MITOCHONDRIAL & METABOLIC MYPATHIES IN CHILDREN

EPNS Training Course

Neuromuscular Diseases

Budapest April 6-7, 2017

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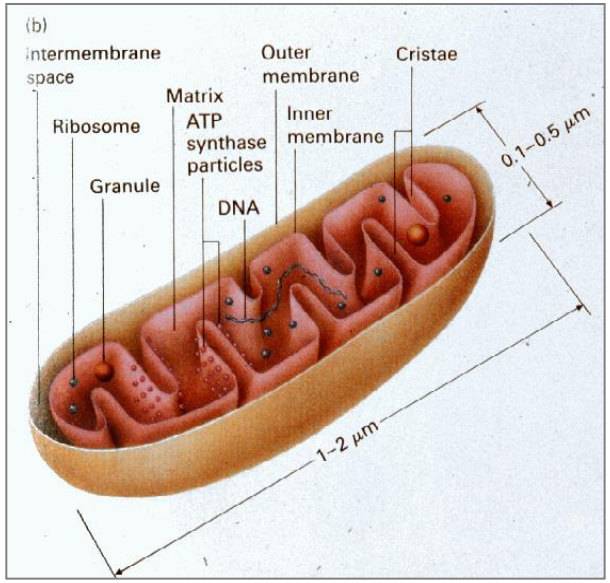
GOTHENBURG, SWEDEN

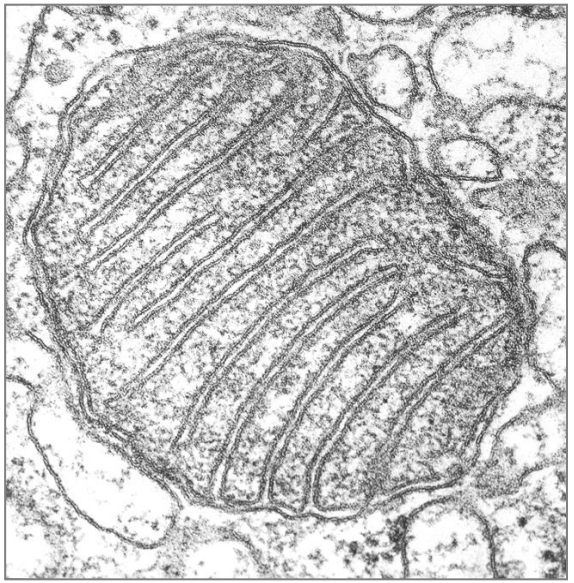
Recent reviews

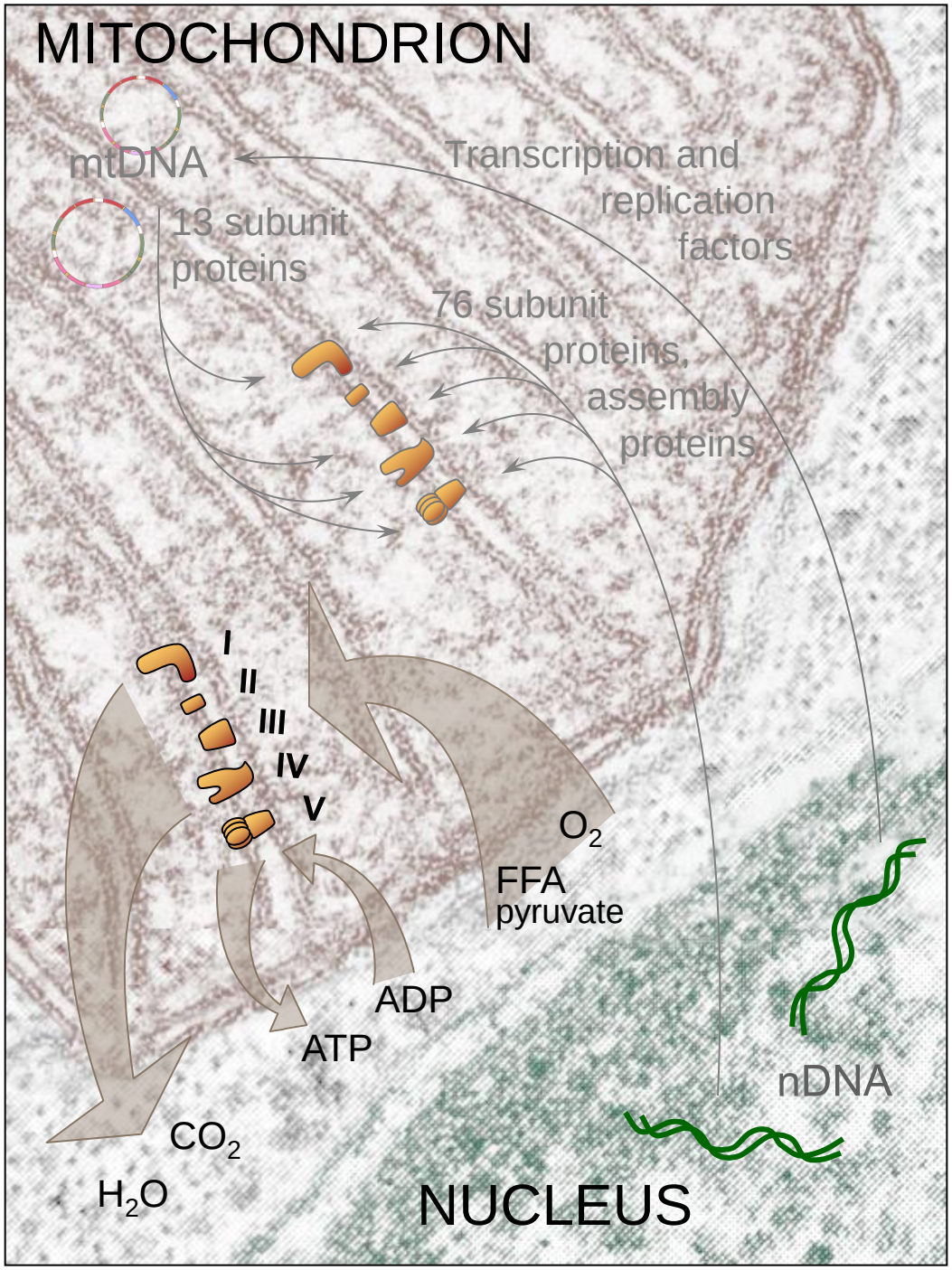
- **Koopman WJH, et al. Mitochondrial disorders in children: toward development of small-molecule treatment strategies. EMBO Mol Med 2016;8:311-27**
- **Nascimento A, et al. Neuromuscular manifestations in mitochondrial diseases in children. Semin Pediatr Neurol 2016;23:290-305**
- **Lake NJ, et al. Leigh syndrome: One disorder, more than 75 monogenic causes. Ann Neurol 2016;79:190-203**
- **Sofou K, et al. A multicenter study on Leigh syndrome: disease course and predictors of survival. Orphanet Journal of Rare Diseases 2014;9:52**

Mitochondrial Diseases

Disorders with dysfunction of the mitochondrial respiratory chain leading to defective oxidative phosphorylation







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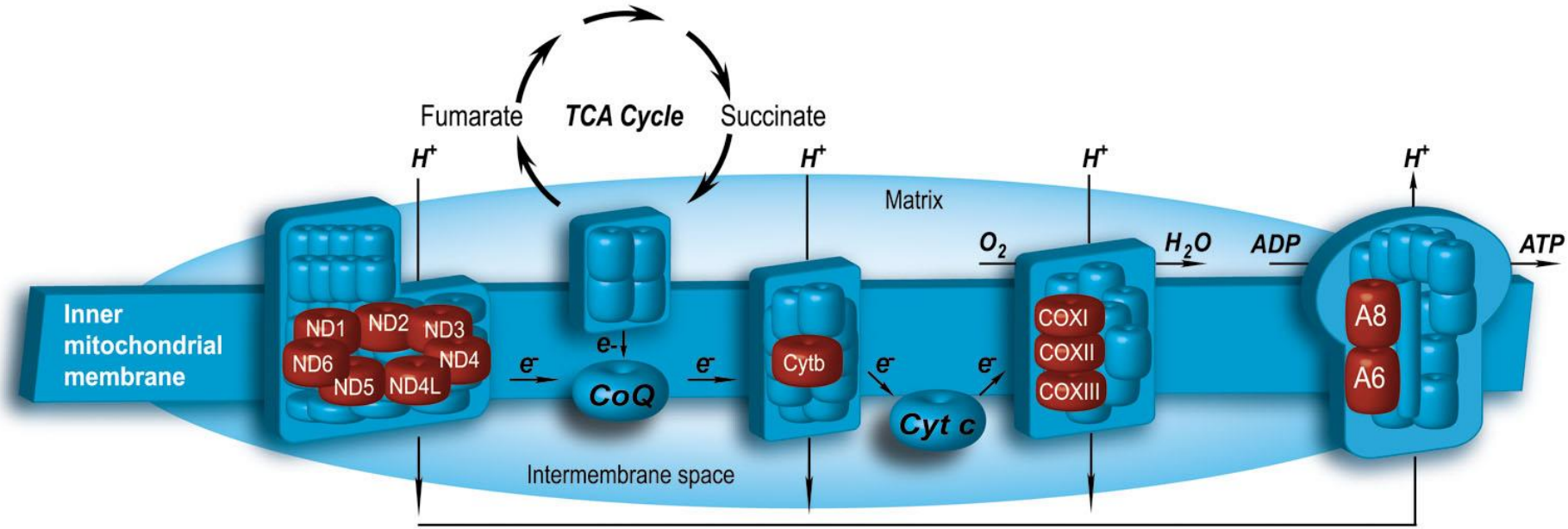
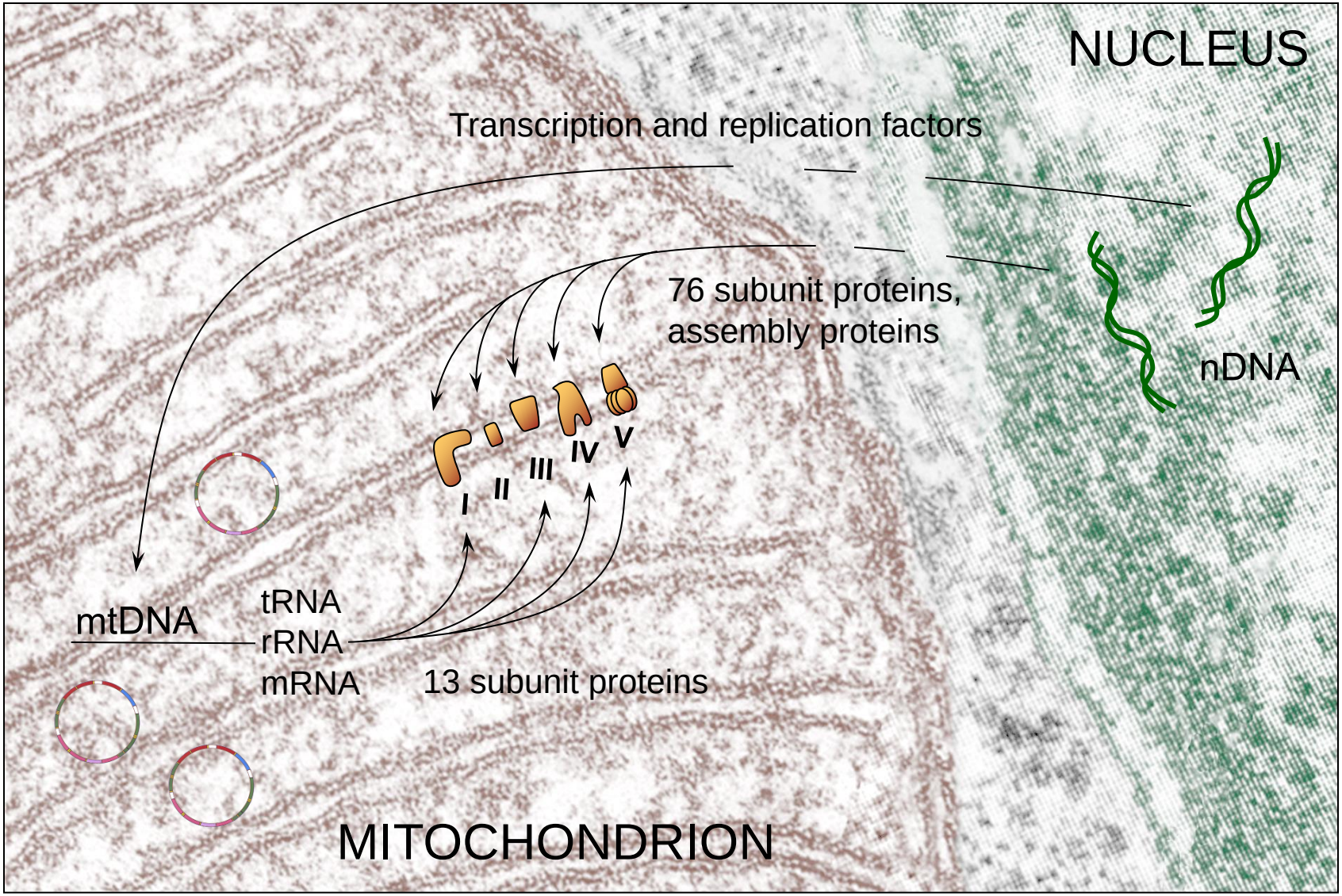
The oxidative phosphorylation system is composed of five complexes including subunits encoded by both the nuclear and mitochondrial genomes



