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Investigating the Phenotypic Proteome of Triple Negative Breast Cancer (TNBC) for Precision Medicine

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The authors have chosen not to publish the abstract.

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Single cell and low cell count spheroid analysis to assess cellular responses to treatment using the cellenONE proteoCHIP EVO 96 workflow on the timsTOF Ultra 2

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Artificial lab environments are altering inter cell connectivity, communication, and microenvironments and thereby responses to treatment. 3D-spheroid-based cell culture simulates structural properties better. Single cell proteomics aims to decipher heterogeneity on cell microenvironment. Therefore, we want to assess treatment responses in small spheroids versus 2D-culture derived single cells.

A549 and HEK293 cells from 2D-culture and spheroids grown for one day, were treated with lipopolysaccharides (LPS) or DMSO. 2D-cultured cells and small spheroids were isolated into the proteoCHIP® EVO 96, using the cellenONE platform, transferred by centrifugation onto Evotips (96 tip box) separated on a 15 cm Aurora Elite C18 column in Whisper Zoom 40 SPD and eluted into a timsTOF Ultra 2. dia-PASEF data were processed with Spectronaut 19 using directDIA+.

Cellular responses to treatment on single cell level from 2D-cultures and small spheroids were compared to responses seen in bulk samples. Sample-to-sample variability decreased with an increasing cell number. However, comparing protein groups identified in the undisturbed non-treated samples, 65% of all proteins identified in bulk were identified at single cell level and increased to 85% on small spheroid level. The protein group identification overlap between Spheroids and single cells was 75% demonstrating good protein coverage depth.

LPS treatment responses on bulk samples, spheroids and 2D-cultured single cell level were comparable showing expected activation of inflammatory pathways. Severity varied more in the single cells and spheroids than in the bulk samples presumably due to drug distribution and accessibility, or cell cycle stage, which is averaged out in the bulk.

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developing targeted single-cell proteomics to dissect complex cellular hierarchies

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The authors have chosen not to publish the abstract.

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Advancing Chronic Liver Disease Diagnosis: Targeted Proteomics for Non-Invasive Detection of Fibrosis

Andrea Villanueva Raisman[1,2], David Kotol[3], Ozlem Altay[1,2], Adil Mardinoglu[1,2], Dila Atak[4], Müjdat Zeybel[5,6], Mathias Uhlén[1,2], Fredrik Edfors[1,2]
[1] SciLifeLab, KTH Royal Institute of Technology, 114 28 Stockholm, Sweden, [2] Department of Protein Science, Division of Systems Biology, School of Chemistry, Biotechnology and Health, KTH Royal Institute of Technology, 114 28 Stockholm, Sweden, [3] ProteomEdge AB, Stockholm, Sweden, [4] Koc University Translational Medicine Research Center, Koç University, Istanbul, Turkey, [5] Department of Gastroenterology and Hepatology, School of Medicine, Koç University, Istanbul, Turkey, [6] NIHR Nottingham Biomedical Research Centre, Nottingham University Hospitals NHS Trust & University of Nottingham, Nottingham, UK

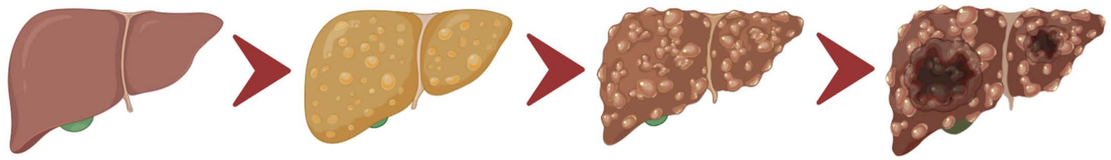
Chronic liver disease poses significant challenges to healthcare systems, which frequently struggle to meet the needs of end-stage liver disease patients. Early detection and management are essential because they can decelerate and potentially reverse liver damage. However, the implementation of population-wide screenings is hindered by the asymptomatic nature of early chronic liver disease, along with the risks and high costs associated with traditional diagnostics, such as liver biopsies.

