Early Changes in the Brain Proteome Associated with Alzheimer's Disease Risk

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Proteomics at the Emory ADRC

Pathological Aggregates → Proteome → Synapse-rich

Cerebral Spinal Fluid (CSF) is analyzed for protein levels

Lumbar puncture performed to obtain cerebrospinal fluid or CSF
Proteomics in Alzheimer’s Disease (AD)

Specific Goal

• Develop accurate and precise method to quantify proteins in brain tissue

Ultimate goal: Can we use proteomics to better define pre-clinical stages of AD and target key molecular pathways that associate with cognitive decline?
Preclinical or Asymptomatic AD: The period between the first appearance of AD neuropathology and the onset of clinically detectable symptoms of disease.
Evidence for Asymptomatic AD

• Post-mortem neuropathological and in vivo PET imaging studies suggest that a substantial proportion of cognitively normal older individuals demonstrate evidence of Aβ and tau tangle accumulation, approximately 30%.

• The distribution of amyloid deposition in these cognitively normal older individuals tends to occur in a pattern similar to that found in AD.

• **Summary:** This suggests that Aβ and neurofibrillary pathologies are necessary, but not sufficient alone to explain the onset of cognitive decline.

Relevance of Asymptomatic AD

- Understanding of the Pathophysiology of Early AD
- Accurate Diagnosis of AD Prior to Onset of Symptoms
- Successful Drug Therapy for Prevention of AD

Novel Biomarkers!
1. Detect a fundamental feature of AD neuropathology.
2. Be validated in autopsy-confirmed cases of the disease.
3. Have a diagnostic specificity for distinguishing and sensitivity for detecting AD from other dementias.
4. Diagnostic laboratory tests should be reliable, reproducible, non-invasive and simple to perform.

Biomarker Criteria in AD

Brain -> Orbitrap MS -> CSF


CSF is analyzed for protein levels
Lumbar puncture performed to obtain cerebrospinal fluid or CSF
Where do we first look for biomarkers in AD?

1) Human brain tissue
2) Detergent insoluble-fraction

- β-amyloid Tau
- α-synuclein
- Huntingtin
- PrP
- SOD1
- TDP-43
- FUS

**Hypothesis:**

Conserved sets of, yet unidentified, proteins are pathologically altered in AD and contribute to disease pathogenesis.

**Approach:**

Analysis of the detergent-resistant (i.e., insoluble) brain proteome from control and seven neurodegenerative disease sub-groups by quantitative MS-based proteomics.
## Enriching for the detergent-resistant pathological proteins

### Methods:

1. **Homogenization buffer (low salt)**
2. **1% Sarkosyl buffer and high salt (Homogenate (H))** (sonicate)
3. Spin 200,000 g for 30 mins (Supernatant = (S))
4. Wash Pellet
5. **8 M Urea, 1% SDS, 50 mM Tris-HCl, pH 7.8**
   - Urea soluble Pellet (U)

### Results:

**WB: Phospho-tau**

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<th>AD Case</th>
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**WB: Tau**
Identifying AD specific markers in the insoluble proteome

Brain Tissue (Pre-frontal cortex) was provided by the Emory ADRC Neuropath Core
Proteomic Workflow

Control  MCI  AD
↓  ↓  ↓
Detergent Insoluble Fractions
↓  ↓  ↓
In-gel trypsin digestion
↓  ↓  ↓
SDS-PAGE

Orbitrap MS
LC-MS/MS based proteomics — identify and quantify

SDS-PAGE

Trypsin digest

peptides

nanoLC

peptide identification

protein identification

protein quantitation
Microtubule Associated Protein Tau

K.IGSLDNITHVPGGGNK.K
(AMINO ACIDS 354-369)

MS/MS

Extracted Ion Intensity

Control

MCI

AD

Relative Abundance

Time (min)
Identifying AD specific markers in the insoluble proteome
**U1 snRNP – structure and function**

**U1 Small Nuclear Ribonucleoprotein Complex**

**Structure**

i. U1 snRNA

ii. U1 proteins
   • U1-A
   • U1-70K
   • U1-C

iii. 7 Sm proteins (SMN complex)

**Function**

i. Binds 5’ splice site of pre-mRNA to initiate intron removal and alternative splicing of 95% of transcripts.

ii. Functional form localized to nucleus
U1 snRNP insolubility is highly specific to AD

U1 snRNP is associated specifically with AD pathology

U1 snRNP is associated specifically with AD pathology

U1 snRNP is associated specifically with AD pathology

Does U1 snRNP directly associated with NFTs in AD?

EM of immunogold-labeled U1-70K aggregates in cytoplasm

2,2,7-trimethylguanosine cap of U1 snRNA evident in tangles

Hales et. al., (2014) *Brain Pathol.* In press
Are there defects in RNA maturation in AD brain?
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Are there defects in RNA maturation in AD brain?

Proteomics at the Emory ADRC

Individually, the process involves:

- Pathological Aggregates
- Synapse-rich
- Proteomics
- Cerebral Spinal Fluid (CSF)
- Plasma/Platelets

Lumbar puncture performed to obtain cerebrospinal fluid or CSF.

CSF is analyzed for protein levels.
Proteomics Collaborators at Emory ADRC

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