Mitochondrial disorders and defects of mitochondrial beta oxidation of fatty acids

Institute of Inherited Metabolic Disorders
1.LF
Mitochondria
Mitochondria structure
OXPHOS (oxidative phosphorylation system)
Respiratory chain
ATP production – molecule for energy transport in cells
Aerobic oxidation in eukaryotic mitochondria

(glycolysis and Krebs cycle)

ATP – adenosine triphosphate
Metabolic pathways in mitochondria

- Krebs cycle
- β oxidation FA
- Ketogenesis
- Urea cycle
- Synthesis of haem and phosphoryrrole
- ...

- A diagram illustrating various metabolic pathways in the mitochondria, including the Krebs cycle, β oxidation of fatty acids, ketogenesis, urea cycle, and the synthesis of haem and phorphyrine.
Mitochondriální transportní systém

- shuttle for NADH.H⁺ transport
  - malate-aspartate shuttle
  - glycerolphosphate shuttle

- substrates and products transporters

- ADP/ATP transocator

- protein import
Oxidative phosphorylation

> 43  4  11  13  16

subunits
Supercomplexes

Tissue specificity
(liver, heart, brown adipose tissue ...)

Ontogenetic changes
(perinatal development, ...)
Diagram of the mammalian mitochondrion showing the relationship between energy production, ROS generation, and regulation of apoptosis.
Mitochondrial disorders

Mitochondrial energy defects
  nuclear genes
  mitochondrial genes (mtDNA)

- Deficiency of respiratory chain complexes; OXPHOS
- Deficiency of Krebs cycle enzyme and pyruvate dehydrogenase metabolism
- Defects of mitochondrial replication, transcription, translation, repair, assembly or structural proteins…; in fusion and fission processes

Incidence 1/3000-4000
Mitochondrial DNA

- 16,569 base pairs
- 13 mRNA, 2 rRNA; 22 tRNA

- 2-20 / mito
- 10-10,000 / bb
Mitochondrial disorders

- Incidence 1:3500 – 1:4000
- High energy dependent tissues
- Mutation in ncDNA and v mtDNA

- 13 mRNA, 2 rRNA
- 22 tRNA - >100 mutation
  - Leu; Lys; Ser
  - MELAS; MERRF

Schon et al., 2001
Mitochondrial disorders

- 1963 Nass S, Nass MHK DNA v mitochondria
- 1974 Berk AS, Clayton DA replication of mtDNA
- 1979 Barrell BG, et al. genetic code of mtDNA
- 1981 Andersson S, et al. Sequence in mtDNA
- 1988 Wallace DC, et al. point mutation of mtDNA
- 2000 [www.gen.emory.edu/mitomap.html](http://www.gen.emory.edu/mitomap.html) > 130 point mutations
  > 70 deletions/duplications
ncDNA

mRNA

nc subunit

nc subunit

mt subunit

mt subunit

mRNA

mtDNA

> 70 nc OXPHOS subunits
13 mt OXPHOS subunits
26 assembly proteins
~ 150 „biogenesis“ proteins
Genetic of mitochondrial disorders

mtDNA
- OXPHOS subunits
- rRNA
- tRNA
- Amount of mtDNA

nDNA
- OXPHOS subunits
- mt biosynthesis system (replication, transcription, translation)
- Protein import and modification
- Assembly factors

OXPHOS defects
Hereditary mtDNA mutation

- Sporadic mtDNA defects
  - _single deletion/duplication_

- Maternally inherited mtDNA mutation
  - _point mutations_
  - _single deletion/duplication_

- Autosomal inherited mtDNA mutations
  - _multiple deletions/depletions_
Point mutations in mtDNA

Homoplasmic form of mtDNA
- OXPHOS proteins (LHON)

Heteroplasmic form of mtDNA
- tRNA (MERRF, MELAS)
- rRNA (cardiomyopathy)
- OXPHOS proteins
  - Leigh, NARP
  - LHON
The symptoms of mtDNA diseases often progressively worsen with age bioenergetic threshold is breached that results in mitochondrial dysfunction.

