PreFrontAIs
Searching for therapeutic interventions in frontotemporal dementia with *C9orf72* repeat expansions

Aim: The aim of this collaborative project is to develop novel biomarkers and therapeutic interventions in presymptomatic and symptomatic *C9orf72*-associated frontotemporal dementia (FTD). *C9orf72* repeat expansions (*C9orf72*RE) are the most common cause of familial FTD and cause significant personal and societal burden. There is an unmet need for sensitive biomarkers to diagnose FTD at an early stage, estimate the prognosis and monitor treatment effect in therapeutic trials. Furthermore, this project may provide an improved understanding of the pathophysiology of *C9orf72*-related neurodegeneration and thereby generate targets for therapeutic interventions.

Results: Several studies were performed to identify and validate biomarkers in blood and CSF. Firstly, longitudinal measurement of serum neurofilament light (NfL) revealed low and stable levels in presymptomatic *C9orf72*RE carriers, a sharp increase around symptom onset, followed by stable, elevated NfL levels in the symptomatic stage, underlining the value of NfL as a marker of disease activity. Secondly, we found increased CSF levels of the neuroinflammatory markers CHIT1, YKL-40 and GFAP in symptomatic (ALS/FTD), but not in presymptomatic mutation carriers. Furthermore, a different neuroinflammatory profile was detected in *C9orf72*-ALS compared to *C9orf72*-FTD, which might drive phenotypic differences.

Thirdly, CSF proteomics revealed downregulation of several proteins not previously described in symptomatic *C9orf72*-associated FTD, indicating dysregulation of synaptic, secretory vesicle and inflammatory proteins. Further validation by immunoassays of the most promising candidate biomarkers, NPTXs, confirmed our findings, and suggests that NPTX2 may be a valuable synapse-derived biomarker.
Fourthly, in CSF proteomics comparing *C9orf72*-FTD with *C9orf72*-ALS we identified and validated eight differentially regulated proteins, including UCHL1, which may explain diverging ubiquitination and autophagy processes. NPTXR was significantly decreased in *C9orf72*-FTD, but not in *C9orf72*-ALS, compared to controls. Affinity-based suspension bead arrays in CSF of *C9orf72* mutation carriers revealed differential expression of VGF, TNR and NfM in symptomatic subjects.
Finally, a newly generated mouse monoclonal poly-GR antibody revealed specific staining of perinuclear inclusions in frontal and temporal cortex, hippocampus and cerebellum of *C9orf72*-FTD patients. We set-up an ELISA that shows high specificity for poly-GR, but could not detect poly-GR in the CSF of *C9orf72*RE carriers, however, we were able to demonstrate the presence of poly-GR in human cortex of C9FTD/ALS cases, and in our zebrafish and mouse models.

To better understand the prodromal stages of *C9orf72*-related disease, we also studied cognitive performance and MRI abnormalities in presymptomatic *C9orf72*RE carriers. We found poorer cognitive performance, as well as loss of white matter integrity in frontal lobe tracts, thalamic radiation and tracts associated with motor functioning, and grey matter volume loss in the thalamus, cerebellum, parietal and temporal cortex.

A worldwide study in collaboration with the Frontotemporal Dementia Prevention Initiative (FPI) determined the mean age at onset (AAO) and age at death (AAD) in 1433 *C9orf72*-FTD patients to be 58.2 years and 65.3 years respectively. Family membership only accounted for 17% of AAO variation, indicating that data from other family members is not helpful to estimate AAO.

Pharmaceutical companies are currently preparing trials with antisense oligonucleotides (ASO) to inhibit the RNA expression of repeat expansions, or to tackle the formation of TDP-43 inclusions. The above-mentioned biomarkers are important tools to evaluate their therapeutic effects.