Optimization of Urinary Extracellular Vesicles Analysis to Study Kidney Disease in People with Cystic Fibrosis

Hayrettin Yavuz, M.D., Ph.D.

Postdoctoral Research Associate

Urban Lab, Pediatric Nephrology

University of Virginia

e-mail: eeu9ng@virginia.edu

Disclosure

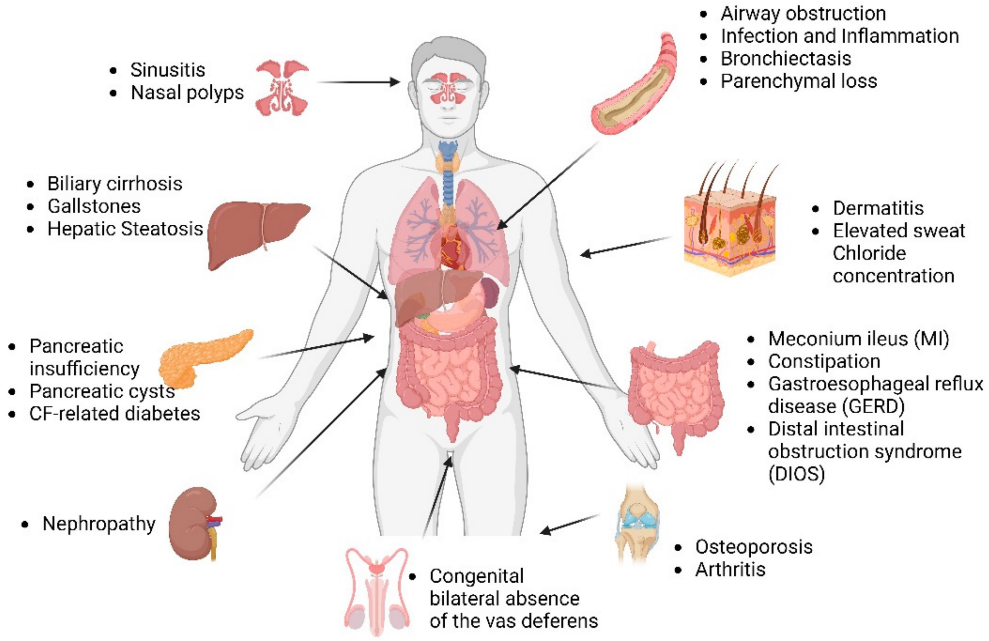
I have NO financial disclosure or conflicts of interest with the presented material in this presentation.

Cystic Fibrosis

A genetic disorder that affects the lungs, kidney, pancreas, and other organs.

Up to 20-fold higher risk of end-stage kidney disease.

No biomarkers predicting kidney disease risk in People with Cystic Fibrosis (PwCF).



Ramananda Y. *Int. J. Mol. Sci.* 2024. https://doi.org/10.3390/ijms25063384

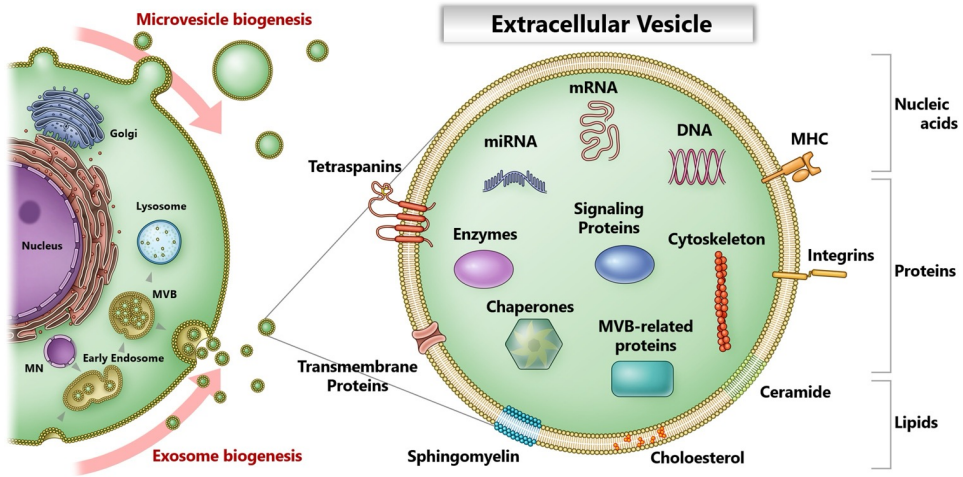
UNIVERSITY of VIRGINIA

Extracellular Vesicles

Extracellular vesicles (EVs):

