Mitochondrial diseases and genetic defects of ATP synthase

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Energy demands are met through ATP production. ADP + Pi is converted to ATP in the mitochondria with a yield of >95%.
Mitochondrial OXPHOS system

respiratory chain + ATP synthase = ~100 proteins

<table>
<thead>
<tr>
<th></th>
<th>CI</th>
<th>CIII</th>
<th>CII</th>
<th>CIV</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>mtDNA</td>
<td>7</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>ncDNA</td>
<td>39</td>
<td>10</td>
<td>4</td>
<td>10</td>
<td>14</td>
</tr>
</tbody>
</table>
~1000 ncDNA genes + 37 mtDNA genes
37 mtDNA genes

13 proteins of RCH
2 rRNA, 22 tRNA,
Multicopy DNA (10-10 000/cell)
Maternal inheritance
High mutation frequency
169 in tRNA, 195 in cod. genes

Essential OXPHOS components
ncDNA → mRNA → precursor → nc subunits

mtDNA → mt subunits → mtOxPhos complex

- 13 mt OxPhos subunits
- > 80 nc OxPhos subunits
- > 40 ancillary factors
- ~ 150 "biogenesis" proteins

..........
One or more OXPHOS enzymes altered
Insufficient energy provision

Mitochondrial diseases

Organs with high energy demands
  brain, muscle, heart, liver, kidney, endocrine

Age of onset, clinical course
  infant - adult; light - severe

Variability of symptoms
  genetic defect with different phenotypes
defifferent genetic defects Identical phenotype
OXPHOS genetic defects

mtDNA mutations predominant in adults
ncDNA mutations prevalent in children
Frequency > 1/5 000
Autosomal recessive carriers 1/5-1/10

Heredity maternal, AR, AD, XR

Altered OXPHOS biogenesis
ATP synthase (F₀F₁ ATPase)
ATP synthase

Proton channel

620 kDa, 17 subunits
2 mtDNA genes

Catalytic part

$\alpha_3, \beta_3, \gamma, \delta, \varepsilon + \text{IF1}$

$a, b, c_{10-12}, d, e, f, g, OSCP, F_6, A6L, FB$
ATP synthase generator

Proton channel

\[ a, b, c_{10-12}, d, e, f, g, OSCP, F_6, A6L, FB \]
\[ \alpha_3, \beta_3, \gamma, \delta, \varepsilon + IF_1 \]

Catalytic part

620 kDa, 17 subunits
2 mtDNA genes
ATP synthase biogenesis is step-wise process

<table>
<thead>
<tr>
<th>Genes</th>
<th>Yeast</th>
<th>Human</th>
<th>Target</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP11</td>
<td>+</td>
<td>+</td>
<td>F1 subunit β</td>
<td>chaperone</td>
</tr>
<tr>
<td>ATP12</td>
<td>+</td>
<td>+</td>
<td>F1 subunit α</td>
<td>chaperone</td>
</tr>
<tr>
<td>FMC1</td>
<td>+</td>
<td>-</td>
<td>Atp12p/ F1-α</td>
<td>co-chaperone</td>
</tr>
<tr>
<td>NAM1</td>
<td>+</td>
<td>-</td>
<td>subunit 6 and 8</td>
<td>mRNA processing</td>
</tr>
<tr>
<td>AEP3, NCA2,</td>
<td>+</td>
<td>-</td>
<td>subunit 6 and 8</td>
<td>mRNA stab./translation</td>
</tr>
<tr>
<td>NCA3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATP22</td>
<td>+</td>
<td>-</td>
<td>subunit 6</td>
<td>translation</td>
</tr>
<tr>
<td>ATP10</td>
<td>+</td>
<td>-</td>
<td>subunit 6</td>
<td>chaperone</td>
</tr>
<tr>
<td>ATP23</td>
<td>+</td>
<td>?</td>
<td>subunit 6</td>
<td>processing/assembly</td>
</tr>
<tr>
<td>NCA1, ATP25</td>
<td>+</td>
<td>-</td>
<td>subunit 9</td>
<td>mRNA stability</td>
</tr>
<tr>
<td>AEP1, AEP2/ATP13</td>
<td>+</td>
<td>-</td>
<td>subunit 9</td>
<td>translation</td>
</tr>
</tbody>
</table>

