Poster #2341

CSF proteomic changes associated with aggregating beta amyloid and tau proteins in Alzheimer's disease

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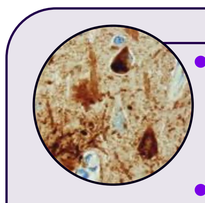
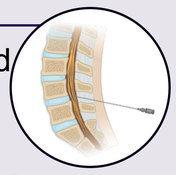
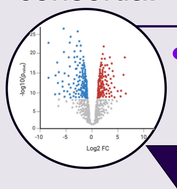


- Alzheimer's disease (AD), Parkinson's disease (PD) and Lewy Body Dementia (DLB) are three of the most common neurodegenerative diseases.
- Despite common pathological processes, neuropathological findings clearly differentiate these diseases post-mortem.



- While single biomarker studies (NfL, pTau, Aβ) in cerebrospinal fluid (CSF) or plasma are informative to help diagnose neurological diseases, they can't accurately predict AD, PD or DLB
- Here, we analyzed over 300 CSF samples from patients with AD, PD, and DLB using the O-link platform, in partnership with the EPND-biomarker consortia.



- 3000 proteins were profiled, revealing significant differences in ~10% of proteins in AD and PD samples, and 3% in DLB samples

Strategy: Conduct a comprehensive proteomic profiling of over 3000 proteins in the CSF to understand the molecular mechanisms underlying these neurodegenerative diseases

Citations
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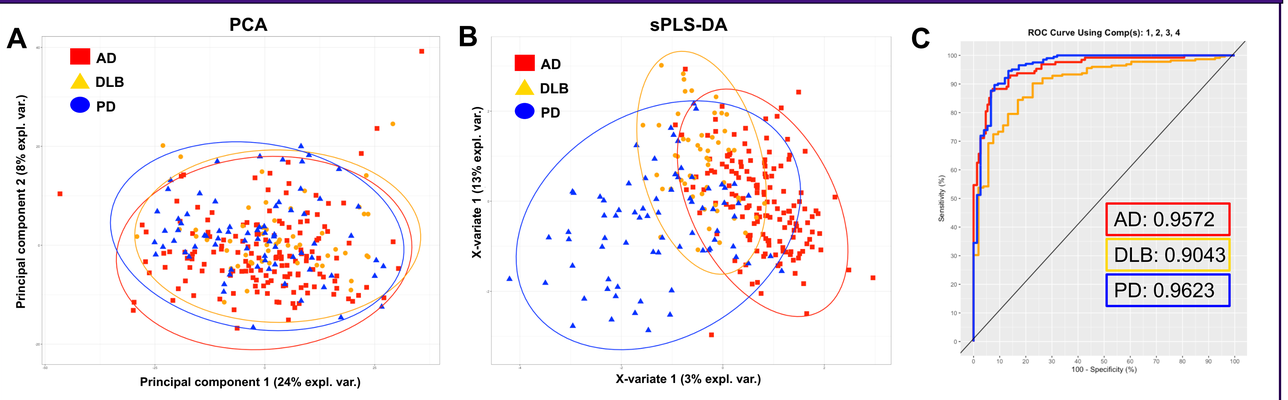
Patient demographics

	AD	DLB	PD
N	150	53	75
Age	69.4 (±8.3)	69.6 (±25.5)	69.9 (±10.2)
Sex (F/M)	67/83	9/44	24/51
BMI	24.5 (±3.4)	25.5 (±3.4)	27.9 (±4.1)
aβ 42:40 ratio	0.034 (±0.009)	0.052 (±0.020)	0.068(±0.013)
pTau181	28.5 (±13.4)	19.5 (±6.5)	15.5 (±5.7)
NfL	198.5	212.9	145.9
α-synuclein	144.4	121.4	112.3

Patient demographics – The cohort was comprised of a mixture of patients with Alzheimer's Disease (AD), Dementia with Lewy Bodies (DLB) and Parkinson's Disease (PD). For all patients, select CSF biomarkers were measured using the Roche Elecsys NeuroToolKit. Measurements are reported in pg/mL.