- nanosized membrane-bound particles
- secreted by the cells to the extracellular space.
- play a critical role in regulating intercellular communication associated with physiological and pathological processes.

Urinary EVs (uEvs) are essential tools for the crosstalk between diverse cell populations during kidney injury, inflammation, fibrosis, and regeneration.



Yokoi A. et al. Seminars in Cancer Biology. 2021.

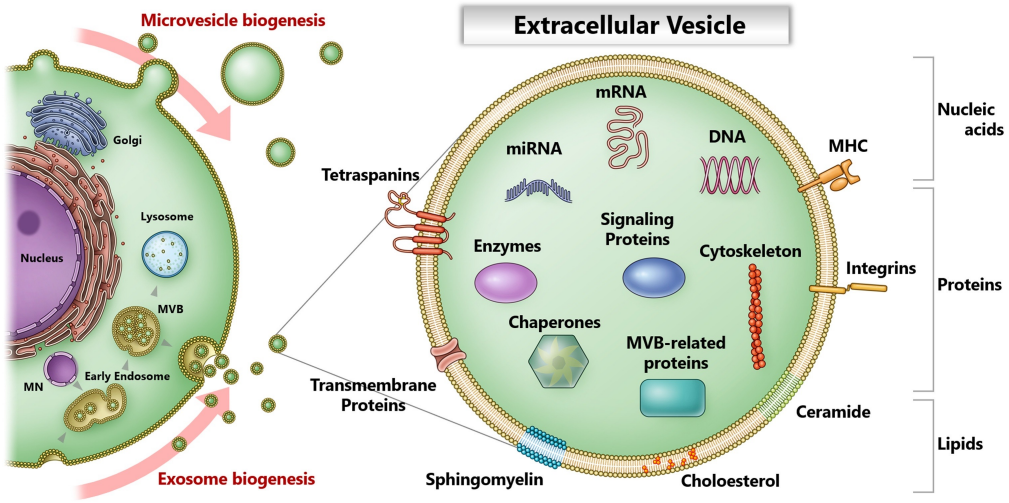
https://doi.org/10.1016/j.semcancer.2021.03.032.

UNIVERSITY of VIRGINIA

Urinary Extracellular Vesicles

uEVs are reliable source of potential biomarkers associated with different disease states.

- Carry a cargo of proteins, lipids, and genetic material such as DNA, mRNA, and microRNA derived from parental cells
- The cargo is protected from urinary enzymes
- Reflects: What is going on in the tissue!
- Non-invasive liquid biopsy
- Unique tools for diagnostic, prognostic, therapeutic, and regenerative purposes.



Yokoi A. et al. Seminars in Cancer Biology. 2021.

