Hereditary mitochondrial diseases
and
disorders of mitochondrial fatty acid oxidation
Biologically universal intracellular energy carrier

ATP – adenosine triphosphate
ATP production

aerobic oxidation in **mitochondria** in eukaryotic cells

photosynthesis

(glycolysis and citric acid cycle)

Synthesis of ATP from ADP: \( \Delta G = 7.3 \text{ kcal/mol} \)
Mitochondria

citric acid cycle

electron transport chain - oxidative phosphorylation

mitochondrial beta oxidation of fatty acids

parts of amino-acid metabolic pathways (urea cycle)

play important role in apoptosis
Glucose $\rightarrow$ pyruvate $\rightarrow$ Substrate oxidation (citric acid cycle) $\rightarrow$ NADH, FADH$_2$ (electron carriers) $\rightarrow$ Electron transport $\rightarrow$ O$_2$, H$_2$O $\rightarrow$ Proton motive force (proton gradient) $\rightarrow$ ATP

(Lipid or sugar)

Cytosol

Mitochondrion

ATP
Diagram of the mammalian mitochondrion showing the relationship between energy production, ROS generation, and regulation of apoptosis.

http://www.mitomap.org/MITOMAP/mito_apop.pdf
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Mitochondrial OXPHOS

Mitochondria generate much of the energy of the cell and this process regulates cellular redox potential, mitochondrial membrane potential, ATP production, and Ca ++ uptake.

Mitochondria generate most of the endogenous reactive oxygen species (ROS) as a toxic byproduct of OXPHOS.

Mitochondria integrate many of the signals for initiating apoptosis through regulating the opening of the mitochondrial permeability transition pore (mtPTP).

Opening of the mtPTP results in the release of cytochrome c and apoptotic enzymes from the mitochondrial intermembrane space, precipitating programmed cell death. All three of these processes use common OXPHOS polypeptides and functions.
Threshold Hypothesis

OXPHOS Capacity vs. mtDNA Damage

% OXPHOS capacity

Damaged mtDNA
Mitochondrial disorders

disrupt mitochondrial respiratory function
  \textbf{nuclear genes}
  genes encoded by \textbf{mtDNA}

Respiratory chain defects
Deficiencies of citric acid cycle and lactate and pyruvate metabolism
Disorders of mitochondrial fusion and fission

Conservative incidence estimate : 11.5/100,000
THE MITOCHONDRIAL GENOME:
mtDNA = 37 genes
Extra-cellular plasmids (chromosomes) ~ 1500 genes

ENCEPHALOMYOPATHIES
Mutations:
Inherited

DEAF 1555
12s rRNA
D-Loop

Regulatory Mutations: Somatic, Inherited?

DEAF 1555
12s rRNA
D-Loop

MELAS 3243
LHON 3460
ADPD 4336

POSTRATE CANCER
Mutations
Inherited & Somatic

PC 6252
PC 6261
PC 6340
PC 6663

America A
America C
America D
America B, Asia B

Inherited
Europe H
Asia F

America L

http://www.mitomap.org

LHON 14484
LDYS 14459
LHON 11778
LHON 10663
MERRF 8344
NARP 8993/Leigh’s 8993
The genetics of mitochondrial disease

Two different genomes, the mtDNA and nDNA.

The mtDNA: maternally inherited, thousands of copies per cell, has a high mutation rate heteroplasmy

16 kb circular dsDNA, 37 genes
slightly different genetic code
13 proteins (complexes I, III, IV, V)
22 transfer RNAs
2 ribosomal RNAs
organised into discrete units: nucleoids
nucleoids merge and divide, evidence for recombination of mtDNA

The nDNA-encoded mitochondrial genes
Proteins are synthesized on cytosolic ribosomes
Transported into the mitochondrial matrix or inner membrane by an outer (Tom) and either of two inner (Tim) membrane transport systems.
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.

Some mutant mtDNA may have a replicative advantage over wild-type 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 heteroplasmy between primary oocytes within the same female and accounts for the variation amongst offspring.
Respiratory chain impairment

1) Increase in reducing equivalents (mito + cytosol) ↑↑ NADH ↓↓ NAD⁺
2) Increased ratio lactate/pyruvate
3) Increased ratio 3-OH butyrate/acetoacetate
4) Changes more pronounced postprandially
5) Paradoxical hyperketonemia
A defect of the mitochondrial respiratory chain should be considered in patients presenting with an unexplained combination of neuromuscular and/or nonneuromuscular symptoms, with a progressive course, involving seemingly unrelated organs or tissues.

