MITOCHONDRIAL FATTY ACID OXIDATION
AND ITS DISORDERS:
THE CARNITINE CYCLE

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BIOCHEMISTRY BASICS

- Fatty acids
- Glucose
- Amino acids

- Glycolysis
- Pyruvate
- Acetyl-CoA
- Citric acid cycle
- Respiratory chain
- ATP
- H₂O
- O₂
- Reductants
- CO₂
THE FED STATE
GLYCOGEN SYNTHESIS & LIPID BIOSYNTHESIS

Glucose

Fatty acids & TG
Glycogen

Fatty acids

CO₂

CO₂

CO₂

CO₂

TG

Fatty acids

Brain

Kidney

Heart
THE FASTED STATE (EARLY PHASE)
GLUCONEOGENESIS

Glucose

Fatty acids

Glycogen
Amino acids
Lactate
Glycerol

CO$_2$

TG

Fatty acids

CO$_2$

CO$_2$

CO$_2$

CO$_2$
THE FASTED STATE (PROLONGED FASTING)
B-OXIDATION & KETOGENESIS

Ketone bodies

Fatty acids

b-oxidation

Ketone bodies

Fatty acids

CO₂

CO₂

CO₂

CO₂

TG
SIMPLIFIED SCHEME OF MITOCHONDRIAL FATTY ACID β-OXIDATION

FA → acylCoA → β-oxidation

Every tissue

Citric acid cycle:
- CO₂
- ATP
- H₂O

Liver, kidney only

acetylCoA → acetoacetCoA → HMGCoA → acetoacetate → ketone bodies

HMGCoA synthase

HMGCoA lyase

acetylCoA
ENZYMOCOLOGY OF THE MITOCHONDRIAL β-OXIDATION SYSTEM

FATTY ACID (FA) → ACYL-CoA → CPT1 → CPT2 → ACYL-CARNITINE → CACT → CARNITINE → Na+ → OCNT2 → CARNITINE → ACYL-CARNITINE → Krebs cycle

CELL MEMBRANE

FATP → FAT/CD36 → CoASH → ACYL-CoA

MOM

CPT1

MIM

CPT2 → CACT → CARNITINE

β-OXIDATION

Ketone bodies

β-OH-butyrate

acetocacetate → HMG-CoA → ACETYL-CoA → Krebs cycle → CO₂ + H₂O + ATP

CARNITINE

CoASH

ACETYL-CoA
ENZYMEOLOGY OF THE MITOCHONDRIAL ß-OXIDATION SYSTEM

MIM → CPT2 → CACT

- **ACYL-CoA**
  - 1. Acyl-CoA dehydrogenase (AD)
    - SCAD
    - MCAD
    - VLCAD
  - 2. Enoyl-CoA hydrolase (EH)
    - SCEH
    - LCEH
  - 3. 3-Hydroxyacyl-CoA dehydrogenase (HAD)
    - SCHAD
    - LCHAD
    - S/MCKT
    - LCKT
  - 4. 3Keto acyl-CoA thiolase (KT)
    - S/MCKT
    - LCKT

- **ACYL-CARNITINE**

- (n-2) ACYL-CoA → ACETYL-CoA

Krebs cycle → CO₂ + H₂O + ATP
## DEFECTS OF THE CARNITINE CYCLE

<table>
<thead>
<tr>
<th>Deficiency</th>
<th>Plasma carnitine (free)</th>
<th>Plasma acylcarnitines</th>
<th>Cardiomyopathy</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPT1</td>
<td>N - ↑</td>
<td>(C0/C16+C18) ↑</td>
<td>-</td>
</tr>
<tr>
<td>CACT</td>
<td>↓</td>
<td>C16, C18 ↑</td>
<td>+/-</td>
</tr>
<tr>
<td>CPT2</td>
<td>↓ - N</td>
<td>C16, C18 ↑</td>
<td>+/-</td>
</tr>
</tbody>
</table>
# Overview of the Mitochondrial Carnitine Palmitoyltransferases (CPT)