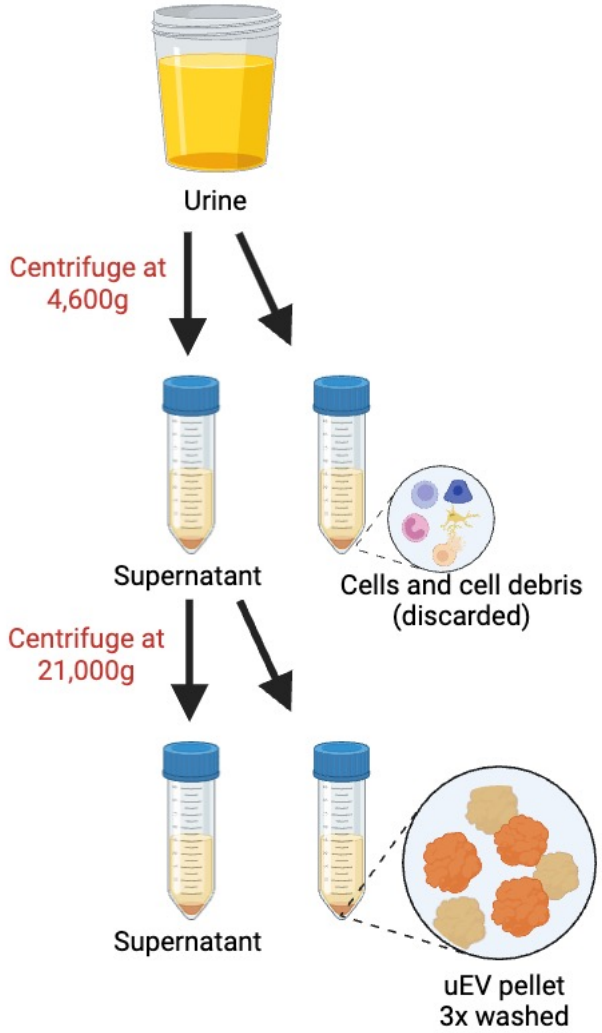
https://doi.org/10.1016/j.semcancer.2021.03.032.

Our hypothesis: Specific uEV markers may identify PwCF at risk of kidney injury.

UNIVERSITY of VIRGINIA

Methods

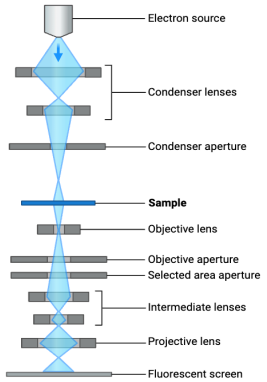
- Differential centrifugation to separate uEVs (common procedure to separate organelles and other particles based on their sedimentation rate)
- Urine samples from 50 PwCF and 50 HCs.
- Differential centrifugation (4,600g and 21,000g)
- uEV separation
- Washing with a low ionic strength buffer to remove **uromodulin.**