This study pioneers the development of innovative, minimally invasive methods capable of improving the outcome of liver disease patients by identifying liver disease biomarkers using quantification methods with translational potential. A targeted mass spectrometry assay based on heavy recombinant peptide isotopes (SIS-PrEST) was employed for absolute quantification of 108 proteins in just two microliters of plasma. The plasma profiles were derived from patients of various liver disease stages and etiologies, including healthy controls. A set of potential biomarkers for stratifying liver fibrosis was identified through differential expression analysis and supervised machine learning. These findings offer promising alternatives for improved diagnostics and personalized treatment strategies in liver disease management. Moreover, our approach is fully compatible with existing technologies that facilitate robust quantification of clinically relevant protein targets via minimally disruptive sampling methods.

a

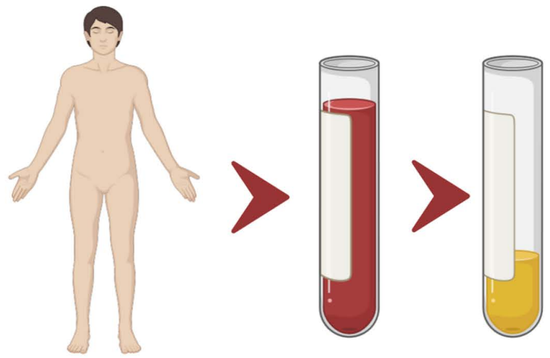
Overview of the dataset

Collection of plasma samples



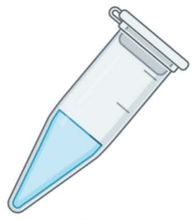
Liver disease
198 patients

Controls
45 patients



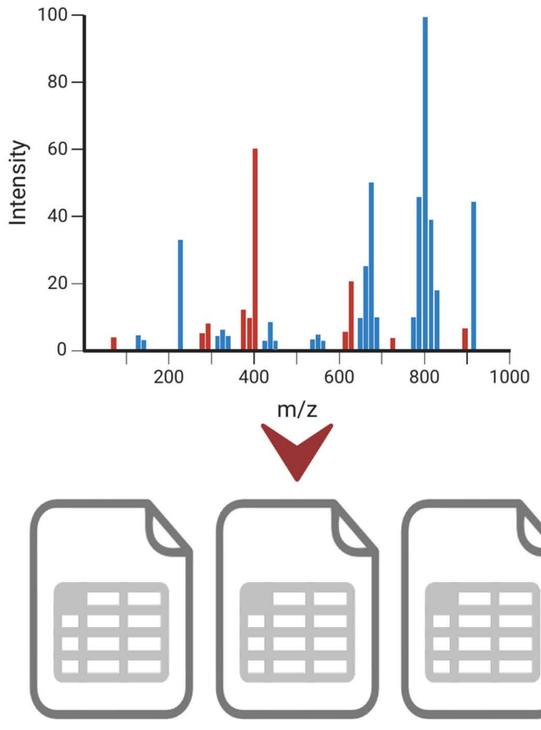
Targeted mass spectrometry

108 SIS-PrESTs



+

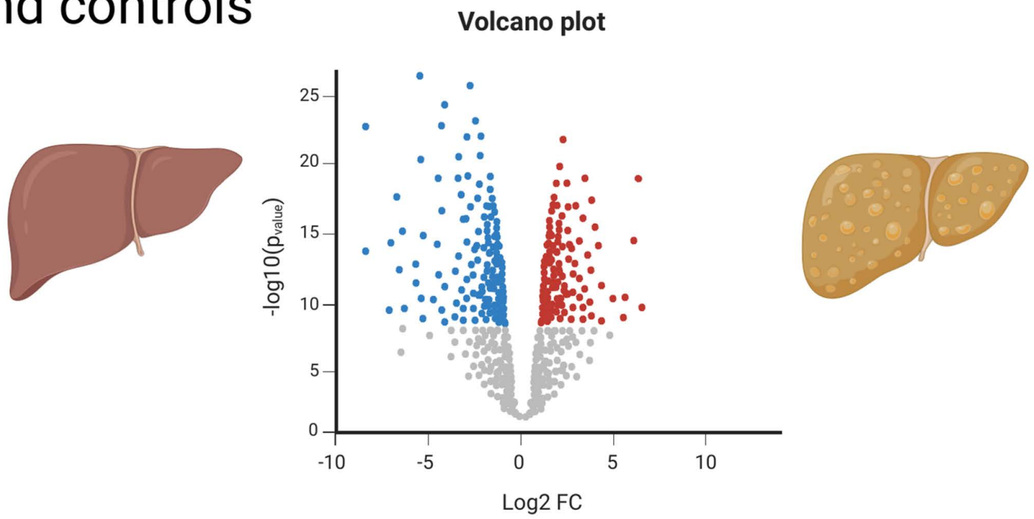
243 samples



Selection of biomarkers

Differential expression analysis

Comparison between different fibrosis levels and controls

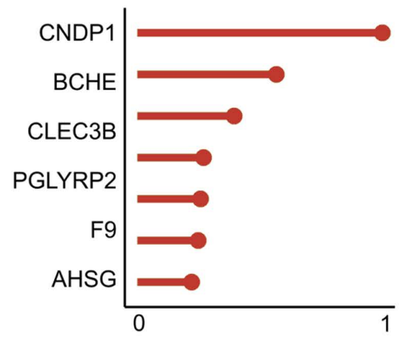


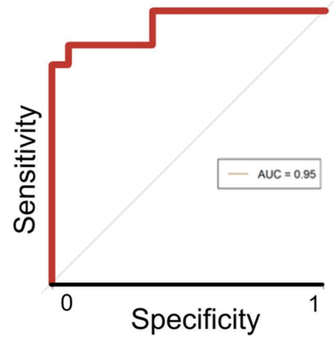
Classification models

Mild, moderate and severe fibrosis

Machine learning classification models

Biomarker panel performance evaluation





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Profiling the Blood Proteome in Autoimmune Disease Using Proximity Extension Assay