Subunits	Complex I	Complex II	Complex III	Complex IV	Complex V
mtDNA-encoded:	7	0	1	3	2
nDNA-encoded:	~36	4	10	10	~14

Nuclear DNA genes encode respiratory chain subunits, assembly proteins and factors important for mtDNA transcription and replication



The epidemiology of mitochondrial disease

Western Sweden

Preschool incidence of mitochondrial disease	1:11 000
Preschool incidence of Leigh syndrome	1:32 000
Preschool incidence of Alpers syndrome	1:51 000
Preschool incidence of infantile mitochondrial myopathy	1:51 000

Darin, Oldfors, Moslemi, Holme, Tulinius. Ann Neurol 2001;49:377

The incidence in the general population is not known. It has been estimated to be higher than 1:5 000 and since most start in childhood this makes mitochondrial disorders the most common inherited metabolic diseases in children

Schaefer, Taylor, Turnbull et al Biochim Biophys Acta 2004;1659:115

Clinical phenotypes of mitochondrial diseases in children

- The newborn child with lactic acidemia
- Benign mitochondrial myopathy with COX deficiency
- Mitochondrial DNA depletion syndromes
  - Myopathic form
  - Encephalomyopathic form
  - Hepatocerebral forms – Alpers-Huttenlocher syndrome
- Leigh syndrome, Pearson syndrome
- Kearns-Sayre, MELAS & MERRF syndromes

The newborn child with lactic acidemia

Prenatal onset of mitochondrial disease

- Girl born preterm, SGA. Congenital lactic acidosis. Severe psychomotor developmental delay, spastic tetraparesis, infantile myoclonic seizures, EEG with hypsarrhythmia
- CT of the brain showed corpus callosum agenesis, frontal cortical dysplasia
- Developed at age 16 years hypertrophic cardiomyopathy, thereafter multiorgan failure & died at 26 years of age
- Complex I deficiency and MTND1 mutation G3481A with 36% mutated mtDNA in muscle tissue

The newborn child with lactic acidemia

Prenatal onset of mitochondrial disease

- Small for gestational age (SGA)
- Dysmorphic features
  - Micrognathia, prominent nasal bridge, low-set ears
  - Arthrogryposis
- Encephalopathy, myopathy, cardiomyopathy and/or hepatopathy
- Cerebral developmental abnormalities
  - Pontocerebellar hypoplasia
  - Porencephaly
  - Corpus callosum dysgenesis

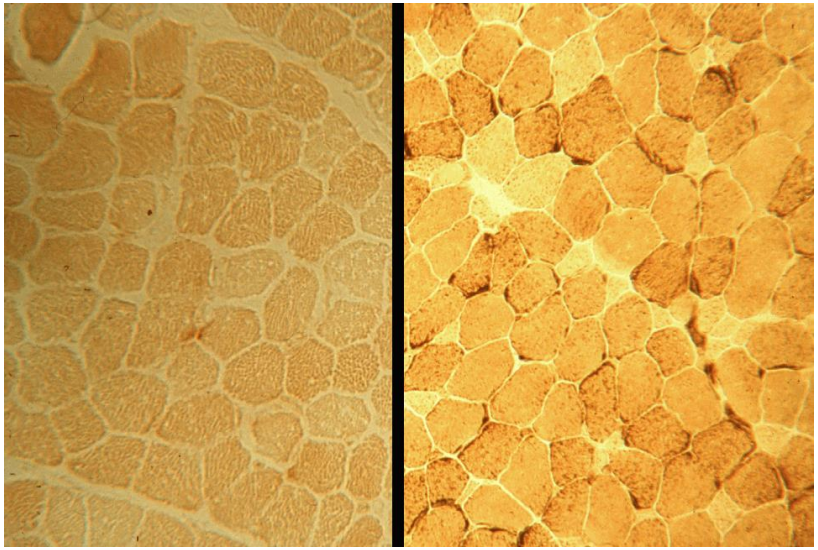
Infantile reversible COX deficiency myopathy

Floppy infant - Lactic acidosis

Mitochondrial myopathy - Complex I and IV deficiency

Muscle weakness and atrophy - Delayed gross motor development

Increased S-creatine kinase



Infantile reversible COX deficiency myopathy

Molecular basis of infantile reversible cytochrome c oxidase deficiency myopathy

17 children from twelve families;

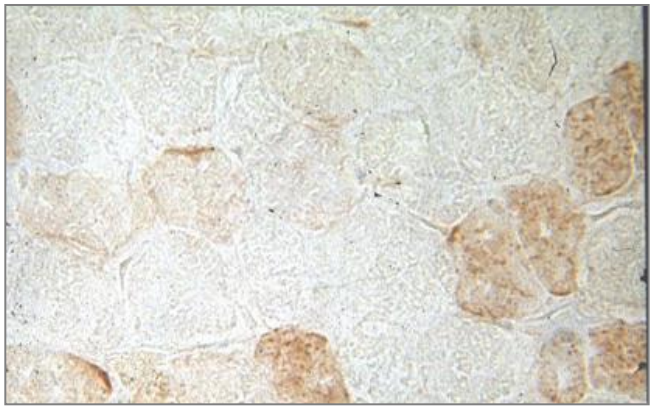
- New York, Newcastle & Gothenburg
- All had a maternally inherited, homoplasmic m.14674T>C mt-tRNA^{Glu} (MT-TE) mutation
- Provides the rationale for a simple genetic test to identify infants with mitochondrial myopathy and good prognosis
- Developmentally regulated

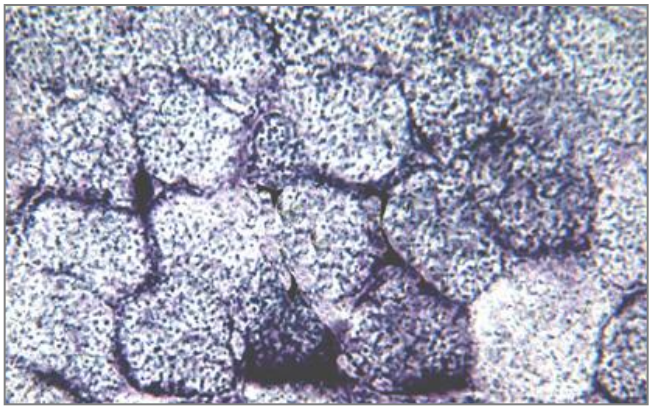
Horvath et al Brain 2009;132:3165

Fatal infantile mitochondrial myopathy

- 2/3 and 3/3 children to healthy, unrelated parents. Oldest sister healthy
- Pregnancy, delivery & early psychomotor development normal
- Onset of disease at 8 & 12 months, respectively, associated with an upper respiratory tract infection
- Progressive muscle weakness and wasting mainly affecting proximal muscles of head and neck, shoulder and pelvic girdle
- Died at 20 & 24 months, respectively

Fatal infantile mitochondrial myopathy with mtDNA depletion





S-CK 6.8 – 25.7 μkat/L (< 3), B-lactate 2.0 – 3.2 mmol/L

Multiple OXPHOS deficiency

Sequencing of the thymidine kinase-2 (TK2) gene revealed two heterozygous missense mutations

Mitochondrial DNA Depletion syndromes
MDDS

- Myopathic form
  - *TK2* mutations
- Encephalomyopathic form
  - *SUCLA2, SUCLG1* mutations
- Hepatocerebral form
  - *DGUOK, POLG, MPV17* & *Twinkle* mutations
- Cerebrorenal form
  - *RRM2B* mutations

Autosomal recessive disorders

Encephalomyopathic form of MDDS

Mutations in SUCLA2

- Age at onset birth – 5 months
- Prominent muscle hypotonia & atrophy
- Failure to thrive needing gastrostomy
- Hyperkinetic movement disorder, dystonia, athetosis
- Sensorineural deafness needing Cochlear implantation
- Many of the patients have died

Elpeleg Am J Hum Genet 2005, Ostergaard Brain 2007, Carrozzo Brain 2007, Carozzo JIMD 2016

Leigh syndrome

Subacute Necrotizing Encephalomyelopathy

A boy with Leigh syndrome due to complex V deficiency with a T9191C mutation in MT-ATP6 gene

3/3 children, normal pregnancy, delivery & early development. At 4 months weakness of the neck muscles with axial hypotonia. At 6 months increasing fatigue, episodes of hyperventilation

Lactic acidosis, CT of the brain showed decreased attenuation of the basal ganglia, thalami and mesencephalon.