<table>
<thead>
<tr>
<th>Feature</th>
<th>L-CPT1</th>
<th>M-CPT1</th>
<th>CPT2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass (kDa)</td>
<td>88</td>
<td>88</td>
<td>70</td>
</tr>
<tr>
<td>Malonyl-CoA (IC₅₀)</td>
<td>2.5 µM</td>
<td>0.03 µM</td>
<td>-</td>
</tr>
<tr>
<td>Carnitine (Kₘ)</td>
<td>30 µM</td>
<td>500 µM</td>
<td>120 µM</td>
</tr>
<tr>
<td>Chromosome</td>
<td>11q13</td>
<td>22q13.3</td>
<td>1p32</td>
</tr>
<tr>
<td>Tissue expression</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>++++</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>(+)</td>
<td>++++</td>
<td>+</td>
</tr>
<tr>
<td>Heart</td>
<td>(+)</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Kidney</td>
<td>++++</td>
<td>(+)</td>
<td>+</td>
</tr>
<tr>
<td>Lung</td>
<td>++++</td>
<td>(+)</td>
<td>+</td>
</tr>
<tr>
<td>Spleen</td>
<td>++++</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Intestine</td>
<td>++++</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Pancreas</td>
<td>++++</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Brown adipose tissue</td>
<td>(+)</td>
<td>++++</td>
<td>+</td>
</tr>
<tr>
<td>White adipose tissue</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Ovary</td>
<td>++++</td>
<td>(+)</td>
<td>+</td>
</tr>
<tr>
<td>Testis</td>
<td>(+)</td>
<td>++++</td>
<td>+</td>
</tr>
<tr>
<td>Fibroblasts</td>
<td>++++</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Human deficiency known</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>
OVERVIEW OF THE CLINICAL AND BIOCHEMICAL FEATURES
OF CPT1-DEFICIENCY IN A SERIES OF 25 PATIENTS

<table>
<thead>
<tr>
<th>Feature</th>
<th>Abnormal/total</th>
<th>%</th>
</tr>
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<tbody>
<tr>
<td>Hypoketotic hypoglycemia</td>
<td>21/23</td>
<td>91</td>
</tr>
<tr>
<td>Renal tubular acidosis</td>
<td>5/10</td>
<td>50</td>
</tr>
<tr>
<td>Seizures</td>
<td>18/20</td>
<td>90</td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>20/20</td>
<td>100</td>
</tr>
<tr>
<td>Hemiplegia</td>
<td>4/4</td>
<td>100</td>
</tr>
<tr>
<td>Coma</td>
<td>12/21</td>
<td>57</td>
</tr>
<tr>
<td>Early death</td>
<td>3/21</td>
<td>14</td>
</tr>
<tr>
<td>Cardiomyopathy</td>
<td>0/6</td>
<td>0</td>
</tr>
<tr>
<td>Elevated free carnitine (&gt;55)</td>
<td>14/21</td>
<td>67</td>
</tr>
</tbody>
</table>
CARNITINE PALMITOYLTRANSFERASE 1 (CPT1) ASSAY

Palmitoyl-CoA + [14C]-carnitine

Palmitoyl-[14C]-carnitine + CoA

Permeabilized with digitonin

Plasma membrane

Works in fibroblasts as well as in lymphocytes
Performed in the absence and presence of malonyl-CoA, a specific inhibitor of CPT1

Mitochondrion

CPT1

Extraction with butanol
CARNITINE PALMITOYLTRANSFERASE 1 (CPT1) ASSAY

Permeabilized with digitonin

[U-\textsuperscript{13C}]-Palmitoyl-CoA + carnitine

[U-\textsuperscript{13C}]- Palmitoyl-carnitine + CoA

Analysis by Tandem-MSMS


Plasma membrane

Mitochondrion

CPT1

Works in fibroblasts as well as in lymphocytes
Performed in the absence and presence of malonyl-CoA, a specific inhibitor of CPT1
A NOVEL SENSITIVE CPT1 ASSAY

In the classic CPT1 assay, C16-carnitine is not the final endproduct!

Van Vlies et al, Mol Genet Metab. 90, 2007, 24
A NOVEL SENSITIVE CPT1 ASSAY

In the new CPT1 assay, which now includes 5 mM KCN, C16-carnitine is the final endproduct!

