Biomarkers in the differential diagnosis of Dementia: which role for Omics?

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AD

- FTD
- VaD
- α-syn

AD
Alzheimer’s disease

- Slowly progressive memory loss and dementia

Neuropathology:
- plaques  β-amyloid
- tangles  phosphorylated tau protein
- neuronal and synaptic degeneration

Very rare (< 0.1%) familial form - very common sporadic and age-related form

USA 2014

- Around 5,200,000 patients with Alzheimer’s
- A new patient every minute
- Around 200,000 patients < 65 years

Costs for society – around 214 billion USD per year
- more than costs of cancer, heart disease and stroke together
IWG Criteria (FDA and EMA approved)

• Clinical signs and symptoms
  (amnestic syndrome of the hippocampal type)
• CSF biomarkers ($A\beta_{1-42}$, Tau, P-tau)
• PET amyloid tracer retention (PiB and others)
• PET bilateral parieto-temporal hypometabolism (FDG)
• MRI medial temporal/hypocampus atrophy

Dubois B, 2014
The “OMICS”

GenOmics  TranscriptOmics  ProteOmics

MetabolOmics  InteractOmics  MirrOmics  EpigenOmics
In Biomarker Discovery we need:

- **Biological samples**
- **Technologies**
- **Decoding Big Data**
The BIOMARKAPD central and virtual biobank (JPND), 2013

Normal Cognition
Mild Cognitive Impairment (MCI)
Alzheimer’s Disease (AD)
Parkinson’s Disease (PD)
Dementia with Lewy Bodies (DLB)
Fronto Temporal Dementia (FTD)
Vascular Dementia (VaD)
Progressive Supranuclear Palsy (PSP)
Multiple System Atrophy (MSA)
Other type of Dementia
Clinical specimens

CNS Tissues samples
Cerebrospinal Fluid
Blood/serum/plasma
Saliva
Urine
PROTEOMICS

- Study of protein functions, interactions, dynamics and structure

- Complement for genomics and transcriptomics

- Clinical Proteomics is useful for the discovery and verification of new diagnostic and prognostic biomarkers, novel drug targets as well as unraveling novel biological mechanisms

- Neuroproteomics (15 years old), understand CNS disorders through the study of protein expression and the discovery of protein biomarkers to facilitate diagnosis, treatment monitoring and prognosis.
Are there significant quantitative differences in protein expression between patients with AD and healthy controls or patients with other non-AD related diseases?
Quantitative Proteomic Methods

Relative Quantitation

Labeled based
- Enzymatic
  - $\text{H}_2^{18}\text{O}$
- Chemical
  - 2D-DIGE
  - ICAT
  - cICAT
  - TMT
  - iTRAQ
  - ICPL
  - DTT
  - Anhydrides
- Metabolic
  - Radiolabeling
  - SILAC
  - sSILAC
  - SILAM

Labeled free
- 2-D-densitometry
- Label-free MS (Peak intensity)
- Label-free (spectral counting)
- MRM/SRM
- SWATH

Absolute Quantitation

Absolute SILAC
- PSAQ
- FlexiQuant
- PrESTs
- QCAT
- AQUA (MRM)
- SWATH
- mTRAQ

By Craft GE 2013 (modified)
<table>
<thead>
<tr>
<th>Protein/Peptide</th>
<th>Method</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neural Cell Adhesion Molecule 1</td>
<td>2-DE LC/MS</td>
<td>Yin GN, Brain Res 2009</td>
</tr>
<tr>
<td>Alpha dystroglycan</td>
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<tr>
<td>Neuronal Pentraxin Receptor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neuronal Cell Adhesion Molecule Chitinase3-like 1, Chromogranin A Carnosinasi 1</td>
<td>2-DE LC-MS</td>
<td>RJ Perrin, PloS One 2011</td>
</tr>
<tr>
<td>ApoA1, ApoE, Transthyretin</td>
<td>2-DE LC-MS</td>
<td>Castano E., Neurol Res 2006</td>
</tr>
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<td></td>
<td></td>
<td>Daviddson P Neureport 2002</td>
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<td></td>
<td></td>
<td>PurchadesM, Brain Res 2003</td>
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<td></td>
<td></td>
<td>Korolainenen MA Clin Chem 2007</td>
</tr>
<tr>
<td>Pattern of CSF protein in FAD with PSEN1 and APP mutations vs relatives non carrier</td>
<td>LC/MS non labeled</td>
<td>Ringman JM, Arch Neurol 2012</td>
</tr>
<tr>
<td>Pattern of Age related changes</td>
<td>LC/MS</td>
<td>Zhang J, Neurobiol Aging 2005</td>
</tr>
</tbody>
</table>
Lack clinical diagnostic or theragnostic marker from Explorative Proteomics
The limited loading capacity is one contributing factor for the limited dynamic range.
Pre-Fractionating samples
One protein and its various modified forms, or a group of specific proteins are selectively purified and subjected to proteomic analysis.
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<tr>
<td>Pattern of Aβ peptides in CSF</td>
<td>Immunoaffinity/MMS</td>
<td>E. Portelius, Curr Pharm Des 2011</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G Brinkmalm 2012</td>
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<tr>
<td>Pattern of Aβ isoforms and peptides during treatment with disease modifying drugs</td>
<td>Immunoaffinity/MMS</td>
<td>E Portelius, Alzhiemers Res Ther 2010</td>
</tr>
<tr>
<td>SNAP 25 in CSF</td>
<td>Immunoaffinity/MMS</td>
<td>G Brinkmalm, Mol Neurodegener 2014</td>
</tr>
<tr>
<td>Neurogranin Peptides pattern</td>
<td>Immunoaffinity/MMS</td>
<td>Kvartsberg H, Alzheimer Dementia 2014</td>
</tr>
<tr>
<td>Quantification of ApoE</td>
<td>MRM-MS</td>
<td>Han SH, Mol Cell Prot 2014</td>
</tr>
<tr>
<td>Quantitation of aβ42, aβ40, Retinol Binding Protein, Cystatin C</td>
<td>MRM-MS</td>
<td>YS Choi, J Chormatogr. B 2013</td>
</tr>
<tr>
<td>Neurogranin</td>
<td>Immunoaffinity/MS</td>
<td>Thorsell A, Brain Res 2010</td>
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<tr>
<td>Quantification of aβ42, aβ40, aβ38</td>
<td>MRM-MS</td>
<td>J Pannee, J Alz Dis 2013</td>
</tr>
</tbody>
</table>
Concerns

