Molecular similarity of renal ageing and CKD revealed by urinary proteomics

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INTRODUCTION AND OBJECTIVES

Ageing is a complex and multi-mechanistic process that is associated with an increased prevalence of age-related chronic conditions affecting organs such as the kidney.

Understanding the molecular mechanisms of normal renal ageing could help provide clues for both renal ageing and kidney diseases management.

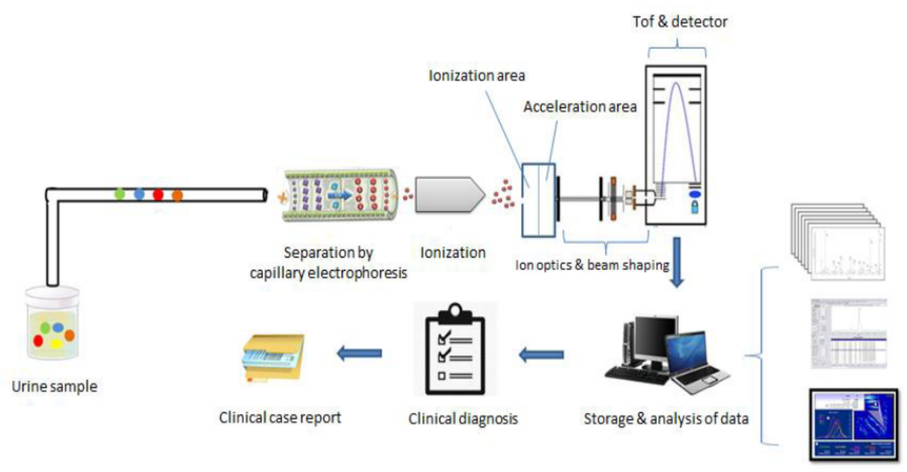
Urinary peptides associated with chronic kidney disease (CKD) onset and progression have been described in multiple studies, and recently correlation of urinary peptides with the estimated glomerular filtration rate (eGFR) and also with the future eGFR decline were investigated in detail [1].

The most prominent molecular change observed in CKD was the reduction of type I collagen peptides, indicating involvement of the extracellular matrix (ECM) in CKD progression. **The purpose of this study was to investigate renal ageing at a molecular level using urinary proteomics** in the context of iMode-CKD and CodeAge projects using a unique cohort of over 10000 datasets.

METHODS

Datasets from **11560 individuals** between 20 and 86 years of age were retrieved from the "Human proteome urinary" database [2].

CE-MS instrumental setup



Correlation analysis and statistical analysis

- Age as a continuous variable
- Spearman's rank correlation was used
- The statistical significance was set at $p<0.05$
- Cut-off value was set for rho at ≥ 0.2 or ≤ -0.2 ($|\rho|\geq 0.2$)

***In silico* protease prediction**

Urinary fragments were linked to the proteases involved using Proteasix software. Proteasix is based on the NCBI-database and SwissProt protease consensus sequences, where hexapeptide consensus sequences were matched with the observed naturally occurring urinary peptides.

RESULTS AND CONCLUSIONS

112 age-correlated peptides were identified and confirmed.

- Decrease of type I collagen fragments
- Increase of uromodulin and fibrinogen

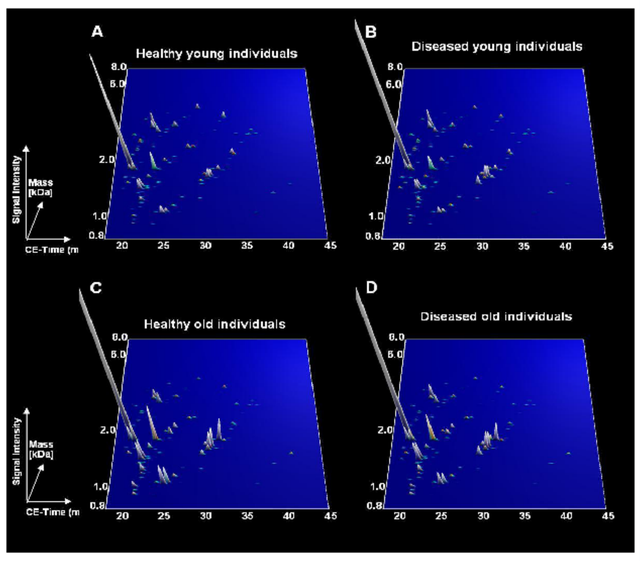


Figure 1: Urinary peptide marker pattern for the differentiation between healthy and diseased individuals. A. Healthy young between 20-29 years of age. B. Diseased young between 20-29 years of age. C. Healthy old from 60 years old of age and above. D. Diseased old from 60 years old of age and above.

Out of 112 peptides identified, 100 peptides were significant in the CKD subgroup.