Munnich et al, OMMBD, Chapter 99
The diversity of organ involvement in respiratory chain deficiency

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

Munnich et al, OMMBID, Ch 99
<table>
<thead>
<tr>
<th>Disorder</th>
<th>Primary features</th>
<th>Additional features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic progressive external ophthalmoplegia (CPEO)</td>
<td>External ophthalmoplegia and bilateral ptosis</td>
<td>Mild proximal myopathy</td>
</tr>
<tr>
<td>Kearns–Sayre syndrome (KSS)</td>
<td>PEO onset before age 20 with pigmented retinopathy</td>
<td>Bilateral deafness</td>
</tr>
<tr>
<td></td>
<td>Plus one of the following:</td>
<td>Myopathy</td>
</tr>
<tr>
<td></td>
<td>CSF protein greater than 1 g l⁻¹</td>
<td>Dysphagia</td>
</tr>
<tr>
<td></td>
<td>cerebellar ataxia, heart block</td>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hypermphathyldism</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dementia</td>
</tr>
<tr>
<td>Pearson's syndrome</td>
<td>Sideroblastic anaemia of childhood</td>
<td>Renal tubular defects</td>
</tr>
<tr>
<td></td>
<td>Pancytopenia</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Exocrine pancreatic failure</td>
<td></td>
</tr>
<tr>
<td>Mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS)</td>
<td>Stroke-like episodes before age 40</td>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td></td>
<td>Seizures and/or dementia</td>
<td>Cardiomyopathy (hypertrophic</td>
</tr>
<tr>
<td></td>
<td>Ragged-red fibres and/or lactic acidosis</td>
<td>leading to dilated)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bilateral deafness</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pigmented retinopathy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cerebellar ataxia</td>
</tr>
<tr>
<td>Myoclonic epilepsy with ragged-red fibres (MERRF)</td>
<td>Myoclonus</td>
<td>Dementia</td>
</tr>
<tr>
<td></td>
<td>Seizures</td>
<td>Optic atrophy</td>
</tr>
<tr>
<td></td>
<td>Cerebellar ataxia</td>
<td>Bilateral deafness</td>
</tr>
<tr>
<td></td>
<td>Myopathy</td>
<td>Peripheral neuropathy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spasticity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Multiple lipomatosa</td>
</tr>
<tr>
<td>Leber's hereditary optic neuropathy (LHON)</td>
<td>Subacute bilateral visual failure (four times more</td>
<td>Dystonia</td>
</tr>
<tr>
<td></td>
<td>common in males than females)</td>
<td>Cardiac pre-excitation syndromes</td>
</tr>
<tr>
<td></td>
<td>Median age of onset 24 years</td>
<td></td>
</tr>
<tr>
<td>Leigh syndrome</td>
<td>Subacute relapsing encephalopathy with cerebellar and</td>
<td>Basal ganglia lucencies</td>
</tr>
<tr>
<td></td>
<td>brain-stem signs</td>
<td></td>
</tr>
</tbody>
</table>
Leigh's 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)
CPEO – Chronic progressive external ophtalmoplegia

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_{Ile}</td>
<td>T4274C</td>
<td>142</td>
</tr>
<tr>
<td></td>
<td>T4285C</td>
<td>143</td>
</tr>
<tr>
<td></td>
<td>G4309A</td>
<td>144</td>
</tr>
<tr>
<td>tRNA_{Asn}</td>
<td>A5692G</td>
<td>145</td>
</tr>
<tr>
<td></td>
<td>G5703A</td>
<td>146</td>
</tr>
<tr>
<td>tRNA_{Leu(CUN)}</td>
<td>T12311C</td>
<td>147</td>
</tr>
<tr>
<td></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's 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
Maternally inherited diabetes and deafness (MIDD), “mitochondrial diabetes”

A3243G mtDNA mutation within the tRNALeu gene
Type 1 or type 2 diabetes
All carriers develop diabetes or IGT before the age of 70 years: 100% penetrance
Progressive, most patients will require insulin
Impaired hearing, reflected by a reduced perception of high tone frequencies

Mitochondrial function is a key component of the glucose sensor.
In response to high blood glucose, pancreatic β cells increase glycolysis and oxidative phosphorylation
Increase in ATP concentration leads to calcium signals that trigger exocytosis of secretory vesicles containing insulin

Why is A3243G more diabetogenic compared to other mtDNA mutations?