Ancillary factors
Isolated disorders of human ATP synthase

**mtDNA mutations**
*ATP6, Fo subunit a*
ATP synthase - content unaffected
- altered structure

**Impaired function of H+ channel**

**ncDNA mutations**
ATP synthase - content low
- structure normal

**Altered enzyme biogenesis**
Missense point mutations in ATP6 gene

- **T8993G (C)**  
  Leu$^{156}$ to Arg/Pro  
  *Leigh syndrome, NARP syndrome*

- **T8851C**  
  Trp$^{109}$ to Arg  
  *FBSN*

- **T9176G (C)**  
  Leu$^{217}$ to Pro  
  *FBSN, sudden death*

**Heteroplasmic, missense mutations**

*Replacement of conserved AA*  
*Brain – devastating encephalopathy*

**Leigh syndrome:** devastating degenerative disorder, striatal necrosis, psychomotor regression, seizures, optic atrophy, peripheral neuropathy.

**NARP syndrome:** neuropathy, ataxia, retinal pigmentary degeneration,

**FBSN:** familiar bilateral striatal necrosis, weaker form of Leigh syndrome
Leu$_{156}$
Family with T8993G mutation disease
ATP Production by T8993G Fibroblasts

<table>
<thead>
<tr>
<th>Condition</th>
<th>ATP (nmoles/30min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>50% mut. mtDNA (mother)</td>
</tr>
<tr>
<td>Glu+MalPyr+MaDg+Mal</td>
<td>98% mut. mtDNA (patient)</td>
</tr>
<tr>
<td>Succ+Rot</td>
<td></td>
</tr>
</tbody>
</table>

The diagram illustrates ATP production under various conditions, comparing control and mutated mtDNA (mother and patient) scenarios.
Accumulation of incomplete forms of ATP synthase in ATP6 mutations

--- $F_1F_0$

--- assembly intermediate

--- $F_1$

B subunit

ATP6 defects
### Manifestation of 8993 mtDNA mutation

<table>
<thead>
<tr>
<th>mut mtDNA</th>
<th>31 %</th>
<th>82 %</th>
<th>93 %</th>
<th>&gt;95 %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onset</td>
<td>adult</td>
<td>children</td>
<td>newborn</td>
<td></td>
</tr>
<tr>
<td>Symptoms</td>
<td>healthy</td>
<td>ataxia</td>
<td>NARP</td>
<td>Leigh</td>
</tr>
<tr>
<td></td>
<td></td>
<td>retinopathy</td>
<td>syndrome</td>
<td>syndrome</td>
</tr>
</tbody>
</table>

**Threshold:**

- mutation is tolerated until 70-80% mutation load
Defect in processing of ATP6 transcript

Short Communication

A mitochondrial DNA microdeletion in a newborn girl with transient lactic acidosis

S. Seneca1*, M. Abramowicz1, W. Linnens1, M. F. Müller2, E. Vanos3 and L. de Meirleir1,2

Departments of Medical Genetics1, and Pediatrics1, University Hospital of the Dutch-speaking Brussels Free University, Brussels and 2Erasme University Hospital, Brussels, Belgium

*Correspondence: Department of Medical Genetics, University Hospital of Dutch-speaking Brussels Free University, 1041 Laarbeeklaan, 1050 Brussels, Belgium

Diminished synthesis of subunit a (ATP6) and altered function of ATP synthase and cytochrome c oxidase due to the mtDNA 2 bp microdeletion of TA at positions 9205 and 9206

Fava JESÍNIA1*, Markéta TESAŘOVÁ1, Daniela FORNKOSKOVÁ1, Alena KOUPÍŠKOVÁ1, Petra PESHLA1, Víra KAPLANOVÁ1, Hanka HANUSOVÁ1, Jitína ZEMÁNKOVÁ1 and Josef HOLŽTÍN1,2

