ADPKD is the most common monogenic renal disease and affects ~1:400-1:1000 people worldwide. It is characterised by bilateral kidney enlargement as a result of progressive expansion of numerous fluid-filled, tubule-derived epithelial cysts and varying amounts of interstitial fibrosis. The rate of renal functional decline leading to end-stage renal disease (ESRD) is highly variable in ADPKD patients and there is no single prognostic test available to predict disease progression. We have analysed the protein profiles of purified urinary exosomes (UEXs) to assess their potential for development of a simple, non-invasive, biomarker test to predict disease progression.

Exosomes are small, nano-sized, endosome-derived vesicles (30-120nm) that are released from all mammalian cells and contain characteristic membrane proteins and intravesicular cargo. They function in intercellular communication and are secreted into body fluids including urine. Since these vesicles have been shown to be released in increased numbers in many cancers, where they can be used as diagnostic aids, they have been predicted to hold specific biomarker potential in acute and chronic progressive renal diseases.

The aims of this study were to characterise the protein profiles of unaffected versus ADPKD patient UEXs and to evaluate the potential of proteomics to identify and stratify patients into slow- or rapid-progression groups. Coordinated clinical data, urine and blood samples have been collected from >350 ADPKD patients attending the Royal Free specialist clinic every 6-12 months for >4 years, allowing for longitudinal sample study. Multiple protease inhibitor-treated urine aliquots are stored at -80oC in the PKD Charity UK-sponsored Biobank.

An ultracentrifugation with filtration protocol for isolation and purification of UEXs from small (5ml) volumes of urine was optimised and validated by nanoparticle tracking analysis, electron microscopy and immunoblot marker analysis. A pilot study using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) comparing ADPKD patient with unaffected control UEXs (n=6/group) identified distinct profiles with 79 shared proteins, 37 up-regulated (>2-fold) and 17 down-regulated (>2-fold) proteins in ADPKD UEXs. Preliminary analyses also suggest differences in the total numbers and size ranges of exosomes excreted by ADPKD patients compared to normal unaffected control subjects.

Quantitative LC-MS/MS using Tandem-Mass-Tags and isoelectric focusing was carried out to compare UEX protein profiles from ADPKD patients with rapidly progressive disease (PG, n=10) versus age- and gender- matched patients with non-progressive disease (NPG, n=10). 291 proteins were up-regulated >2-fold (involved in focal adhesion and growth factor-mediated proliferation and signalling) and 15 down-regulated >2-fold (involved in cell differentiation and apoptosis) in PG-UEXs compared to NPG-UEXs. Comparisons between the ADPKD PG and NPG patients, dependent on their initial status of renal function (eGFR >70; 50-69; <49 ml/min/1.73m2) at onset of the 4-year clinical observation period, revealed that the most common pathways to be upregulated in PG-UEXs were those associated with proliferation-related, growth factor-mediated intracellular signalling as well as cell and matrix adhesion.

We conclude that UEXs show potential as a ready source of biomarkers with new prognostic and therapeutic potential to stratify ADPKD patients at high risk of progression to ESRD dependant on their eGFR at presentation.