ReMiND

Role of metals and metal containing biomolecules in neurodegenerative diseases such as Alzheimer’s disease

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Motivation

- Approximately 10.5 million patients with dementia in EU
- Number predicted to double in next 20 years
- Estimated costs for health care systems €286.1 billion in 2015
- Lack in comparability between results of different kits and laboratories ⇒ hampers large-scale studies
- Traceability in accordance with EC-directive 98/79/EC required (2017 evolved into the EU regulation 2017/746)

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Median ng/L</th>
<th>Ref ng/L</th>
<th>normal</th>
<th>borderline</th>
<th>pathological</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aβ1-42 Kit 1</td>
<td>39</td>
<td>517</td>
<td>med. 500</td>
<td>18</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>Aβ1-42 Kit 2</td>
<td>8</td>
<td>330</td>
<td>651</td>
<td>0</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>tot Tau Kit 1</td>
<td>42</td>
<td>442</td>
<td>med. 450</td>
<td>21</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>tot Tau Kit 2</td>
<td>8</td>
<td>501</td>
<td>466</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>P Tau Kit 1</td>
<td>40</td>
<td>33</td>
<td>40</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Reiber et al., J Alzheimers Dis Parkinsonism 2014, 4(3)
Aim

- Development of **new and accurate methods** for measuring peptide and protein biomarkers
- Development of methods for the **traceable quantification** of metals and metal containing biomolecules
- Production and characterisation of isotopically labelled spike materials
- Characterisation of uptake, metabolism and transport of metals and metal containing biomolecules to the brain
- Facilitation of uptake of the technology and measurement infrastructure
Underlying measurement principle

Double Isotope Dilution

sample x

blend bx and bz, resp.

reference z

\[ w_x = w_z \cdot \frac{m_y}{m_x} \cdot \frac{m_z}{m_yz} \cdot \frac{R_y - R_{bx}}{R_{bx} - R_x} \cdot \frac{R_{bz} - R_z}{R_y - R_{bz}} \]

Ideally, spike and analyte have the same chemical form.
Technical Work Packages of ReMiND

**WP1**
Reference measurement procedures for β-amyloid, T-tau and P-tau

**WP2**
Reference measurement procedures for total metal content and isotope ratios

**WP3**
Reference measurement procedures for metalloproteins

- Correlation metal content ↔ established biomarkers
- Correlation metal content ↔ isotope ratio
- Correlation metalloprotein ↔ established biomarkers
WP1
Quantification of established peptide and protein biomarkers
Quantification of established biomarkers

Established biomarkers

- **β amyloid**
  - Physiological function unknown
  - Formation of plaques in the brain during aging
  - Decreased in Alzheimer patients

- **τ-protein**
  - Stabilisation of microtubules in neurons
  - Formation of tangles in the brain during aging
  - Increased in Alzheimer patients

Kindly provided by Prof. Theuring (Charité)
**τ-Protein quantification with ID-Raman**

- Proteins
- Gold NP Raman reporter and m-antibody
- Magnetic NP and p-antibody

Spike with isotopic enriched Raman reporter

Specific interactions

Separation

Detection, quantification
t-Protein quantification with ID-Raman

- Spike with isotopic enriched Raman reporter
- Specific interactions
- Separation
- Detection, quantification