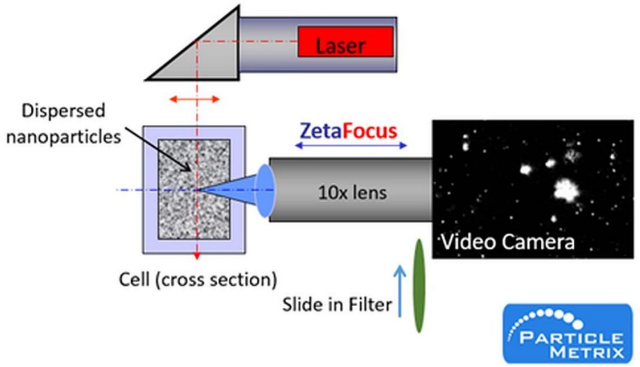


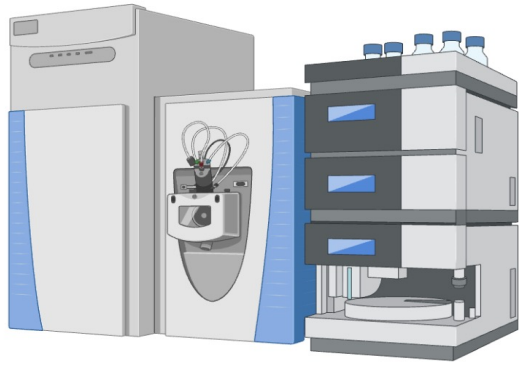
Created with **BioRender**.com

UNIVERSITY of VIRGINIA

Methods







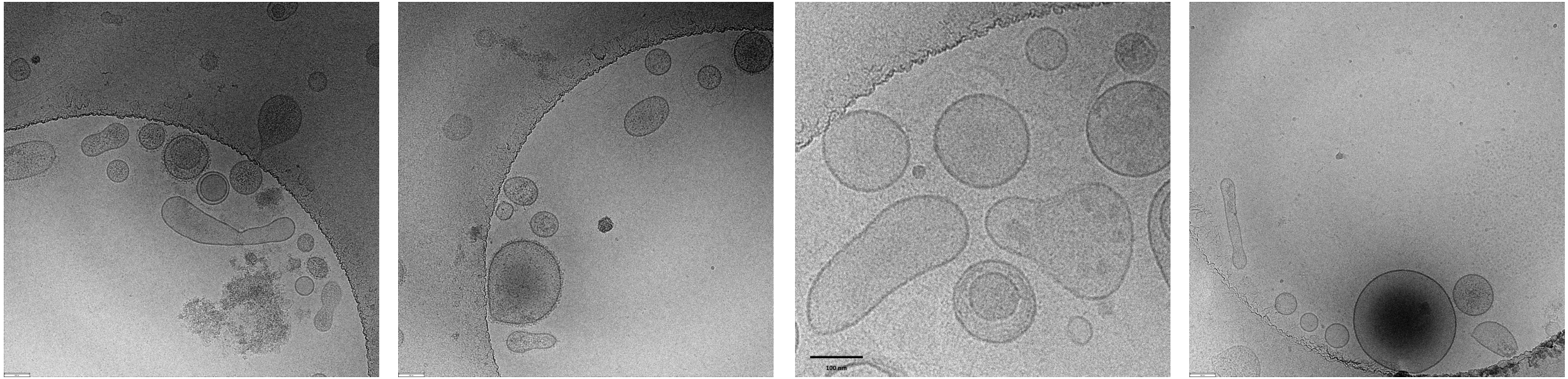
Cryogenic electron microscopy (Cryo-EM) was used to examine the size and morphology of uEVs.
Cryo-EM is a technique involves flash-freezing solutions of biomolecules to produce microscope images with electrons.

Nanoparticle tracking analysis (NTA) was used to determine size and concentration.
NTA is a technique that uses an ultramicroscope and laser illumination to visualize and analyze particles in liquid suspension.

Proteomics approach (LC-MS/MS) was used to study the uEV's protein cargo.
Proteomics is a technique for identifying proteins in complex samples.