Josefin Kenrick[1], Fredrik Edfors[1], Mathias Uhlén[1], Peter Nilsson[1], Sofia Bergström[1], Elisa Pin[1]
[1] KTH

Autoimmune diseases are heterogeneous diseases characterized by dysregulation of the immune system. They often result in chronic inflammation and damage to overall health. Due to the complex nature of these diseases, they are frequently difficult to diagnose and present with comorbidities which increase mortality risk. There is a pressing need for the discovery of novel biomarkers to facilitate early diagnosis, stratification and treatment evaluation of patients within these disease populations.

In this study, five autoimmune diseases were selected for plasma profiling as part of the Human Disease Blood Atlas program, including myositis (n=210), rheumatoid arthritis (n=84), systemic sclerosis (n=100), Sjögren's syndrome (n=99), and systemic lupus erythematosus (n=99).

In total, 592 plasma samples were analysed using the Olink Explore 1536 platform, a highly sensitive and multiplexed antibody-based technology, resulting in expression data of 1163 unique proteins.

Differential expression identified potential prognostic biomarkers; some of these have previously been found to be associated with autoimmune disease, and others are novel. Pathway analysis provides further insights into the underlying biological processes and molecular interactions involved in the pathogenesis of these autoimmune disorders. Many identified proteins are involved in pro-inflammatory response and have suggested immune system functions. A portion of identified proteins have strong associations with cancer as well as infectious disease.

In summary, this study provides a comprehensive, exploratory analysis with the aim to identify distinct protein profiles both within and across five autoimmune diseases and their subgroups.