proteins

gold NP
Raman reporter
and m-antibody

magnetic NP
and p-antibody
Protein quantification with ID-Raman

β-amyloid quantification using MS/MS

**ID MS Analysis of Amyloid 1-40**

- **Equation:** \( y = 9 \times 10^{-5}x - 0.039 \)
- **R²:** 0.9996

**ID MS Analysis of Amyloid 1-42**

- **Equation:** \( y = 0.000153x - 0.135735 \)
- **R²:** 0.998168

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Expected Concentration (ng/mL)</th>
<th>Measured Concentration (n=4)(ng/mL)</th>
<th>Combined Uncertainty ( U ) (ng/ml) ( k=2 )</th>
<th>Precision (%CV)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>aβ 1-40</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>QC-1</td>
<td>2.41</td>
<td>2.64</td>
<td>0.32</td>
<td>6.65</td>
<td>109.58</td>
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<tr>
<td>QC-2</td>
<td>6.93</td>
<td>6.97</td>
<td>0.61</td>
<td>2.66</td>
<td>100.61</td>
</tr>
<tr>
<td>Pooled CSF</td>
<td>2.00-4.00</td>
<td>4.95</td>
<td>0.47</td>
<td>3.35</td>
<td>N/A</td>
</tr>
<tr>
<td>aβ 1-42</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>QC-1</td>
<td>2.03</td>
<td>2.08</td>
<td>0.35</td>
<td>7.98</td>
<td>102.40</td>
</tr>
<tr>
<td>QC-2</td>
<td>5.84</td>
<td>5.26</td>
<td>0.57</td>
<td>6.74</td>
<td>90.03</td>
</tr>
<tr>
<td>Pooled CSF</td>
<td>0.35-0.70</td>
<td>0.70</td>
<td>0.12</td>
<td>7.63</td>
<td>N/A</td>
</tr>
</tbody>
</table>
WP2
Multielemental and isotopic analysis
Total metal amounts

Results

- Generally, higher Fe, Cu and Zn concentrations in disease model brains compared to healthy controls
- Highest metal concentrations in the disease model that has both disease pathologies present

WT, n = 13. NMRI, n = 5. PLB1 - Dbl, n=5. PLB2 – APP, n = 5. 5X, n=7. APP/PS1, n = 3. L66, n = 13.

‡ - None of the individual samples are perfused
† - All individual samples are perfused
Isotopic signatures in AD models

Brain tissue of APP and L66 tau mice vs. matched WT controls

Brain Cu, Fe and Zn isotopic composition respectively for A, C and E: APP mice (n=10) vs. matched wild-type (n=10); and B, D and F: L66 tau mice (n=13) vs. matched wild-type (n=5). Significant difference (p < 0.05, Student’s t-test) between the Tg-L66 and the WT tau-transgenic mice for Cu and Fe (B and D).
WP3
Metal containing biomolecules as potential biomarkers
<table>
<thead>
<tr>
<th>Protein/Metal</th>
<th>Function/Property</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transferrin</td>
<td>Fe transport protein, Fe is suspected to be involved in plaque formation, free Fe causes oxidative stress.</td>
</tr>
<tr>
<td>Ferritin</td>
<td>Fe storage protein, incorporation also of other metals (including toxic ones) with similar properties.</td>
</tr>
<tr>
<td>Cu, Zn-superoxide dismutase</td>
<td>Oxidative stress prevention, acute phase protein, indication for inflammation processes.</td>
</tr>
<tr>
<td>Ceruloplasmin</td>
<td>Cu storage protein, ferroxidase, Cu is suspected to be involved in plaque formation.</td>
</tr>
</tbody>
</table>
Metalloproteins

species-specific IDMS

column

detection

sample preparation and separation

dialysis

Metalloproteins

65Cu-SOD1

sample

\[ ^{65}\text{Cu-SOD1} \]

\[ ^{63}\text{Cu} \]

\[ ^{65}\text{Cu} \]

\[ ^{nat}\text{Zn} \]

\[ ^{nat}\text{Cu} \]

\[ ^{67}\text{Zn} \]

\[ ^{65}\text{Cu} \]
Metalloproteins

- Mass fraction of SOD1 in erythrocytes (63.95 ± 0.93) µg g⁻¹
- Mass fraction of SOD1 in CSF much smaller
- Dilution series for the determination of LOD and LOQ

\[ y = 51153167 \times x + 556 \]

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Blank / µg g⁻¹</th>
<th>LOD / µg g⁻¹</th>
<th>LOQ / µg g⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD1 in CSF</td>
<td>0.013</td>
<td>0.039</td>
<td>0.117</td>
</tr>
</tbody>
</table>
Impact

Clinical Impact

- Support in establishing **global cut-off values** for biomarkers by providing comparable and reliable measurement procedures for interlaboratory studies

- Investigation of **potential biomarkers** such as metalloproteins and isotope ratio analysis may lead to new approaches of dementia treatment

Social Impact

- Potential biomarkers investigated in proposed project can enable **earlier diagnosis**

- Earlier diagnosis allows for earlier intervention and, thus, delay of severe symptoms and hospitalisation
  ⇒ **improvement of quality of life** for patients and their caretakers
Thank you to all the partners…

Coordination, lead WP1
Determination of proteins using ICP-MS and Raman

Lead WP2
Multielemental and isotopic analysis

Lead WP3
Quantification of metal containing biomolecules

Lead WP4 (Impact)
Quantification of potential biomarkers, provision of animal models

Multielemental and isotopic analysis

Quantification of metals and metalloproteins

Quantification of metal and provision of animal models

Isotopic analysis in metalloproteins
Thank you for your attention!