Developed hypertrophic cardiomyopathy & died at age 28 months

T9191C mutation 94% in muscle tissue which could not be detected in blood samples from the patient's parents or siblings

Leigh syndrome

Subacute Necrotizing Encephalomyelopathy

- Age at presentation
  - Usually infantile onset, juvenile and adult less common
- Major clinical features
  - Failure to thrive, recurrent vomiting, hypotonia, psychomotor regression, brainstem dysfunction, involuntary movements, ataxia, dystonia, spasticity, optic atrophy
- Major neuroradiologic features
  - Lesions of the basal ganglia, brainstem, thalami, cerebellum and white matter

Leigh syndrome

Subacute Necrotizing Encephalomyelopathy

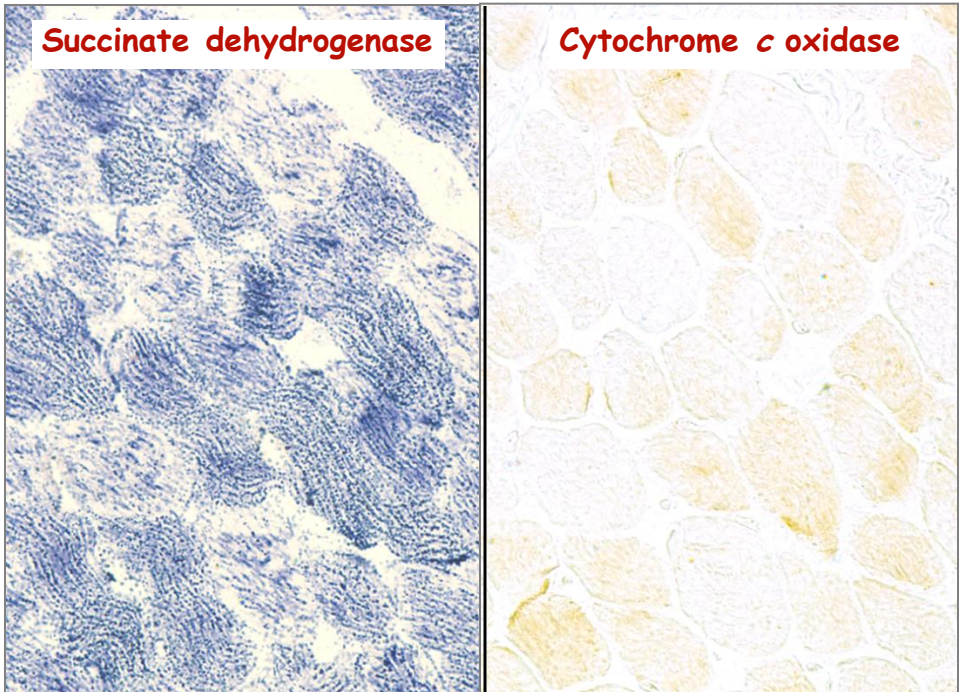
- mtDNA mutations
  - tRNA point mutations
  - Mutations in polypeptide subunits of OXPHOS
    - MTND1-6, MTATP6 (T8993G/C), COX genes
- Nuclear DNA mutations
  - Mutations in polypeptide subunits of OXPHOS
    - NDUFS1-8, NDUFV1-2, SDHA
  - Mutations in proteins important for assembly
    - COX assembly gene SURF1
    - CI assembly genes NDUFAF2, C8ORF38, C20ORF7, FOXRED1
  - SLC19A3 encoding for a Thiamine transporter
- Mutations in PDHA1, the E1a catalytic subunit of PDHC

Leigh syndrome

Subacute Necrotizing Encephalomyelopathy

LS with COX deficiency and SURF1 mutations

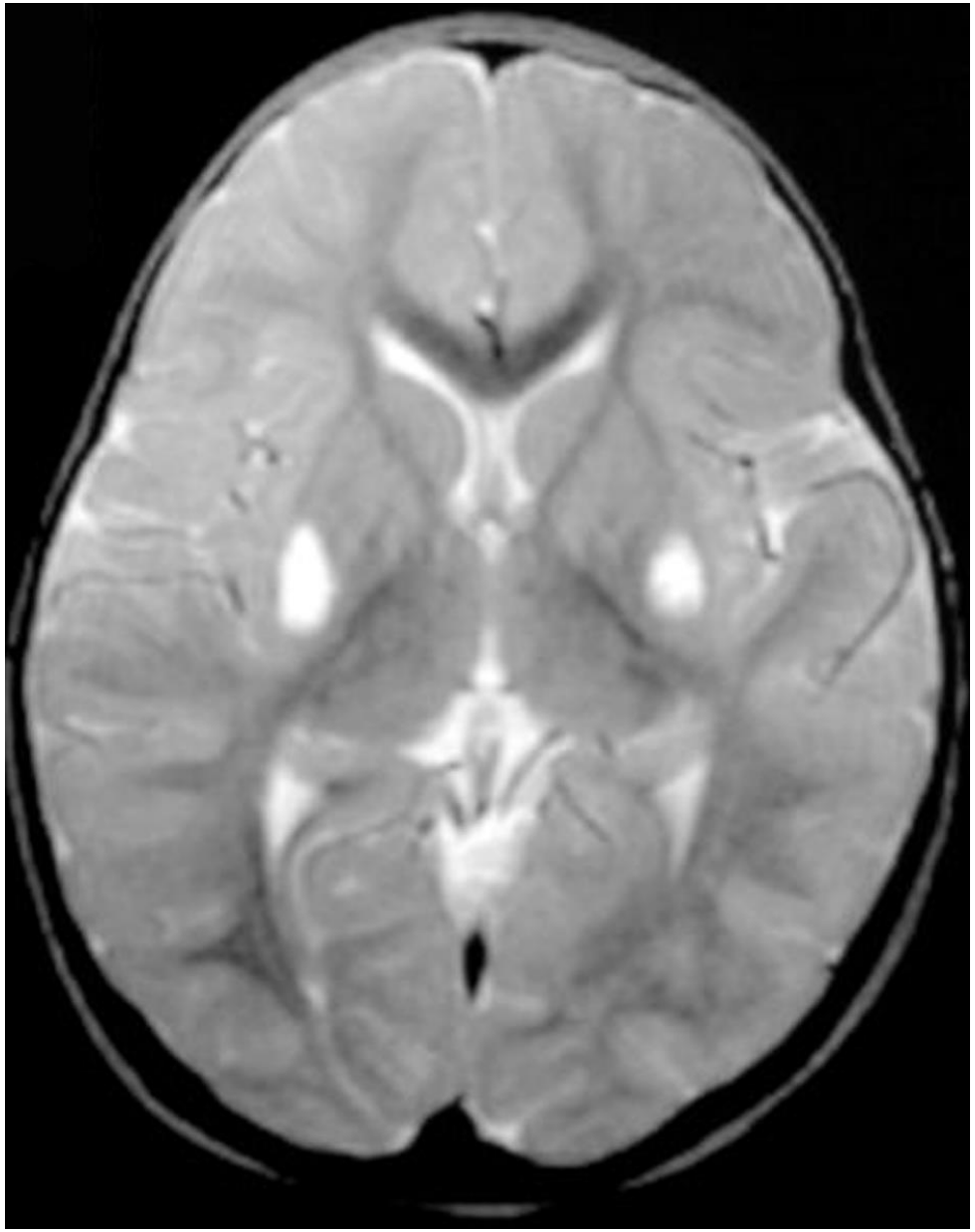
- Onset of disease between 6-12 months
- Failure to thrive, vomiting, feeding difficulties and hypotonus are common early symptoms, followed by rapid neurological deterioration
- Death usually occurs during the first years of life, although exceptions have been reported



SURF1 mutation causing COX deficiency and Leigh syndrome

Leigh syndrome

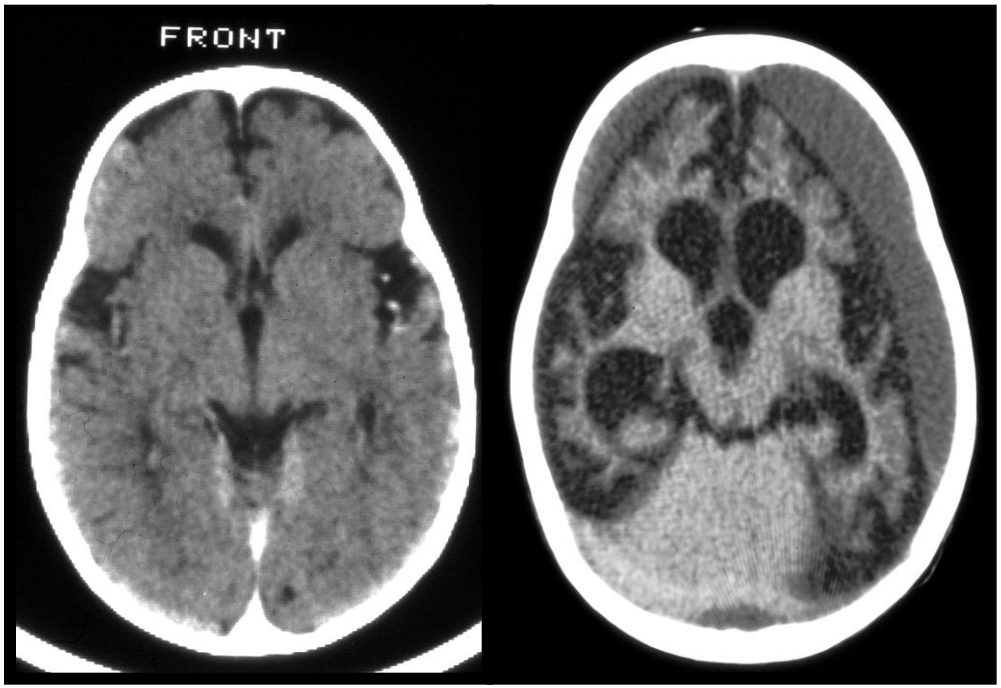
MRI of the brain showing hyperintensive signals in the dorsal parts of the putamen in a 18 month old girl with COX deficiency and *SURF1* mutation



