

Plasma proteomic signatures in chronic kidney disease versus healthy controls utilising data-independent acquisition mass spectrometry

Rowena Brook¹⁻⁴, Laura F Gianni⁵⁻⁶, Jumana Yousef⁵⁻⁶, Prahlad Ho^{1-4,7-8}, Hui Yin Lim^{1-4,7-8}

1. Northern Clinical Diagnostics & Thrombovascular Research (NECTAR), Northern Health 2. Northern Pathology Victoria 3. Hematology Department, Northern Health 4. Department of Medicine – Northern Health, University of Melbourne 5. Proteomics Facility, Walter and Eliza Hall Institute of Medical Research 6. Department of Medical Biology, The University of Melbourne 7. Australian Centre for Blood Diseases, Monash University 8. School of Health and Biomedical Sciences, RMIT University

Northern Health



RESEARCH WEEK
19-22 OCTOBER 2026
INSPIRED RESEARCHERS
Northern Health

AIMS

We aim to characterise plasma proteomic differences between CKD patients and healthy controls to identify potential biomarkers of CVD.

MATERIALS & METHODS

Comparative Biomarker Discovery Study:

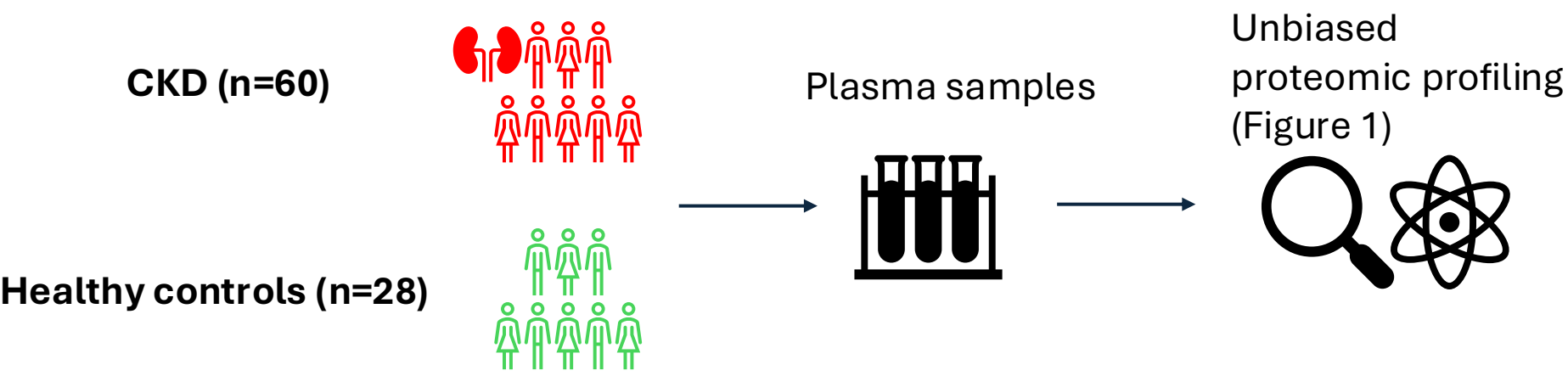
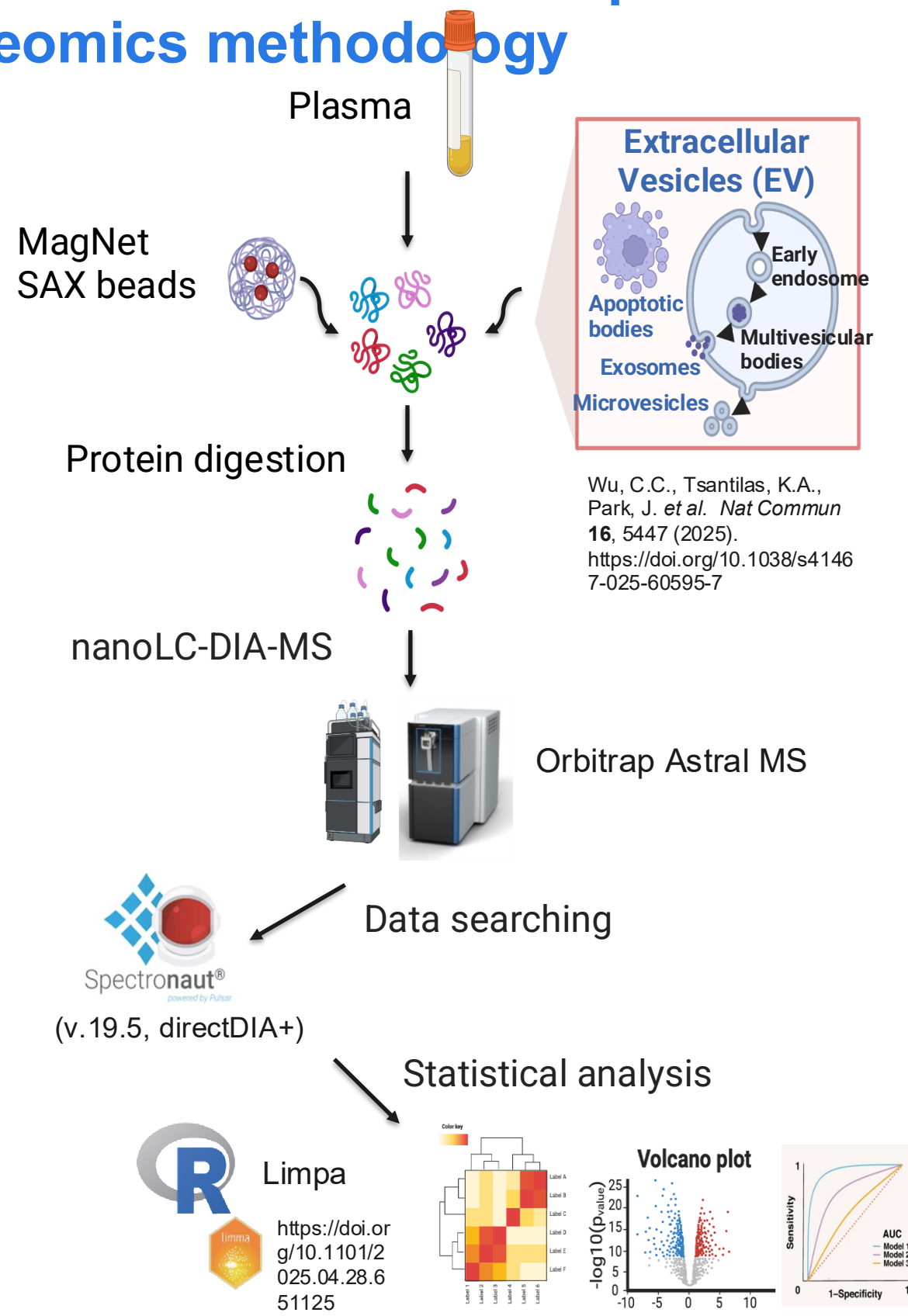


