Molecular and biochemical resolution of pyridoxine-dependent epilepsy

alpha-amino adipic semialdehyde as diagnostic marker
Pyridoxine-dependent seizures

- First described in 1954

- Neonatal seizures beginning in the first few days of life

- Cessation of seizures after administration of 50-100mg pyridoxine
  Continued seizure control on 15 mg/kg/d pyridoxine
  Seizure recurrence if pyridoxine is withdrawn

- Autosomal recessive

- No gene has been identified; favoured hypothesis was defect in glutamate decarboxylase (GAD)

- maps to 5q31.2 – q31.3 (Cormier-Daire et al., Am J Hum Genet, 2000)
Pyridoxine-dependent seizures

- Elevated concentrations of pipecolic acid in plasma and CSF

- Defect in PLP-dependent step in lysine degradation pathway?

```
\[
\text{L-Lysine} \quad \xrightarrow{2\text{-keto-6-amino caproic acid}} \quad \text{Saccharopine} \quad \xrightarrow{\Delta 1\text{-Piperideine 2-carboxylic acid}} \quad \text{Pipecolic acid} \\
\quad \xrightarrow{\Delta 1\text{-Piperideine 6-carboxylic acid}} \quad \text{L-2-Aminoadipate 6-semialdehyde} \\
\quad \xrightarrow{\text{L-2-Aminoadipate}} \quad \text{2-Oxoadipate} \\
\quad \xrightarrow{\text{PLP} \quad \alpha\text{-aminoadipate transaminase}} \quad \text{Acetyl-CoA}
\]
```
Pyridoxine-dependent seizures

• similar scenario to that of hyperprolinaemia type II

L-Proline

P5C

L-Glutamic-\gamma\text{-semialdehyde}

P5C dehydrogenase

L-Ornithine

L-Glutamic acid

P6C

L-2-Aminoadipate 6-semialdehyde

Pipecolic acid

L-2-Aminoadipate

L-Glutamic acid

P5C reacts with PLP to inactivate it
Pyridoxine-dependent seizures

- L-lysine
- L-2-aminoadipate 6-semialdehyde
- L-2-aminoadipate

\[ \text{COOH} \]

- \( \Delta^1 \)-piperideine-6-carboxylic acid (P6C)
- L-\( \Delta^1 \)-pyrroline-5-carboxylic acid (P5C)

- 2-keto-6-amino caproic acid
- \( \Delta^1 \)-piperideine 2-carboxylic acid
- \( \Delta^1 \)-piperideine 6-carboxylic acid

- Saccharopine
- L-2-amino adipate 6-semialdehyde
- L-2-amino adipate
- 2-oxoadipate
- Acetyl-CoA

**Pipecolic acid**

Diagram showing the metabolic pathway involving pyridoxine-dependent seizures.
Pyridoxine-dependent seizures

P6C dehydrogenase (S. clavuligerus)

FAAVGTAGQRCTTLRRL

C. elegans P6C; P. sativum P6C
B. subtilis P5C

ANTIQUITIN
(ATQ1; ALDH7A1)
Maps to 5q31

L-Lysine

2-keto-6-amino caproic acid

Δ¹-Piperideine 2-carboxylic acid

Saccharopine

Pipecolic acid

Δ¹-Piperideine 6-carboxylic acid

L-2-Aminoadipate 6-semialdehyde

L-2-Aminoadipate

2-Oxoadipate

Acetyl-CoA

∆¹-Piperideine 2-carboxylic acid
Antiquitin (ALDH7A1)

- First described in peas – cellular turgor pressure
- Highly conserved across species
- Belongs to the superfamily of aldehyde dehydrogenases
- Shown to have acetaldehyde dehydrogenase activity
- Exact physiological role has not previously been elucidated
Antiquitin (ALDH7A1)

- Mutations in the antiquitin gene cause pyridoxine-dependent epilepsy in man.
Pathophysiologic mechanism in PDE: analogy to hyperprolinemia type II

Piperideine-6-carboxylic acid (P6C) + Pyridoxal 5-Phosphate

Mw 127 → Mw 247

Complex A

Mw 374

Complex B

Mw 356

2-keto-6-amino caproic acid

Δ1-Piperideine 2-carboxylic acid

L-pipeolic acid

Saccharopine

Δ1-Piperideine 6-carboxylic acid

L-2-Aminoadipate 6-semialdehyde

L-2-Aminoadipate

2-Oxoadipate

Acetyl-CoA
Pathophysiologic mechanism in PDE

P6C + PLP $\rightarrow$ complex A + complex B

Experiment: Aqueous solution

- 1 mmol/L P6C
- 1 mmol/L PLP
- pH 7.5
- 37°C, 24 hours

Hypothesis of PLP deactivation by complexation with P6C appeared to be true \((in-vitro)\).
L-2-Aminoadipate 6-semialdehyde