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Plasma Proteomics-Based Multi-Label Classification of Co-occurring Metabolic Diseases

Konstantinos Antonopoulos[1,2], María Bueno Álvez[1,2], Mathias Uhlén[1,2], Fredrik Edfors[1,2]
[1] KTH Royal Institute of Technology, [2] Science for Life Laboratory

Background

While current studies focus mostly on case-control comparisons, the complex interplay and co-occurrence of cardiometabolic diseases necessitate the development of robust and sophisticated multi-label classification methods. This study aims to identify novel biomarker signatures that will facilitate early diagnosis and improved stratification of patients with multiple metabolic and cardiovascular conditions using advanced machine learning techniques.

Methods

3,000 plasma samples were analyzed using the Olink Explore 1536 platform, a highly sensitive and multiplexed antibody-based technology. This dataset consists of two independent cohorts of patients aged 50-65 from the general Swedish population that will be used as discovery (n=2,000) and validation (n=1,000) cohorts. We employed and evaluated the performance of a range of machine learning methods, starting with binary lasso classifiers as a baseline and expanding to more complex approaches including Classifier Chains, Neural Networks, and unsupervised techniques.

Results

By applying our multifaceted machine learning approach to a comprehensive cardiometabolic plasma proteomics dataset, we identified novel biomarker signatures for multiple co-occurring metabolic and cardiovascular conditions. By integrating these biomarker signatures with clinical metadata, we defined phenotypic subtypes within our multi-diseased cohort. This provides a deeper understanding of co-occurring disease patterns and uncovers new insights into disease mechanisms and interactions.

Conclusions

Our study demonstrates the power of combining plasma proteomics with advanced machine learning for multi-disease classification. The developed multi-label classification methods offer a more nuanced understanding of complex disease profiles compared to traditional case-control approaches. Ultimately, our findings offer insights into disease mechanisms and pave the way for personalized therapeutic strategies.

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Proximity labeling identifies a neuronal Alk interactome in the Drosophila CNS

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Background: Anaplastic lymphoma kinase (Alk) is a receptor tyrosine kinase (RTK) of the insulin receptor family. In addition to its role in oncogenesis, studies on model organisms have revealed a role for Alk signaling in the central nervous system (CNS) including axon targeting, synapse development, growth and body size regulation, brain sparing, memory formation and learning, circadian rhythm, and longevity. Although the Alk receptor is associated with a wide range of processes, downstream effectors and regulators are not characterized. Our aim is to identify Alk interactome in Drosophila CNS using TurboID-based proximity labeling.

Materials and methods: BirA is a biotin protein ligase which converts biotin to reactive biotinoyl-5'-AMP and labels exposed lysine residues of substrates with this reactive biotin. TurboID is a mutant form of BirA which has lower affinity for reactive biotinoyl-5'-AMP compared to wild-type BirA and thereby prematurely releases reactive biotin resulting in biotinylation of proximal proteins. Biotinylated proteins are isolated by streptavidin pull-down and identified by TMT-based LC-MS3.

Results and conclusion: We described the core neuronal "Alk proximitome" by introducing TurboID into the endogenous Drosophila Alk locus by CRISPR/Cas9-mediated genome editing. In addition, we revealed the effect of Alk-activation, via either Jelly belly (Jeb) ligand overexpression, or gain-of-function mutation of the Alk locus (AlkY1355S) on the Alk interactome in the CNS. We identified known signaling cassettes downstream of Alk as well as novel activity-dependent components of Alk signaling.

