Fatty Acid Oxidation Defects and Its Clinical Significance

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Izumo-Taisha Shrine, Shimane, Japan
Outline

Fatty Acid Oxidation Disorder (FAOD)
Energy production in mitochondria

Glycolysis → Pyruvate → acetyl-CoA → TCA cycle

mitochondrial β-oxidation → Respiratory chain → ATP
Mitochondrial β-oxidation

1. OCTN2
   - Long chain FA
   - CoA
   - CoA
   - Carnitine
   - Octyl carnitine
   - Long chain FA

2. CPT1
   - Medium chain FA
   - Medium chain FA
   - Outer membrane
   - CACT
   - Acyl carnitine
   - Acyl-CoA
   - CPT2
   - Medium chain FA

3. VLCAD
   - TFP
   - Long chain FA
   - CAD
   - Medium chain FA
   - EH
   - HAD
   - Acetyl-CoA

4. Respiratory chain (CoQ)
   - TCA
   - FADH2
   - ATP
   - Respiratory chain (CoQ)
### Fatty acid β-oxidation defects (FAODs)

<table>
<thead>
<tr>
<th>β-Oxidation Metabolic Step</th>
<th>Disorders</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Carnitine cycle</td>
<td>Carnitine uptake defect (CUD)</td>
</tr>
<tr>
<td></td>
<td>CPT1 deficiency</td>
</tr>
<tr>
<td></td>
<td>CPT2 deficiency</td>
</tr>
<tr>
<td></td>
<td>CACT deficiency</td>
</tr>
<tr>
<td>2) Long-chain β-oxidation</td>
<td>VLCAD deficiency</td>
</tr>
<tr>
<td></td>
<td>TFP deficiency</td>
</tr>
<tr>
<td>3) Medium to short chain</td>
<td>MCAD deficiency</td>
</tr>
<tr>
<td>4) Electron transfer</td>
<td>Glutaric acidemia type II (GA2)</td>
</tr>
</tbody>
</table>
# CLASSIFICATION of FATTY ACID DISORDERS

<table>
<thead>
<tr>
<th>CLINICAL FORM</th>
<th>FINDING</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Severe form</td>
<td>Death in early infancy</td>
</tr>
<tr>
<td></td>
<td>Hypoglycemia</td>
</tr>
<tr>
<td></td>
<td>Liver failure</td>
</tr>
<tr>
<td></td>
<td>Cardiomyopathy</td>
</tr>
<tr>
<td>2) Intermediate form</td>
<td>Episodic attacks</td>
</tr>
<tr>
<td></td>
<td>Acute encephalopathy</td>
</tr>
<tr>
<td></td>
<td>Sudden infant death</td>
</tr>
<tr>
<td>3) Late onset form (myopathic)</td>
<td>Intermittent Episodes of:</td>
</tr>
<tr>
<td></td>
<td>Lethargy</td>
</tr>
<tr>
<td></td>
<td>Myopathy</td>
</tr>
<tr>
<td></td>
<td>Myalgia</td>
</tr>
<tr>
<td></td>
<td>Myoglobinuria</td>
</tr>
<tr>
<td></td>
<td>Liver dysfunction</td>
</tr>
</tbody>
</table>
Diagnostic Approaches for Mitochondrial fatty acid oxidation defects (FAOD)
Diagnostic Methods

1) Blood acylcarnitine analysis (MS/MS)
2) Organic acid analysis (GCMS)
3) In vitro probe assay (enzyme determination)
4) Molecular analysis (immunoblot or gene analysis)
5) Muscle biopsy: lipid myopathy
Tandem MS (MS/MS)

Blood filter paper

acylcarnitine • amino acids
(mass screening)

Urine

organic acids
(confirmative)
1) Acylcarnitine (MS/MS)

**Blood filter paper**

A) MCAD deficiency

![](image1)

B) CPT2 deficiency

![](image2)

C) Control

![](image3)