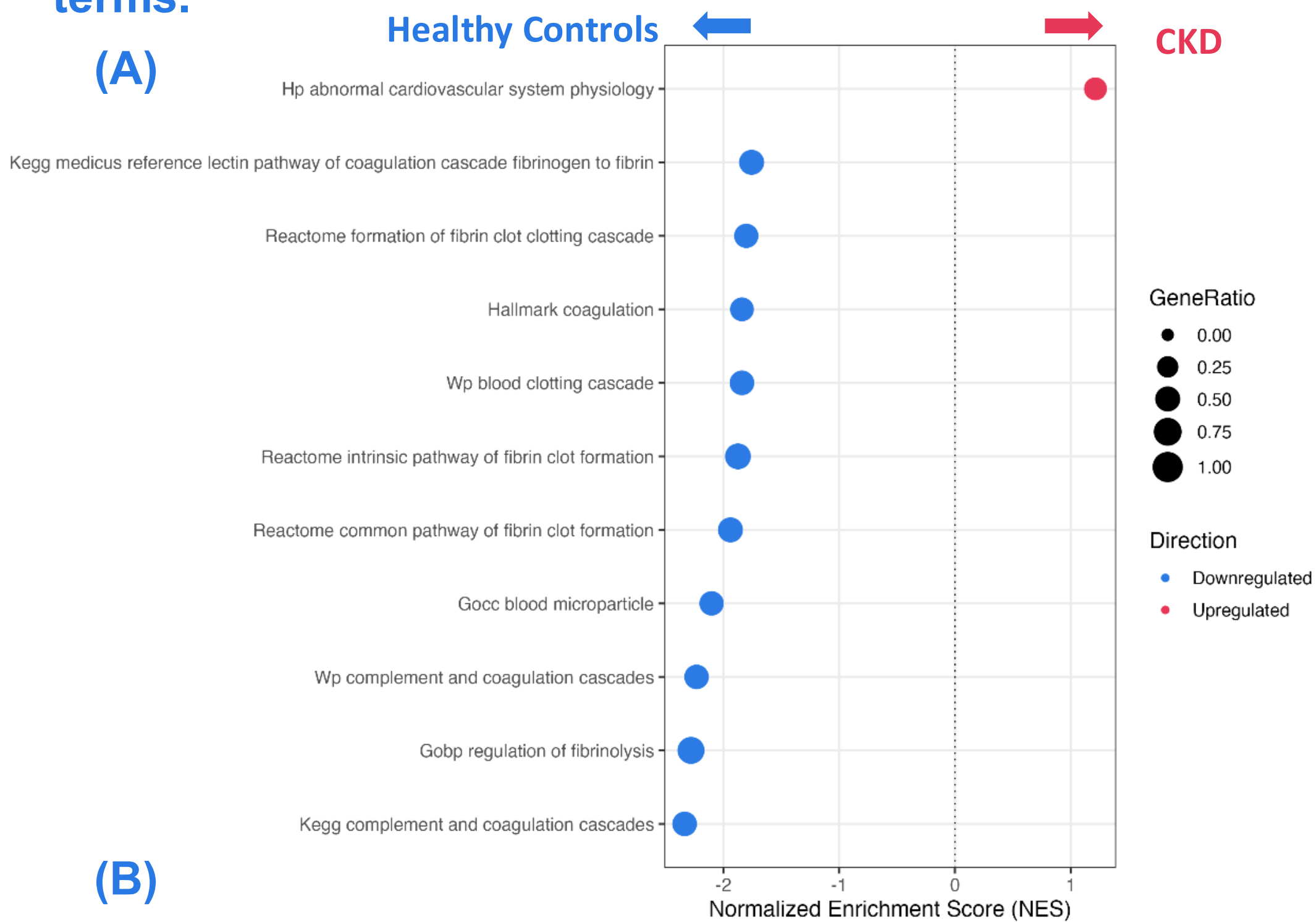
Figure 1. Unbiased mass spectrometry based proteomics methodology