Institute of Biomedical Genetics, Academy of Sciences of the Czech Republic, Vídeňská 1351, 142 21 Prague, Czech Republic, and Department of Pediatrics, Medical Faculty, Charles University, 720 00 Prague, Czech Republic.
2bp microdeletion in *ATP6/COX3* gene

- Deletion of TA at position 9205, 9206
- Altered *ATP6* stop codon and *ATP6/COX3* mRNA cleavage site

```
.....ACACA TA ATGA......
.....ACACA ATGA....
```

```
ATP6

ATP8

ATP8/ATP6 RNA

UAA

UAAUG

AUG

COX3 RNA

AAAAAn

AAAAAn
```
Altered biosynthesis of ATP6 subunit protein

**Western blot**

9205ΔTA Control

- Anti SDH70
- Anti F1α
- Anti OSCP
- Anti F0α
- Anti F0c
- Anti COX1
- Anti COX4
- Anti COX6c

ATP6 content decreased 10-fold

**methionine labeling**

[35S]methionine labelling

- COX1
- ND4
- Cytb
- ND2
- ND1
- ND3
- ATP6
- ATP8

ATP6 synthesis decreased 9-fold
Modulation of mtDNA heteroplasmy in cybrids

Enucleated cells with mtDNA heteroplasmy mutation

Control cells devoid of mtDNA by EB (Rho cells)

Fusion and selection

Cybrid clones
Modulation of mtDNA heteroplasm in cybrids
Threshold in ATP6 mRNA processing

Gene

80% Mutation load

ATP6 mRNA

ATP6 protein

ATP synthase complex

Respiration

ATP synthesis

ATP6 Protein

Respiration

ATP synthesis
Nuclear genetic defects of ATP synthase
Case report

Boy (1950 g, 45 cm), cardiomyopathy, hepatomegaly, metabolic acidosis
Died in 2d due to fatal LA and heart failure
Parents – gypsy ethnic group, first degree cousins, 9 pregn., 1 miscarriage
2 sisters died in the 1st wk, 1 brother with CM died in 3y

* 3 children healthy, 3 children died after birth
** 4 children healthy, 2 children died after birth
ATP synthase deficiency in heart, muscle and fibroblasts

Normal activity of RC enzymes (CS, CI, CII, CIV)
ATPase hydrolytic activity $\leq 30$

Decreased content in ATP synthase complex
Complementation of ATP synthase defect in cybrids by replacement of ncDNA

Fibroblasts                   Cybrids

ATPase

COX

* Methionine labeling of ATP synthase complex

Control

Patient

Time (h)

Restored content of ATP synthase

Restored de novo biosynthesis of ATP synthase
Metabolic consequences of ATP synthase deficiency
Low ADP-stimulated respiration and ATP synthesis

High mitochondrial membrane potential $\Delta \Psi_m$ at state 3-ADP

Digitonin-treated fibroblasts labeled with 20 nM TMRM in 80 mM KCl, 10 mM Tris-Cl, pH 7.4, 3 mM MgCl2, 1 mM EDTA, 5 mM KH2PO4, 10 mM succinate, 0.1 mM ADP.

**ADP-induced decrease $\Delta \Psi_m$**
- Control – 24.4±2.1 mV
- Patients – 15.7±4.2 mV
Increased ROS production in ATPase-deficient patient cells

1uM CM-H2DCFDA, Leica TCS SP2 AOBS, excitation - two-photon laser Mira + Verdi (Coherent) tuned at 800 nm (2 mV at the level of objective), emission 500-533 nm.

Mracek et al., 2007
Two components of pathogenic mechanism, energy deprivation and ROS production

Houstek BBA 2006, 1757:1400
How to survive with 10-30% of ATP synthase?