* Internal standard
Abnormal metabolites in impaired β-oxidation

\[
\text{acyl-CoA} 
\xrightarrow{AD} \text{enoyl-CoA} 
\xrightarrow{EH} \text{3-hydroxyacyl-CoA} 
\xrightarrow{HAD} \text{3-ketoacyl-CoA} 
\xrightarrow{KAT} \text{acyl-CoA, acetyl-CoA}
\]

- **ω-oxidation**
  - Dicarboxylic acid
  - (n-1)-hydroxy-acid
  - acylglycine
  - acylcarnitine
- **[ω-1]-oxidation** + glycine
  - acylglycine
- **ω-oxidation** + carnitine
  - Unsaturated dicarboxylic acid
- **ω-oxidation** + carnitine
  - 3-hydroxy-dicarboxylic acid
  - 3-hydroxy-acylcarnitine
Urinary organic acid profile of MCAD deficiency (GC/MS)

- 7-OH-octanoic
- octenedioic
- adipic
- suberic
- sebacic
- 3-OH-sebacic
- 3OH-dodecanedioic

MCAD deficiency

Control

IS-1
IS-2
C24
HG
SG
C24

TIC

31230722
In vitro probe acylcarnitine (IVP) assay
In vitro Probe Assay (enzyme determination)

Palmitate (C16) loading

Glucose low  
Fatty acid free  
Enriched carnitine

(β-oxidation)

block

M

C2

C10
C8
C6
C4

block

L

C16
C14
C12

Acylcarnitines (MS/MS)
Results of
In vitro probe assay

A

Normal

B

MCAD def
(Medium)

C

VLCAD def
(long-chain)

D

Glutaric acidemia type II
(short~long)
<3>

Treatment

Fatty acid Oxidation Disorders
Treatment for Fatty Acid Disorder

1) Avoid “long fasting”
   (ex.) < 8 hrs: before 1 yr of age; < 10 hrs: before 2 yrs

2) Early infusion of “glucose”
   In particular, during stress (infection, over-exercise)

3) “Carnitine therapy” for some cases
   Except for CPT1 deficiency

4) Some drugs
   Riboflavin, CoQ₁₀

5) Dietary therapy
   (ex.) high carbohydrate / low lipid diet
   (ex.) MCT milk (oil)
CASE Presentation
CASE 1

1-year-old boy with MCAD deficiency
Sudden death following common cold
MCAD deficiency: **Sudden infant death**

**Case:** 1 yr 8 m, boy. **Pyrexia** due to infection, followed by unconsciousness and convulsion, and **suddenly died**.

