CSF proteomic signatures in Alzheimer's disease across amyloid and tau biomarker subgroups

SUMMARY

The advances in AD biomarker research over the last years have greatly improved our understanding of the early course of AD. It is now known that AD pathological changes in the brain can manifest decades before the onset of clinical symptoms. Future disease-modifying interventions for AD are most likely to be effective in the earliest stages of the disease. However, the development of treatment strategies is hampered by substantial gaps in our understanding of the pathogenesis and pathophysiology of AD. In this thesis, we investigated the pathogenesis and underlying pathophysiology of AD and related amyloid and tau biomarker subgroups, including suspected non-Alzheimer's disease (SNAP). In **Part I**, we described the clinical features and the underlying pathophysiological processes implicated in SNAP, as well as its diagnostic and prognostic importance. In **Part II**, we focused on the pathophysiology of predementia AD. We investigated the processes associated with different AD neurodegeneration markers and the role of the choroid plexus (ChP) in AD pathogenesis.

In **Chapter 2**, we provided a literature review and meta-analysis of the clinical features and pathophysiology of SNAP. We showed that SNAP was common in individuals with normal cognition (NC; ~20%) and mild cognitive impairment (MCI; ~16%), but less common in persons with dementia (~10%). NC-SNAP individuals have a low risk for cognitive decline over seven years, while MCI-SNAP persons had an increased risk for clinical AD dementia, though lower than MCI-AD. Globally, SNAP individuals showed a lower frequency of *APOE*-ε4 carriers compared to AD, and changes in choroid plexus (ChP) functioning. SNAP individuals also presented higher Aβ40 and Aβ42 levels compared to controls and AD, suggesting a potential disturbance in amyloid metabolism. Neuropathological studies indicate that SNAP is a heterogeneous biomarker-based concept that can represent early AD pathology, presumed AD-spectrum pathologies such as primary age-associated tau pathology (PART), as well as non-AD pathologies that occur with increased frequency in AD, such as limbic predominant, age-related TDP-43 encephalopathy neuropathological changes (LATE-NC).

In **Chapter 3**, we compared the CSF proteomic and genetic profiles of individuals with normal cognition and SNAP (NC-SNAP) to those of controls (NC and normal biomarker levels) and individuals with NC-AD (abnormal amyloid with or without abnormal tau). NC-SNAP showed mostly increased levels of CSF proteins compared to controls, which were mainly associated with neuroplasticity, angiogenesis, and protein modification and degradation. The CSF decreased protein levels in NC-SNAP compared to controls, while not predominant, were associated with changes in ChP functioning and BBB permeability. The proteomic profile of NC-SNAP was similar to that of NC A+T+, while distinct from that of NC A+T-, which showed mainly decreased levels of proteins associated with neuroplasticity. In polygenic risk scores (PGRS) analyses based on AD risk GWAS data, NC-SNAP and NC-AD were similar, while NC-SNAP showed some genetic differences from controls, which were driven by *APOE*.

In **Chapter 4**, we compared the CSF proteomic and genetic profiles of individuals with mild cognitive impairment and SNAP (MCI-SNAP) to those of controls (NC and normal biomarker levels) and individuals with MCI-AD (abnormal amyloid and tau). We found that individuals with MCI-SNAP showed decreased CSF protein levels compared to controls and MCI-AD, which were associated with extracellular matrix (ECM), hemostasis, immune system, lipids, protein processing/degradation and synapses, and with a predominant expression in the ChP. In PGRS analyses based on AD risk GWAS data, MCI-SNAP was similar to controls. MCI-AD showed higher PGRS compared to controls and MCI-SNAP. These differences were driven by *APOE*.

In **Chapter 5**, the pathophysiological processes associated with distinct AD neurodegeneration markers, i.e., neurogranin (Ng), neurofilament light (NfL) and hippocampal volume (HCV), were investigated and compared in persons without dementia (NC and MCI) and with AD pathophysiology (amyloid pathology with or without tau pathology). We found that CSF Ng, CSF NfL and HCV were each associated with distinct CSF proteomic profiles. Ng+ showed predominantly increased levels of proteins, which were related to hyperplasticity, and protein modification and degradation. Ng, t-tau and p-tau correlated highly and incongruency was uncommon. This suggests that Ng may rather serve as a tau-related marker of synaptic dysfunction

in AD, which precedes neurodegeneration. NfL+ showed mainly decreased levels of proteins, which were related to hypoplasticity, ECM, lipids, protein processing and hemostasis, and with a predominant expression in the ChP. HCV+ was associated with relatively few proteomic changes, that were related to oxidative stress.

In **Chapter 6**, we investigated the involvement of the ChP in the pathogenesis of AD using ChP tissue proteomics in an AD mouse model exhibiting amyloid pathology and compared this to mice and human CSF proteomic findings. ChP tissue proteome was dysregulated in APP^{NL-G-F} mice relative to wild-type mice at both 7 and 40 weeks. At both ages, ChP tissue proteomic changes were associated with epithelial cells, mitochondria, protein modification, extracellular matrix and lipids. Nonetheless, some ChP tissue proteomic changes were different across the disease trajectory; pathways related to lysosomal function, endocytosis, protein formation, actin and complement were uniquely dysregulated at 7 weeks, while pathways associated with nervous system, immune system, protein degradation and vascular system were uniquely dysregulated at 40 weeks. Similar results were observed in the CSF of APP^{NL-G-F} mice, as well as of human AD patients with amyloid but without tau pathology (A+T–).

In **Chapter 7**, we provided a general discussion of our findings, its implications for future proteomic research, current theoretical framework, clinical practice and clinical trials, and recommendations for future research. Overall, the studies presented in this thesis have improved our understanding of the pathophysiology of SNAP and AD and highlight its heterogeneity.