UNIVERSITY of VIRGINIA

Cryo-Electron Microscopy

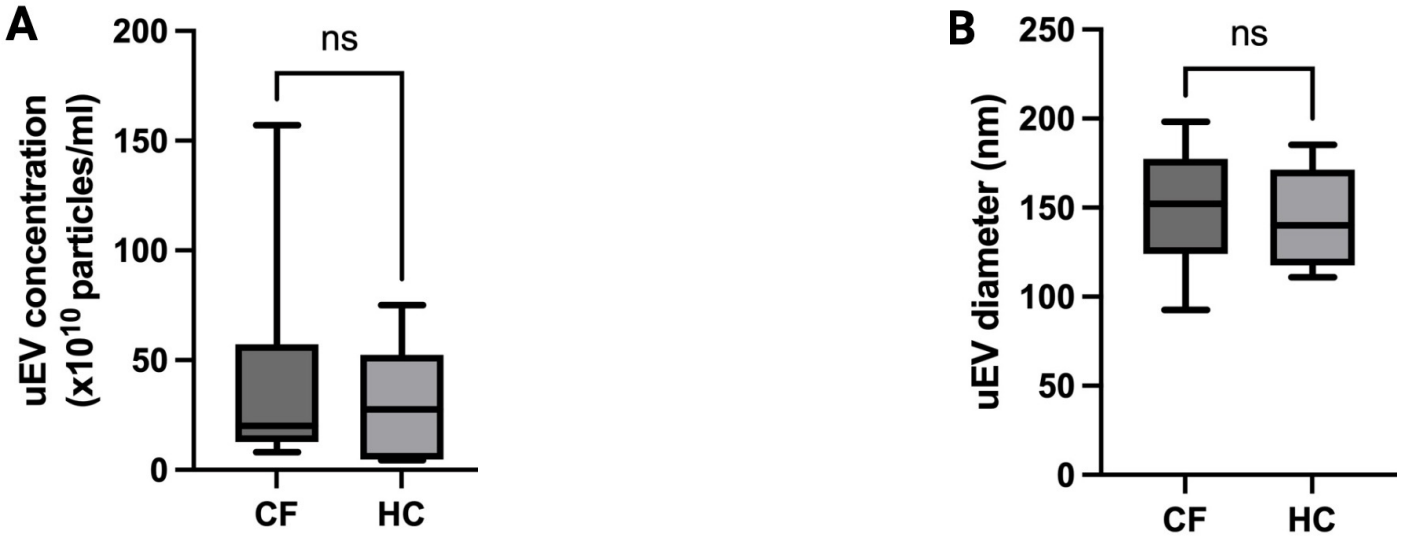


Cryo-EM image of uEVs with different shapes and sizes.

UNIVERSITY of VIRGINIA

Nanoparticle Tracking Analysis

- There was no statistically significant difference in uEV's size and concentration between PwCF and HCs.



Comparison of uEVs concentration (A) and diameter (B) in PwCF and HC. Data analyzed by NTA. (N = 7/group)

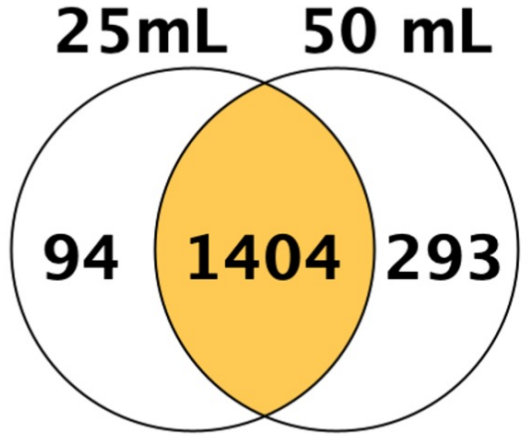
University of Virginia

Proteomics Analysis

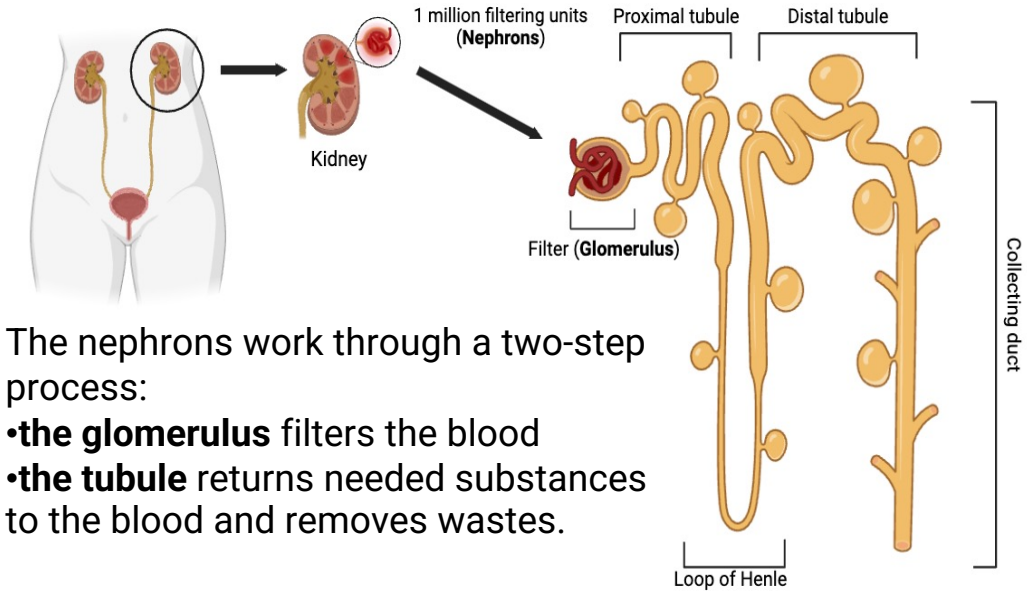
- We compared protein coverage in uEVs separated from different starting volumes of **HC urine.**

- We identified 1404 common proteins in uEV pellet from different amount of HC's urine (1498 proteins from 25ml urine and 1697 proteins from 50ml urine).