**Family history:** His sister (5 yr old) is **severely handicapped**, since **acute encephalopathy** at 1 year of age.

**Acylcarnitine analysis**
*(blood filter paper, tandem MS)*
- Elevation of **C6, C8, C10, C10:1**

**Urinary organic acid (acute stage)**
- Hypoketotic dicarboxylic aciduria
- Elevation of hexanoylglycine etc.

**Acute (1y8m)**

**Newborn paper**
### Japanese MCAD deficiency  Clinical onset, outcome and genotype  (Shimane Univ. 2012)

<table>
<thead>
<tr>
<th>Case</th>
<th>Age at onset</th>
<th>Age at diagnosis</th>
<th>Hypoglycemia</th>
<th>Genotype</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8m</td>
<td>8m</td>
<td>(n.a)</td>
<td>c.449-452del</td>
<td>Develop. delay</td>
</tr>
<tr>
<td>2</td>
<td>1y</td>
<td>1y</td>
<td>(+)</td>
<td>IVS4+1G&gt;A</td>
<td>Sudden death</td>
</tr>
<tr>
<td>3</td>
<td>1y</td>
<td>8y 10m</td>
<td>(+)</td>
<td>c.449-452del</td>
<td>Develop. delay</td>
</tr>
<tr>
<td>4</td>
<td>1y 1m</td>
<td>1y 1m</td>
<td>(+)</td>
<td>del. ex 11-12</td>
<td>Develop. delay</td>
</tr>
<tr>
<td>5</td>
<td>1y 3m</td>
<td>1y 3m</td>
<td>(n.a)</td>
<td>del. ex 11-12</td>
<td>Develop. delay</td>
</tr>
<tr>
<td>6</td>
<td>1y 4m</td>
<td>1y 4m</td>
<td>(+)</td>
<td>c.449-452del</td>
<td>Develop. delay</td>
</tr>
<tr>
<td>7</td>
<td>1y 7m</td>
<td>1y 7m</td>
<td>(+)</td>
<td>c.275C&gt;T</td>
<td>Develop. delay</td>
</tr>
<tr>
<td>8</td>
<td>1y 8m</td>
<td>1y 8m</td>
<td>(+)</td>
<td>c.449-452del</td>
<td>Sudden death</td>
</tr>
<tr>
<td>9</td>
<td>2y 2m</td>
<td>2y 2m</td>
<td>(+)</td>
<td>c.449-452del</td>
<td>normal</td>
</tr>
<tr>
<td>10</td>
<td>—</td>
<td>5d</td>
<td>(-)</td>
<td>c.1085G&gt;A</td>
<td>normal</td>
</tr>
<tr>
<td>11</td>
<td>—</td>
<td>5d</td>
<td>(-)</td>
<td>c.449-452del</td>
<td>normal</td>
</tr>
<tr>
<td>12</td>
<td>—</td>
<td>5d</td>
<td>(-)</td>
<td>IVS3+2T&gt;C</td>
<td>normal</td>
</tr>
<tr>
<td>13</td>
<td>—</td>
<td>5d</td>
<td>(-)</td>
<td>c.449-452del</td>
<td>normal</td>
</tr>
<tr>
<td>14</td>
<td>—</td>
<td>5d</td>
<td>(-)</td>
<td>c.449-452del</td>
<td>normal</td>
</tr>
<tr>
<td>15</td>
<td>—</td>
<td>5d</td>
<td>(-)</td>
<td>c.1085G&gt;A</td>
<td>normal</td>
</tr>
<tr>
<td>16</td>
<td>—</td>
<td>5d</td>
<td>(-)</td>
<td>c.449-452del</td>
<td>normal</td>
</tr>
<tr>
<td>17</td>
<td>—</td>
<td>5d</td>
<td>(-)</td>
<td>c.449-452del</td>
<td>normal</td>
</tr>
<tr>
<td>18</td>
<td>—</td>
<td>5y 5m</td>
<td>(-)</td>
<td>c.449-452del</td>
<td>normal</td>
</tr>
</tbody>
</table>

*a-a, b-b: sibling cases  (n.a) data, not available*
## MCAD deficiency
Medium-chain acyl-CoA dehydrogenase deficiency

| Incidence                  | 1: 10,000 (Caucasian, a common mutation, 985A>G)  
|                           | 1: 110,000 (Japanese, a common mutation, 449delCTGA) |
| Acute symptoms triggered by long fasting | Vomiting, lethargy, acute encephalopathy  
|                           | Sudden death                                      |
| Laboratory test (acute)    | Hypoglycemia, hyperammononemia                    |
| Biochemical Markers (mass spectrometry) | MS/MS (blood AC)  
|                           | C8, C6, C10, C8/C10  
|                           | GC/MS (urinary OA)  
|                           | Hexanoylglycine (HG)  
|                           | Suberylglycine (SG)  
|                           | Dicarboxylic acids                                      |
| Prognosis                  | As many as 35% have no episodes lifelong  
|                           | The 1st attack occurs before 3 to 4 years  
|                           | 25% of children suddenly die during the 1st attack |
Common mutation in MCAD deficiency

Caucasian
985A>G (90%)

Japan
449delCTGA (45%)
CASE 2

19-year-old woman with VLCAD deficiency
Repeated episodes of myopathy-like illness after adolescence
VLCAD deficiency: adult onset, myopathic form

