

Quantitative neuroproteomics for biomarker discovery in Alzheimer's disease

Akademisk avhandling

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Avhandlingen baseras på följande arbeten:

- I. **Hölttä M**, Zetterberg H, Mirgorodskaya E, Mattsson N, Blennow K, Gobom J. Peptidome analysis of cerebrospinal fluid by LC-MALDI MS. PLoS One. 2012;7(8):e42555.
- II. **Hölttä M**, Minthon M, Hansson O, Holmén-Larsson J, Pike I, Ward M, Kuhn K, Zetterberg H, Blennow K, Gobom J. Multiplexed quantitative proteomics and peptidomics of cerebrospinal fluid identify potential biomarkers for Alzheimer's disease. Submitted.
- III. **Hölttä M**, Dean R, Siemers E, Mawuenyega K, Sigurdson W, May P, Paul S, Holtzman D, Portelius E, Zetterberg H, Bateman R, Blennow K, Gobom J. Effects of γ -secretase inhibition on endogenous peptides in human cerebrospinal fluid. Manuscript.



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ABSTRACT

Alzheimer's disease (AD) is the most common form of dementia characterized by amyloid plaques and neurofibrillary tangles in the brain. Novel biomarkers for AD are needed that reflect disease progression and could identify subjects at risk of developing AD at an asymptomatic stage. The aim of this thesis was to develop methods that can be used to quantify endogenous peptides and proteins in cerebrospinal fluid (CSF) to identify potential biomarkers for AD.

A workflow was developed for preparation of peptide extracts from CSF. The endogenous peptides from CSF were identified by tandem mass spectrometry, and several novel endogenous peptides were found. To quantify the endogenous peptides in CSF, isobaric labeling for relative quantification was incorporated into the workflow. A clinical cohort with CSF samples from AD patients and controls was then analyzed with the method to identify potential biomarkers for AD. Several alterations among the endogenous peptides and proteins were found in the AD group. Altered endogenous peptides derived from proteins that affect e.g. A β aggregation, such as integral membrane protein 2B, and from proteins that have been reported as associated with AD, such as neurosecretory protein VGF, metallothionein-3 and secretogranin-1. Increased levels of the protein YKL-40 were found in the CSF of AD patients as well as alterations in novel potential protein biomarkers.

Human CSF samples from a γ -secretase inhibitor (GSI) trial were also analyzed with the developed workflow to identify potential biomarkers for γ -secretase activity. Endogenous peptides from amyloid precursor-like protein 1 (APLP1), apolipoprotein E, proSAAS, secretogranin-1 and metallothionein-3 were significantly lowered in subjects who received the GSI compared to those who received a placebo. Two peptides from APLP1, which is a known γ -secretase substrate, were identified as decreased and could be potential biomarkers for γ -secretase activity. The other endogenous peptides were derived from proteins that are not known γ -secretase substrates but were nevertheless decreased.

In summary, the developed method could be used to identify novel biomarkers for diseases affecting the brain, and for monitoring treatment effects of substances which have their target in the brain. Several potential CSF biomarkers were identified for AD and for γ -secretase activity.

Keywords: cerebrospinal fluid, proteomics, peptidomics, Alzheimer's disease, biomarker discovery

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