Van Vlies et al, Mol Genet Metab. 90, 2007, 24
A NOVEL SENSITIVE CPT1 ASSAY

- Malonyl-CoA sensitivity of CPT1 using the new modified assay

Van Vlies et al, Mol Genet Metab. 90, 2007, 24
CARNITINE PALMITOYLTRANSFERASE 1 (CPT1) ASSAY

n=27

100%

controls patients

n=27
Patient U

- Girl, born January 2003
- Consanguineous parents (nephew-niece)
- December 2008: upper airway infection, food refusal
- Lethargy progressing into coma
- Hypoglycemia (1.8 and 2.2 mmol/l)
- ASAT, CRP and leukocytes ↑
- Complete and rapid recovery upon i.v. glucose.
Patient U

METABOLITE INVESTIGATIONS

- Plasma (crisis):
  - Acylcarnitines
  - Amino acids

- Urine (crisis):
  - Acylcarnitines
  - Organic acids
Relatively normal profile but high carnitine with low long-chain acylcarnitines
Patient U

URINARY ACYLCARNITINE PROFILE

Dicarboxylic acylcarnitines
Patient U

FASTING TEST

**Hypoketotic hypoglycemia**

- **Plasma 3-HB (mmol/l)**
- **Blood glucose (mmol/l)**

Graph showing plasma 3-HB levels and blood glucose levels for controls and a patient with hypoketotic hypoglycemia.
Patient U

ENZYME AND DNA STUDIES

- CPT1-activity in fibroblasts: 0.08 nmol/(min.mg protein)
  Controls: 1.34 ± 0.57 nmol/(min.mg protein)

- Homozygous 1318G>A (A440T)

Brother and sister of patient both homozygous for the same mutation!!
1. Belongs to the family of mitochondrial solute carriers
2. 33 kDa integral membrane protein
3. Six transmembrane spanning elements
4. Single protein present in mitochondria of all tissues
5. Official gene name: *SLC25A20*
MITOCHONDRIAL CARNITINE/ACYLCARNITINE TRANSPORTER (CACT) ASSAY

Digitonin permeabilized plasma membrane

*Acetyl-carnitine

Carnitine

CAT

CoASH

Carnitine

*Acetyl-CoA

Krebs Cycle

*CO₂

MOM

MIM

CACT

MITOCHONDRION

Works in fibroblasts as well as in lymphocytes
MITOCHONDRIAL CARNITINE/ACYLCARNITINE TRANSPORTER (CACT) ASSAY

controls  patients

n=25
1. Most patients present in the neonatal period with seizures, heart problems (arrhythmias, cardiomyopathy, heart block) and apnea.

2. Often triggered by fasting or infections

3. Primary organs involved: heart, liver, skeletal muscle, and brain

4. Patients with presentations later in life also described

5. Most patients show hypoketotic hypoglycemia, hyperammonemia with elevated CK and liver enzymes

6. Acyl-carnitine profile: C16:0, C18:0, C18:1 and C18:2
   
   PS Pattern indistinguishable from CPT2 deficiency
Molecular basis of carnitine acyl-carnitine translocase (CACT) deficiency in a patient with severe presentation and with mild presentation
Patient 1: severe presentation

Presented at 36 hours of age:
- sudden cardiorespiratory insufficiency
- extreme hypoglycaemia
- hyperammonaemia
carnitine and low fat diet
died at 24 months of age
(hypotrophic cardiomegaly)
Patient 2: mild presentation

Severe neonatal condition with:
- hypoglycaemia
- cardiac arrest
- hepatomegaly and hepatic disfunction
- lethargic during mild viral infections

At present (9y), physical and neurophysiological development essentially normal
Biochemical investigations in fibroblasts

<table>
<thead>
<tr>
<th></th>
<th>fatty acid oxidation</th>
<th>CACT activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>controls</td>
<td>7.2 ± 3.2</td>
<td>51 ± 15</td>
</tr>
<tr>
<td>patient 1 (severe)</td>
<td>&lt; 0.1</td>
<td>not detectable</td>
</tr>
<tr>
<td>patient 2 (mild)</td>
<td>2.4</td>
<td>&lt; 0.1</td>
</tr>
</tbody>
</table>
patient 1 (severe): G81R  
patient 2 (mild): 21 aa extension
Expression of human CACT in *S. cerevisiae*

![Graph showing CACT activity for different variants](image-url)
Expression of human CACT in *S. cerevisiae*

- \( \Delta \text{cact} \)
- CACT \text{wt}
- CACT +21aa
- CACT G81R

Fatty acid oxidation activity, nmol/h/mg

0 1 2 3 4 5 6
Patient 1 (severe phenotype)
Findings in fibroblasts:
• no CACT activity
• very low fatty acid oxidation rate
Molecular findings:
• G81R mutation
• expressed protein shows no activity
Patient 2 (mild phenotype)

Findings in fibroblasts:
• very low CACT activity
• fatty acid oxidation partly impaired