Alpers syndrome

Infantile, rapidly progressive disorder with severe seizures leading to mental retardation, tetraparesis, and blindness

Investigations showed a Complex I deficiency



This boy lived until the age of 16 years. He had severe myoclonic seizures and developed renal tubular nephropathy

Alpers syndrome

Classical form

- Onset during the first months of life with irritability, within weeks followed by onset of severe myoclonic seizures
- During 2nd half year of life their EEG is characterized by focal, often multifocal spikes mixed with high voltage low frequency activity, i.e. hypsarrhythmic appearance
- Myoclonic jerks are common and often independent of epileptic seizures
- Mutations in *NARS2* and *PARS2*

Alpers-Huttenlocher syndrome

- Infantile, diffuse cerebral degeneration with liver cirrhosis
  - *Huttenlocher et al Arch Neurol 1976*
- Distinct autosomal recessive disease
- ”Only coherent group to emerge from the miscellany of Alpers reports in the 1960’s and 1970’s”
  - *Harding J Child Neurol 1990*

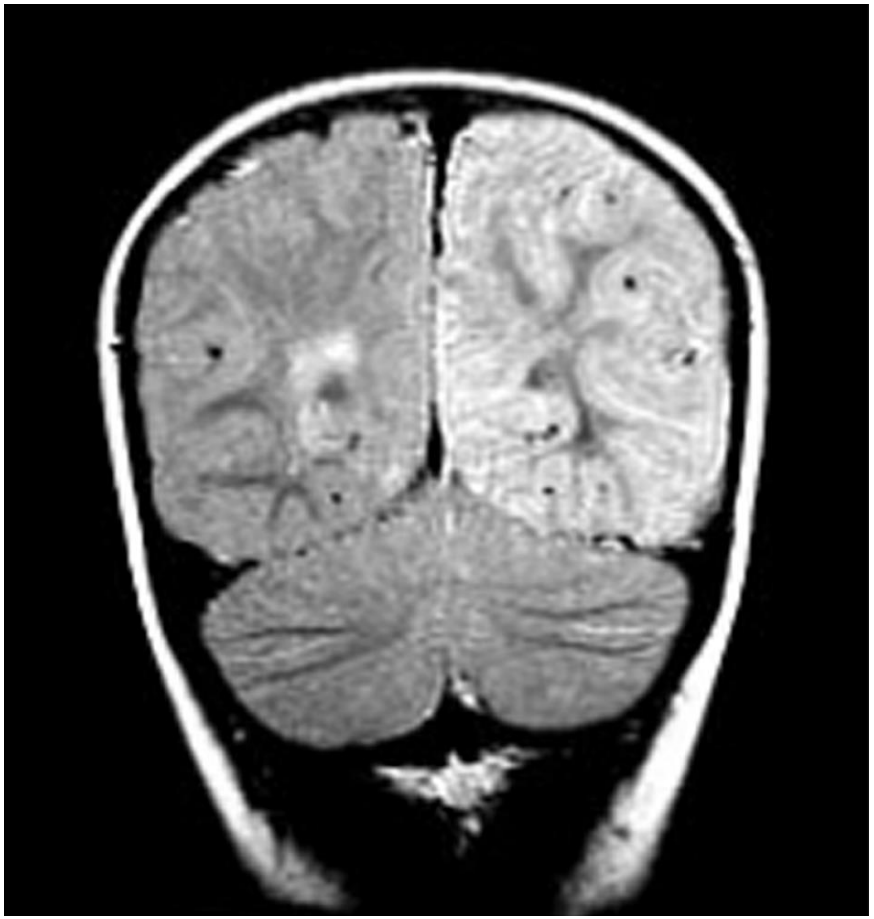
Alpers-Huttenlocher syndrome

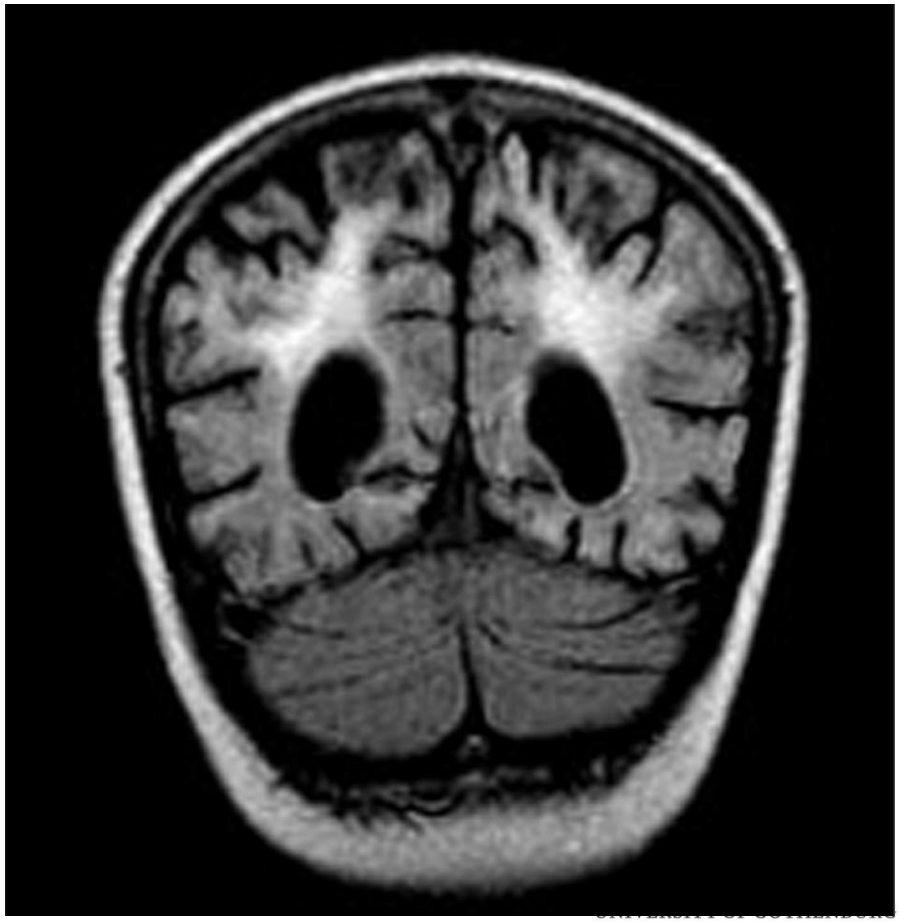
- Seizures – severe, refractory
  - Status epilepticus, epilepsia partialis continua, epileptic crises, multifocal partial seizures, myoclonic seizures
- Liver failure
  - Valproate toxicity documented in 14 cases
- Other
  - Failure to thrive, hypotonia, sudden head drop, psychomotor regression, ataxia, persistent vomiting

Mutations in mtDNA polymerase (POLG)

Alpers-Huttenlocher syndrome

MRI of the brain of a girl with mutations in *POLG* at 3 and 4 years of age showing on T2 FLAIR initial signal abnormalities in the cortex of the left occipital lobe and one year later progressive atrophy of both occipital lobes





Pearson syndrome

Infantile onset marrow-pancreas syndrome

- Sideroblastic anemia, neutropenia and thrombocytopenia, with vacuolization of marrow precursors
- Exocrine pancreatic dysfunction
- Failure to thrive, ataxia, dementia, liver steatosis/cirrhosis, endocrinopathies
- Usually fatal during early life
- Large deletions of mtDNA
- Most often a sporadic disease. However, at least two women with CPEO have given birth to children with Pearson syndrome

Kearns-Sayre syndrome

- Progressive external ophthalmoplegia
- Pigmentary degeneration of the retina
- Cardiac conduction defects

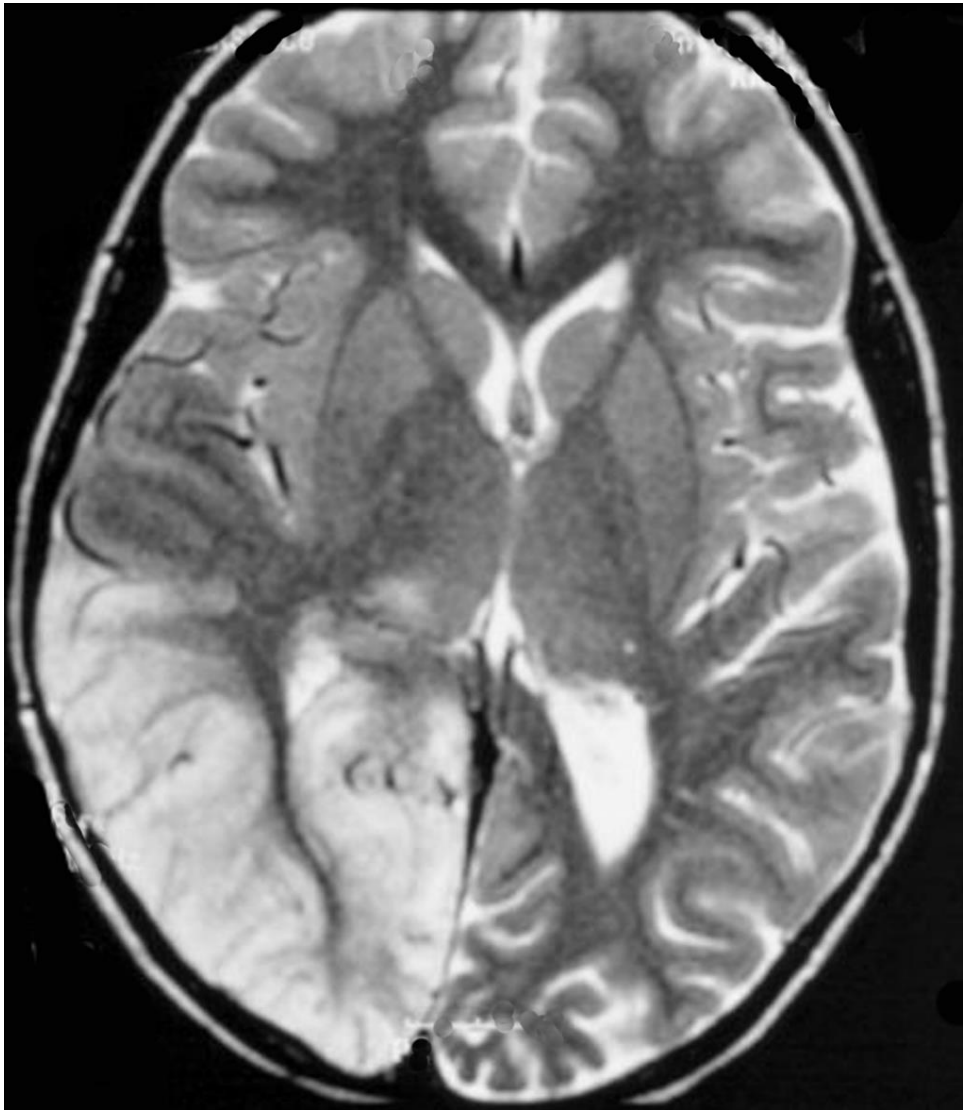
MELAS syndrome

Mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes

- Onset during early childhood or adolescence
- Headache and vomiting
- Seizures and stroke-like episodes
- Short stature, sensorineural hearing loss, dementia
- Muscle weakness
- Maternal inheritance
- Mitochondrial myopathy with Complex I deficiency
- A3243G mutation in mtDNA – most common

