Pump-Priming Grant Report

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Title: Assessing Biological Age in Liver Transplantation: Proteomic Markers for Predicting Graft Viability and Patient Survival.

Objective: This study assessed proteomic profiles in donor livers at the time of retrieval to evaluate their correlation with biological age and their predictive value for graft viability and patient survival following transplantation. By analysing global protein expression patterns, we identified proteomic signatures associated with donor biological age and post-transplant outcomes.

Liver tissue samples from Donation after Brain Death (DBD) and Donation after Circulatory Death (DCD) donors were examined to determine the impact of donor age, graft function, and donor type on protein expression. Through large-scale proteomic analyses and computational modelling, this study improved organ selection strategies, reduced unnecessary organ discard, and contributed to better long-term transplant outcomes. The identification of reliable proteomic markers of donor liver quality refined risk stratification and enhanced objective decision-making in liver transplantation.

Method: A total of 80 biopsy samples from Donation after Brain Death (DBD) and Donation after Circulatory Death (DCD) donors were evaluated, representing different post-transplantation outcomes (Table 1). Tissue biopsy samples were first assessed for size and weight, followed by total protein recovery using a combination of chemical and mechanical extraction methods.

Clinical characteristics		DBD	DCD
Donor_Type (%)		39 (57.4)	29 (4.6)
Donor Age	Young (Mean ± SD)	27.5 ± 3.5	33 ± 3.5
	Old (Mean ± SD)	63 ± 3.7	62.5 ± 4.7
GSTATUS	IF (%)	20 (50)	20 (50)
	Graft failure (%)	19 (68)	9 (32)

Table 1: Clinical Characteristics of donors.

Protein concentration was estimated using the Bicinchoninic Acid (BCA) assay, and equal amounts of protein from each sample were subsequently digested and labelled with Tandem Mass Tag (TMT) 11-plex reagents. Phosphopeptides were then enriched using the high-select TiO_2 Phosphopeptide Enrichment Kit and analysed on an Orbitrap Fusion Mass Spectrometer. All data processing was performed using Proteome Discoverer, with protein identification conducted against the Uniprot Human database. The Motif Analysis tool from PhosphoSitePlus was used to confirm phosphorylation sites in DCD and DBD groups. Differentially regulated phosphopeptides and proteins in each group were identified using the Bioconductor Software Package (LIMMA) in R Studio.

To explore the underlying biological pathways, over-representation analyses of Gene Ontology (GO) terms, including cellular components, biological processes, and molecular functions, were conducted. These analyses provided insights into the signaling pathways differentially regulated between DCD and DBD donor groups.

Results: A total of 6,950 proteins were identified in liver tissue samples. After applying stringent quality control measures, 6,440 proteins were confirmed based on the presence of at least one unique peptide, the detection of that peptide in at least two independent analyses, and high-confidence identification with a statistical significance threshold of $p < 0.05$. Proteomic analysis revealed both unique and overlapping protein signatures associated with Donor Age, Graft Function, and Donor Type. A Venn diagram (Figure 1) illustrates the distribution of these proteins, with 175 proteins uniquely associated with donor age, 339 proteins linked exclusively to graft function, and 66 proteins associated with donor type. Notably, 13 proteins were common to both donor age and graft function, suggesting that these markers may play a crucial role in aging-related changes that affect graft viability. This overlap highlights the biological interplay between age-related liver function decline and graft performance, emphasizing the need for further exploration of these proteins as potential biomarkers for transplantation outcomes.

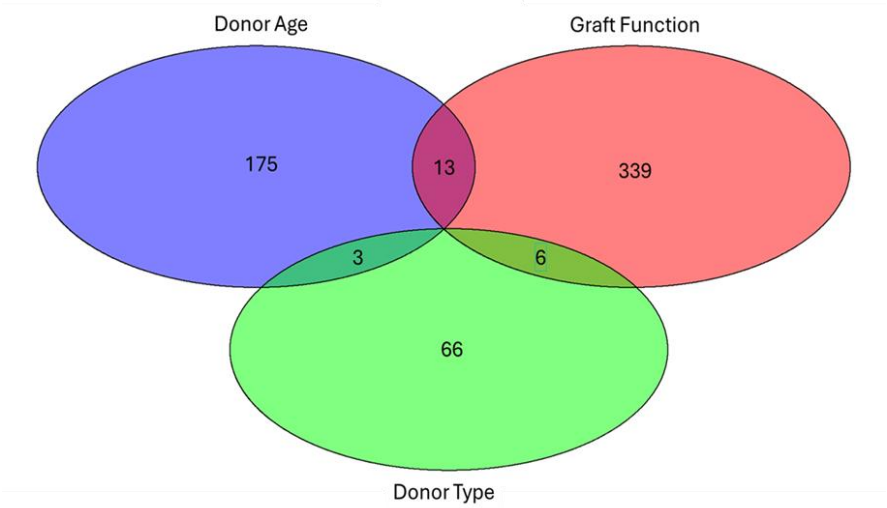

Figure 1: Key Overlaps in Liver Proteome: Donor Age, Graft Function, and Donor Type show both unique and shared protein signatures, with 13 proteins related to age and Graft function.