【Case】 18y female
【Family history】 nothing special

【Clinical history】
Since around 10 yrs of age,
   Exercise-induced myalgia at legs
14 yrs~  Episodic general fatigue
17 yrs~  Frequent episodes of the fatigue.
   Sometimes red-colored urine (myoglobinuria)
18 yrs  Entered a university, and a year off.
   Administered for thorough medical check
## Routine laboratory tests

### Biochemistry
- **AST** 104
- **ALT** 55
- **LDH** 726
- **CK** 2,520
- **ALD** 1.9 (2.7~5.9)
- **BUN** 13

### Blood carnitine:
- **total** 62.6 μmol/L
- **free** 44.5 μmol/L

### Urine myoglobin
- 11 ng/mL (<4)

### Total ketone
- 634 μmol/L (<150)

### Acetoacetate
- 195 μmol/L

### 3-OH-butyrate
- 439 μmol/L

### Urinary organic acid:
- WNL

### ECG, Cardiac echo, X-ray:
- no abnormalities
MS/MSによる血清アシルカルニチン分析

患者

対照
First discoverer of VLCAD and TFP (JBC, 1992) and many of peroxisomal enzymes

Reported VLCAD deficiency
Yamaguchi S et al (Ped Res, 1993)

Professor Takashi Hashimoto, Department of Biochemistry, Shinshu University, Japan
Identification of VLCAD deficiency

<table>
<thead>
<tr>
<th>C</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
</table>

Identification of Very-Long-Chain Acyl-CoA Dehydrogenase Deficiency in Three Patients Previously Diagnosed with Long-Chain Acyl-CoA Dehydrogenase Deficiency

SEIJI YAMAGUCHI,¹ YASUHIRO INDO,² PAUL M. COATES, TAKASHI HASHIMOTO, AND KAY TANAKA
CASE 3

2-year-old boy with glutaric acidemia type II
Bezafibrate (BEZ) showed dramatic effects
Case: 2y9m boy (intermediate form)

• Past history
  - Detected by NBS using MS/MS
  - 1y ~ : Repeated episodes of unconsciousness
  - 1y7m : Lethargy due to hypoglycemia
  - 2y2m : Respiratory failure following RSV infection
    (→ ICU for 3 weeks)

• Clinical findings
  - Muscle weakness : moderate
  - Unstable gait
  - Developmental delay : DQ 59 (Enjouji system)
PATHOPHYSIOLOGY
Glutaric aciduria type II (GA2)

FAO pathway
- SCAD, MCAD, LCAD, VLCAD

BCAA pathway
- Isovaleryl-CoA DH
- Isobutyryl-CoA DH
- Methylbutyryl-CoA DH

Lysine & tryptophan
- Glutaryl-CoA DH

Others
- Sarcosine DH
- 2-Hydroxyglutarate DH

ETFα, ETFβ, ETFDH

ETF: electron transfer flavoprotein
ETFDH: ETF dehydrogenase

DH: dehydrogenase

Respiratory chain

ATP
Topics in treatment

Bezafibrate

- Hypolipidemic drug
- PPAR agonist

(peroxisome proliferation activated receptor)

Bezafibrate can be a new treatment option for mitochondrial fatty acid oxidation disorders: Evaluation by in vitro probe acylcarnitine assay

Seiji Yamaguchi a,*, Hong Li a,b, Jamiiyan Purevsuren a, Kenji Yamada a, Midori Furui a, Tomoo Takahashi a, Yuichi Mushimoto a, Hironori Kobayashi a, Yuki Hasegawa a, Takeshi Taketani a, Toshiyuki Fukao c, Seiji Fukuda a
Changes of Acylcarnitines Before and After Bez addition

In vitro probe assay

**VLCAD deficiency**
(long chain defect)

**Glutaric acidemia type 2**
(broad range defect)

Before addition of Bezaflibrate (BEZ)

In the presence of BEZ

※ **Bez** may up-regulate mitochondrial β-oxidation enzymes.

