Follow-up of 5 early-onset cbLC deficiency patients after hydroxocobalamin dose intensification treatment: how in cbLC but not in cbLE patients to bypass MMACHC in order to allow Cobalamin to be allocated to the MetCbI and AdocbI cycles?

1Scalais E., 2Schlesser V., 3Gilson G., 3Pierron C., 3Geron C., 4Cardillo S., 5Borde P., 6Regal L. Pediatric department3, division of Pediatric Neurology1, ophthalmology4, chemical laboratory2, Centre Hospitalier de Luxembourg, Laboratoire National de Santé5, Luxembourg, Universiteit Zienkenhuis6 – VUB, Brussels, Belgium

Background : cbLC
The most common inborn error of intracellular cobalamin (B12) disorders

- MMACHC: Co3+ ≠ Co2+ ≠ Co1+
- [methylcobalamin] ≠ [adenosylcobalamin]: Δ methionine synthase ≠ Δ methylmalonyl-CoA mutase ≠ [homocysteine] ≠ [methylmalonic acid]
- Neurodevelopmental, hematologic, ophthalmologic, dermatologic and renal abnormalities.

From fetal to adulthood period: early-onset (<1 year), childhood onset (>1 year) and adolescent and adult onset
OH-cobalamin dose intensification (OHCbI-DI) ➔ better biochemical and clinical outcome (Scalais, 2019)
cbLE deficiency: impaired methionine synthase reductase (MSR) ➔ isolated homocystinuria.

Aim- Hypothesis:
By means of OHCbI-DI
- Control of biomarkers (Hcy, MMA, Met)
- Better cognitive and ophthalmological outcome
- Bypass MMACHC: 2 different one-electron reductions
- Co3+ → Co2+ via chemical, but not enzymatic reduction
- Co2+ → Co1+ via 2 repair enzymes: MSR + adenosyl-transferase (ATR)

Methods
5 early onset cbLC patients (p1-p5) - 1 cbLE patient (p6)

- Daily OHCbI-DI: (50 mg/ml): e-porth or Clea Smith drink
- estimated B12: 34,6 ± 10

Early: p1-p2: at age 5 months
p3: at age 1 months (pomp Crono Super Pid)
p4: at age 1/2 month
Late: p5: at age 5 years (pomp Crono Super Pid)
p6: at age >10 years

Molecular analysis: cDNA
Biochemical biomarkers : Hcy, MMA, Met

B12 (Cob(III)alamin) levels in serum (pg/ml)
Biochemical Data

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age Year</th>
<th>Hcy (µmol/l)</th>
<th>MMA (μmol/l)</th>
<th>Met (µmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>p1</td>
<td>6-7 years</td>
<td>177</td>
<td>2,5</td>
<td>0,58</td>
</tr>
<tr>
<td>p2</td>
<td>8/10</td>
<td>6,4</td>
<td>1,7</td>
<td>0,8</td>
</tr>
<tr>
<td>p3</td>
<td>1 month</td>
<td>5,78</td>
<td>4,4</td>
<td>1,2</td>
</tr>
<tr>
<td>p4</td>
<td>1 month</td>
<td>177</td>
<td>2,5</td>
<td>0,58</td>
</tr>
<tr>
<td>p5</td>
<td>1 month</td>
<td>14,4</td>
<td>63</td>
<td>15</td>
</tr>
<tr>
<td>p6</td>
<td>&gt;10 years</td>
<td>177</td>
<td>2,5</td>
<td>0,58</td>
</tr>
</tbody>
</table>

BYPASS MMACHC: 2 different one
Excellent metabolic control (Hcy, MMA, Met) in p1

OHCbl 5,78 μmol/mmol creat (normal: < 8)

Hypothesis:

- Co3+→ Co2+, 2,5 μmol/l (normal < 10).
- 1,2 mg /day
- Better cognitive and ophthalmological outcome
- Much better neurological and ophthalmological outcome in early

Summary

- Excellent metabolic control (Hcy, MMA, Met) in p1-p5, but not p6
- Much better neurological and ophthalmological outcome in early versus late OHCbI-DI (p1-p4 versus p5)
- High levels of cob(II)alamin chemically but not enzymatically reduced to cob(I)alamin, subsequently rescued by repair enzymes such as MSR and ATR and therefore bypassing MMACHC.
- OHCbI-DI not effective in p6 (cbLE) because of MSR deficiency
- No serious side effects observed in the 5 cbLC and 1 cbLE patients following OHCbI-DI.

Results

Follow-up: p1-p3: 6-7/2 years, p4: 22 months, p5: 22 months

c.DNA: p1, p2: c.271dupA/c.271dupA
p4: c.271dupA/AC: 435_436del
p5: c.271dupA/AC: 394C>T
p6: MTHFR c.323G>A/AC: 766G>T

Biochemical Data (mean ± SD)
B12: 34.6 ± 10 µg/mmol (normal 200-900),
estimated-Cob(II)alamin: 501993 ± 286569 pg/ml (normal 2.8-13.0)
p1-p5: Hcy: 14.4 ± 2.5 µmol/l (normal < 10),
MMA: 0.58 ± 0.1 µmol/l (normal = 0),
uminary MMA 12.75 ± 5.78 µmol/mmol creat (normal: < 8)
P6: Hcy: 177 ± 10 µmol/l.

Cognitive: p1, p2: mild delay, p3: WISC-V (verbal 130), p4 normal
p5: severe delay

Ophthalmological F-U: Nystagmus: p3, p5
- Eye Fundus: p1-p2, p4: peripheral irregularity (Fig.1)
p3: normal eye fundus, p4: pallor

Summary of ellipsoid portion of photoreceptors (Fig1), p3 (age 6 years) normal

Figure 1

- Perifoveal irregularity (white arrow)
- Normal laminae except mild disruption of ellipsoid portion of photoreceptors (Fig1), p3 (age 6 years) normal

Optical Coherence Tomography

- NFL: nerve fiber layer
- GGL: ganglion cell layer
- I-ONL: inner –outer nuclear layer
- I-OLP: inner-outer photoreceptor layer
- RPL: retinal pigmented layer
- ELM: external limiting membrane