Muscle 65-87 %
Heart 50-77 %
Liver 50-75 %
Kidney 37-64 %
Brain 32-58 %

Rossignol et al., JBC
1999, 274:33426
Search for the mutated gene
### 14 patients with isolated deficiency of ATP synthase in 2006

<table>
<thead>
<tr>
<th>Condition</th>
<th>Patients Affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonatal onset</td>
<td>14/14</td>
</tr>
<tr>
<td>Died</td>
<td>7/14</td>
</tr>
<tr>
<td>Cardiomyopathy</td>
<td>13/14</td>
</tr>
<tr>
<td>Hypotonia</td>
<td>12/13</td>
</tr>
<tr>
<td>MRI brain unspecific</td>
<td>5/10</td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>6/14</td>
</tr>
<tr>
<td>Facial dysmorphy</td>
<td>5/14</td>
</tr>
<tr>
<td>Psychomotor retardation (longer surviving patients)</td>
<td>10/10</td>
</tr>
<tr>
<td>Hyperlactataemia &gt;2.5 mmol/l</td>
<td>14/14</td>
</tr>
<tr>
<td>3-Methylglutaconic aciduria &gt;20 mmol/mol creat.</td>
<td>12/12</td>
</tr>
<tr>
<td>ATPase activity &lt;30 % of control</td>
<td>13/13</td>
</tr>
<tr>
<td>ATPase complex content &lt;30 % of control</td>
<td>13/13</td>
</tr>
</tbody>
</table>

*BBA 2006, 1757:1400-5*

*Neuromuscular Disorders 2006, 16:821-9*
Biosynthesis terminated at early stage of enzyme assembly
No mutation in enzyme subunits

Mutation or expression of ancillary factors (helper proteins)
homozygous TGG-AGG missense mutation in exon 3 changing Trp94 to Arg

ATP12 mutation in 1 case
Absent in all other patients
Specific phenotype
# Expression profiling of ATP synthase deficient fibroblasts

<table>
<thead>
<tr>
<th>Patient</th>
<th>Survival</th>
<th>Clinical and biochemical presentation</th>
<th>Lab</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1-m</td>
<td>9y</td>
<td>PMR, M, LA</td>
<td>CZ</td>
</tr>
<tr>
<td>P2-f</td>
<td></td>
<td>GR, LA</td>
<td>Be</td>
</tr>
</tbody>
</table>

**mtDNA mutation 9205ΔTA**

<table>
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<tr>
<th>Patient</th>
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<th>Lab</th>
</tr>
</thead>
<tbody>
<tr>
<td>P3-f</td>
<td>20y</td>
<td>PMR, Hy, PNP, HCMP, LA, ↓ATPase</td>
<td>A</td>
</tr>
<tr>
<td>P4-m</td>
<td>†2d</td>
<td>HCMP, Dy, Hps, LA, 3MGA, ↓ATPase</td>
<td>CZ</td>
</tr>
<tr>
<td>P5-m</td>
<td>†4y</td>
<td>PMR, M, Hy, HCMP, Dy, Hps, LA, 3MGA, ↓ATPase</td>
<td>CZ</td>
</tr>
<tr>
<td>P6-m</td>
<td>4y</td>
<td>PMR, M, Hy, HCMP, Dy, Hps, LA, 3MGA, ↓ATPase</td>
<td>CZ</td>
</tr>
<tr>
<td>P7-m</td>
<td>†18m</td>
<td>PMR, M, Hy, HCMP, Dy, Hps, LA, 3MGA, ↓ATPase</td>
<td>CZ</td>
</tr>
<tr>
<td>P8-m</td>
<td>2y</td>
<td>PMR, M, Hy, HCMP, Dy, Hps, LA, 3MGA, ↓ATPase</td>
<td>CZ</td>
</tr>
<tr>
<td>P9-f</td>
<td>17y</td>
<td>PMR, M, Hy, HCMP, Dy, LA, 3MGA, ↓ATPase</td>
<td>CZ</td>
</tr>
<tr>
<td>P10-m</td>
<td>†10d</td>
<td>PMR, M, Hy, HCMP, Dy, Hps, LA, 3MGA, ↓ATPase</td>
<td>CZ</td>
</tr>
<tr>
<td>P11-m</td>
<td>7y</td>
<td>PMR, M, Hy, HCMP, GR, Dy, LA, 3MGA, ↓ATPase</td>
<td>CZ</td>
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<tr>
<td>P12-f</td>
<td>8y</td>
<td>PMR, Hy, HCMP, LA, 3MGA, ↓ATPase</td>
<td>A</td>
</tr>
<tr>
<td>P13-f</td>
<td>5y</td>
<td>PMR, M, He, GR, LA, 3MGA, ↓ATPase</td>
<td>CZ</td>
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</tbody>
</table>