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Morphological and molecular profiling as a pilar of precision medicine: tailoring treatment in late-stage cancer patients

Nicole Woldmar[1], Jéssica Guedes[1], Leticia Szadai[2], András Kriston[3], Ede Migh[3], Ferenc Kovacs[3], Henriett Oskolás[1], Roger Appelqvist[1], Zsolt Megyesfalvi, Peter Horvath[3], Balázs Döme, István Balázs Németh[2], Johan Malm[1], Jeovanis Gil[1], György Marko-Varga[1]
[1] Lund University, Lund, Sweden, [2] University of Szeged, Szeged, Hungary, [3] Biological Research Centre, Szeged, Hungary

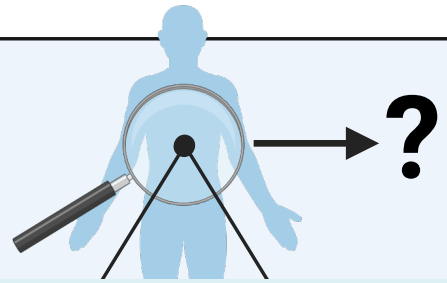
Background: Treating metastatic cancer is challenging due to therapy resistance and partial effectiveness. Comprehensive profiling of individual tumors can identify actionable targets, allowing selection of the most beneficial treatment for the patient.

Methods: We developed a workflow for aiding treatment decision in late-stage cancer patients, incorporating AI-based morphological characterization of FFPE tissues; laser microdissection of tumor area, stroma regions, and different subclones; and molecular profiling of the samples, using gene mutation panels, transcriptomics, and proteomics.

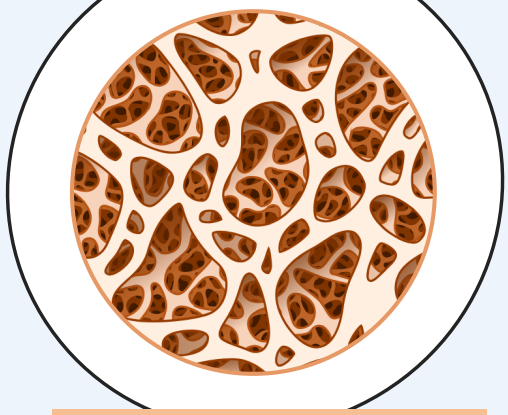
Results: The established workflow was applied to multiple metastatic cancer patients, revealing new therapeutic directions. Patient 1 (melanoma) exhibited significant intra-tumor heterogeneity with distinct molecular subclones, low immune cell infiltration, and high metabolic activity in both primary and metastatic tumors, suggesting likely resistance to immunotherapy but potential responsiveness to targeted therapies. Patient 2 (prostate cancer) showed elevated mitochondrial metabolic activity in tumor regions, along with upregulated PI3K/AKT/MTOR signaling in stromal rich regions. This data suggests that combining mitochondrial complex inhibitors with targeted therapies may be effective. Patient 3 (melanoma) demonstrated high intra-tumor heterogeneity presenting two different subclones in the primary tumor, but only one in the metastatic sites. The patient had a high proliferative profile overall, with greater metabolic activity in metastases, indicating potential benefit from metabolic inhibitors.

Conclusion: High-throughput molecular profiling combined with morphology proved crucial for implementing precision medicine in metastatic cancer patients, improving treatment response prediction, aiding clinical decision-making, and extending patient survival. Our approach sets the ground for omic analysis inside clinical surroundings.

Workflow for tailored patient treatment



Late-stage cancer patient



Molecular profiling

- mutation panel
- transcriptomics
- proteomics

Morphological profiling

- subclones
- tumor
- stroma

Treatment selection

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Mapping Antibody-Antigen Interactions with Sidewinder: An XL-MS and Structural Modeling Approach

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Antibodies are critical to the host's immune defense against bacterial pathogens. Understanding the mechanisms of antibody-antigen interactions is essential for developing more targeted therapeutic interventions. Developing computational workflows that can deconvolute the complexities inherent to these interactions in a high-throughput manner is critical for advancing this field. Cross-linking mass spectrometry integrated with structural modeling offers a powerful strategy to map protein-protein interactions in complex molecular environments. However, the inherent complexity of the data requires robust analytical workflows. Here, we introduce Sidewinder, a modular, high-throughput pipeline combining state-of-the-art structural prediction, molecular docking, and rapid XL-MS analysis, enabling comprehensive interrogation of antibody–antigen systems. We validated this pipeline on murine antibodies targeting two Streptococcus pyogenes virulence factors. Using recently published data, we identified a well-defined monoclonal antibody epitope on Streptolysin O by generating and querying a large population of interaction models in a probabilistic manner. We also showcased Sidewinder's potential by challenging it with a more complex system, involving monoclonal antibodies that target the membrane-anchored M protein. The flexibility and robustness of Sidewinder provide a powerful framework for future studies of complex antibody-antigen systems, potentially leading to new therapeutic strategies.

