

Identification of a proteomic signature linked to major adverse cardiovascular events in chronic kidney disease using data-independent acquisition mass spectrometry

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Northern Health



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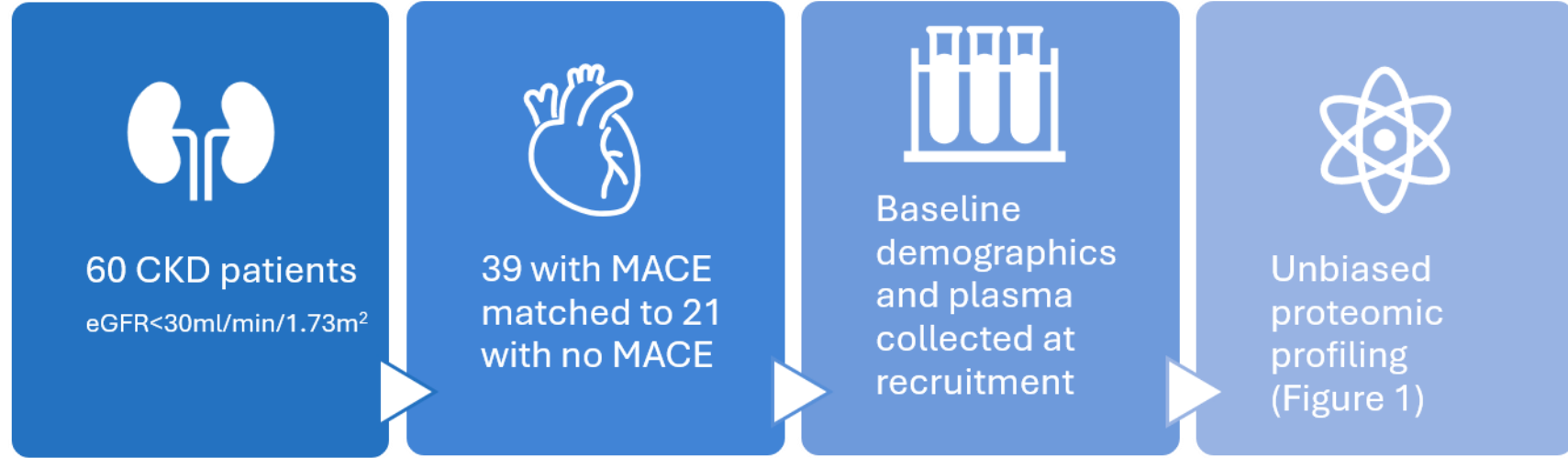
BACKGROUND & AIMS

Cardiovascular disease (CVD) is the leading cause of mortality in chronic kidney disease (CKD), yet accurate risk prediction remains elusive.