Molecular findings:
• C-terminal 21 amino acid extension
• expressed protein has residual activity
Conclusions -3-

The activity of both mutant CACTs as determined by expression in the \( \Delta cact \) yeast mutant, reflects the findings in the corresponding patients.
KEY FEATURES OF THE MITOCHONDRIAL CARNITINE PALMITOYLTRANSFERASE 2

1. 80 kDa peripheral membrane protein
2. No transmembrane spanning elements
3. Single CPT2 present in mitochondria of *all* tissues
4. Catalyzes the reversible reaction between free carnitine and acyl-CoAs
CARNITINE PALMITOYLTRANSFERASE 2 (CPT2) ASSAY

• Originally measured in the reverse direction

\[ ^{14}\text{C} \text{ palmitoyl-CoA} + \text{carnitine} \rightarrow ^{14}\text{C} \text{ palmitoyl-carnitine} + \text{CoASH} \]

• Now modified and measured in the physiological direction using HPLC with or without tandem MS

\[ \text{palmitoyl-carnitine} + \text{CoASH} \rightarrow \text{palmitoyl-CoA} + \text{carnitine} \]
CLINICAL AND BIOCHEMICAL FEATURES OF CPT2 DEFICIENCY

1. Three distinct phenotypes described although intermediate forms have also been published (CPT2 spectrum).

2. Classical muscular form: onset in teenagers/young adults, recurrent episodes of rhabdomyolysis triggered by (prolonged) exercise, fasting or febrile illness.

3. Infantile, hepato-cardio-muscular form: hypoketotic hypoglycemia, liver failure, cardiomyopathy and peripheral myopathy often with sudden death.


   PS Reminiscent of Zellweger syndrome and GA2.

5. Acylcarnitine profile: elevated C16:0, C18:0, C18:1, and C18:2
FATTY ACID OXIDATION DEFICIENCIES IN THE MOUSE

Several mouse models with specific defects in the β-oxidation have been generated

<table>
<thead>
<tr>
<th>Disease</th>
<th>Human deficiency</th>
<th>Mouse model</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>OCTN2 / Primary carnitine deficiency</td>
<td>+</td>
<td>+</td>
<td>Koizumi et al. 1988; Kuwajima et al. 1991</td>
</tr>
<tr>
<td>CPT1a</td>
<td>+</td>
<td>†</td>
<td>Nyman et al. 2005</td>
</tr>
<tr>
<td>CPT1b</td>
<td>?</td>
<td>†</td>
<td>Ji et al. 2008</td>
</tr>
<tr>
<td>LCHAD / MTP</td>
<td>++</td>
<td>++</td>
<td>Ibdah et al. 2001</td>
</tr>
<tr>
<td>VLCAD</td>
<td>++</td>
<td>+/-</td>
<td>Cox et al. 2001; Exil et al. 2003</td>
</tr>
<tr>
<td>LCAD</td>
<td>?</td>
<td>+</td>
<td>Kurtz et al. 1998</td>
</tr>
<tr>
<td>MCAD</td>
<td>-(++)</td>
<td>+/-</td>
<td>Tolwani et al. 2005</td>
</tr>
<tr>
<td>SCAD</td>
<td>-(?)</td>
<td>+/-</td>
<td>Wood et al. 1989; Schiffer et al. 1989</td>
</tr>
<tr>
<td>DCI</td>
<td>?</td>
<td>+/-</td>
<td>Janssen and Stoffel 2002</td>
</tr>
<tr>
<td>DECR</td>
<td>++ (?)</td>
<td>+/-</td>
<td>Miinalainen et al. 2009</td>
</tr>
</tbody>
</table>

† Embryonic lethal  ? Unknown – No phenotype or asymptomatic +/- Mild, + moderate, ++ severe

Such models are of great importance to investigate pathophysiological mechanisms and test new treatment strategies.
Such single enzyme deficiencies in the mouse may also be very helpful for the identification of still unidentified FAO disorders.

