Urinary proteome analysis of autosomal polycystic disease patients selected by a TKV imaging classification method

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Background:

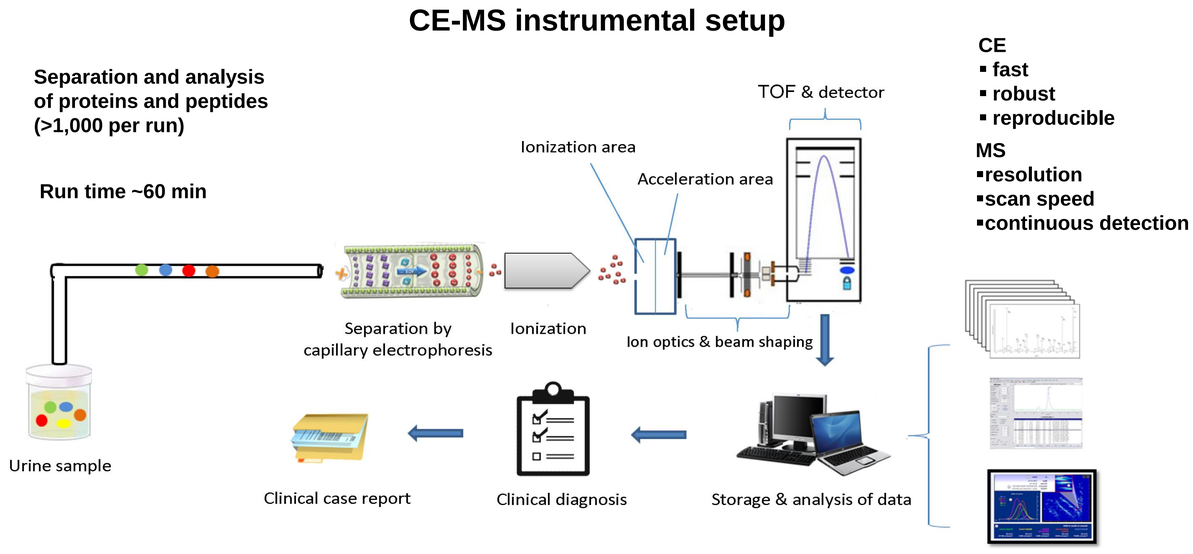
The rate of disease progression in individuals with autosomal polycystic kidney disease (ADPKD) is highly variable and its prediction remains difficult. Enlargement of the numerous fluid-filled epithelial cysts developing in both kidneys leads to progressive loss of renal function and ultimately end stage renal disease (ESRD). In the Consortium for Radiologic Imaging Studies of Polycystic Kidney Disease (CRISP) cohort, total kidney volume (TKV) has been established as a prognostic marker for selection of patients appropriate for enrolment into clinical trials and/or effective therapy. In our current study, we aimed to investigate the changes of the urinary proteome of ADPKD patients selected by the TKV imaging classification method of Irasabal et al[1].

Materials and Methods:

Urine proteome analysis:

Electronically available abdominal computer tomography scan (CT) or magnetic resonance imaging (MRI) from 177 ADPKD CRISP participants were reviewed. 173 of them were classified as having typical ADPKD symptoms and were subclassified into five classes (1A-1E) based on age, gender, baseline TKV and baseline serum creatinine concentration. The classes typically related to different kidney growth rates. 1A <1.5%, 1B 1.5% - 3%, 1C 3% - 4%, 1D 4.5% - 6%,1E > 6%/year increase in TKV. Urine samples of these patients were analyzed by capillary electrophoresis online coupled to mass spectrometry (CE-MS) technology. This method allows to **simultaneously** quantify > 1.000 low molecular weigh peptides in a single urine sample with high degree of reproducibility. Sequence identification was attempted by using LC-MS/MS.

CE-MS instrumental setup



On-line coupling of CE for peptide m/z separation and ESI-TOF-MS for mass detection. CE-MS coupling uses a coaxial sheath-flow system. The setting allows sensitive and fast mapping of low-molecular weight urinary proteomes. Spectra of identified polypeptide peaks are transduced to peptide lists for comparative analysis and support vector machine (SVM) learning to generate multimarker models.

Statistical analysis:

Comparison of their urinary proteome between each of the group (1A-1E) was performed in order to identify peptide markers that were significantly associated with disease severity. Peptide distribution differences between the subclasses were calculated on the bases of natural logarithm transformed intensities and the Wilcoxon rank sum test. Statistical adjustment for the multiple testing was performed by the method of Benjamini and Hochberg.