Some organs are particularly dependent on respiratory function: brain, skeletal muscle, heart muscle, and endocrine glands are particularly dependent on respiratory function.

Cells do not lose respiratory function until high loads of pathogenic mtDNA are present, ranging from 60% to 90% depending on the specific mutation.
Inter- a intramitochondrial heteroplasmacy
Segregace mtDNA
Figure 1. The mitochondrial genetic bottleneck. The mitochondrial genetic bottleneck provides an explanation for the different percentage of mutant mtDNA that can occur in siblings. It is thought that there is a restriction in the number of mtDNA molecules within the cell early in the development of the female germ line. This leads to marked differences in the level of heteroplasmacy between primary oocytes within the same female and accounts for the variation amongst offspring.
Segregations and distribution of mtDNA mutations

Germinative cells
(“bottleneck” effect)
Speed segregation
(homoplasy trends)

Somatic cells
slow segregation
tissue heteroplasmy
7512T>C mutation in mtDNA

Muscle: 95%
Blood: 91%
Fibroblasts: 92%

Muscle: 95%
Blood: 93%
Fibroblasts: 94%
Bucal cells: 92%
Hair fol.: 95%

Blood: 71%
Bucal cells: 74%
Hair fol.: 87%

Blood: 68%
Bucal cells: 59%
Hair fol.: 73%
Segregation of mtDNA
NARP (8993), MELAS (3243) a MERRF (8344). Of
Threshold effect of OXPHOS defects

- 0-100% of mutated mtDNA
- Non-linear relation between heteroplasmy and dysfunction - 90%
- Age dependent OXPHOS activity
- Tissue and cell specificity – energetic demands
Threshold Hypothesis

OXPHOS Capacity vs. mtDNA Damage

Mitochondrial disease patient
Premature senescence
Normal

Central nervous system
Heart/muscle
Endocrine
Kidney

Proportion damaged mtDNA (arbitrary units)

% OXPHOS capacity

Mitochondrial disease patient
Premature senescence
Normal

Damaged mtDNA
Patogenetic mechanisms

- mtDNA mutations
- OXPHOS defects
- Decreased ATP production
- ncDNA mutations

ROS

- apoptosis
- necrosis
- Cell death

Various cellular dysfunctions
“Any symptom, in any organ, at any age, and with any mode of inheritance”

Munnich et al, OMMBID, Ch 99
MtDNA defects

**LHON** – Leber hereditary optic neuropathy
- 11778 G>A; 3460G>A; 14484 T>C – complex I subunits
- akute/subakute blindess in adults
  - 4x in men

**NARP/Leigh syndrome** – neuropathy, ataxy, retinitis pigmentosa
- 8993 T>G and others
- psychomotor retardation, lactic acidosis, basal ganglia nekrosis

**MERRF** – myoclonic epilepsy, ragged red fibres
- 8344 G>A (tRNA pro Lys) and others
- deafness sensorineural

**MELAS** – mit. encephalomyopathy, laktic acidosis, stroke-like episodes
- 3243 A>G (tRNA pro Leu) and others
- diabetes mellitus

……..
Leigh syndrome

A neurodegenerative disorder usually starting before 1 year of age and leading to death within months or years.

„subacute necrotizing encephalomyelopathy“

Degeneration of basal ganglia, progressive course with motor and developmental decline ( „plateaus“), irregular breathing, ataxia, hyperlactacidemia, muscle weakness, seizures

Intermediate phenotypes

Defects of OXPHOS:
pyruvate dehydrogenase complex (PDH)(E1α gene),
cytochrome c oxidase (complex IV) -often putative complex IV assembly gene SURF-1

NADH-ubiquinone oxidoreductase (complex I).
Both nuclear gene defects and mtDNA mutations