- We repeated the analysis under similar conditions and achieved very high reproducibility of protein coverage.



UNIVERSITY of VIRGINIA



The nephrons work through a two-step process:

- **the glomerulus** filters the blood
- **the tubule** returns needed substances to the blood and removes wastes.

Created with **BioRender**.com

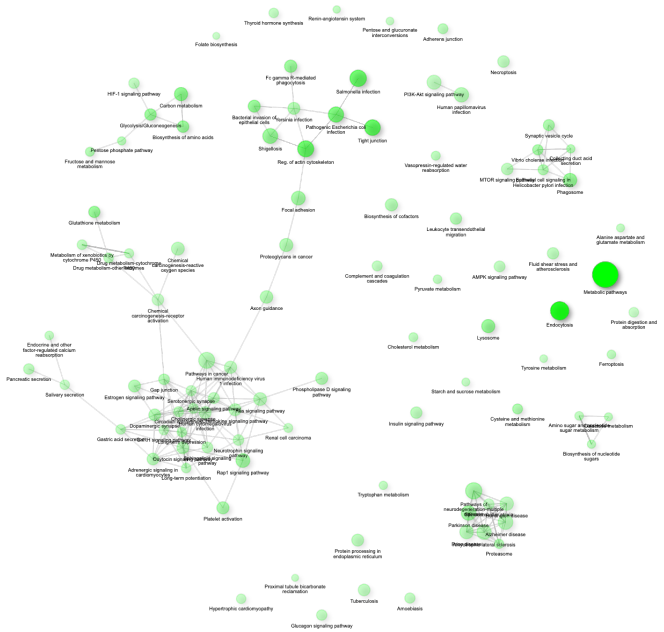
uEV marker	Origin at the urinary tract
Podocin Podocalyxin Complement receptor 1 (CR1) Nephrin	Glomerulus (podocytes)
Angiotensin-converting enzyme (ACE)	Glomerulus/proximal tubules
Aminopeptidase N (APN) Cubilin Sodium/glucose cotransporter 2 (SGLT 2) Carbonic anhydrase (CA IV)	Proximal tubules
CD133 (Prominin 1)	Renal progenitor cells
Aquaporin 1 (AQP1)	Proximal tubules/Henle's loop
Uromodulin (Tamm-Horsfall Protein)	Henle's loop
Klotho	Proximal/distal tubules
Prominin 2	Distal tubules
Aquaporin 2 (AQP2)	Distal tubules/collecting duct
Mucin-1	Collecting duct
Uroplakin-1 Uroplakin-2 Mucin-1	Transitional epithelial cells
Prostatic acid phosphatase (PPAP) Prostate transglutaminase (TGM4)	Epithelial cells