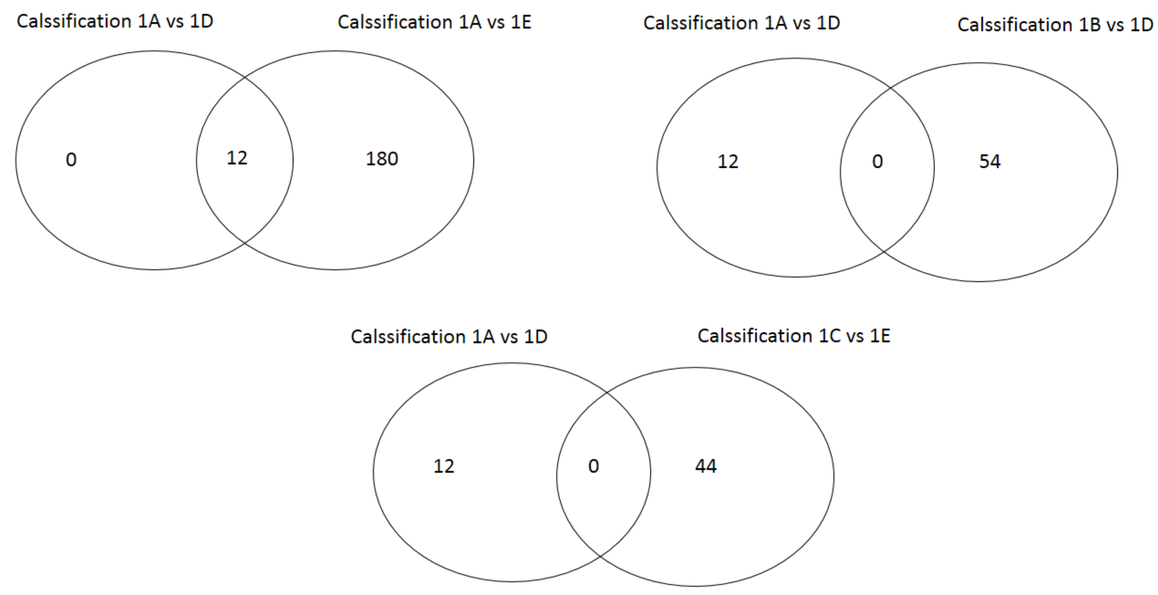
Clinical proteome data processing system

Storage and retrieval of CE-MS peptide spectra, peptide sequences and patient clinical records in a central database for appropriate sample selection and comparative proteome analysis.

Results:

- Urine proteome analysis and comparisons of the subclasses 1A vs 1D, 1A vs 1E, 1B vs 1D, 1C vs 1E revealed 12, 192, 54 and 44 peptide markers significantly differentiated between the investigated groups (figure 1A).
- All 12 peptide markers that differed from subclasses 1A vs 1D were also among the 179 differentiating classes 1A vs 1E (figure 1A). We found 18 and 17 overlapping peptides by comparing subclasses 1A vs 1E with both 1B vs 1D and 1C vs 1E patients. There were few peptide markers when comparing just 1B vs 1D and 1C vs 1E (figure 1B).

A)



B)

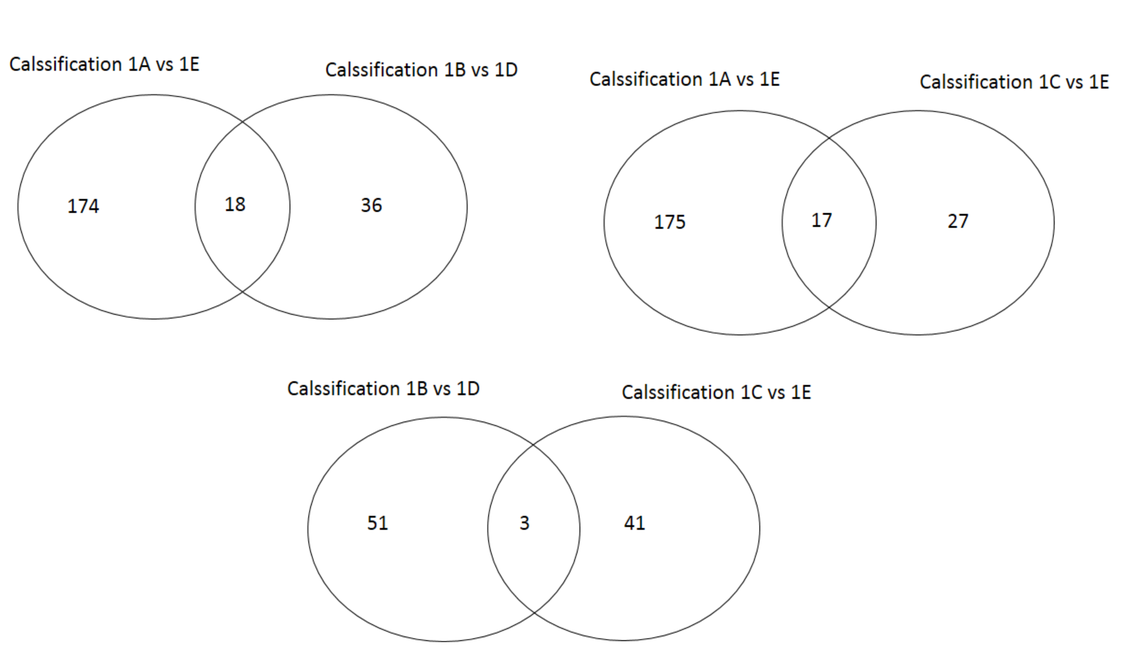


Figure 1. A) Overlap of the urinary peptide markers of the subclasses 1A vs 1D, 1A vs 1E, 1B vs 1D, 1C vs 1E and B) overlap of the urinary peptide markers of the subclasses 1A vs 1E, 1B vs 1D and 1C vs 1E.

- We were able to obtain sequence information for 9, 103, 32 and 19 peptide markers from the comparison of classes 1A vs 1D, 1A vs 1E, 1B vs 1D and 1C vs 1E. Majority of the peptides were belonging to collagen alpha (I) chain and collagen alpha (III) chain fragments. These results imply alternation of extracellular matrix (ECM) remodeling during cysts enlargement. Other common identified peptides were fragments of fibrinogen alpha chain, serum albumin, hemoglobin, uromodulin and membrane associated progesterone receptor component 1.

Conclusion:

Our study shows that urine proteome analysis may add biological and possibly prognostic information in the risk stratified ADPKD patients based on the Irasabal classification system.

Reference:

[1] Irazabal MV et al., J Am Soc Nephrol. 2015 Jan;26(1):160-72

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