(other complexes of respiratory chain)
Threshold effect

<table>
<thead>
<tr>
<th>Mutant mtDNA Level</th>
<th>31%</th>
<th>82%</th>
<th>93%</th>
<th>&gt;95%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onset</td>
<td>-</td>
<td>Adult</td>
<td>Infancy</td>
<td>Newborn</td>
</tr>
<tr>
<td>Symptoms</td>
<td>Healthy</td>
<td>Ataxy</td>
<td>NARP Syndrome</td>
<td>Leigh Syndrome</td>
</tr>
<tr>
<td>Retinopathy</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Mutant mtDNA level:

- 31%: Healthy symptoms
- 82%: Ataxy
- 93%: NARP syndrome
- >95%: Leigh syndrome

Defect:

- Mutant mtDNA level increases, leading to defects.
CPEO – Chronic progressive external ophthalmoplegia

Point mutations in mtDNA

<table>
<thead>
<tr>
<th>Gene</th>
<th>MtDNA mutation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>tRNA^Leu(UUR)</td>
<td>A3243G</td>
<td>21</td>
</tr>
<tr>
<td>tRNA^Leu</td>
<td>T4274C</td>
<td>142</td>
</tr>
<tr>
<td>tRNA^Leu</td>
<td>T4285C</td>
<td>143</td>
</tr>
<tr>
<td>tRNA^Leu</td>
<td>G4309A</td>
<td>144</td>
</tr>
<tr>
<td>tRNA^Asn</td>
<td>A5692G</td>
<td>145</td>
</tr>
<tr>
<td>tRNA^Leu(CUN)</td>
<td>G5703A</td>
<td>146</td>
</tr>
<tr>
<td>tRNA^Leu(CUN)</td>
<td>T12311C</td>
<td>147</td>
</tr>
<tr>
<td>tRNA^Leu(CUN)</td>
<td>G12315A</td>
<td>22</td>
</tr>
</tbody>
</table>

Abbreviations: tRNA = transfer RNA, Leu = Leucine, Ile = Isoleucine, Asn = Asparagine.
Kearns-Sayre syndrome

Ophthalmoplegia, ptosis, and mitochondrial myopathy prior to age 20 additional symptoms: retinitis pigmentosa and at least one of the following: cardiac conduction defects, cerebellar ataxia, or elevated cerebral spinal fluid protein above 100 mg/dl.

Commonly caused by mtDNA deletions
Leber hereditary optic neuropathy-LHON

LHON is a **maternally inherited, late-onset, acute, optic atrophy.** In some families also there is also optic neuritis. Incomplete penetrance (40% males, 10% females develop symptoms) Caused by homoplasmic missense mutations in mtDNA (complex I).

More than 90 percent of European and Asian LHON cases result from three mtDNA missense mutations.

G to A mutation in the MTND4 gene at nucleotide 11778 (MTND4*LHON11778A) about 50 percent of European cases and about 95 percent of Asian LHON patients. MTND1*LHON3460A (ND1 Ala52Thr) and MTND6*LHON14484C (ND6 Met64Val) . A number of rare mutations also appear to cause LHON.
LHON pedigree - maternal inheritance
MtDNA defects– MERRF (myoclonic epilepsy, RRF)
Muscle biopsy

Ragged red fibres
MtDNA defects
Muscle biopsy

Subsarcolemal accumulation of SDH

COX negative fibres
Nuclear defects of the mtDNA

nuclear DNA - replication, transcription, translation, repair assembly or structural proteins...