36
A Clinical trial of Bezafibrate for a 2-year-old boy with Glutaric acidemia type 2

Changes of Blood acylcarnitine

C4

C10, C8
Clinical Trial of Bezafibrate in a 2y9m old boy with glutaric acidemia type 2

<table>
<thead>
<tr>
<th>Developmental test (Enjoji system)</th>
<th>Before</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DQ 59</td>
</tr>
<tr>
<td></td>
<td>(2y9m)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gait</th>
<th>unstable</th>
</tr>
</thead>
<tbody>
<tr>
<td>hypoglycemia</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>(frequent)</td>
</tr>
<tr>
<td>General fatigue</td>
<td>+</td>
</tr>
</tbody>
</table>
CASE 4

Neonatal osent form of TFP deficiency
Prenatal diagnosis of the next 2 pregnancy
Prenatal diagnosis of mitochondrial trifunctional protein (TFP) deficiency

**Outer membrane**
- CPT2
- ACS
- CACT
- VLCAD
- TFP
- Carnitine
- Acyl-CoA
- Acylcarnitine
- Long-chain fatty acid (長鎖脂肪酸)

**Inner membrane**
- Long-chain β-oxidation (C18~C12)
- Medium-chain β-oxidation (C12~C4)
- ETF
- 3-OH-acyl-CoA
- 3-ketoacyl-CoA
- C16-OH
- C18:1-OH
- Acetyl-CoA
- Carnitine
Clinical course of the proband

Normal delivery:
- at 38wks of gestation, Birth weight 2588g

2 days after birth:
- hypotonia, hypoglycemia, cardiomyopathy

6 days of age
- Died, despite no response of any treatments

Postmortem investigation:
- diagnosis of TFP deficiency
Immunoblotting

C: control
T: TFP deficiency
V: VLCAD deficiency
E: rat enzyme

Enzymes and antibodies are gifted by Professor T. Hashimoto, Biochemistry, Shinshu University
Prenatal diagnosis for OA and FAOD in Shimane University

**Amniocentesis**

- Aminiotic fluid Collected at around 16 wks of gestation
- Centrifuge
- Organic acid (GC/MS)
- acylcarnitine (MS/MS)
- supernatant
- pellet
- Gene analysis
- Cultured amniotic cells
- Enzyme assay
- Immunoblotting
- Gene analysis
Prenatal Diagnosis of TFP deficiency

28才

① IVS16+2  T>G
② IVS13+1  G>A

Case 1

Case 2

6才

生後6日死亡

① IVS16+2  T>G
② IVS13+1  G>A

① wild
② wild
Acylcarnitine analysis of amniotic fluid

- **C16-OH**: 3.09 DBS
- **C18-OH**: 0.76 DBS
- **C18:1-OH**: 1.9 DBS

**Case 1**
- C16-OH: 0.11
- C18-OH: 0.08
- C18:1-OH: 0.14

**Case 2**
- C16-OH: 0.007
- C18-OH: 0.0045
- C18:1-OH: 0.017

Normal
- n=6
### Results of inherited metabolic disease of organic and fatty acids

(1998 to 2014, Shimane University)

<table>
<thead>
<tr>
<th>Disease</th>
<th>No. of case</th>
<th>affected</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>(Organic acidemia)</em></td>
<td>(112)</td>
<td>(43)</td>
</tr>
<tr>
<td>Methylmalonic acidemia</td>
<td>80</td>
<td>32</td>
</tr>
<tr>
<td>Propionic acidemia</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>Glutaric acidemia type 1</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>Multiple carboxylase</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td><em>(Fatty acid oxidation defect)</em></td>
<td>(10)</td>
<td>(2)</td>
</tr>
<tr>
<td>TFP deficiency</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>VLCAD deficiency</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>CACT deficiency</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Glutaric acidemia type 2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><em>(Others)</em></td>
<td>(21)</td>
<td>(5)</td>
</tr>
<tr>
<td>Citrulinemia type 1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Infant death of unknown origin (from Asia)</td>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td><em>(total)</em></td>
<td>(143)</td>
<td>(50)</td>
</tr>
</tbody>
</table>

Courtesy by Dr. Hasegara, Shimane
Thank you

Iwami Kagura Dancing, Shimane