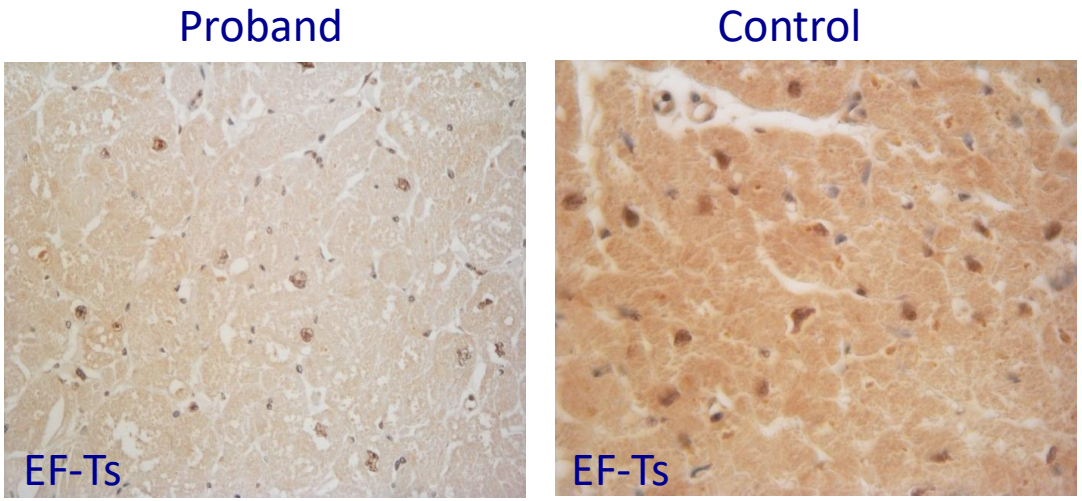


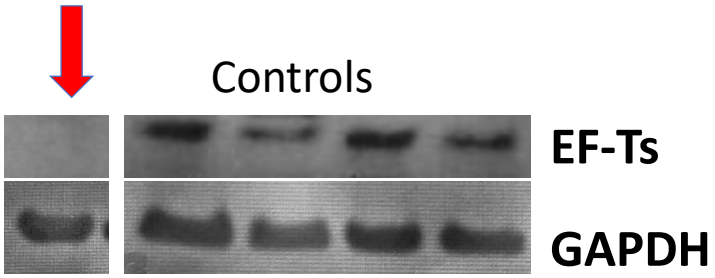


Whole exome sequencing detected two heterozygous variants (one truncating and one missense mutation) in the gene for mitochondrial translation factor EF-TS



Marked decrease of the mutant EF-Ts protein in cardiac tissue from the proband confirm the pathogenicity of the mutation.





Novel compound mutations in the mitochondrial translation elongation factor (TSFM) gene cause severe cardiomyopathy with myocardial fibro-adipose replacement

Perli E et al. Sci Rep. 2019 Mar 25;9(1):5108.

Conclusions

- The heart is commonly involved in most cases of NMDs with a wide spectrum of phenotypes
- Cardiomyopathies can be the first or predominant manifestations of NMDs.
- Simple biomarkers (e.g., serum creatine kinase, lactic acidemia) should be systematically tested because they can provide preliminary clues for exploring skeletal muscle disease
- Pathologists play a major role in unraveling the specific etiology, provided that a detailed diagnostic flowchart, including both morphologic and molecular analysis of heart muscle, is followed.

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Wellcome centre for Mitochondrial Research, institute of Neuroscience, Newcastle University

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