Examples:

- 2,4-dienoyl-CoA reductase deficiency
- 3,2-trans-enoyl-CoA isomerase deficiency
FATTY ACID OXIDATION DEFICIENCIES IN THE MOUSE
2,4-DIENOYL-CoA REDUCTASE DEFICIENCY

Most diagnostic metabolite: 2,4-decadienoyl-carnitine
Accumulation of 2-trans,4-cis-decadienoyl carnitine

Table I. 2,4-Dienoyl-CoA Reductase Activity in Liver and Psoas Muscle of a Patient with a Suspected Deficiency in the Oxidation of Polyunsaturated Fatty Acids

<table>
<thead>
<tr>
<th>Tissue and source</th>
<th>2t,4c*</th>
<th>2t,4t*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver, control</td>
<td>13±2.6 (100%)</td>
<td>8.4±2.2 (100%)</td>
</tr>
<tr>
<td>Liver, patient</td>
<td>5.1 (40%)</td>
<td>5.5 (65%)</td>
</tr>
<tr>
<td>Muscle, control</td>
<td>4.6±0.8 (100%)</td>
<td>3±0.28 (100%)</td>
</tr>
<tr>
<td>Muscle, patient</td>
<td>0.8 (17%)</td>
<td>1.24 (43%)</td>
</tr>
</tbody>
</table>
Disruption of Mitochondrial β-Oxidation of Unsaturated Fatty Acids in the 3,2-trans-Enoyl-CoA Isomerase-deficient Mouse*

Received for publication, November 16, 2001, and in revised form, March 11, 2002
Published, JBC Papers in Press, March 26, 2002, DOI 10.1074/jbc.M110993200

Uwe Janssen and Wilhelm Stoffel
Beta-oxidation of oleoyl-CoA (C18:1)

Cis-3-C12:1-CoA

trans 2-C12:1-CoA

CPT2

Cis-3-C12:1-carnitine

Cis-3-C12:1-CoA

trans 2-C12:1-CoA

\[ \text{cis-3-C12:1-CoA} \]

\[ \text{trans-2-C12:1-CoA} \]

\[ \text{CPT2} \]

\[ \text{Cis-3-C12:1-carnitine} \]

**mECI-KO**

**Δ³,Δ²-enoyl-CoA isomerase**

**Δ³,Δ²-enoyl-CoA isomerase**

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**Δ³,Δ²-enoyl-CoA isomerase**

**Δ³,Δ²-enoyl-CoA isomerase**

**Δ³,Δ²-enoyl-CoA isomera
ACAD9

- First identified by Zhang et al. (2002) BBRC 297, 1033-1044
- Member of the ACAD-family
- Closest homologue: VLCAD ($M_w \approx 70$ kDa; amino acid sequence: 47% identical, 65% similar to VLCAD)
- Like VLCAD, ACAD9 is a dimer bound to the mitochondrial inner membrane
- Maximal activity with long-chain unsaturated acyl-CoA's
- Tissue distribution: ACAD9 expression especially high in brain (frontal cortex, hippocampus, cerebellum) where VLCAD expression is low to absent.
- PS: ACAD9 expression mimicks that of LCHAD/MTP.
Patient 1 was a 14-year-old, previously healthy boy who died of a Reye-like episode and cerebellar stroke triggered by a mild viral illness and ingestion of aspirin.

Patient 2 was a 10-year-old girl who first presented at age 4 mo with recurrent episodes of acute liver dysfunction and hypoglycemia, with otherwise minor illnesses.

Patient 3 was a 4.5-year-old girl who died of cardiomyopathy and whose sibling also died of cardiomyopathy at age 21 mo. Mild chronic neurologic dysfunction was reported in all three patients.

Defects in ACAD9 mRNA were identified in the first two patients, and all patients manifested marked defects in ACAD9 protein.

Additional information: plasma acyl-carnitine analysis only performed in patient 2: no abnormalities.

Fibroblasts β-oxidation studies performed in patient 1 (normal) and patient 2 (low normal).
THE MITOCHONDRIAL OXIDATIVE PHOSPHORYLATION MACHINERY

<table>
<thead>
<tr>
<th>Complex</th>
<th>CI</th>
<th>CI I</th>
<th>CI II</th>
<th>CI IV</th>
<th>CI V</th>
<th>CV</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>45</td>
<td>4</td>
<td>11</td>
<td>13</td>
<td>16</td>
<td>1</td>
<td>89</td>
</tr>
<tr>
<td>subunits</td>
<td>nDNA</td>
<td>38</td>
<td>4</td>
<td>10</td>
<td>10</td>
<td>14</td>
<td>76</td>
</tr>
<tr>
<td>subunits</td>
<td>mtDNA</td>
<td>7</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>13</td>
</tr>
</tbody>
</table>

