DEEP PROTEOMIC PROFILING OF AD CSF FOR UNBIASED BIOMARKER DISCOVERY AND SUBJECT STRATIFICATION

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INTRODUCTION

Cerebrospinal fluid (CSF) is established as a key matrix that enables interrogation of biological processes within the central nervous system. The need for better biomarkers and biological understanding is evidenced by the lack of success of disease modifying drugs in late-stage clinical trials.

Here, we seek to address this unmet need by applying an optimized discovery mass spectrometry (HRM™/DIA-MS) workflow, to deeply characterize the proteomes of CSF from subjects with Alzheimer's' Disease (AD).

CSF samples were obtained from AD patients and age-matched control subjects. Across all samples, 1924 proteins were identified and quantified. The pool of quantified proteins comprises well-characterized biomarkers associated with AD and other neurological disorders. By this example, we demonstrate the utility of an unbiased method for biomarker discovery as well as subject stratification.

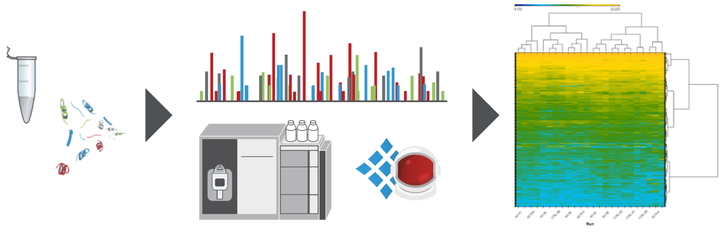
Diagnosis	Normal Controls	Alzheimer's Disease
N (%)	8 (33%)	16 (67%)
Median MMSE (0-30; 95% CI)	30 (28-30)	16 (15-17)
Median Age (yrs; 95% CI)	66 (55-74)	63 (53-74)

CONCLUSIONS

- **A robust proteomics workflow that quantifies close to 2'000 proteins in routine CSF analysis**
- **Functional analyses associate AD with dysregulation of innate immunity, oxidative stress, lipoprotein remodeling and proteostasis**
- **Unbiased profiling identified a panel of proteomic signatures which drives subject stratification**
- **The presented proteomics workflow is scalable to 100s-1000s of samples, thus applicable for the profiling of large cohorts**

RESULTS

A



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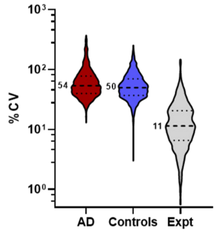

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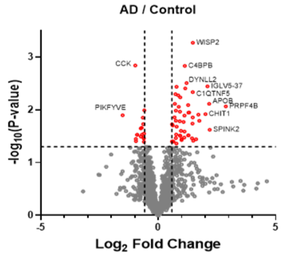
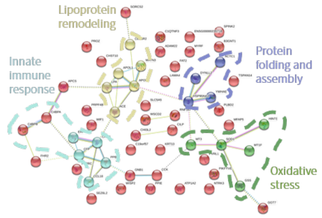


(A) Schematic of the DIA-MS workflow
Protein are extracted from CSF and processed into tryptic peptides for analysis. Single-shot quantification was performed for each sample by DIA-MS. Data analysis was performed using Spectronaut™ software. Unsupervised clustering of 1924 quantified proteins does not show clear separation of AD and control.

(B) Quantitative robustness of DIA-MS
The biological variances within the sample sets are larger than the technical variance estimated by quantitative CV

(C) 73 significantly regulated proteins
Univariate t-test identifies significantly regulated proteins with the cutoff: p-value < 0.05, $\log_2$(fold change) > 0.58

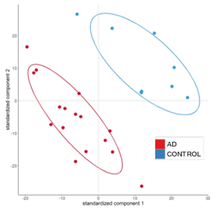
D



(D) Candidate proteins fall into 4 major functional categories
Innate immune response, lipoprotein remodeling, oxidative stress and protein folding are identified as main clusters among the 73 regulated proteins.

(E) Multivariate PLS-DA analysis of the entire cohort using all quantified proteins

E



(F) Subject stratification based on a panel of CSF protein markers
PLS-DA analysis yields a panel of 25 signature proteins based on their contribution in differentiating AD vs. control. This signature panel fully reconstructs the two groups of subjects and identifies two subpopulations within the AD group (indicated by the red, dotted line).

F