Toxification, Detoxification: Urinary Porphyrin Survey in Autism Spectrum Disorders

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INTOXICATION
Environnemental toxic exposure:
Etiopathogenic factor in Autism

• Autism is a disorder of reciprocal social interaction, behaviour repertoire, language and communication.

• A significant role for genetics in aetiology is supported by a high concordance of autism between monozygotic twins and increased incidence in siblings.

• However, increasing « epidemic » prevalence of autism during this last decade, ecologic reports, and studies suggestive of accumulation or defective elimination of heavy metals in these children, have pointed to the contribution of environnemental toxicants, especially mercury, in the pathogeny of this disease.
Urinary Porphyrin Levels: A Biomarker of Environmental Toxicity
Porphyrins are cruciform compounds synthesized by all the cells of the living world which constitute the active sites of the hemoproteins which transport oxygen Hb & Mb, ensure the energy production Cytochromes A3, B, C and the detoxication of xenobiotic, Cytochromes P450.
8 enzymes ensured in mitochondrion and the cytoplasm, the synthesis of porphyrins starts from the glycine and the succinyl-CoA. They have a different sensitivity to heavy metals and derivative organic compounds which allows to find the intoxication type.
Glycine + Succinyl CoA

Delta Aminolevulinoic acid (ALA)

Porphobilinogen

Coproporphyrinogen III

7-carboxyproporphyrinogen III

6-carboxyproporphyrinogen III

Protoporphyrinogen IX

Protoporphyrinogen IX

Coproporphyrinogen III

5-carboxyproporphyrinogen III

Heme
Hemogloblin & Myoglobin

Respiratory Chain Cytochromes: CYT A3, CYT B, CYT C and CYT C1

Cytochrome P450 of Phase I detoxification
Toxic sensitivity of different porphyrins

- Polychorinated Biphenyl (PCB)
- Arsenic (As)
- Aluminium (Al)
- Uroporphyrin (UroP, 7.42)
- Heptacarboxylic porphyrin (8.87)
- Pentacarboxylic porphyrin (11.23)
- Hemin porphyrin (12.123)
- Mercury (Hg)
- 5 cxP
- PcP
- CoP
- Lead (Pb)
- CoP

URO-D = Uroporphyrin Decarboxylase
COP-O = Coproporphyrin oxidase
Xenobiotic compounds
Aluminium (Al)
Arsenic (As)
Lead (Pb)
Mercury (Hg)
Effects of consecutive DMPS injections on urinary porphyrin concentration in MMH-exposed rats

- Saline injection
- 1st DMPS injection
- 2nd DMPS injection
- 3rd DMPS injection

Urine Porphyrin Concentration (% control)

- Penta
- Pre-copro
- Copro

Date: 14/02/2006
Linear Relationship of Prechelation Urinary Porphyrin Concentrations with Urinary Mercury Concentrations

### Following a single DMPS Injection

<table>
<thead>
<tr>
<th>Porphyrin</th>
<th>Total Hg</th>
<th>Hg $^{2+}$</th>
<th>CH$_3$Hg$^+$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penta</td>
<td>0.927</td>
<td>0.984</td>
<td>0.876</td>
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<tr>
<td>Precopro</td>
<td>0.961</td>
<td>0.884</td>
<td>0.987</td>
</tr>
<tr>
<td>Copro</td>
<td>0.998</td>
<td>0.989</td>
<td>0.984</td>
</tr>
</tbody>
</table>

### Following MMH Exposure for 6 Weeks

<table>
<thead>
<tr>
<th>Porphyrin</th>
<th>Total Hg</th>
<th>Hg $^{2+}$</th>
<th>CH$_3$Hg$^+$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penta</td>
<td>0.967</td>
<td>0.996</td>
<td>0.879</td>
</tr>
<tr>
<td>Precopro</td>
<td>0.895</td>
<td>0.987</td>
<td>0.762</td>
</tr>
<tr>
<td>Copro</td>
<td>0.989</td>
<td>0.908</td>
<td>0.984</td>
</tr>
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</table>
Mercury exposure elicits characteristic changes in the urinary porphyrin excretion pattern. Urinary porphyrin levels in MMH-exposed rats were highly correlated with kidney mercury levels and declined with consecutive DMPS treatments.