- defects in genes for structural subunits
  complex I – Leigh syndrome, cardiomyopathy, encefalomyopathy, myoclonic epilepsy
  complex II – ataxy, optical atrophy
    - hereditary paragangliomas

- defects in assembly proteins
  COX – SURF-1, SCO2, SCO1, COX10, COX15, ...
  ATPase – ATP12, TMEM70
Leigh syndrome

- SURF1 defect (assembly protein)
- severe neurodegenerative disease
- newborn, infancy
- basal ganglia necrosis
- fatal prognosis
COX (cytochrom c oxidase) deficiency due to Surf1 protein defect

Non-complet froms of COX
- absence of regulating nc-encoded subunits
- Labilita of subcomplexes
- H+transport defect
**ATPase defects – nuclear origin**

**TMEM70 protein**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newborn onset</td>
<td>14/14</td>
</tr>
<tr>
<td>Death</td>
<td>7/14 (4; 3)</td>
</tr>
<tr>
<td>Alive (≥3;≥5;≥10 let)</td>
<td>7/14 (3;2;3)</td>
</tr>
<tr>
<td>Cardiomyopathy</td>
<td>13/14</td>
</tr>
<tr>
<td>Hypotony</td>
<td>12/13</td>
</tr>
<tr>
<td>Psychomotor retardation</td>
<td>10/10</td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>6/14</td>
</tr>
<tr>
<td>Facial dysmorphy</td>
<td>5/14</td>
</tr>
<tr>
<td>Hyperlactacidemia</td>
<td>14/14</td>
</tr>
<tr>
<td>3-methylglutakon. aciduria</td>
<td>12/12</td>
</tr>
<tr>
<td>ATP hydrolysis &lt;30%</td>
<td>13/13</td>
</tr>
<tr>
<td>ATP production &lt;30%</td>
<td>4/4</td>
</tr>
<tr>
<td>Decrease of ATPase level</td>
<td>13/13</td>
</tr>
</tbody>
</table>


Study of mitochondrial proteins was based on electrophoretic and immunochemical analysis. Blue-Native PAGE showed a decreased content of the whole ATPase complex which retained normal size of approximately 640 kDa. It was detected in fibroblasts, skeletal and heart muscle tissue, liver and in one case in brain.
ATPase defects

Mutations in mtDNA          in nuclear DNA

„qualitative“                  „quantitative“
Structural modified ATPase complex with dysfunction  X  Decreased content of ATPase

Maternally inherited  X  Mendelian inherited
neurologic symptomatology  X  cardiomyopathy

Mitochondrial ATP synthase
Disorders of mitochondrial pyruvate metabolism and citric acid cycle

**Pyruvate dehydrogenase deficiency**
Dihydrolipoamid dehydrogenase, E3 subunit of PDH, multiple 2-oxo acid dehydrogenase deficiency: PDH, 2-ketoglutarate deficiency, branched-chain 2 oxo-acid deficiency

**Pyruvate carboxylase deficiency**

**Phosphoenolpyruvate carboxykinase deficiency**

**Fumase deficiency** - In heterozygotes: predisposition to leiomyomas of skin and uterus, kidney carcinoma

**Succinate dehydrogenase deficiency**

**Pyruvate transporter deficiency**

Lactic acidosis, progressive course, frequent neurological symptoms, muscle symptoms

Autosomal recessive disorders, deficiency of α-subunit of PDHE1 is X-linked
Pyruvate dehydrogenases complex

Pyruvate → acetyl-CoA

Dehydrogenase component : E1, subunit α is X-linked PDHE1α
Psychomotor retardation, ataxia, seizures
**Phenotypes:**
neonatal lactic acidosis,
Leigh encephalopathy: abnormal breathing, apnoe, ataxia, muscle, developmental delay,
Females: facial dysmorphism, seizures, subcortical and cortical atrophy,
Deficiencies of other subunits are rare

Lactic acidosis, increase of lactate after meals, during fasting lowering of lactate levels

Treatment: ketogenic diet, thiamin, dichloroacetate (inhibition of pyruvate kinase)

Unfavourable prognosis
Defects of nuclear DNA

nuclear DNA - replication, transcription, translation, repair ... assembly, structural subunits...