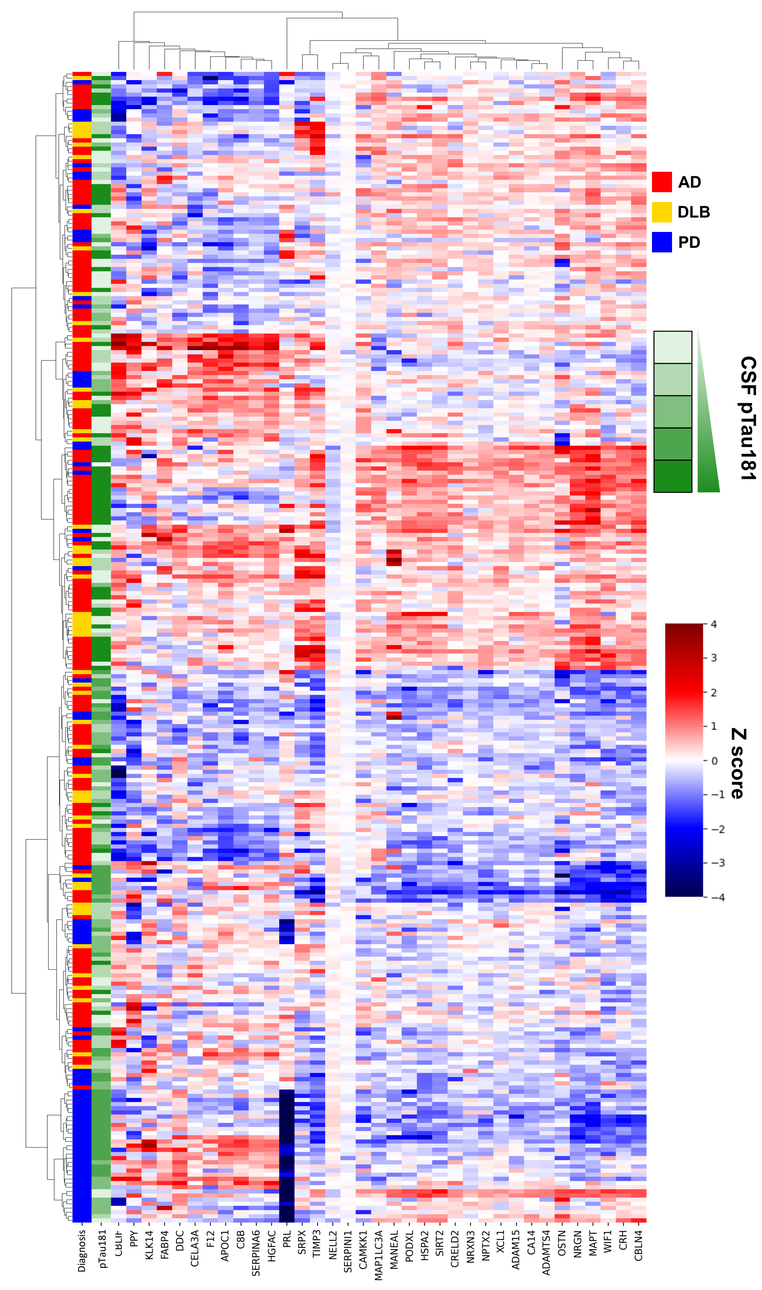
Patients belonged to one of six cohorts: ADC - from VUMC; DANCER, DELCODE, DESCRIBE, and MiGAP - from DZNE; COSCODE/gMAD - from UNIGE; Luxembourgish Parkinson study - from UNILU; NOR-DLB - from Stavanger University Hospital; DDI – from Akershus University Hospital