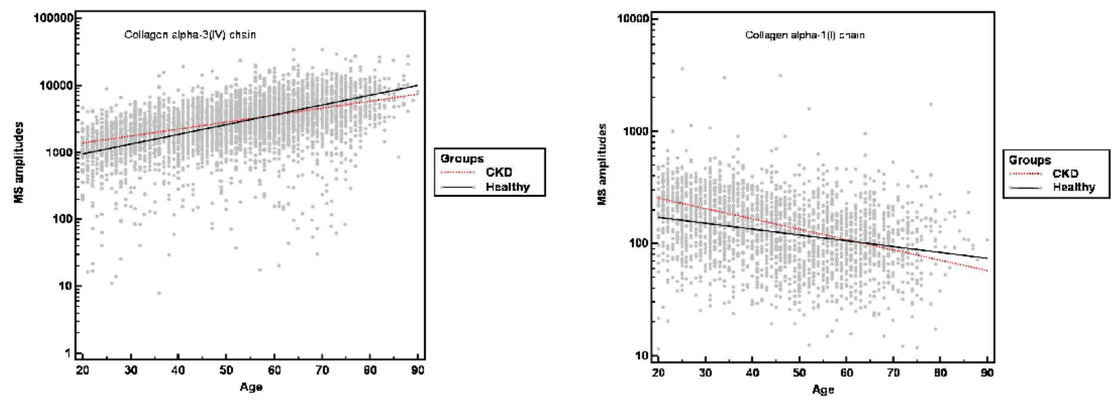


Figure 2: Correlation analysis of individual urinary peptides in healthy and CKD with age. Collagen alpha-3(IV) chain (ρhealthy=0.504, $p<0.0001$ and ρCKD=0.399, $p<0.0001$). Collagen alpha-1(I) chain (ρhealthy=-0.324, $p<0.0001$ and ρCKD=-0.472, $p<0.0001$).

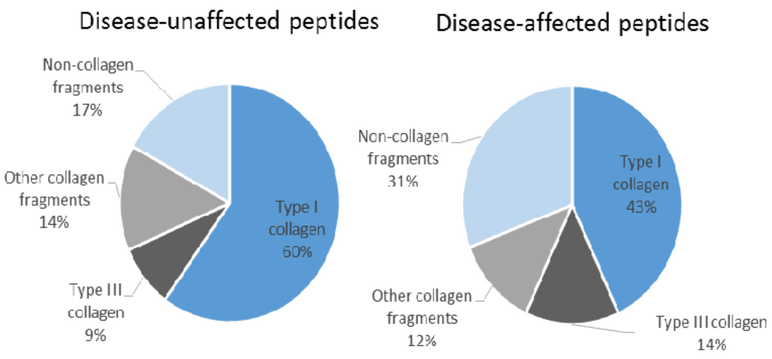


Figure 3: Comparison of age-related peptides identified in the healthy and CKD.

Comparison of age-correlated peptides with the previously established CKD273 classifier depicted overlapping peptides including type I collagen I, uromodulin and fibrinogen and unique peptides in both ageing and CKD273 classifier.

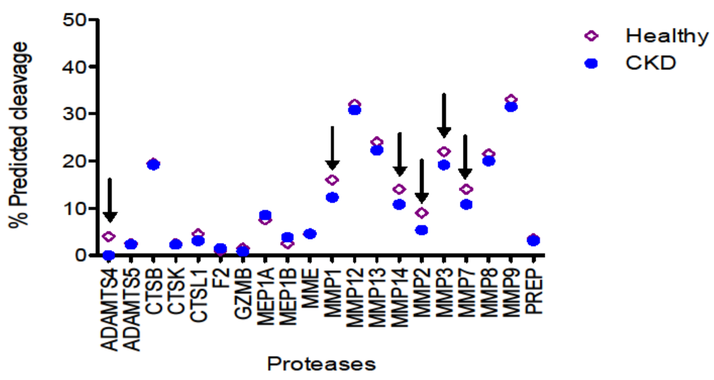


Figure 4: Comparison of age-correlated proteases between healthy individuals and CKD. Arrows underscore the main changes in predicted protease activity between age-correlated disease-affected peptides in the healthy group the disease subgroups. ADAMTS4: A disintegrin and metalloproteinase with thrombospondin motifs 4; CTSB: cathepsin B; CTSK: cathepsin K; CTSL1: cathepsin L1; F2: thrombin; GZMB: granzyme B; MEP1A: meprin A subunit alpha; MEP1B: meprin A subunit beta; MME: neprilysin; PREP: prolyl endopeptidase

- Similarities in molecular mechanisms between ageing and CKD.
- Fibrosis observed in ageing and CKD characterised by ECM alterations and decreased in type I collagen fragments.
- Protease analysis revealed a greater influence of CKD.
- Perturbations in basement membrane ECM was uniquely observed in ageing whereas inflammation processes were distinct to CKD.

REFERENCES

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2. *Siwy J, Mullen W, Golovko I, Franke J, Zürbig P. Human urinary peptide database for multiple disease biomarker discovery. Proteomics Clin Appl 2011; 5: 367-374.*

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