The A3243G mutation was originally detected in patients with mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS syndrome) in Japan
Diagnostic techniques - an example

COX deficiency due to mutations in SCO2 gene


<table>
<thead>
<tr>
<th>Patient</th>
<th>Onset of symptoms</th>
<th>Inspiratory stridor</th>
<th>Infantile encephalopathy</th>
<th>Hypotonia</th>
<th>HCMP*</th>
<th>Brain atrophy</th>
<th>Respiratory insufficiency</th>
<th>Age at death</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>3 mo</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>-/+</td>
<td>microcephaly</td>
<td>last 2 mo</td>
<td>8 mo</td>
</tr>
<tr>
<td>2.</td>
<td>6 wk</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>-/+</td>
<td>?</td>
<td>last 2 mo</td>
<td>12 mo</td>
</tr>
<tr>
<td>3.</td>
<td>4 mo</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>++</td>
<td>last mo</td>
<td>15 mo</td>
</tr>
<tr>
<td>4.</td>
<td>6 mo</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>++</td>
<td>last mo</td>
<td>8 mo</td>
</tr>
<tr>
<td>5.</td>
<td>5 mo</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>++</td>
<td>last mo</td>
<td>9 mo</td>
</tr>
<tr>
<td>6.</td>
<td>birth</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>since birth</td>
<td>7 wk</td>
</tr>
</tbody>
</table>

* HCMP: hypertrophic cardiomyopathy.
** artificial ventilation in an intensive care unit.
Cox deficiency – histology, COX activity (mutations in SCO2 gene)

Table 2. Activities of cytochrome c oxidase, citrate synthase and their ratio in skeletal muscle homogenate, cultivated fibroblasts, isolated skeletal and heart muscle, liver and brain mitochondria in six children with mutations in the SCO2 gene.

<table>
<thead>
<tr>
<th>Case</th>
<th>Tissue*</th>
<th>Cytochrome c oxidase (COX) (nmol/min/mg protein)</th>
<th>Citrate synthase (CS) (nmol/min/mg protein)</th>
<th>COX/CS ratio (nmol/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Patients</td>
<td>Controls**</td>
<td>Patients</td>
</tr>
<tr>
<td>1.</td>
<td>Skeletal muscle (H)</td>
<td>12</td>
<td>25–100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fibroblasts (S)</td>
<td>10.6</td>
<td>18–40</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Skeletal muscle (M)</td>
<td>334</td>
<td>658–1552</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fibroblasts (S)</td>
<td>17</td>
<td>18–40</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Heart (M)</td>
<td>175</td>
<td>650–1500</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Liver (M)</td>
<td>119</td>
<td>140–580</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Brain (M)</td>
<td>117</td>
<td>483</td>
<td></td>
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<tr>
<td>3.</td>
<td>Skeletal muscle (H)</td>
<td>18</td>
<td>25–120</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fibroblasts (S)</td>
<td>33</td>
<td>18–40</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Skeletal muscle (H)</td>
<td>11</td>
<td>25–100</td>
<td></td>
</tr>
<tr>
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<td>Fibroblasts (S)</td>
<td>48</td>
<td>18–40</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Skeletal muscle (M)</td>
<td>349</td>
<td>658–1552</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Liver (M)</td>
<td>113</td>
<td>140–580</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fibroblasts (S)</td>
<td>22.7</td>
<td>18–40</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Skeletal muscle (M)</td>
<td>47</td>
<td>658–1552</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fibroblasts (S)</td>
<td>31</td>
<td>18–40</td>
<td></td>
</tr>
</tbody>
</table>

The data represent mean of two independent measurements.

* investigated tissues: M: isolated mitochondria; H: homogenate; S: suspension.

** controls: 27 age-related “disease-free controls” (20).

*** frontal cortex.
Disorders of mitochondrial pyruvate metabolism and citric acid cycle

Pyruvate dehydrogenase deficiency
Pyruvate carboxylase deficiency
Phosphoenolpyruvate carboxykinase deficiency
Pyruvate dehydrogenase deficiency (PDH)
Dihydrolipoamide dehydrogenase, E3 subunit of PDH, multiple 2-oxo acid dehydrogenase deficiency: PDH, 2-ketoglutarate deficiency, branched-chain 2 oxo-acid 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 $\rightarrow$ acetyl-CoA

Dehydrogenase component : E1, subunit $\alpha$ is X-linked
PDHE1$\alpha$
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
Pyruvate carboxylase deficiency