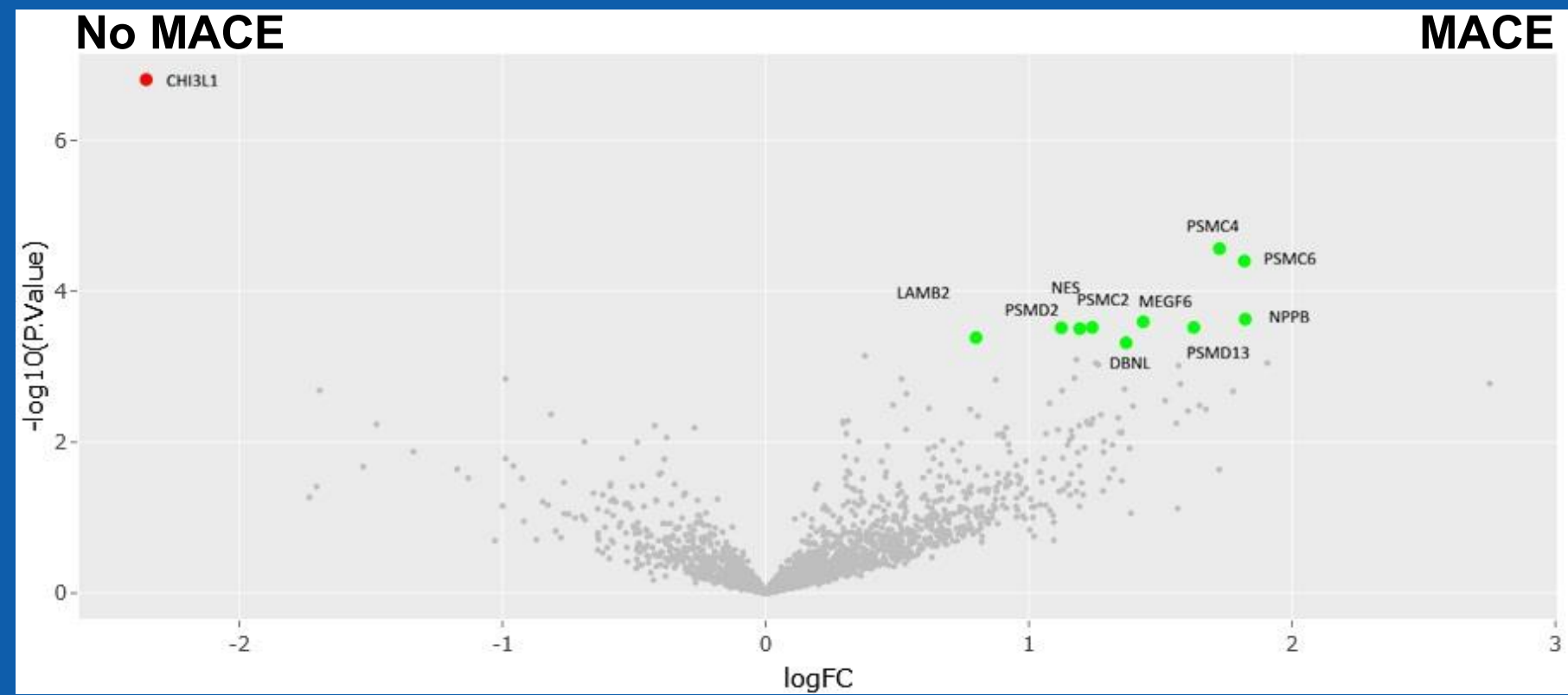
We aim to characterise plasma proteomic differences between CKD patients who experienced a major cardiovascular adverse event (MACE) and those who did not.

MATERIALS & METHODS

Case-control biomarker discovery study:



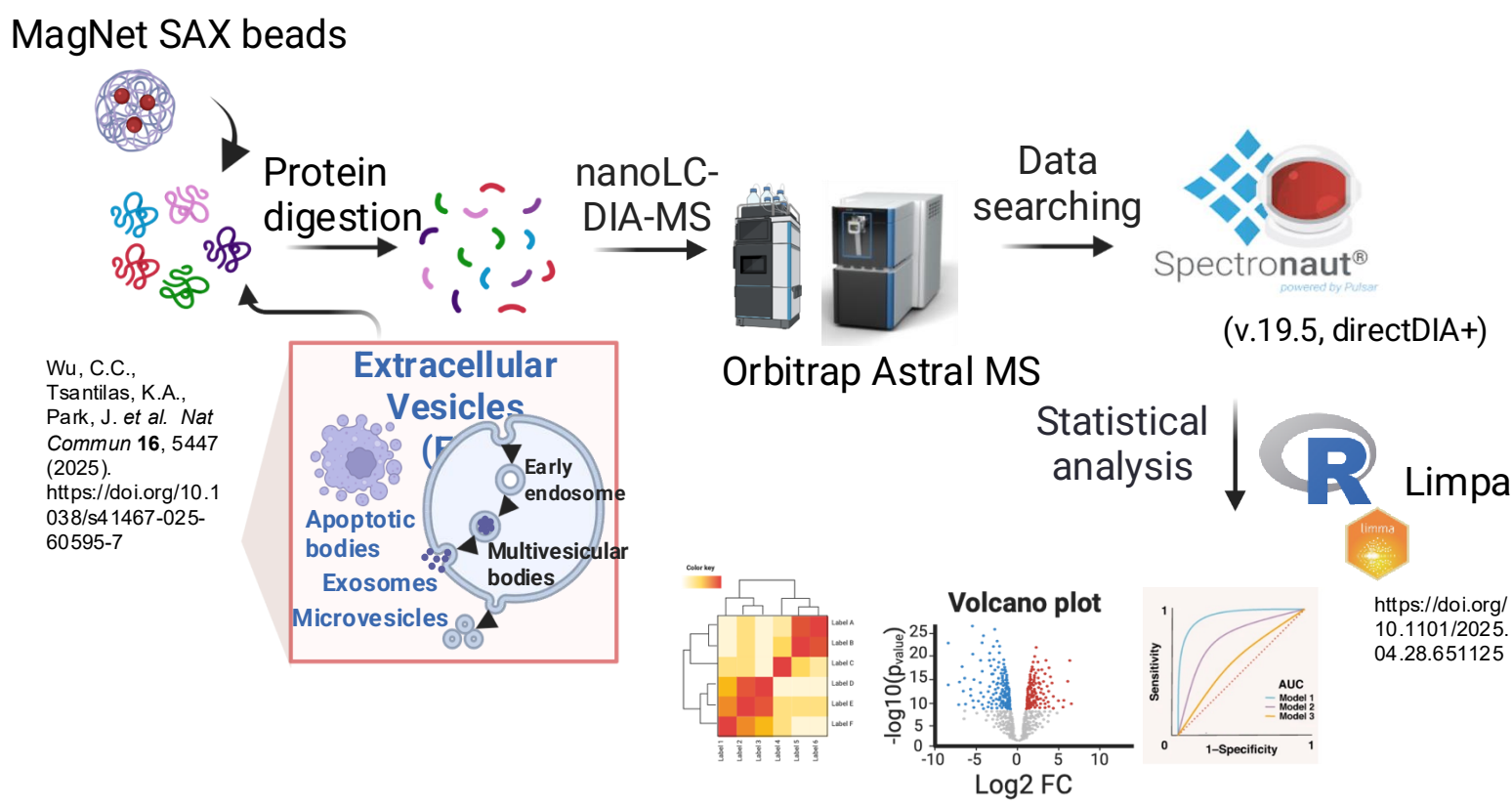
Median time to MACE event 356 days.
Median follow up time 741 days.



Differential expression analysis of MACE vs no event populations identified 11 significant proteins. Comparing MACE to no MACE populations, significant downregulated proteins are indicated with red dots, unchanged in grey and significantly upregulated in green (adj p<0.05).

MATERIALS & METHODS cont.