MELAS syndrome

MRI of the brain showing an infarct-like lesion with swelling and hyperintensive cortical and subcortical parenchyma in the right parieto-occipital lobe of a ten-year old girl with MELAS syndrome and the A3243G mutation



MERRF syndrome

Myoclonus Epilepsy and Ragged Red Fibres

- Myoclonus and Seizures
- Ataxia and Dementia
- Optic atrophy and Sensorineural hearing loss
- Short stature
- Maternal inheritance
- Mitochondrial myopathy with Complex I & IV deficiency
- A8344G mutation in mtDNA – most common

Metabolic Myopathies

- The two major energy sources for muscle contraction are glycogen and fatty acids, whose metabolic pathways converge into acetyl-CoA for final intramitochondrial oxidation through the Krebs cycle and the respiratory chain

» DiMauro et al 2007

Metabolic Myopathies

- Disorders of lipid metabolism
- Disorders of carbohydrate metabolism
- Mitochondrial disorders

Disorders of lipid metabolism

- Disorders of carnitine metabolism
- Disorders of fatty acid oxidation
- Disorders of electronic transfer
- Disorders of ketone synthesis

Boy with recurrent infections, fever and high transaminases

- 2.5 year old boy admitted to a pediatric clinic because of tonsillitis with high fever and dehydration
- 5/5 children, nonconsanguinous parents. Older brother born 1981 hospitalized at age 18 years because of suspected myositis with myoglobinuria.
- Psychomotor development normal. Walked unsupported at age 9 months.
- Has had frequent fever episodes since early age. During fever episodes he did not want to eat or drink.

Boy with recurrent infections, fever and high transaminases

- S-ASAT 28 μkat/L and S-ALAT 9 μkat/L, (ref int <1)
- S-CK 864 μkat/L, ref int <3, S-LDH 66 μkat/L
- Hepatitis A, B, C, CMV, EBV negative, S-urea and creatinine, B-lactate and U-organic acids normal
- Muscle morphology, Muscle & Serum carnitine normal
- Muscle CPT II activity 42 nmol/min x g protein
  - reference interval 334-810

Carnitine palmitoyl transferase II (CPT II) deficiency

- Mediates transport of fatty acid CoA across inner mitochondrial membrane
- Chromosome 1p32 – autosomal recessive
  - Most cases with common mutations + heterozygous for 2nd mutation
- Carnitine levels normal
- Muscle morphology – normal
- Muscle CPT II activity decreased

Carnitine palmitoyl transferase II (CPT II) deficiency

Clinical features

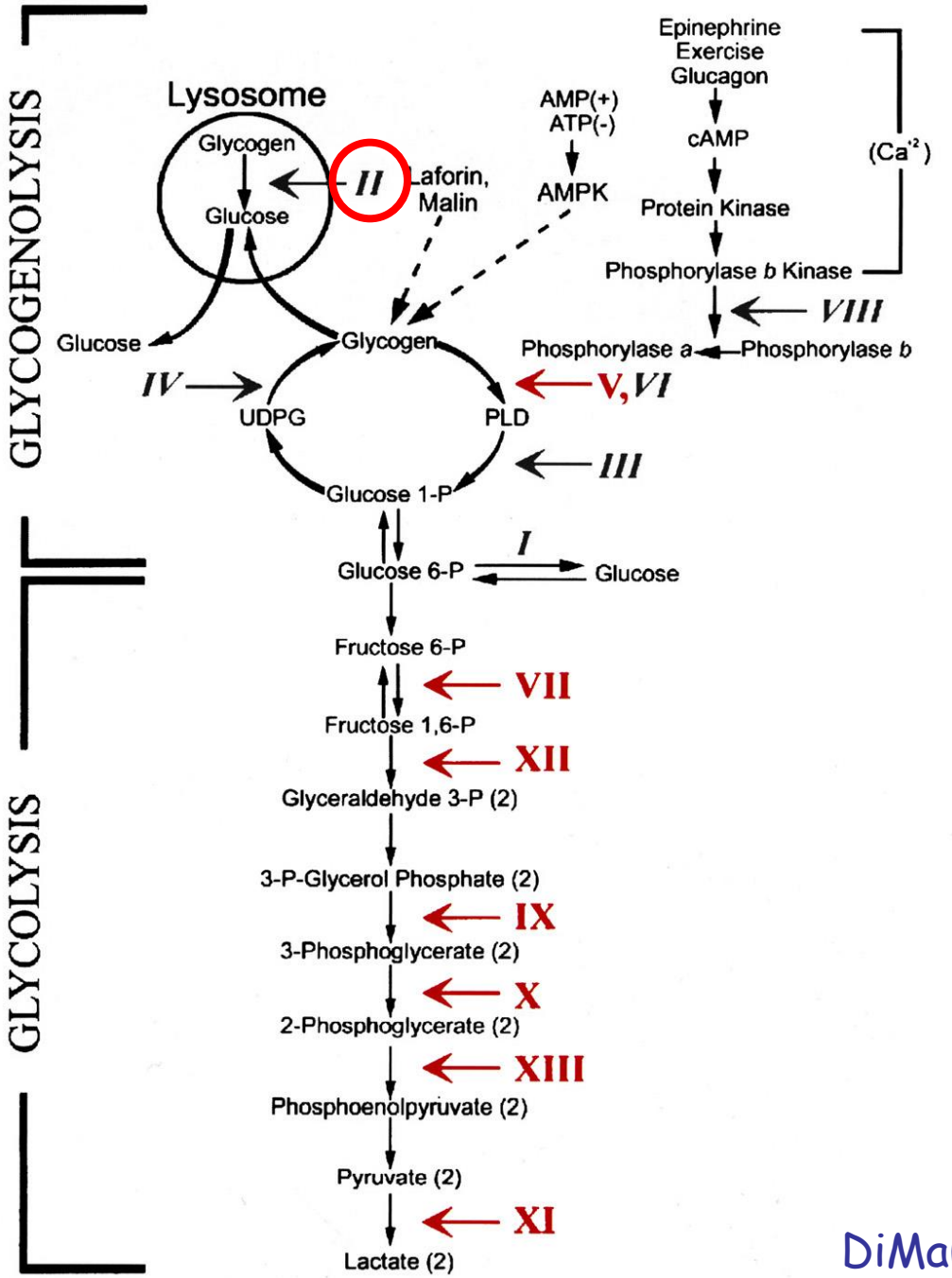
- Onset – childhood, adolescence, adulthood
- Most common metabolic cause of repeated myoglobinuria
- Rhabdomyolysis – after prolonged exercise, cold, fasting, infections, valproate treatment
- Myopathy – normal strength between attacks
- Male predominance

Disorders of carbohydrate metabolism

There are two main clinical presentations;

- Acute, recurrent, reversible muscle dysfunction, manifesting as exercise intolerance, myalgia with or without painful cramps (contractures), often culminating in muscle breakdown and myoglobinuria

- Fixed, often axial and proximal limb weakness, sometimes simulating dystrophic or inflammatory processes



DiMauro et al 2007

Glycogenoses causing progressive weakness

Acid maltase deficiency

Glycogenosis type 2 = Pompe disease

α-1,4 glucosidase on Chr 17q13

Frequency 1: 40000

Muscle biopsy shows massive accumulation of glycogen in both infantile and childhood forms, but may be unimpressive in adult cases

Enzyme analysis performed on fibroblasts or blood

Many different mutations described

Infantile onset Pompe Disease

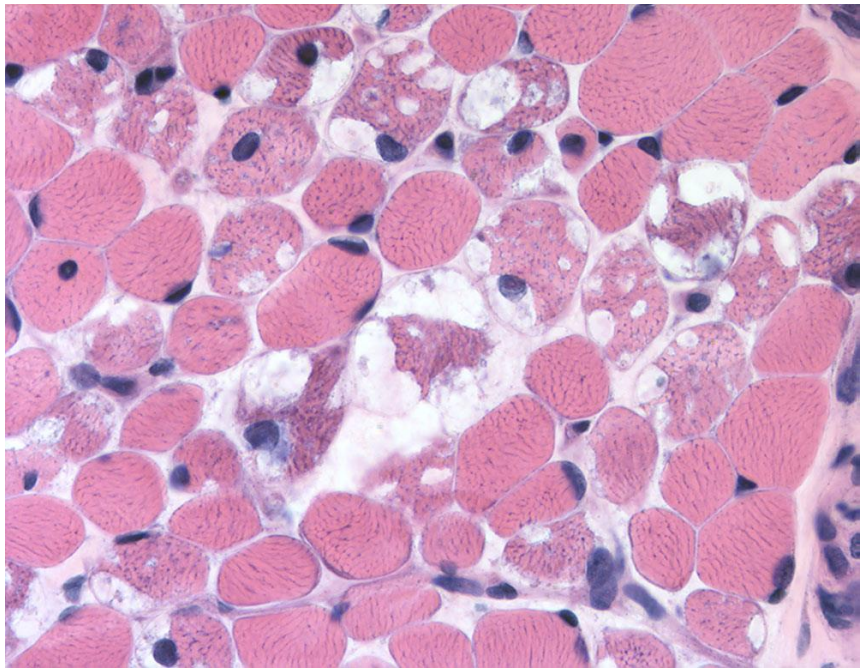
Clinical picture

- Onset < 6 months
- Hypotonia – floppy infant 88%
- Cardiomegaly (>90%) with congestive heart failure. Arrhythmia
- Respiratory distress
- Death <1 year 80-95%, median 6-8 months, without treatment