Prechelation urinary porphyrin levels are highly correlated with postchelation urinary.

Prechelation urinary porphyrin levels are highly correlated with renal mercury contents.
A study on urinary porphyrin levels in 256 children with neurodevelopmental and related disorders including 106 with autistic disorder.

Nataf R, Skorupka C, Amet L, Lam A, Springbett A, Lathe R, 
Porphyrinuria in childhood autistic disorder
## 269 Study Subjects (2002-2004)

<table>
<thead>
<tr>
<th>Condition/diagnosis</th>
<th>M</th>
<th>F</th>
<th>Total</th>
<th>Mean age (yr)</th>
<th>M/F</th>
<th>% total</th>
<th>% ASD group</th>
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</thead>
<tbody>
<tr>
<td>Allergy</td>
<td>5</td>
<td>3</td>
<td>8</td>
<td>7,3</td>
<td>1,67</td>
<td>3</td>
<td></td>
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<tr>
<td>Asperger</td>
<td>10</td>
<td>1</td>
<td>11</td>
<td>10</td>
<td>10</td>
<td>4,1</td>
<td>5,8</td>
</tr>
<tr>
<td>Attention deficit</td>
<td>2</td>
<td>7</td>
<td>9</td>
<td>9,4</td>
<td>0,29</td>
<td>3,3</td>
<td></td>
</tr>
<tr>
<td><strong>Autism (autistic disorder)</strong></td>
<td>79</td>
<td>27</td>
<td>106</td>
<td>6,4</td>
<td>2,9</td>
<td>39</td>
<td>55,5</td>
</tr>
<tr>
<td>Autism+epilepsy</td>
<td>7</td>
<td>2</td>
<td>9</td>
<td>9,3</td>
<td>3,5</td>
<td>3,3</td>
<td>ASD=91%</td>
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<tr>
<td>Cerebral palsy</td>
<td>6</td>
<td>6</td>
<td>12</td>
<td>8,3</td>
<td>1</td>
<td>4,4</td>
<td>total sample (M/F=3,34)</td>
</tr>
<tr>
<td>Epilepsy</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>10</td>
<td>na</td>
<td>0,7</td>
<td></td>
</tr>
<tr>
<td>Hyperactivity</td>
<td>27</td>
<td>2</td>
<td>29</td>
<td>9,1</td>
<td>13,5</td>
<td>10,7</td>
<td></td>
</tr>
<tr>
<td>MR+ epilepsy</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>6</td>
<td>1</td>
<td>0,7</td>
<td></td>
</tr>
<tr>
<td>PDD-NOS</td>
<td>51</td>
<td>12</td>
<td>63</td>
<td>6,6</td>
<td>4,3</td>
<td>23,4</td>
<td>33</td>
</tr>
<tr>
<td>Psychomotor retardation</td>
<td>1</td>
<td>3</td>
<td>4</td>
<td>7,3</td>
<td>0,33</td>
<td>1,5</td>
<td></td>
</tr>
<tr>
<td>Rett</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>2,5</td>
<td>0</td>
<td>0,7</td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>7</td>
<td>5</td>
<td>12</td>
<td>10,3</td>
<td>1,4</td>
<td>4,4</td>
<td>1</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>198</td>
<td>71</td>
<td>269</td>
<td>7,4</td>
<td>2,8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
neurodevelopmental and related disorders
Coproporphyrin levels in urines of children with
Elevated urinary Coproporphyrin (COPRO) levels in ASD expressed as absolute values normalised to creatinine (left) or as an internal ratio with uroporphyrin (URO) (right)
Precoproporphyrin and pentacarboxy porphyrin: Markers of heavy metal toxicity

Precoproporphyrin plotted against baseline uroporphyrin : the ratio is independent of age-related creatinine variation

14/02/2006
Spectrum of mean porphyrin excess, expressed as a ratio of control group (CTL) value for the different porphyrin subtypes.
Reduction in urinary coproporphyrin on chelation with DMSA

DMSA
(Dimercaptosuccinic Acid)
A Metabolic Phenotype in Autism?