Tetrameric enzyme, biotin
Gluconeogenesis, forms intermediates of citric acid cycle
Pyruvate + CO2 → oxalacetate

Deficiency: autosomal recessive
**French type (type B)**
In the newborn period vomiting, hypotonia, death in infancy
**North American phenotype (type A)**
In early infancy repeated attacks of vomiting, hypotonic, tachypnoe, metabolic acidosis, often during infections
Subdural haematomas, brain atrophy, progressive course with death in infancy

Lactate acidosis, hypoglycemia, hyperammonemia, high ratio lactate/pyruvate
Low aspartate (aspartate important in urea cycle → hyperammonemia)
Elevated plasma citrulin, lysin, low glutamine
Mitochondrial fusion and fission

Coordinated fusion of both the outer and inner mitochondrial membranes.

**Mitofusins** are GTPases localized to the outer mitochondrial membrane. Mfn1 and Mfn2

Mutations **mitofusin 2** cause **Charcot-Marie-Tooth neuropathy type 2A** (Hereditary Motor and Sensory Neuropathy)

Dynamin family GTPase **OPA1** : mutated in **autosomal dominant optic atrophy** linked to chromosome 3q28.

Kjer's disease – loss of visual acuity, loss of retinal ganglion cells
Mitochondrial fusion and fission
Brown adipose tissue

Thermogenesis in the absence of shivering

Abundant in newborns and hibernating animals

Present in young adults

Uncoupling protein 1 (UCP1)

The brown adipocyte has very high density of mitochondria
Brown-Adipose-Tissue Activity as Assessed by PET-CT with 18F-FDG

Fatty acids and mitochondria

Diagram:
- Long-chain fatty acid
  - Fatty acid
  - Fatty acyl-CoA
  - Fatty acyl-carnitine
- Medium-chain fatty acid
  - Fatty acyl-CoA
  - Acetyl-CoA → (HMG-CoA) → Ketones → CO₂
- Plasmic fatty acid
  - Fatty acyl-CoA → Acetyl-CoA → (HMG-CoA) → Ketones → CO₂

Mitochondria

Cytoplasm

Plasma
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)
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
OCTN2 - organic cation transporter 2 (SLC22A5), carnitine transporter
Carnitine cycle deficiencies

CPT1 - carnitine palmitoyl transferase I deficiency (CPT1A liver/kidney specific isoform):
Hypoketotic hypoglycemia after fasting, heart and skeletal muscle are not affected

CPT2 - carnitine palmitoyl transferase 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

OCTN2 -organic cation transporter 2 (SLC22A5),
carnitine transporter deficiency:
Hypetrophic cardiomyopathy leading to heart failure (onset 12 mo -7 years), muscle weakness. Fasting leads to hypoketotic hypoglycemia, coma, sudden death. Treatment with carnitine.
Carnitine metabolism – diagnostic findings

**CPT1 - carnitine palmitoyl transferase I deficiency** (CPT1A liver/kidney specific isoform): Total carnitine is elevated (150-200% of controls)

**OCTN2 - organic cation transporter 2 (SLC22A5),**
- carnitine transporter deficiency:
  Very low carnitine levels in plasma, cardiac and skeletal muscle <2-5% „primary carnitine deficiency“

In other BOX disorders are carnitin levels 25-50% of normal levels ("secondary carnitine deficiency")
Mitochondrial-beta oxidation of fatty acids

1. Dehydrogenation

\[
\text{Acyl-CoA} \xrightarrow{\text{FAD, FADH}_2} \text{trans-} \Delta^2\text{-Enoyl-CoA}
\]

2. Hydration

\[
\text{trans-} \Delta^2\text{-Enoyl-CoA} \xleftrightarrow{\text{Enoyl-CoA-Hydrolase}} \text{L-3-Hydroxyacyl-CoA}
\]

3. Dehydrogenation

\[
\text{L-3-Hydroxyacyl-CoA} \xrightarrow{\text{NAD}^+, \text{H}^+} \text{3-Ketoacyl-CoA}
\]

4. Thiolysis

\[
\text{3-Ketoacyl-CoA} \xrightarrow{\text{Thiolase}} \text{Acyl-CoA} + \text{Acetyl-CoA}
\]
Disorders of mitochondrial beta-oxidation I:

A: Deficiencies of acyl-CoA dehydrogenases

**VLCAD deficiency** (very-long-chain acyl-CoA dehydrogenase): Hypoketonemic hypoglycemia, cardiomyopathy, muscle weakness, severe metabolic decompensation with coma