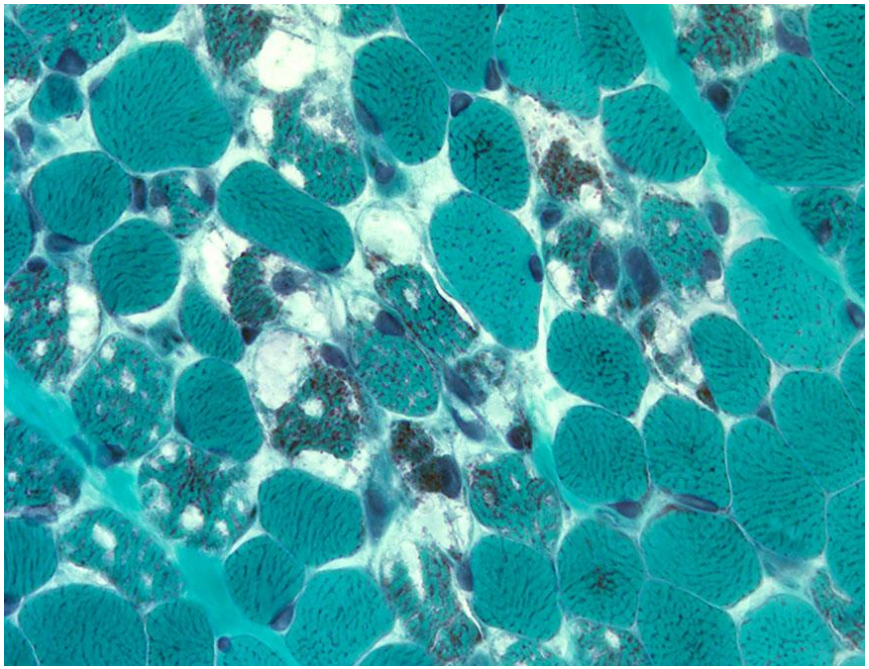
Myozyme treatment in a boy with a mild infantile-onset form of Pompe disease

- 3/3 children. Older sisters healthy
- Pregnancy & delivery normal, bw. 3200 g
- Onset of disease at age 3-4 months
  - Admitted to hospital because of vomiting and acidosis
  - S-Ck 15 – 20 μkat/L (ref int < 3)
- Mental and fine motor development normal
- Gross motor developmental delay
  - At 9 months he learned to roll over
  - He learned to sit at 12 months
- Muscle biopsy was performed at age 17 months

Myozyme treatment in a boy with a mild infantile-onset form of Pompe disease

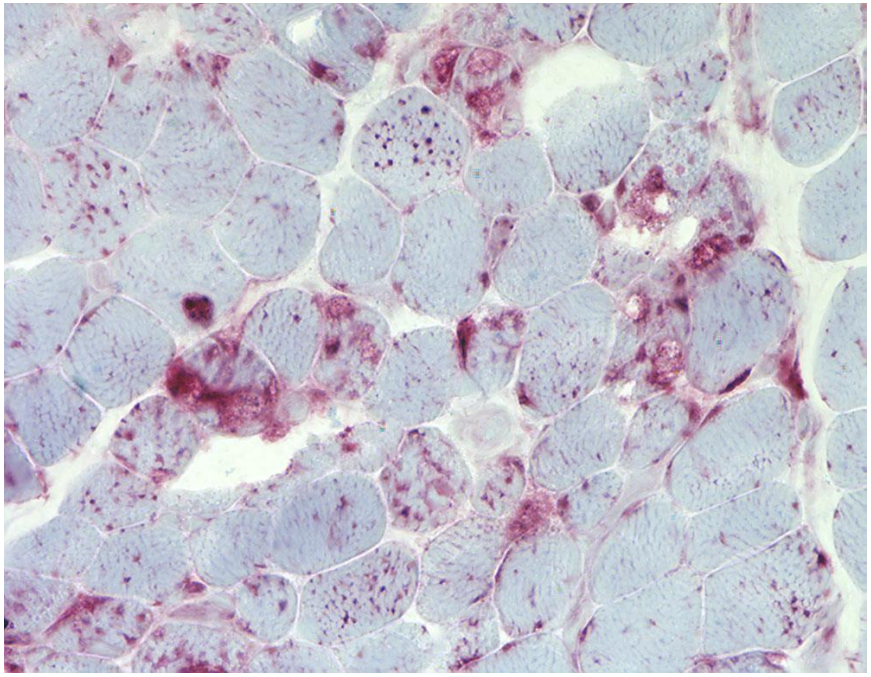


Hematoxylin-eosin

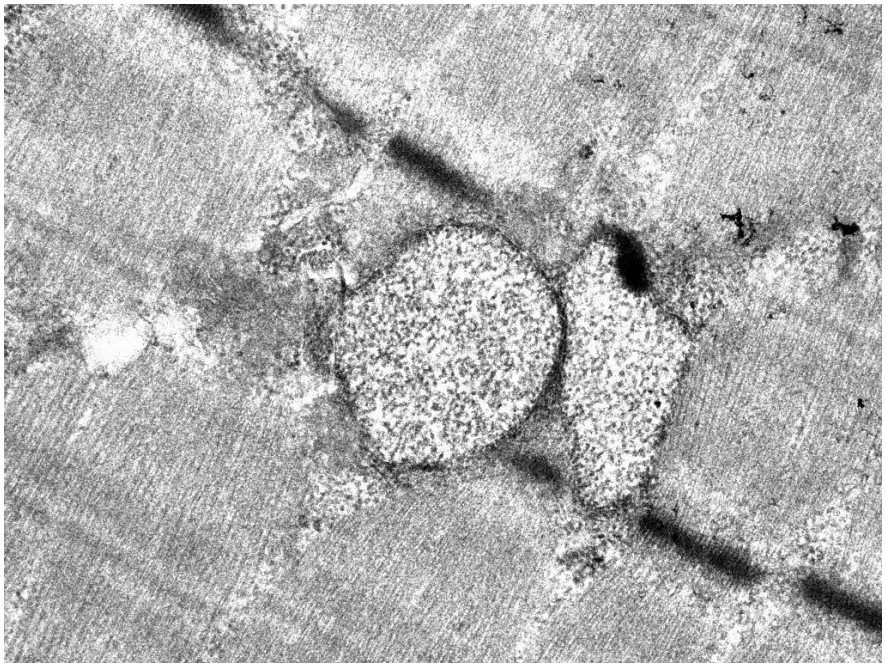


Gomori-trichrome

Myozyme treatment in a boy with a mild infantile-onset form of Pompe disease



Acid phosphatase



Electron microscopy

Myozyme treatment in a boy with a mild infantile-onset form of Pompe disease

- Alfa-glucosidase activity in fibroblasts was markedly decreased!

1,5 μkat/kg protein

reference interval 9-19 μkat/kg protein

Myozyme treatment in a boy with a mild infantile-onset form of Pompe disease

- Regular follow-ups between 18 months and 5 years showed
  - Gross motor developmental delay
  - Muscle weakness
  - Cardiac function normal
  - No severe respiratory tract infections

Myozyme treatment in a boy with a mild infantile-onset form of Pompe disease

- Myozyme treatment started at age 5 years

- At age 17 years
- Normal lungfunction
- Normal cardiac function
- Mild muscle weakness

The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

Cardiomyopathy and Exercise Intolerance in Muscle Glycogen Storage Disease 0

Gittan Kollberg, Ph.D., Már Tulinius, M.D., Ph.D., Thomas Gilljam, M.D., Ph.D., Ingegerd Östman-Smith, M.D., Ph.D., Gun Forsander, M.D., Ph.D., Peter Jotorp, M.D., Anders Oldfors, M.D., Ph.D., and Elisabeth Holme, M.D., Ph.D.

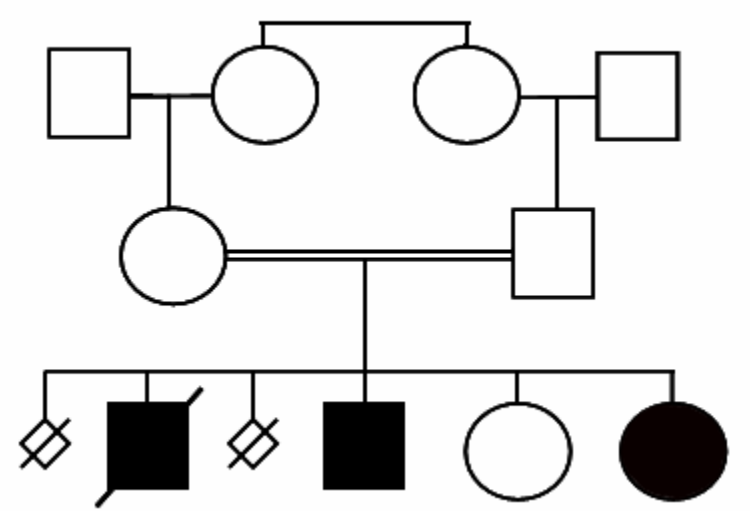
Glycogen metabolism

- Glycogen is a glucose reservoir in the body that rapidly can be utilized as energy supply
- Glycogen is most abundant in skeletal muscle and liver
- Liver glycogen is utilized when blood glucose level is too low and muscle glycogen when the muscle itself needs energy

Glycogen metabolism

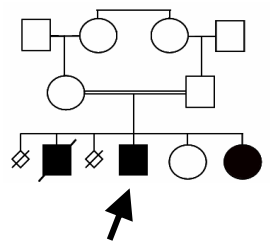
- The final step in glycogen synthesis is catalyzed by the enzyme glycogen synthase
- There are two isoforms encoded by separate genes; liver glycogen synthase (*GYS2*) and muscle glycogen synthase (*GYS1*)
- The synthesis and degradation of glycogen is mainly regulated at the protein level by phosphorylation

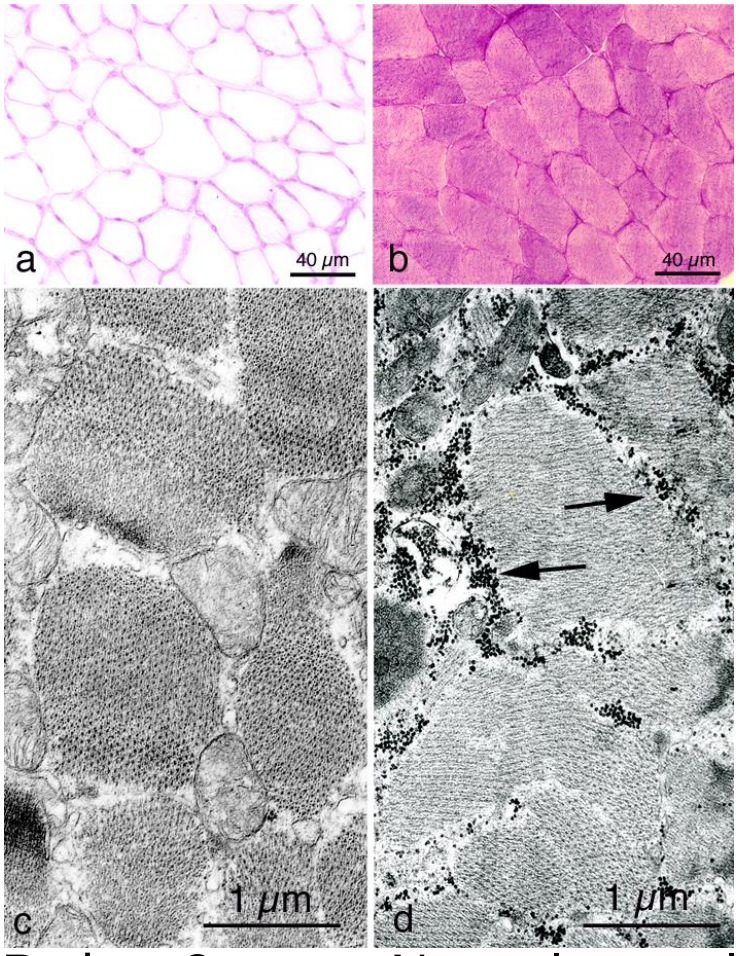
Family background



- The family was Assyrians and the parents were cousins
- A 10-year-old boy died in sudden cardiac arrest while playing in the schoolyard
- The autopsy showed hypertrophic cardiomyopathy
- A younger brother displayed the first symptoms at six years of age when he couldn't keep up with his peers
- He is now extremely exercise intolerant and suffers from hypertrophic cardiomyopathy
- Two younger siblings were free of symptoms, but subtle signs of cardiomyopathy was revealed in the youngest at two years of age