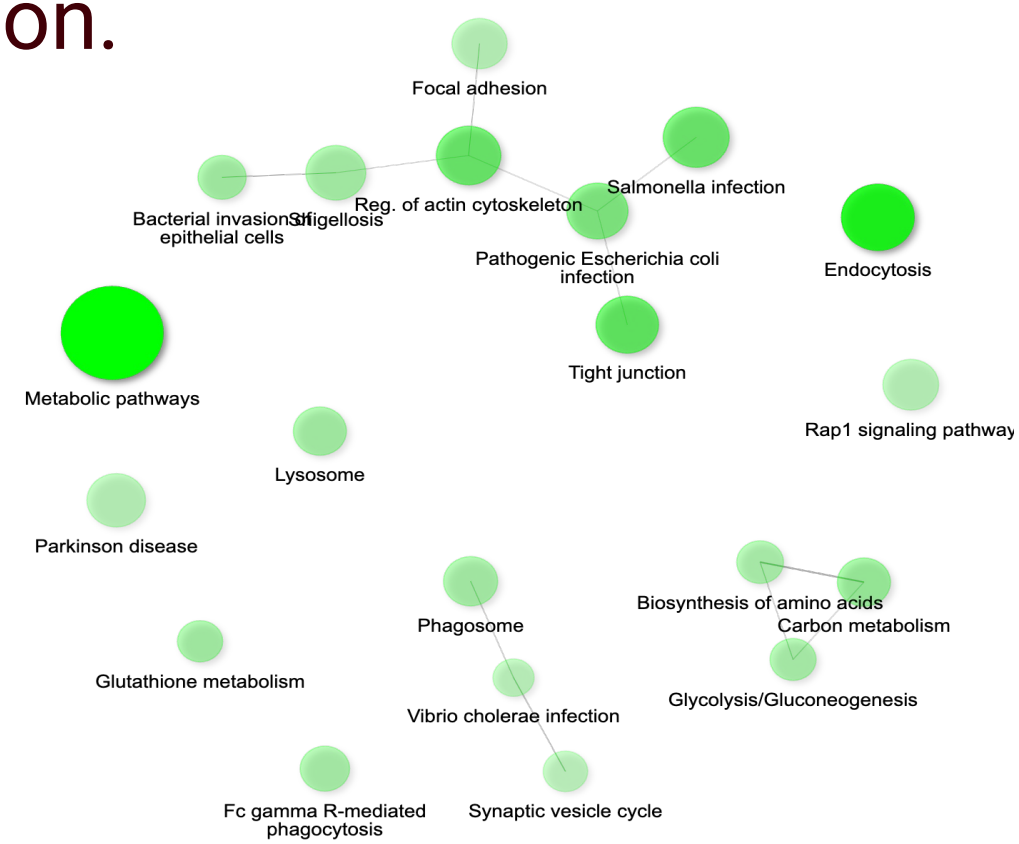
List of uEV markers identified by proteomics characterizing different kidney cell populations*.

*Erdbrügger U. et al. J Extracell Vesicles. 2021. https://doi.org/10.1002/jev2.12093

UNIVERSITY of VIRGINIA

Network of enriched pathways provides a systemic view of the biological question under investigation.