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Two sides of plasma proteome analysis: mass spectrometry versus proximity extension assay

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Recent advances in proteomic technologies have expanded the depth and scale of plasma proteome analysis, providing new opportunities for biomarker discovery and insights into health and disease. Given the diversity of plasma proteomic platforms, understanding their performance is essential for making informed decisions regarding study design and platform selection. In this study, we evaluated the proteome coverage, precision, and agreement of mass spectrometry (MS) and the Olink Explore 3072 proximity extension assay (PEA), based on 88 plasma samples analyzed with both methods and a total of more than 4,300 proteins, with 1,132 overlapping between the methods. Our analysis revealed a complementary plasma proteome coverage, high precision for both platforms, and concordance in differential expression analysis. Cross-platform protein correlations were variable, with a median of 0.59 (IQR 0.33-0.75). Low correlations were associated with various data quality factors, most notably missing values in MS and values below the limit of detection in Olink Explore 3072. The complementary proteome coverage and poor quantitative agreement for a notable proportion of proteins underscore the added value of combining these platforms for a more comprehensive and reliable profiling of the plasma proteome.

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Inflammation links in Alzheimer's Disease: connecting fluid proteomics and TSPO PET.

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Background: This study aims to characterize immune-related proteins in cerebrospinal fluid (CSF) and plasma in the AD context.

Methods: Participants from the Translational Biomarker for Aging and Dementia Cohort (TRIAD) incorporating within the AD spectrum, and with available amyloid Aβ ([18F]AZD4694), tau ([18F]MK6240) and TSPO ([11C]PBR28) PET data (positivity = 2.5 SD > mean ROI-SUVR of young participants), had plasma (n= 151) samples analyzed with the NULISA technology (Alamar Biosciences®). Inflammation-related proteins (n=72) were selected for the analysis. Differential expression was evaluated with linear models (LIMMA) contrasting TSPO groups. After FDR correction for multiple comparisons, the differentially expressed proteins were selected for further analysis, where protein levels were correlated with the PET uptake of 45 anatomical brain regions.

Results: The differential expression analysis unveiled 5 proteins that are in higher concentrations in the plasma of TSPO positive in comparison with TSPO negative participants (Figure 1): GFAP, CHI3L1, CST3, FABP3, and CHIT1. The correlation analysis between the plasmatic proteins and PET uptake showed significant overlapping correlations with TSPO, Aβ and tau PET in brain regions such as inferior frontal gyrus, inferior occipital gyrus and amygdala (Figure 2).

Conclusion: These preliminary findings underscore the relevance of GFAP, CHI3L1, CST3, FABP3, and CHIT1 at proxying immune-related processes in AD, by linking peripherally quantified proteins to brain pathology, as suggested by the colocalized correlations with amyloid, tau and TSPO PET uptake. Further analysis of CSF proteomic data will help validate these proteins as valuable indicators of neuroinflammation in AD pathology.