**MCAD deficiency** (medium-chain acyl-CoA dehydrogenase): Most common BOX disorder, without signs of cardiomyopathy of myopathy. 60-80% of patients are homozygous for A985G(K235E) mutation (North Europe). Incidence in GB and USA is 1:10 000. Patients appear healthy, attacks of hypoglycemia are induced by fasting, infection (usually 3-24 months). The tolerance of fasting improves with age. Lethargy, vomiting, coma, seizures. Misdiagnosis: Reye sy, SIDS

**SCAD deficiency** (short-chain acyl-CoA dehydrogenase): Two common polymorphisms:
Disorders of mitochondrial beta-oxidation II:

B: Deficiencies of 3-hydroxyacyl-CoA dehydrogenases

**LCHAD deficiency** (Long-chain 3-hydroxy acyl-CoA dehydrogenase/Trifunctional protein deficiency):
*Mitochondrial trifunctional protein (TFP):* 4 α and 4 β subunits
*Alpha-Subunits* contain long-chain enoyl-CoA hydratase and LCHAD activities
*Beta subunits* contain long-chain 3-oxo acyl CoA thiolase activity
Some patients have isolated LCHAD deficiency, other have mutations in both subunits
Variable phenotype with severities similar to MCAD up to VLCAD
Some patients have retinal degeneration, peripheral neuropathy

Heterozygous mothers: acute fatty liver of pregnancy (AFLP sy), or hemolysis, elevated liver enzymes, and low platelet count syndrome (HELLP)

**3-SCHAD, medium chain 3-oxo-acyl dehydrogenase deficiency (MCKT) deficiency:** very rare
<table>
<thead>
<tr>
<th>Deficiency</th>
<th>Plasma acylcarnitins</th>
<th>Urinary acylcarnitins</th>
<th>Urinary organic acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLCAD</td>
<td>Tetradecenoyl-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCAD</td>
<td>Octanoyl-Decenoyl-</td>
<td>Hexanoyl-Suberyl-(Phenylpropionyl-)</td>
<td></td>
</tr>
<tr>
<td>SCAD</td>
<td>Butyryl-</td>
<td>Butyryl-</td>
<td>Ethylmalonic aciduria</td>
</tr>
<tr>
<td>LCHAD</td>
<td>3-hydroxypalmitoyl-3-hydroxyoleoxyl-3-hydroxylinoleoyl</td>
<td></td>
<td>3-hydroxy-dicarboxylic aciduria</td>
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<tr>
<td>ETF and ETF-dehydrogenase</td>
<td>Butyryl-Isovaleryl-Glutaryl-</td>
<td>Isovalryl-Hexanoyl-</td>
<td>Ethylmalonic, glutaric and isovaleric aciduria</td>
</tr>
<tr>
<td>HMG-CoA lyase</td>
<td>Methylglutaryl-</td>
<td></td>
<td>3-hydroxyl3-methylglutaric aciduria</td>
</tr>
</tbody>
</table>
Diagnostic tests

**Acylcarnitines**
Tandem mass spectrometry

**Acylglycines**

**Fatty-acids in plasma**
cis-4-decenolic acid is specific for MCAD

**Organic acids**
In fed state usually normal findings
After fasting – dicarboxylic aciduria, in some disorders specific organic acids

**Oxidation of fatty acids in vitro**
Lymphocytes or cultured skin fibroblasts

**Tests for individual enzyme activities, DNA diagnostics**
Dicarboxylic aciduria in MCAD

Fig. 2. Dual capillary column chromatogram of a urine extract from a patient with medium-chain acyl-CoA dehydrogenase deficiency. The upper chromatogram was from an SPB-35 column, and the lower from SPB-1. Column temperature was from 60 to 250°C with a 4°C/min rate increase and a 1 ml/min helium flow. A 50 to 1 split ratio injection was used. The peaks were identified as: 1, adipic; 2, trans-2-hexedioic; 3, 7-hydroxyoctanoic; 4, pimelic; 5, ρ-hydroxyphenylacetic; 6, cis-3-octenedioic; 7, cis-4-octenedioic; 8, trans-3-octenedioic; 9, suberic; 10, aconitic; 11, hippuric; 12, cis-5-decenedioic; 13, cis-4-decenedioic; 14, trans-5-decenedioic (?); 15, sebacic; and 16, pentadecanoic (internal standard) acids.