Plasma samples were subjected to the SAX EV enrichment protocol. Peptides were analysed via nanoLC-DIA-MS analysis on an Orbitrap Astral MS on a 22 minute gradient on a 15 cm Aurora column (IonOpticks). Data were searched using directDIA (Spectronaut v19.5) prior to in-house statistical analysis using available metadata.

RESULTS

Figure 2. (A) GSEA reveals significantly enriched of biological pathways in CKD vs healthy controls. (B) Description of GSEA standard Molecular Signatures Database terms.



The dot plot displays significantly enriched terms with only biological processes with a false discovery rate (FDR) <0.10 shown. The x-axis represents the normalised enrichment score (NES). Red dots indicate processes enriched among upregulated proteins in CKD patients compared to healthy controls, while the blue dots indicated those enriched down-regulated proteins. Dot size reflects the number of proteins contributing to each term.

(B)

Standard Name	Description
Hp abnormal cardiovascular system physiology	Abnormal cardiovascular system physiology
Kegg medicus reference lectin pathway of coagulation cascade fibrinogen to fibrin	Lectin pathway of coagulation cascade
Reactome formation of fibrin clot clotting cascade	Formation of fibrin clot
Hallmark coagulation	Genes encoding components of blood coagulation system
Wp blood clotting cascade	Blood clotting cascade
Reactome intrinsic pathway of fibrin clot formation	Intrinsic pathway of fibrin clot formation
Reactome common pathway of fibrin clot formation	Common pathway of fibrin clot formation
Gocc blood microparticle	A phospholipid microvesicle derived from any of several cell types (e.g. platelets, blood cells, endothelial cells) and contains membrane receptors as well as other proteins characteristic of the parental cell
Wp complement and coagulation cascades	Complement and coagulation cascades
Gobp regulation of fibrinolysis	Any process that modulates frequency, rate or extent of fibrinolysis
Kegg complement and coagulation cascades	Complement and coagulation cascades

Median age 64 years with 62.5% males (n=55). Comparable age (p=0.45) and male sex percentage (p=0.48) between healthy controls and CKD cohort (Table 1).

CKD Cohort had:
Lower median haemoglobin (p<0.001) and higher white cell count (p=0.001).
Median eGFR of 7ml/min/1.73m2 and creatinine of 543µmol/L.
78.3% (n=47) dialysis dependence with the majority on haemodialysis (66%, n=31).

Gene set enrichment analysis (GSEA) was performed on 34 molecular signature database pathways relevant to CVD to identify functional impacts of the proteomic differences (Figure 2).

CKD patients demonstrated:

- **Enrichment** of Hp abnormal cardiovascular system physiology which is associated with **cardiovascular dysfunction**.
- **Suppression** of genes associated with **coagulation pathways** were identified, involving intrinsic and common pathways, fibrinogen and fibrinolysis and with blood microparticles (phospholipid microvesicles) was identified.

This likely **reflects mechanisms of the coagulopathy seen in CKD patients contributing to their CVD risk**.

CONCLUSION

We identified a plasma proteomic signature in the CKD cohort reflecting abnormalities of cardiovascular physiology along with altered coagulation pathways including multiple fibrin and fibrinolysis pathways. This data identifies likely mechanisms leading to the high CVD risk in CKD patients and presents future opportunities for development of risk stratifying targeted biomarkers.