Figure 1

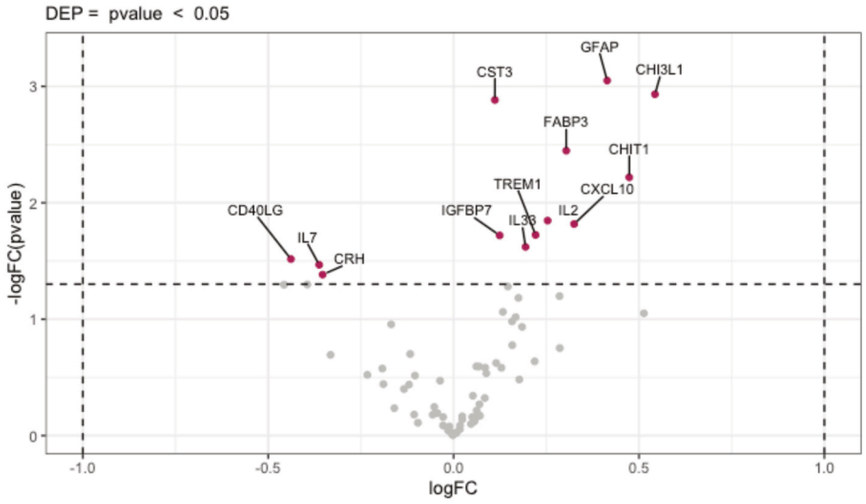
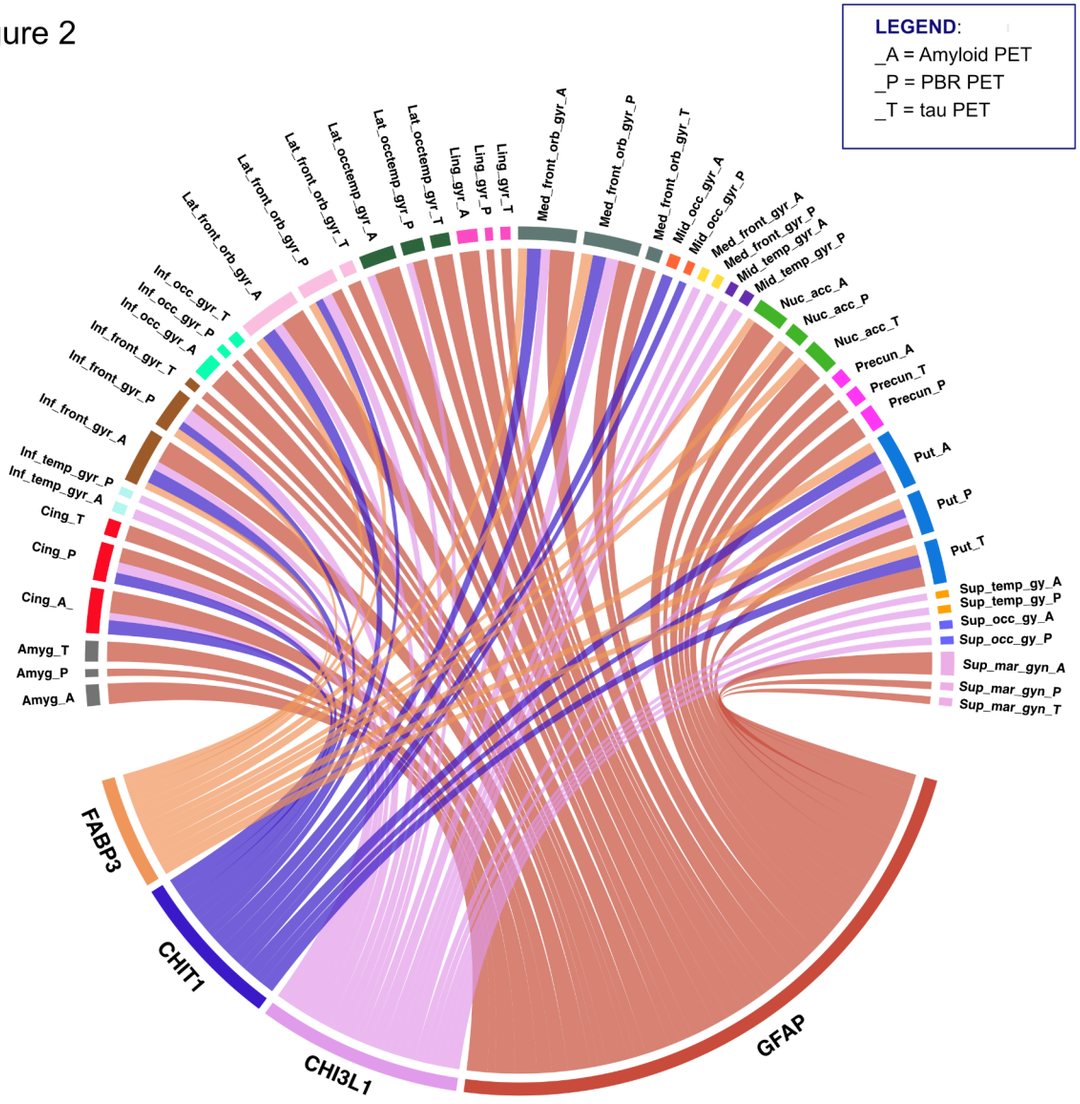