• The association of Autistic syndromes with enzyme deficits has raised the possibility that some genetic component in primary autism, could be expressed as a metabolic imbalance that impairs brain and immune system development and functioning.

• Recent studies have focused on increased oxidative stress and decreased methylation capacity in these children.
Target Molecules and Associated Pathology of Methylation

**Methyltransferase**

- creatine
- neurotransmitter
- catechol
- phospholipids
- protein
- ARN
- ADN

**SAM**
(s-adenosyl methionine)

14/02/2006
Homocysteine

Thiol functions of protein active sites

Cysteine

Metallothioneine

SO₄

taurine

GSH

14/02/2006
METHYLATION without oxidative stress

- Methionine Adenosyl Transferase
  - MET
  - + adenosyl
  - + CH₃
  - Methionine synthase
  - HCYS
  - Cystathionine β-synthase
  - + serine
  - S-Adenosylmethionine methyltransferase
  - - CH₃
  - S-Adenosylhomocysteine hydrolase
  - - adenosyl
  - SAH

Methylation

Detoxication

- Cystathionine Lyase
- CYSTATHIONINE
- Sulfoxidation
- SO₄
- Thiol functions of protein active sites
- Metallothioneine
- GSH synthesis
- GSH
- Taurine

14/02/2006
Cellular Glutathione Function & Synthesis

1. **Cysteine** + **Glutamate** → **γ-Glu-Cys**
   - Glutathione Synthetase

2. **γ-glutamyl Cysteine Synthetase**

**Flavonoids**

1 mM

10 mM

**GSH**

- Redox Potential
- Glutathionylation
- Oxides and oxidant species
- Detoxification
Utilisation and Regeneration du Cellular Glutathione

- Redox Potential
- Glutathionylation
- Oxides and oxidants species
- Detoxication

GGT - Gamma Glutanyl Transferase

Glutathione Reductase
Enzyme B2 (Riboflavin → FAD)

PyroGlu or 5-Oxoproline

Cys-Gly

Glutathion Erythrocyte 2 to 5 mM/l reduced with the effect.

Interpretation of Urinary Thiol Profile

Urinary GSH + CYS-GLY — related to cellular depletion by overutilization and overwhelmed regeneration
Urinary GLU-CYS — index of Glutathione neosynthesis
Urinary Cysteine — index of cellular cysteine availability.
Urinary SO4 — related to the cellular sulfate availability
Urinary SO4/Cys ratio — index of sulfoxidation activity

14/02/2006
Normal Urinary Thiols Profile with average oxidative stress
Oxidative Stress

Urinary Thiols Profile with increased Oxidative Stress
Sulfur Metabolism

Slow transmethylation and marginal status of Folates &/or B12
HOMOCYSTEIN
10.0  5.9

Slow transsulfuration & marginal status of B6
Ratio HCY / CYS ratio
7.0  4.7

Mean Status of tissular glutathion
GLUTATHIONE
0.9  0.4-1.6
GSH-CYS-GLY
3.4  4.8

average neosynthesis of Glutathion
gamma Gla-Cysteine
1.5  1.5-3.5

neosisynthesis/degradation related to GSH equilibrium
gGlu-cys/Cys+GSH ratio
0.44  0.2-0.45

Adequated statut of Cysteine
CYSTEINE
151  130-310

Average capacity of sulfoxidation
Ratio S04/CYSTEINE
98  70-160

Adequated status of Sulfates
SULFATE
15  15-35

*Transmethylation is synthesis of cysteine, low tissular available amino acid, from homocysteine through two B6 dependant enzyme. Cystathionine-beta-synthase & Cystathionine -lyase. This overall pathway activity evaluated by Hcys/Cys ratio is a reliable index of tissular functional need in B6.

*Sulfodetoxication is the most protective and effective way of elimination of xenobiotics, readily excreted by the kidney without entero-hepatic recycling. It requires, on one hand adequate sulfate disponibility provided by sulfoxidation, endogenous cysteine oxidation in sulfate, showing a great interindividual variability, and sulfo transferves phase II. For instance, sulfoxidation deficit is found with a prevalence of 30% in neurodegenerative diseases, Alzheimer and Parkinson’s diseases against 5% in general population.