Muscle morphology - absence of glycogen





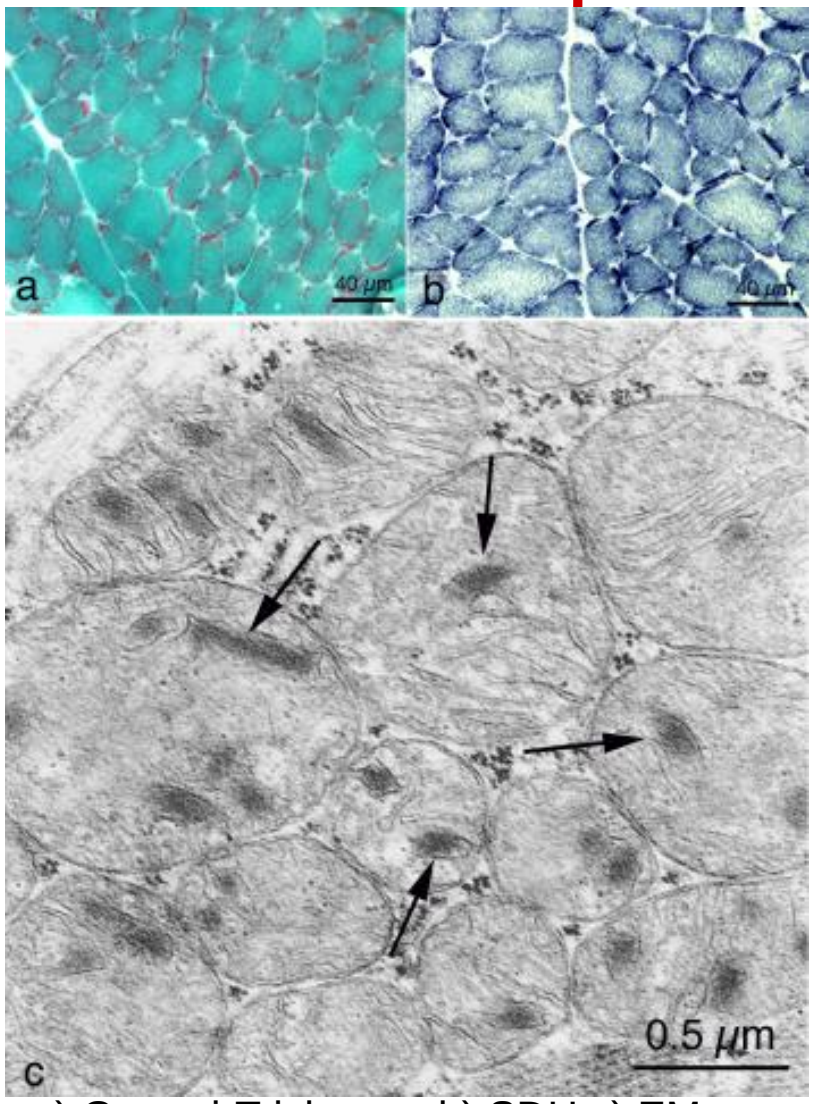
Patient 2 Normal control

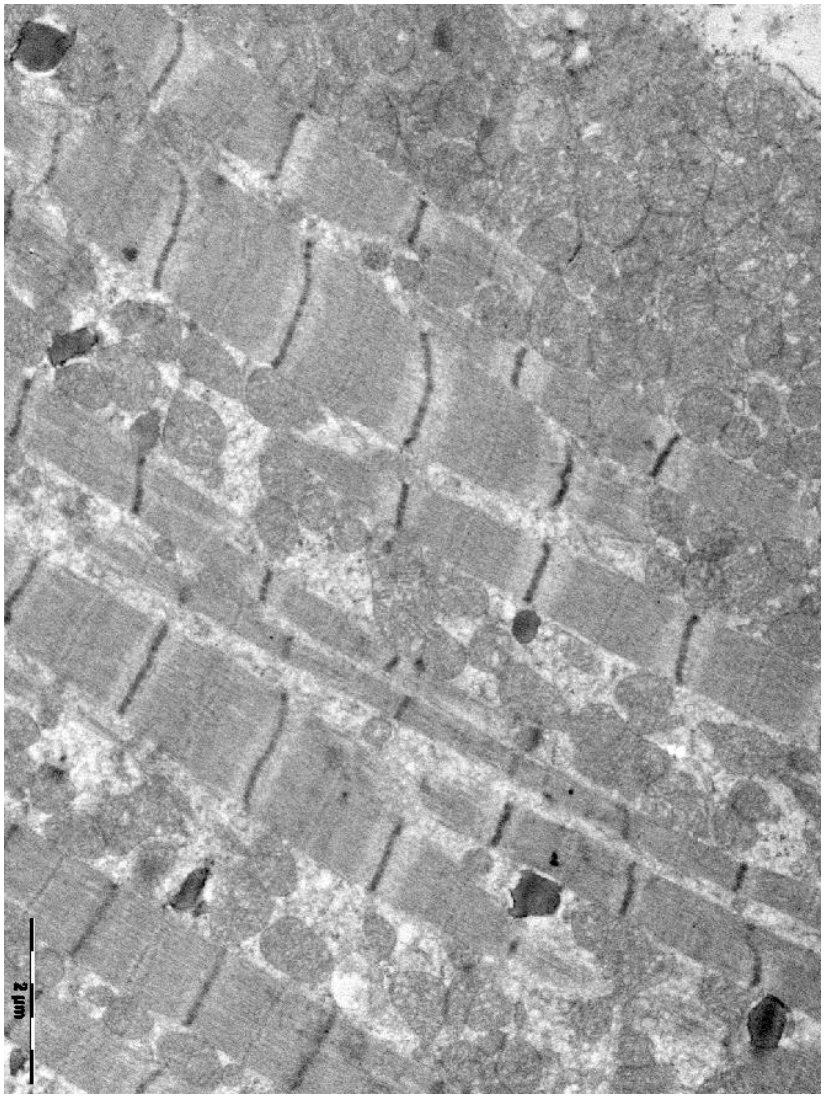
Periodic Acid and Schiff's reagent (PAS)

Electron Microscopy (EM)

Kollberg et al.
N Engl J Med 2007;357:1507-14

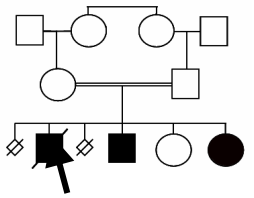
Muscle morphology - mitochondrial proliferation