sPLS-DA model of accurately classify patients by disease



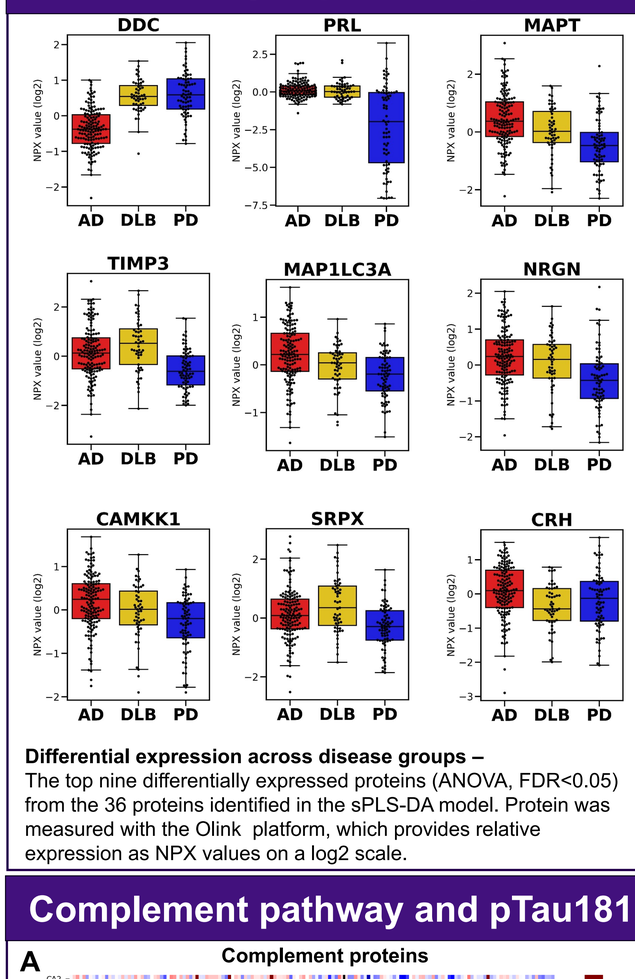
A) PCA of protein expression – Of the ~3000 proteins measured, 1792 had a mean expression value above the lower limit of detection. A PCA was conducted for these proteins and no natural clustering by disease group was observed.
B) sPLS-DA of protein expression – A sparse PLS-DA model was generated to discriminate across the three diagnoses. Tuning and cross-validation of the model selected 36 features across 4 components to accurately classify patients by disease group.
C) ROC of sPLS-DA model– The sPLS-DA model shows robust performance in classifying patients into their respective disease groups. The AUROC for each disease falls above 0.90.
Analysis and plots were generated using mixOmics (Rohart 2017)

Hierarchical clustering of proteins



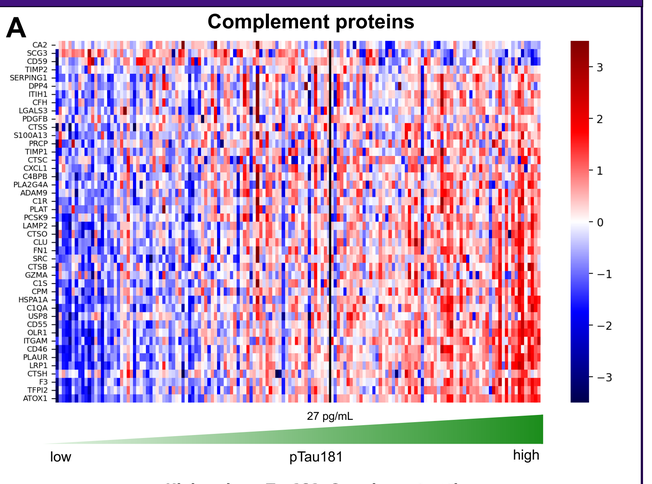
Hierarchical clustering – The 36 proteins identified in the sPLS-DA model were clustered using the Ward method, with each row representing an individual patient. Much of the clustering was driven by patient diagnosis but some sub-clustering appears associated with other biomarker metrics, such as CSF pTau181 levels. Patients were subdivided into quintiles based on pTau181 abundance, with the lowest quintile represented in pale green and the highest in dark green.

