Protein kinase activity profiling to find diagnostic biomarkers for Alzheimer’s disease

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Introduction
Alzheimer’s disease (AD) is a chronic neurodegenerative disease and the most common form of dementia. It affects a constantly growing proportion of our aging population. Disease-modifying treatments might be at their most effective when initiated early in the course of AD. Hence there is a need to identify molecular biomarkers which can be used for diagnosis. In different diseases, for example cancer, protein kinases are used as diagnostic, prognostic and predictive biomarkers. Therefore they may have a high potential as diagnostic biomarkers in AD. The human cerebrospinal fluid (CSF) is in direct contact with the extracellular space of the brain and thus an optimal source for discovery of biomarkers. In this study protein kinase activity will be measured in CSF to investigate the diagnostic potential for AD.

Research questions
• Is it possible to detect protein kinase activity in CSF?
• Can differences in protein kinase activity be observed between AD and no AD cases?

Method - Workflow
Human CSF samples were obtained from AD and non-demented patients by lumbar puncture. The 0.5 µg protein solution was analyzed using the PamChip® peptide micro-array, which comprises 144 serine/threonine-containing peptides derived from known human phosphorylation sites (a).

Peptide phosphorylation is detected using a mixture of fluorescently labelled antibodies against phosphoserine and/or phosphothreonine containing peptides.

Results
• ATP-dependent phosphorylation of peptides is seen in CSF.
• Different phosphorylation patterns between AD and non-demented cases are identified.

Heatmap and Clustering
Comparison of profiles revealed a number of peptides that were significantly different phosphorylated between groups.

The heatmap shows phosphorylation profiles from six patients, three diagnosed AD cases (AD1, AD2 and AD3) and three non-demented cases (no AD1, no AD2 and no AD3).

Cases diagnosed with the same condition are nicely clustered together and can easily be distinguished.

Conclusion
Using the PamChip® peptide array, protein kinase activity is detected in clinical CSF. Furthermore significantly different phosphorylation profiles are shown between AD cases and non-demented cases. This information can also be used to reveal novel signalling pathways and targets in AD.