*Urinary loss of sulfates can be related to high protein diet and some inflammatory diseases as HIV infection where cysteine tissular pools are depleited by excessive catabolism in sulfates excreted by the kidney.

urinary
2385 mg/l
Thank you
DETOXIFICATION
Detoxification Process in cell

The toxics which were oxidized by the CYP450 and could not combined by Phase II transferases "attack" the target cells: DNA, mitochondria, membranes.

Detoxification

Caffeine Test (Phase I)

Acetaminophen Test (Phase II)

Transferase

Pyroosome CYP450

Urine 8OHdG

Urine 8OHdG

Glucuronide

Sulfate

GSH

Sulfur Metabolism Profile

Detoxification Process in cell
Numerous hypotheses in immunological, allergic, toxic and digestive syndrome in young autists, supports the emergence of a dysmicrobism or a microbial pullulation in the psycholeptic metabolites circulation.

Beta-Keto glutarate
Citramalate
P-hydroxy-phenylacetate
P-hydroxy-phenylpropionate

Urinary microbial metabolites profile (dysbiosis) and sulphur compounds (methylation) (Homocysteine, Cysteine, Glutathion, and Sulfate) related to neurotoxic and antitoxic, show intoxication and detoxification of organism.
### Urinary Dysbiosis

**Bacterial dysbiosis markers**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Reference</th>
<th>Reference ranges</th>
</tr>
</thead>
<tbody>
<tr>
<td>PARACRESOL</td>
<td>&lt;1</td>
<td>0.7</td>
</tr>
<tr>
<td>BENZOATE</td>
<td>1.0</td>
<td>0.2-3.8</td>
</tr>
<tr>
<td>2 OH BENZOAOTE</td>
<td>0.3</td>
<td>3.6-14</td>
</tr>
<tr>
<td>4 OH BENZOAOTE</td>
<td>0.5</td>
<td>1.4-3.4</td>
</tr>
<tr>
<td>HIPPURATE</td>
<td>&lt;78</td>
<td>180-400</td>
</tr>
<tr>
<td>3 OH PHENYLACETATE</td>
<td>590-7</td>
<td>40-80</td>
</tr>
<tr>
<td>3 OH PHENYL PROPAionate</td>
<td>&lt;0.2</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>3 OH PHENYL 3 OH PROPION ATE</td>
<td>40</td>
<td>1-5</td>
</tr>
<tr>
<td>HYDROCAFFEATE</td>
<td>0.3</td>
<td>0-7</td>
</tr>
<tr>
<td>TRICARBALLYLATE</td>
<td>0.6</td>
<td>&lt;3</td>
</tr>
<tr>
<td>GLYCERATE</td>
<td>0.4</td>
<td>0-1.6</td>
</tr>
</tbody>
</table>

**Protozoa dysbiosis markers**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-OH PHENYLACETATE</td>
<td>590</td>
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</tbody>
</table>

**Fungal Dysbiosis markers**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>BETA KETOGLUTARATE</td>
<td>13.9</td>
</tr>
<tr>
<td>CITRAMALATE</td>
<td>6.9</td>
</tr>
<tr>
<td>TARTRATE</td>
<td>13.2</td>
</tr>
<tr>
<td>ARABINOSE</td>
<td>1.2</td>
</tr>
<tr>
<td>ARABINITOL</td>
<td>0.6</td>
</tr>
<tr>
<td>2.5 FURANCARBOXYL TE</td>
<td>4.3</td>
</tr>
<tr>
<td>2.5 FURANDICARBOXYLATE</td>
<td>10.1</td>
</tr>
</tbody>
</table>

**Indican test**

- **Obermeyer reagent**
  - INDOXYL: 0 colorless
  - urinary creatinin: 2.4"4"8"g"i

---

**ProtopathIC Dysbiosis Markers**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phloretin</td>
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**Metabolite bacterial detoxication by liver**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAPROXETIN</td>
<td>276</td>
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<tr>
<td>276</td>
<td>180-400</td>
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</table>
Urinary Porphyrin Levels: A Biomarker of Environmental Toxicity

Thank you