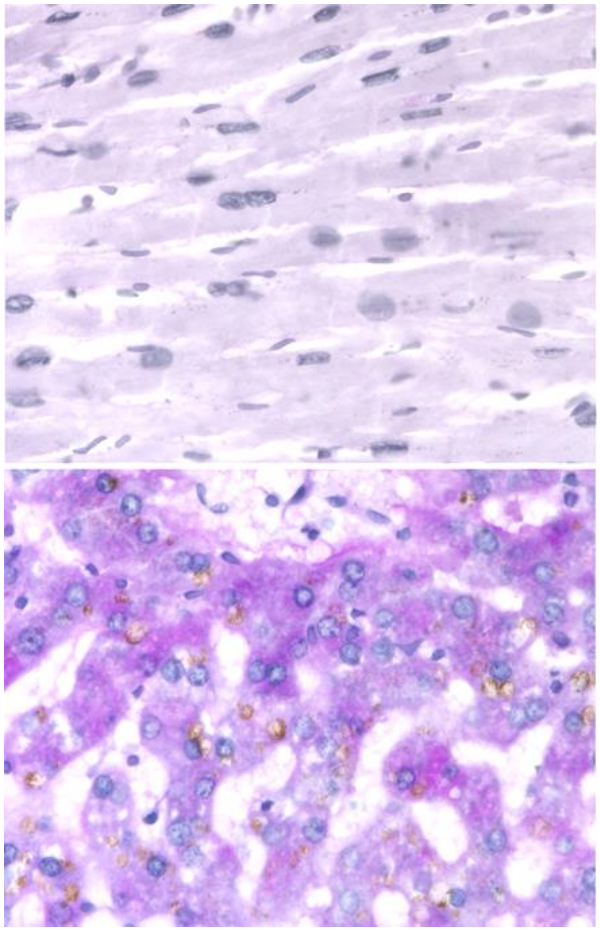
a) Gomori-Trichrome b) SDH c) EM

Glycogen content in heart and liver



Heart

Liver

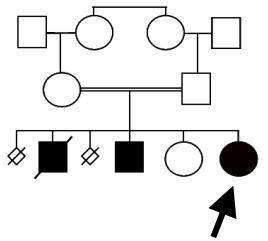


PAS staining

In heart muscle tissue there was an obvious glycogen deficiency

In liver cells there were apparently normal glycogen levels

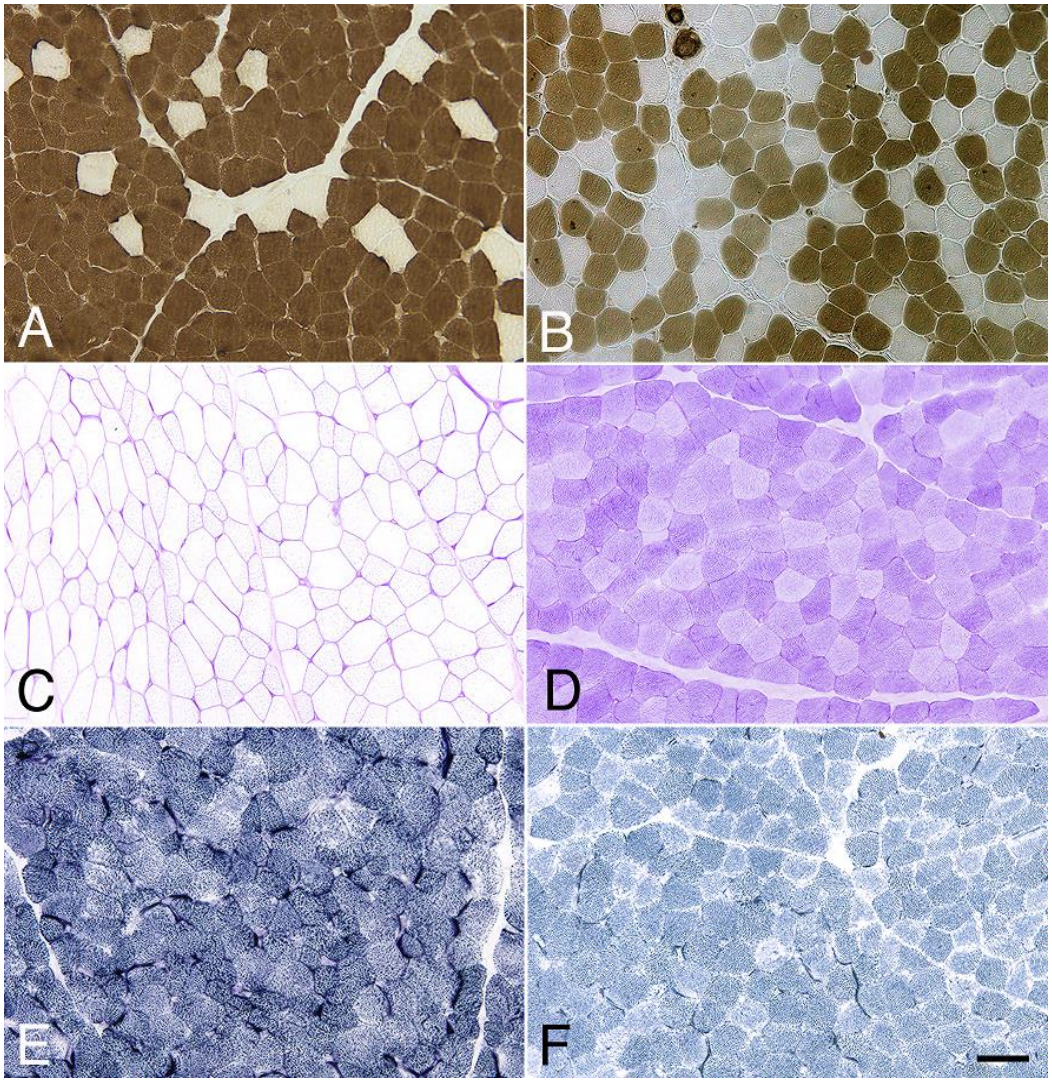
Muscle morphology



Type I fiber predominance

Glycogen deficiency

Abnormal mitochondrial *proliferation*



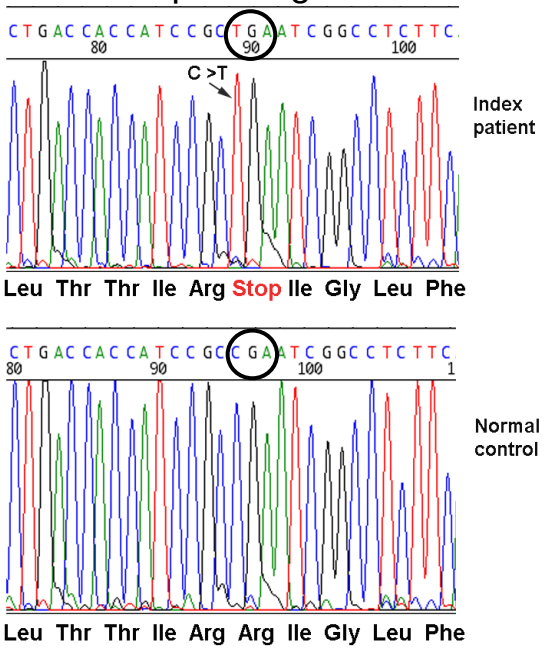
Patient 3 Normal control

Kollberg et al.
N Engl J Med 2007;357:1507-14

Genetic studies and protein analysis

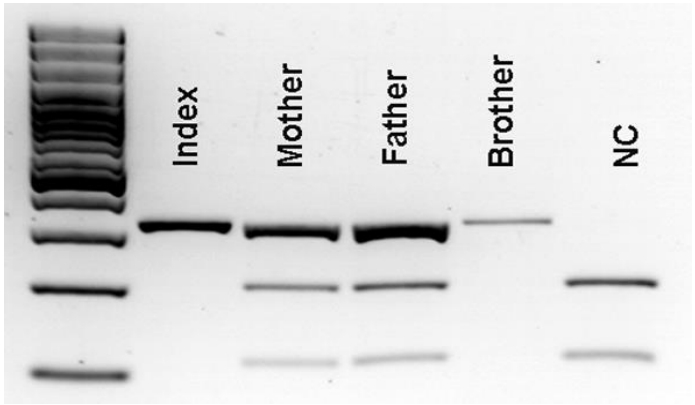
The three siblings had a homozygous nonsense mutation in *GYS1* and both parents were carriers

Sequencing

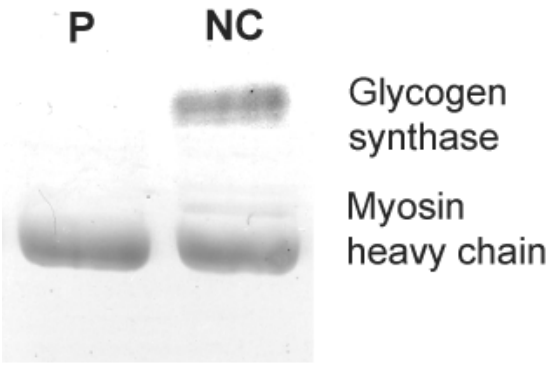


→ **R462X**

RFLP

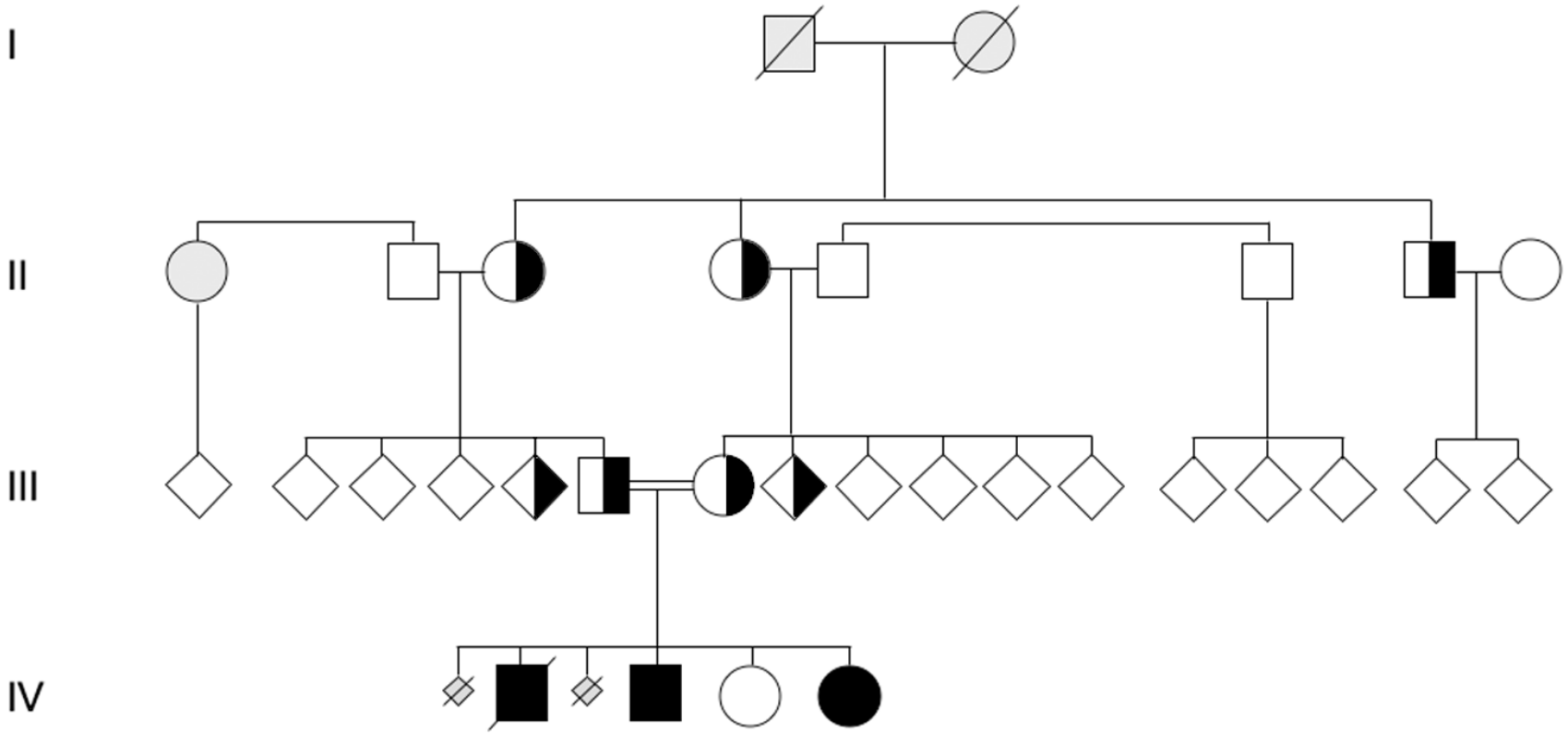


Western Blot



Kollberg et al.
N Engl J Med 2007;357:1507-14

Segregation of the *GYS1* mutation in the family



Carrier frequency in an ethnically matched control group: 1/200 alleles

Kollberg et al.
N Engl J Med 2007;357:1507-14

Muscle Glycogen Storage Disease zero

- Impaired glycogen synthesis due to mutations in *GYS1* has been suggested as a cause of type II diabetes but no actual relation has been found
- MGSKO mice have increased cardiac mass, absence of muscle glycogen, predominance of oxidative skeletal muscle fibers and normal to improved glucose clearance
- There was no difference in exercise capacity in MGSKO mice compared to their wild type littermates
- The patients with MGSD0 had cardiomyopathy, absence of muscle glycogen, predominance of oxidative skeletal muscle fibers and severe exercise intolerance but normal glucose tolerance

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