- Integrity and replication of mtDNA – thymidin fosforylase ANT1, twinkle, polymerase γ (POLG)
  MNGIE (mitochondrial neuro-gastro-intestinal encephalopathy);
  Alpers-Huttenlocher syndrome

- Transport of proteins

- Fission / fuse of mitochondria – OPA1

- Stability of mitochondrial membrane – Barth syndrome
Mitochondrial fusion and fission
Disorders of mitochondrial-beta oxidation of fatty acids

Carnitine cycle
Beta oxidation
Electron transfer to complex II (glutaric aciduria type II)
Synthesis of ketone bodies, ketolysis

Beta oxidation deficiencies:
Symptoms often develop after fasting (12-16h)
Hypoglycemia
Low ketones

(In some disorders muscle weakness, rhabdomyolysis, cardiomyopathy)
Disorders of mitochondrial-beta oxidation of fatty acids

- Disorders of FA transport
- Disorders of b-oxidation
- Cardiomyopathy
- Hepatopathy
- Nonketot.hypoglycaemia
- Myopathy

www.annualreviews.org (Bennett MJ, Fatty acid oxidation disorders, 2002)
Carnitine cycle

*Long chain free fatty acids* are “activated” to acyl-CoA esters in cytosol and are imported to mitochondria via carnitine cycle

*Medium- and shortchain fatty acids* are imported to mitochondria directly and are activate to CoA esters in mitochondrial matrix

CPT1 - carnitine palmitoyl transferase I
CPT2 - carnitine palmitoyl transferase II
CACT – carnitine /acylcarnitine translocase
Disorders of mitochondrial-beta oxidation of fatty acids

- **CPT I** – cardiomyopathy, arytmia, liver dysfunction
- **CPT II** – *Mild adult form:* attacks of rhabdomyolysis after exercise, fasting old cold. Myoglobinuria. *Severe neonatal form:* coma, cardiomyopathy, muscle weakness, congenital malformations of brain and kidneys
- **CACT** – carnitine /acylcarnitine translocase: Hypoketotic hypoglycemia after fasting, coma, arrythmias, apnoe, often death in early infancy
Disorders of mitochondrial-beta oxidation of fatty acids

- **VLCAD** – infant. form – SIDS
  - late onset – rhabdomyolysis, hypotony
- **LCHAD a MTP (trifunctional protein)** – cardiomyopathy, hepatopathy, retinopathy, rhabdomyolysis; neuropathy, Reye-like episodes
  - HELLP (Hemolysis, Elevated Liver enzymes, Low Platelets) syndrome in pregnancy
  - dg – OH-acylcarnitine
  - th – diet, frequent meals, avoid fasting
Disorders of mitochondrial-beta oxidation of fatty acids

- **MCAD** – most common – 1:6000 in Europe
  - prevalent mutation p.K329E
  - newborn screening
  - clinical - Reye like (after fasting, during illness)
    - vomiting, lethargy, seizures, cardiac arrest, hypoglycaemia, hyperammonaemia
    - no primary muscle involvement
    - frequently asymptomatic (p.Y67H)
  - laboratory – C6 and C8acyl carnitine, dicarboxylic aciduria, glycine conjugate
  - therapy – avoidance of fasting
Disorders of mitochondrial-beta oxidation of fatty acids

- **SCAD** – possible non disease
  - mild myopathy
- **Glutaric aciduria II type** – (ETF) – deficient electron transport from FAD dehydrogenases to respiratory chain
  - severe form (Reye syndrome, hypoglycemia, progr.encephalopathy, (cardio)myopathy)
  - mild myopatic form
  - cystic renal disease
  - laboratory – hypoglycemia, metabolic acidosis, elevated lactate
Disorders of mitochondrial-beta oxidation of fatty acids

- **Main symptoms** - chronic. Weakness, hypotony, exercise intolerance, rhabdomyolysis

- Fasting, infection, surgery, other catabolic state – trigger rhabdomyolysis

- **Wide clinical spectrum** – acute x chronic
  - infantil x late onset
  - isolated (myopathy) x multisystem impairment

(cardiomyopathy, encefalopathy)
Thank you.