- L-Lysine
  - 2-keto-6-amino caproic acid
    - \[\Delta^1\text{-Piperidine 2-carboxylic acid}\]
    - Saccharopine
      - L-pipocotic acid
        - \[\Delta^1\text{-Piperidine 6-carboxylic acid}\]
          - \[\text{L-2-Aminoadipate 6-semialdehyde}\]
            - L-2-Aminoadipate
              - \[\text{2-Oxoadipate}\]
                - \[\text{Acetyl-CoA}\]

\[
O=\overset{\text{C}}{\text{C}}-\overset{\text{H}}{\text{CH}}_2-\overset{\text{H}}{\text{CH}}_2-\overset{\text{H}}{\text{CH}}-\text{COOH}
\]

\[
\overset{\text{H}}{\text{H}} \quad \overset{\text{NH}_2}{\text{NH}_2}
\]
L-2-Aminoadipate 6-semialdehyde determination

- Matrices: cerebrospinal fluid, plasma, and urine.

- $^{15}$N-aminoadipic acid used as internal standard.

- Convert AASA and IS to corresponding FMOC-derivatives.

- Measurement performed by LC-MS/MS in MRM mode.
# L-2-Aminoadipate 6-semialdehyde determination

AASA in bodyfluids of patients with pyridoxine-dependent seizures

Cohort 1

<table>
<thead>
<tr>
<th></th>
<th>patients</th>
<th>controls</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF (µmol/L)</td>
<td>1; 28; 19</td>
<td>&lt;0.1</td>
<td></td>
</tr>
<tr>
<td>3/12 available</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>plasma (µmol/L)</td>
<td>3.5 ± 1.2</td>
<td>&lt;0.2</td>
<td></td>
</tr>
<tr>
<td>5/12 available</td>
<td>range 1.5 – 4.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>urine (mmol/mol creat.)</td>
<td>14 ± 8</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>9/12 available on treatment</td>
<td>range 8 – 28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/12 available before treatment</td>
<td>168</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
L-2-Aminoadipate 6-semialdehyde
a novel biomarker for PDE?

Increase of biomarker in bodyfluids of PDE patients
2 cohorts

<table>
<thead>
<tr>
<th></th>
<th>AASA</th>
<th>L-pipecolic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF</td>
<td>↑↑</td>
<td>↑↑</td>
</tr>
<tr>
<td>plasma</td>
<td>↑↑</td>
<td>↑</td>
</tr>
<tr>
<td>urine</td>
<td>↑↑</td>
<td>nl.-↑*</td>
</tr>
</tbody>
</table>

* nl. on-therapy, elevated off- (before) therapy

Yes, a non-invasive specific diagnostic metabolite for PDE, independent whether patients are on/off therapy!
AASA is consistently increased in body fluids derived from PDE patients (now found in two cohorts).
CONCLUSIONS

AASA is consistently increased in body fluids derived from PDE patients.

Since AASA is in equilibrium with P6C, P6C is expected to be increased.
AASA is consistently increased in body fluids derived from PDE patients.

Since AASA is in equilibrium with P6C, P6C is expected to be increased.

P6C complexate with PLP in-vitro: supporting the hypothesis of PLP-deactivation in PDE.
CONCLUSIONS

AASA is consistently increased in body fluids derived from PDE patients.

Since AASA is in equilibrium with P6C, P6C is expected to be increased.

P6C complexate with PLP in-vitro: supporting the hypothesis of PLP-deactivation in PDE.

Increases of AASA revealed that antiquitin acts on AASA, and that the metabolic impairment is located at the level of the conversion of AASA into alpha-amino adipic acid.
CONCLUSIONS

AASA is consistently increased in body fluids derived from PDE patients.

Since AASA is in equilibrium with P6C, P6C is expected to be increased.

P6C complexate with PLP in-vitro: supporting the hypothesis of PLP-deactivation in PDE.

Increases of AASA revealed that antiquitin acts on AASA, and that the metabolic impairment is located at the level of the conversion of AASA into alpha-aminoadipic acid.

AASA is a novel non-invasive diagnostic metabolite for PDE.

No need for test of pyridoxine withdrawal.
Participants

Cornelis Jakobs
Philippa Mills
Peter Clayton
Michel AAP Willemsen
Levinus A Bok
Barbara Plecko
Peter Baxter
Matthias Baumgartner
Heymut Omran
Uta Tacke
Birgit Uhlenberg
Berhard Weschke
Kerra Pearce
Liz Bland