THE MITOCHONDRIAL OXIDATIVE PHOSPHORYLATION MACHINERY

Complex | \(4H^+_{in}\) | \(4H^+_{out}\) | \(2H^+_{out}\) | \(3H^+_{out}\) | ATP |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NADH + H(^+)</td>
<td>QH(_2) (\rightarrow) Q</td>
<td>QH(_2) (\rightarrow) Q</td>
<td>QH(_2) (\rightarrow) Q</td>
<td>ADP + P</td>
</tr>
<tr>
<td></td>
<td>Succinate (\rightarrow) Fumarate</td>
<td>(\frac{3}{2}O_2)</td>
<td>(H_2O)</td>
<td>(3H^+_{in})</td>
<td>(3H^+_{in})</td>
</tr>
</tbody>
</table>

\(4H^+_{in}\) and \(4H^+_{out}\) indicate the movement of protons across the mitochondrial membrane.

\(3H^+_{out}\) indicates the transfer of protons out of the mitochondrial matrix.

\(2H^+_{out}\) indicates the transfer of protons out of the mitochondrial matrix.

\(4H^+_{out}\) indicates the transfer of protons out of the mitochondrial matrix.

\(3H^+_{in}\) indicates the transfer of protons into the mitochondrial matrix.

ATP (Adenosine Triphosphate) is produced at the end of the process.
IDENTIFICATION OF ACAD9 AS A KEY PROTEIN INVOLVED IN THE ASSEMBLY OF COMPLEX I


- NDUFAF1 and Ecsit are known assembly factors of complex I
- Immunoprecipitation studies with anti-NDUFAF1 and anti-Ecsit followed by Western-blotting and nano-LC-MS/MS:
  CONSISTENT COPURIFICATION OF ACAD9!
- Blue-native SDS-PAGE analysis: ACAD9 co-migrates in a high molecular weight complex of 500-850 kDa (Complex I)
SUB-UNIT STRUCTURE OF COMPLEX 1 OF THE RESPIRATORY CHAIN
RNA INTERFERENCE MEDIATED KNOCKDOWN OF ACAD9 (A#1, A#2), ECSIT (E) AND NDUFAF1 (N) IN HEK293 CELLS.

U: untreated, M: mock transfected.

(A) SDS-PAGE western blot immunodetections of ACAD9, Ecsit, and NDUFAF1.

(B) Blue Native-PAGE analysis of complex I in gel activity (CIIGA) and western blot immunodetection of loading control SDHA.

(C) Blue Native-PAGE western blot immunodetection of oxidative phosphorylation complexes I-V.
The specific requirement of ACAD9 for complex I assembly prompted sequence analysis of ACAD9 in a large cohort of patients with isolated complex I deficiency (Nijmegen cohort).

Two unrelated patients with complex I deficiency identified:

- Patient 1: homozygous for c.1553G>A mutation (R518H)
- Patient 2: compound heterozygous for two mutations including c.187G>T (stop codon) and c.1237G>A (E413K).

Transduction of wildtype ACAD9 into the two patients’ fibroblasts gave full restoration of complex I activity.
Patients enzyme activities of the mitochondrial respiratory chain complexes measured in cultured skin fibroblasts.

<table>
<thead>
<tr>
<th>Enzyme activities(^a)</th>
<th>Patient I-1</th>
<th>Patient II-1</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>NADH:O(_4) oxidoreductase(^a)</td>
<td>0.053</td>
<td>0.058</td>
<td>0.10 – 0.26</td>
</tr>
<tr>
<td>Succinate: cytochrome c</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>oxidoreductase(^a)</td>
<td>0.24</td>
<td>0.32</td>
<td>0.16 – 0.44</td>
</tr>
<tr>
<td>Decylubiquinol: cyt c</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>oxidoreductase(^a)</td>
<td>1.46</td>
<td>2.82</td>
<td>1.25 – 2.62</td>
</tr>
<tr>
<td>Cytochrome c oxidase(^b)</td>
<td>0.95</td>
<td>0.79</td>
<td>0.68 – 1.19</td>
</tr>
<tr>
<td>Citrate synthase(^c)</td>
<td>165</td>
<td>190</td>
<td>144 – 257</td>
</tr>
</tbody>
</table>

\(^a\) Values are given in mU per mU cytochrome c oxidase.

\(^b\) Values are given in mU per mU citrate synthase.
ACAD9 AND ITS INVOLVEMENT IN COMPLEX I BIOGENESIS AND FATTY ACID OXIDATION
ACKNOWLEDGEMENTS

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