1. Number of participants to the studies and stratification of participants
2. Different panels of metabolites
3. Low throughput proteomics methods
4. Relative “abundancy” of candidate biomarkers in the analytical samples
5. Harmonization of the preanalytical and analytical steps (different analytical platforms)
6. Limited dynamic range
7. Quality of the study design (STARD)
8. Interpretation of the Big Data
Peptidomics

- CSF, 100 μl/sample
- Reduction/alkylation
- TMT 6-plex labeling
- Combine
- 30 kDa filtration
- On filter digestion
- \( C_{18} \), desalting and concentration

Höltä M 2015
N-glycoproteomics

Palmigiano A, 2016
Metabolomics

I

Alzheimer Disease

Control

III

Capd 106. Arachidonic acid. C20 H32 O2. 11.372. MFE Spectrum (11.372 min)

343.2
343.3
343.4
343.5
343.6
343.7
343.8
343.9
344.0
344.1
344.2

Counts vs. Mass-to-Charge (m/z)

II

IV

Alzheimer Disease

Control

M Jové, 2015
## Lipidomics

Table 10. Lipid biomarker discovery in Alzheimer’s disease

<table>
<thead>
<tr>
<th>Species</th>
<th>Sample</th>
<th><strong>Increase (↑)</strong></th>
<th><strong>Decrease (↓)</strong></th>
<th>Analytical method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>Prefrontal cortex</td>
<td>DGs, Ceramides</td>
<td>PEs, LPCs, TG58:7 (AA-containing)</td>
<td>LC-Qtrap/MS</td>
<td>[95]</td>
</tr>
<tr>
<td>Human</td>
<td>Entorhinal cortex</td>
<td>CE{s}, SM{s}, ganglioside GM3, TG56:7 (DHA-containing)</td>
<td></td>
<td>LC-Qtrap/MS</td>
<td>[95]</td>
</tr>
<tr>
<td>Mouse</td>
<td>Forebrain</td>
<td>CE{s}, ganglioside GM3</td>
<td>PG{s}, PS{s}, PI{s}, LPE{s}</td>
<td>LC-Qtrap/MS</td>
<td>[95]</td>
</tr>
<tr>
<td>Mouse</td>
<td>Brain</td>
<td>PC34:2</td>
<td>PC{s}E{s}, pPE{s}, SM{s}(acyl chain C16 ~ 20)</td>
<td>UPLC-ESI-TOF MS</td>
<td>[96]</td>
</tr>
<tr>
<td>Mouse</td>
<td>Plasma</td>
<td>TG{s} (DHA-containing)</td>
<td></td>
<td>UPLC-ESI-TOF MS</td>
<td>[96]</td>
</tr>
<tr>
<td>Human</td>
<td>Hippocampus</td>
<td>GalCer (hydroxy-FA-containing)</td>
<td></td>
<td>UPLC-Q-TOF MS</td>
<td>[100]</td>
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<tr>
<td>Cell</td>
<td>Aβ treated PC12 cells</td>
<td>PC32:0, PC34:1 ~ 2, PC36:2 ~ 3</td>
<td></td>
<td>ESI-MS/MS</td>
<td>[16]</td>
</tr>
<tr>
<td>Human</td>
<td>Cerebrospinal fluid</td>
<td>LPC/PC ratio</td>
<td>pPE{s}</td>
<td>ESI-MS/MS</td>
<td>[2]</td>
</tr>
<tr>
<td>Human</td>
<td>Brain</td>
<td>pPE{s}</td>
<td></td>
<td>LC-MS</td>
<td>[102]</td>
</tr>
<tr>
<td>Human</td>
<td>Plasma</td>
<td>PC36:5 ~ 6, PC40:6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>