To estimate donor biological age, an SVM model was developed using the 13 significant proteins identified in the proteomic analysis. The model demonstrated strong predictive ability, achieving a Root Mean Square Error (RMSE) of 10, with optimal parameters set at C = 0.001 and σ = 1. A scatter plot (Figure 2) depicting the correlation between predicted biological age and actual donor age reveals a general alignment between the two, with some variance observed. The red dashed line represents a perfect 1:1 correlation, and while individual data points deviate slightly, the model successfully captures aging-related proteomic changes in the liver.

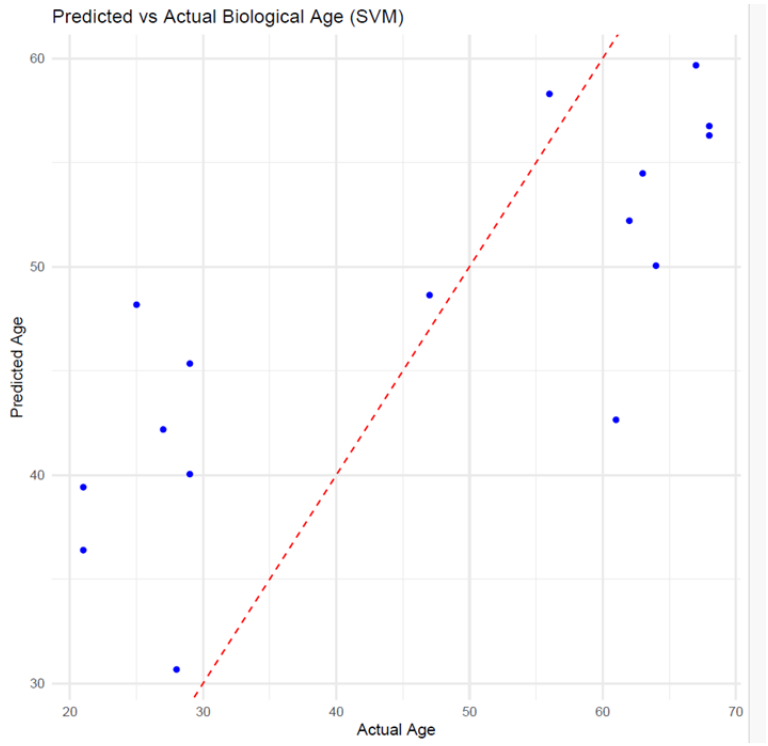

Figure 2: Prediction of Biological Age Using Proteomic Markers

Pathway enrichment analysis using Reactome provided further insights into the biological mechanisms underlying these proteomic changes. Older donors exhibited significant upregulation of amino acid metabolism (p = 2.72E-19), biological oxidations (p = 7.62E-18), and fatty acid metabolism (p = 8.97E-09), indicating increased metabolic activity in aging livers. Additionally, dysregulation of mRNA splicing (p = 3.77E-15) was observed, suggesting potential links to chronic diseases commonly associated with aging. These findings indicate that age-related metabolic alterations may influence liver function and graft viability, potentially impacting long-term transplant success.

Overall, these results demonstrate that liver proteomics can provide valuable insights into biological aging and graft function, with specific protein markers serving as potential predictors of graft viability. The identified proteins and pathways represent promising targets for further investigation, which could improve donor selection strategies and enhance liver transplantation outcomes.

Outputs (publications/presentations)

The abstract titled 'Assessing Biological Age in Liver Transplantation: Proteomic Markers for Predicting Graft Viability and Patient Survival' has been accepted for a poster presentation in the Clinical Proteomics session at the American Society for Mass Spectrometry 73rd Conference, taking place from 1st to 5th of June 2025, at the Baltimore Convention Center, Baltimore, Maryland.

Next Steps (what is it leading to)

The next steps involve advancing the research towards developing a more robust Support Vector Machine (SVM) model for assessing the biological age of the liver. Additionally, the focus will be on investigating post-translational modifications (PTMs) of proteins, including deamidation and carbamylation, which are currently undergoing data analysis, while phosphorylation is in the process of data acquisition. Oxidation, on the other hand, is in the method development phase. Furthermore, the validation of 13 signature proteins associated with age and graft function will be carried out using targeted mass spectrometry through the Liquid Chromatography - Parallel Reaction Monitoring (LC-PRM) assay.