Figure 1. Unbiased mass spectrometry based proteomics methodology

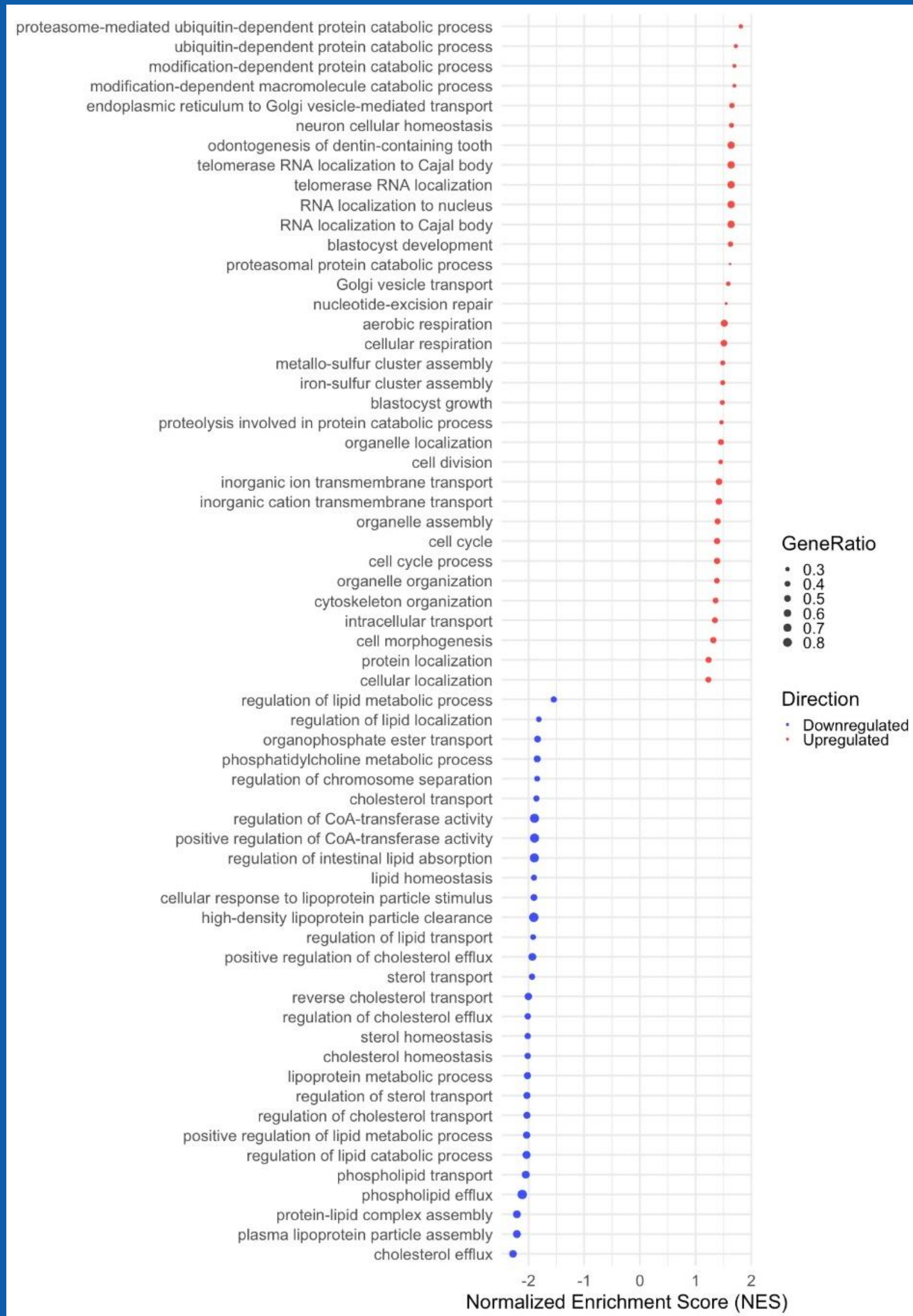


Plasma samples were subjected to the SAX EV enrichment protocol. Peptides were analysed via nanoLC-DIA-MS analysis on an Orbitrap Astral MS on a 22 minute gradient on a 15 cm Aurora column (IonOpticks). Data were searched using directDIA (Spectronaut v19.5) prior to in-house statistical analysis (custom R pipeline including limpa v.1.1.7) using available metadata.

RESULTS FIGURES

Figure 3. Gene set enrichment analysis (GSEA) pathway analysis reveals significantly enriched biological processes in MACE vs no MACE.

The dot plot displays significantly enriched gene ontology terms with only biological processes with a false discovery rate (FDR) <0.05 shown. The x-axis represents the normalised enrichment score (NES). Red dots indicate processes enriched among proteins up-regulated in MACE, while blue dots indicate those enriched among proteins down-regulated in MACE. Dot size reflects the number of proteins contributing to each term.



RESULTS

60 patients with median age 65.4 years, 65% males with median follow up of 2 years.

- Between groups no difference in eGFR (p=0.28) but more MACE patients were dialysis-dependent (57.1% vs 89.7%, p=0.003). There were **11 differentially expressed proteins** (adj p-value <0.05) (Figure 2).

To elucidate **functional implications** of findings GSEA was performed on the differentially expressed proteins (Figure 3).

We observed significant upregulation of key biological pathways related to proteasome-mediated ubiquitin-dependent protein catabolic processes, cell cycle regulation, and mitochondrial repair pathways.

- Suggesting **heightened proteostatic and cellular stress**.

Conversely, lipid and cholesterol transport pathways including cholesterol efflux and HDL particle remodelling were significantly downregulated.

- Indicating **impaired HDL functionality and dysregulated lipid metabolism**.

CONCLUSION

CKD patients with MACE demonstrated **distinct plasma proteomic signatures** marked by: **Impaired lipid metabolism, increased proteasome activity and cellular stress pathways**.

Importantly, this study utilised an **unbiased mass spectrometry proteomics** technique offering **hypothesis-free biomarker discovery** in contrast to other targeted proteomics techniques. These findings highlight potential mechanisms of CVD and opportunities for biomarker development and targeted intervention.