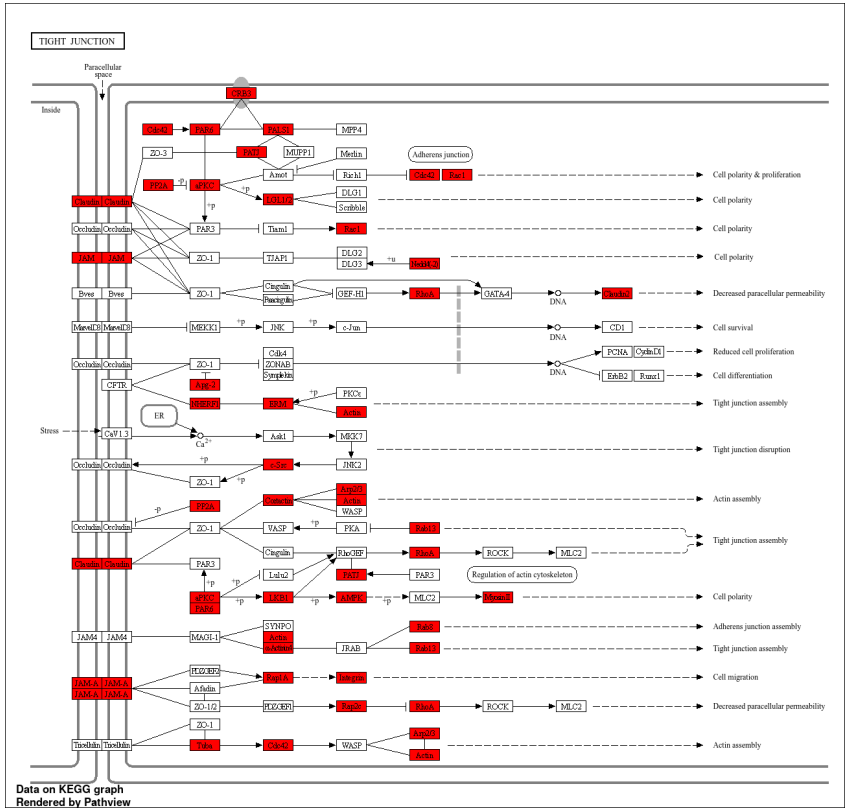


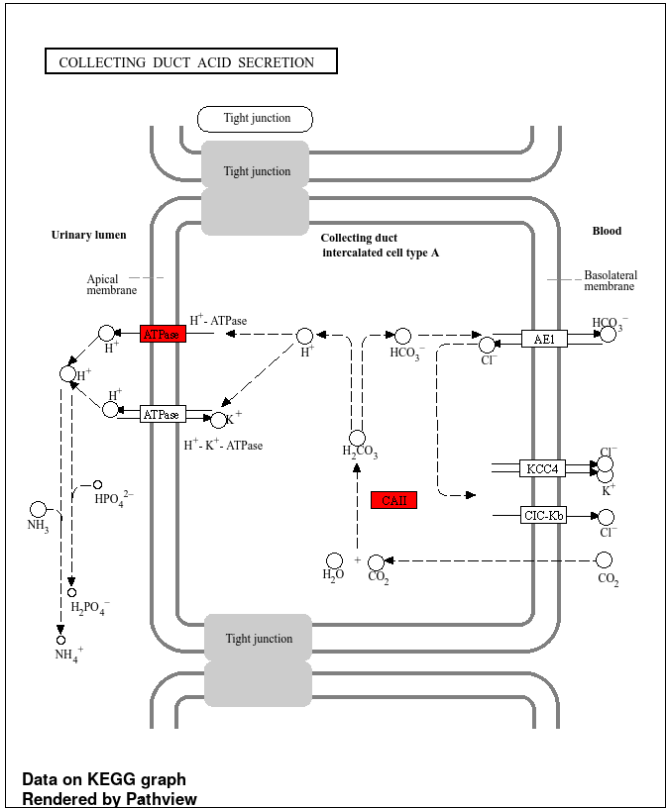
Xijin GS. et al. Bioinformatics, 2020, https://doi.org/10.1093/bioinformatics/btz931

Obtained from ShinyGO online tool.
It becomes **a standard tool** in the analytic pipeline for Omics data,
It reduces the complexity
It provides a systemic view of the biological question under investigation.
Each node represents an enriched pathway.
Related pathways are connected by a **line**.
Bigger nodes corresponds to higher number of genes.

UNIVERSITY of VIRGINIA

KEGG pathway maps are graphical representations of molecular interaction, reaction or relation networks.





Kanehisa M. et al. KEGG: integrating viruses and cellular organisms, Nucleic Acids Research, 2021, https://doi.org/10.1093/nar/gkaa970

KEGG pathways of tight junction and acid secretion in the collecting duct.
Proteins identified in uEVs are highlighted in **red**.
We will compare the uEVs' proteome (PwCF vs HCs in terms of kidney diseases)

UNIVERSITY of VIRGINIA

Conclusions

- We optimized EV isolation method from urine samples.
- We achieved a high reproducibility of protein coverage by proteomics analysis.
- Using this validated approach, we will compare the uEVs' proteome from PwCF with normal or decreased kidney function to HCs.
- We predict that our future studies will identify signatures associated with reduced kidney function in PwCF.

UNIVERSITY of VIRGINIA

Acknowledgements

- **Urban Lab**

Agnieszka Swiatecka-Urban, MD

Hayrettin Yavuz, MD, PhD

Joshua E. Donovan

Ewelina D. Hejenkowska

Mateusz Miszczuk

- **Erdbrügger Lab**

Uta Erdbrügger, MD

Michael Harding, PhD

Hanine El Itawi, PhD

Samantha Upson

Morgan Greig

- **Funding**

Supported by,

NIH R01HL144539 (A.S-U.)

5U2CDK129500 (A.S-U.)

5TL1DK132771 (U.E.)

Contact: Hayrettin Yavuz
eeu9ng@virginia.edu