**nuclear defect**

<table>
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<td>CZ</td>
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</table>

**Clinical and biochemical presentation**
Expression profiling of ATP synthase deficient fibroblasts

- Human oligonucleotide microarray 950 mito-genes
- Agilent 44K Array

- No down regulation of ATP synthase subunits
- No down regulation of ATP synthase assembly factors
## Linkage analysis, homozygosity mapping

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<td>P13-f</td>
<td>5y</td>
<td>PMR, M, He, GR, LA, 3MGA, ↓ATPase</td>
<td>CZ</td>
</tr>
</tbody>
</table>

### Genetic defect

- mtDNA mutation 9205ΔTA
- Nuclear defect

### Genotyping

- Of 6 families
- Affymetrix GeneChip Mapping 250K

### Clinical and biochemical presentation

- Survival: Patient's age or duration of symptom
- Clinical and biochemical presentation: Symptoms and laboratory findings

### Lab

- CZ: Czech Republic
- A: Another location
- Be: Belgium
Overlapping homozygosity regions in ATP synthase-deficient index patients

A genome map showing the number and location of overlapping homozygosity regions identified in eight index patients. Physical position and gene content of the top-candidate region on chromosome 8 is shown above.
Gene expression changes between the patient and control fibroblast cell lines. The logarithm of the **probability that the gene is differentially expressed** (Log Odds) is **plotted as a function** of the logarithm of the gene expression **fold change** (Log Fold Change) between the patient and control samples.
Expression matrix of the genes located in the candidate region showing reduced $TMEM70$ transcript amount in all but one patient (P3) with a nuclear defect. Normal $TMEM70$ transcript amount is present in patients with ATP synthase disorder due to the mt9205ΔTA microdeletion. The results are shown as log base 2 ratios of gene expression signal in each sample to common reference sample.
c.317-2A>G substitution in *TMEM70* genomic DNA sequence

Mutation in splicing site at the end of intron 2

Location: 8q21.11Se
Homozygous **TMEM70 c.317-2A>G** mutation was found in another 13 patients from CZ, SK and A, all cases of Gypsy ethnic group.
Complementation of ATP synthase deficiency by wtTMEM70
Increase of the full size assembled ATP synthase 650 kDa complex

BN-PAGE/WB

- $F_0F_1$ ATP synthase
- RCCIII
- $F_1$ subcomplexes
- RCCIV
Restored ADP-stimulated respiration in patient cells and resulting ATP synthesis
Mitochondrial proteome
Systematic identification of human mitochondrial disease genes through integrative genomics

Sarah Calve1,2, Mehdi Jaim1,2, Xiaohui Xie1, Sunil A Sheth1,2, Betty Chang1, Olga A Goldberger1,2, Antonella Spinazzola4, Massimo Zeviani4, Steven A Carr1 & Vanni K Moehla1,3

Maestro - eight genome-scale data sets
- expanded collection of 1,451 human mitochondrial proteins
- 368 new

TMEM70 - 260 AA  28,969 DA
- two transmembrane regions
- putative IMM protein
TM1, TM2 denote putative transmembrane regions. Core domain represents central, the most homologous part of the protein.

Level of homology red>blue>green>white
Phylogenetic analysis:

TMEM70 is specific for higher eukaryotes.
TMEM70 is a novel 29 kDa factor of ATP synthase biogenesis

TMEM70 is the first ATP synthase factor specific for higher eukaryotes

Mutation of TMEM70 causes isolated deficiency of ATP synthase with neonatal mitochondrial encephalopathy

„TMEM70 disease“ is frequent in gypsy ethnic group

Cizkova et al., Nature Genetics 2008
geneticist William Bateson, 1908:

„Treasure Your Exceptions“…in studing unique challenges of rare genetic diseases….

… because exceptions provide a unique opportunity to learn about the generality..
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