Differentially expressed proteins



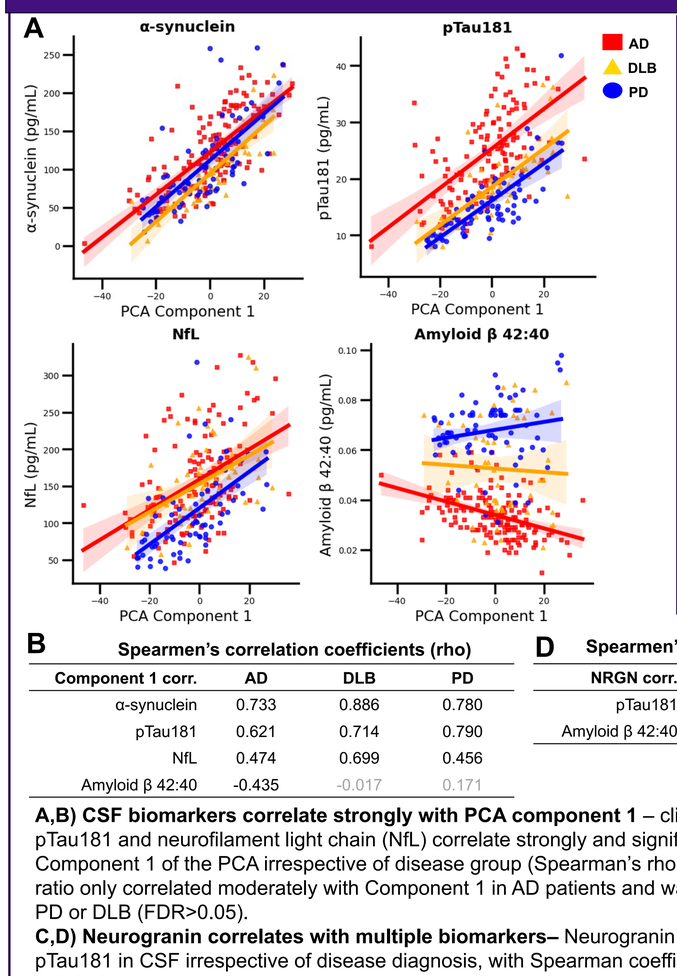
Differential expression across disease groups –
The top nine differentially expressed proteins (ANOVA, FDR<0.05) from the 36 proteins identified in the sPLS-DA model. Protein was measured with the Olink platform, which provides relative expression as NPX values on a log2 scale.

Complement pathway and pTau181



Many complement pathway proteins correlate with pTau181– AD patients were categorized as high or low pTau181 using a 27pg/mL cutoff and differential protein expression was assessed (volcano plot B, with complement proteins in red). Many genes within the complement pathway are significantly altered between high and low pTau patients, with protein expression correlating strongly with the biomarker.

Disease biomarker correlations



B Spearmen's correlation coefficients (rho)

Component 1 corr.	AD	DLB	PD
α-synuclein	0.733	0.886	0.780
pTau181	0.621	0.714	0.790
NfL	0.474	0.699	0.456
Amyloid β 42:40	-0.435	-0.017	0.171

D Spearmen's correlation coefficients (rho)

NRGN corr.	AD	DLB	PD
pTau181	0.863	0.897	0.882
Amyloid β 42:40	-0.628	-0.265	0.227

A,B) CSF biomarkers correlate strongly with PCA component 1 – clinical biomarkers α-synuclein, pTau181 and neurofilament light chain (NfL) correlate strongly and significantly (FDR<0.005) with Component 1 of the PCA irrespective of disease group (Spearman's rho listed in B). The amyloid β 42:40 ratio only correlated moderately with Component 1 in AD patients and was not significantly correlated in PD or DLB (FDR>0.05).
C,D) Neurogranin correlates with multiple biomarkers– Neurogranin exhibits a robust correlation with pTau181 in CSF irrespective of disease diagnosis, with Spearman coefficients (D) greater than 0.86 (FDR<0.0001) for all disease cohorts. A similarly strong correlation is also observed for α-synuclein but not NfL (not pictured). In AD patients, the amyloid β 42:40 ratio is also strongly negatively correlated with neurogranin. This correlation appears to be disease-specific and was not observed in PD or DLB patients.

EPND, a public-private partnership funded by the Innovative Medicines Initiative (IMI), is a joint undertaking between the EU and European pharmaceutical industries. EPND has established an efficient sample and data sharing platform, leveraging existing European research infrastructures, to accelerate the discovery of biomarkers, new diagnostics and treatments for neurodegenerative diseases.

Towards this end Work Package 5 (WP5) is carrying out 5 biomarker case studies. They are designed to test the functionalities of the platform, and delivering results that will be integrated back into EPND to benefit the wider research community:
- Case Study 1: ATN staging system
- Case Study 2: Complement assays
- Case Study 3: Microbiome feasibility
- Case Study 4: Molecular subtypes validation
- Case Study 5: Pilot of full functionality

For Case Studies 1, 2 and 4, EPND has identified 350 plasma & serum samples from healthy individuals, participants with AD, PD or DLB. Sanofi carried out the o-link analysis for these samples.
Case Study 5 allows partners to bring in new assays, identify cohorts of patients and test the full functionality of the platform. For more information: www.epnd.org, info@epnd.org.

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