Figure 2



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Precision Medicine of Sepsis with Integrative Proteomics

Thanadol Sutantiwanichkul[1], Elin Palm[1], Fredrik Edfors[1], Kristoffer Strålin[2]

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Infections can be widely present in daily life however insufficient response can lead to organ dysfunction and consequently sepsis. It is still uncertain on the causation and prevention of it. In this work, we utilized a sepsis cohort from the human disease blood atlas with 1,536 protein targets from proximity extension assay (PEA). Additionally, several proteomics data from other studies were combined to remedy the specificity of the detection issues. The work focused on main types of bacterial pathogens causing sepsis including Escherichia coli and Streptococcus pneumoniae. Several clinical assays were also combined to resolve the complexity. From traditional case-control studies, there were several differentially expressed proteins from different sets of the cohort. However, as the sample size is relatively small to the number of detectable proteins, there were a few hundreds of highly different protein expressions in each group. This could lead to misinterpretation of the data. Concurrently, we found several clinical factors that can be useful for modeling the onset of sepsis with the admission time period. In conclusion, the combination of both clinical biomarkers and factors can potentially for the future of the study. On the one hand, there still need to combine several datasets from sepsis despite the sample rarity. This will allow us to analyze a much better ground truth of the causation and hopefully provide a hypothetical way for prevention. This work can be substantially helpful for practitioners at the frontline to utilize several high-throughput techniques to prevent sepsis within time.

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Uncovering proteomic alterations in Parkinson's disease using patient-specific iPSC-derived brain cells

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Parkinson's disease (PD) is characterized by motor impairment due to the degeneration of dopaminergic neurons in the substantia nigra pars compacta. Despite significant research, the mechanisms driving its initiation and progression remain unclear. This study aims to identify early disease mechanisms leading to neuronal injury by investigating the link between patients' genetic profiles and dysfunctional brain cell networks using induced pluripotent stem cells (iPSCs).

Preclinical studies suggest that iPSC-based patient stratification may play a crucial role in improving patient management and therapeutic interventions. iPSC-derived brain cell types from patients with idiopathic PD have demonstrated an intrinsic capacity to exhibit disease phenotypes similar to those observed in well-defined genetic cases.

We performed MS-based proteomic and phosphoproteomic analyses on dopaminergic neurons, astrocytes, and midbrain spheroids derived from iPSCs of 50 patients from the Parkinson's Progression Markers Initiative cohort. Over 7,500 proteins and 9,000 phosphopeptides were quantified per sample. Distinct proteomic and phosphoproteomic profiles were observed across different brain cell types and patient groups with varying genetic makeup. Several processes were dysregulated across all PD groups compared to controls, such as mitochondrial translation and the integrin β3 pathway. Furthermore, patient group-specific alterations, including differential kinase activity, were noted among the disease subgroups.

Our findings suggest that iPSC-derived brain cells from PD patients may serve as a valuable model for identifying early cellular dysfunctions preceding neuronal damage and clinical symptom onset. These results provide insights into the pathogenic processes underlying PD, which may inform improved patient stratification and the development of personalized therapeutic strategies.