Modeling X-linked Alport syndrome with deep-intronic variations in kidney organoids for antisense oligonucleotide-based therapy development

No. 275

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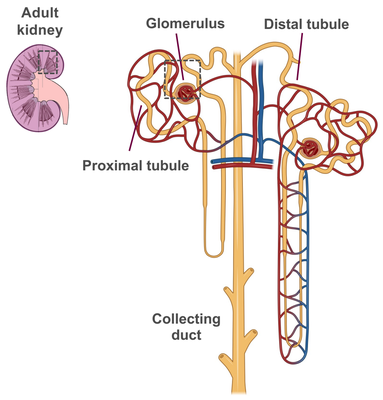
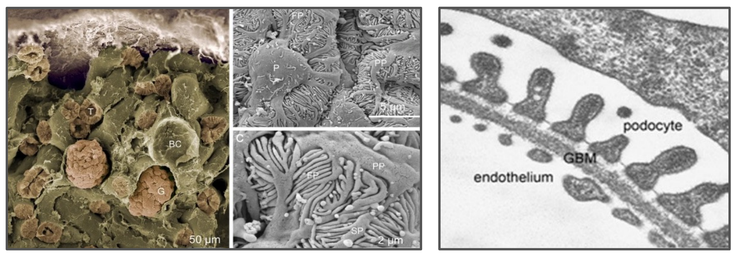
X-linked Alport Syndrome

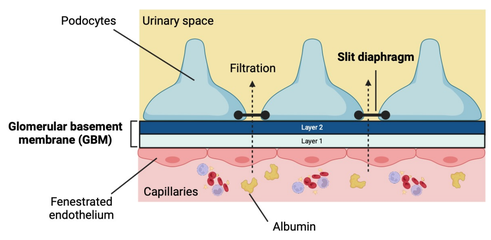
X-linked Alport syndrome (XLAS) is a hereditary glomerulopathy arising from genetic mutations in the *COL4A5* gene, encoding the α5 chain of the collagen IV [α5(IV)] in the glomerular basement membrane (GBM).

In a study on a cohort of **19 patients** with clinically proven XLAS, we identified deep-intronic variants responsible for the aberrant splicing events (17/19) using a **targeted RNA sequencing approach**.

The objective of this study is to develop a robust *in vitro* model for XLAS to characterize the disease and to test different therapeutic approaches including ASO therapy.

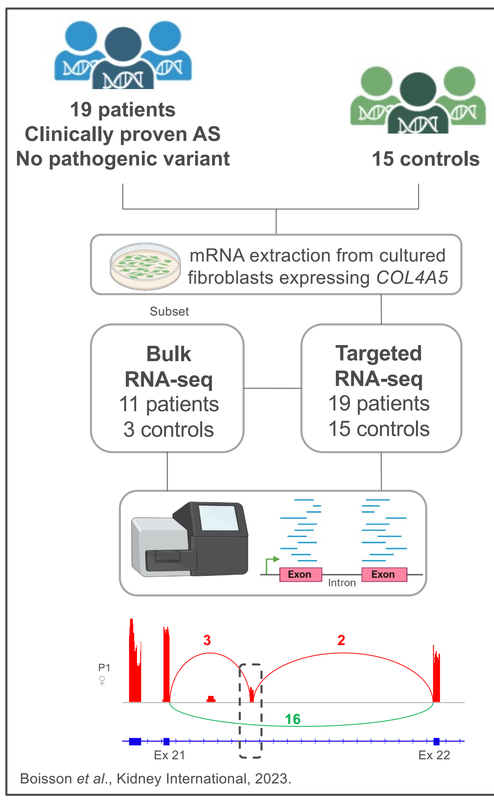


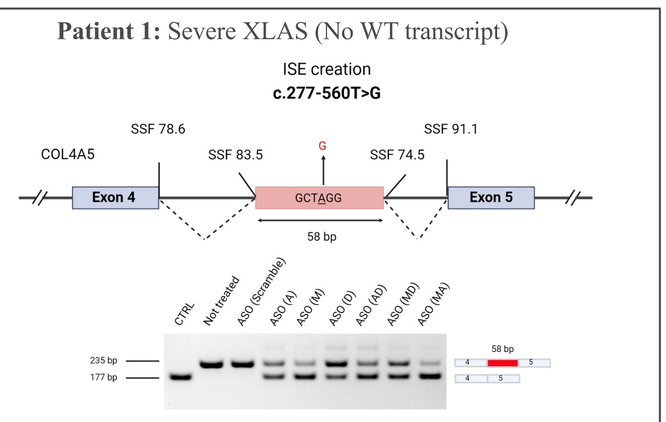




Andres *et al*., 2023; Naylor RW *et al*. Nat Rev Nephrol, 2021

Identification of Missing Variant Amenable to ASO Therapy

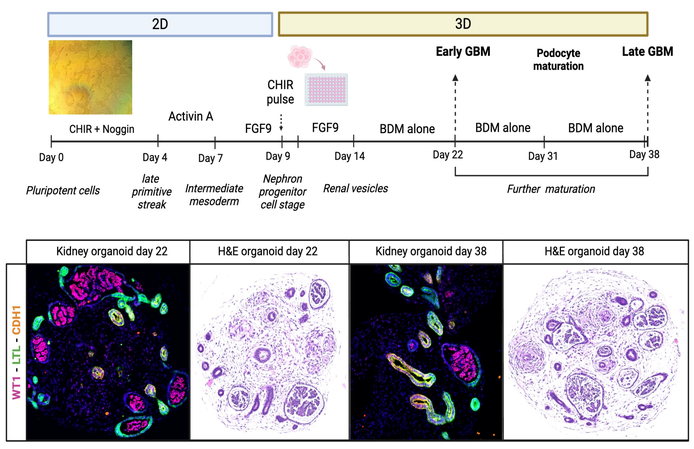


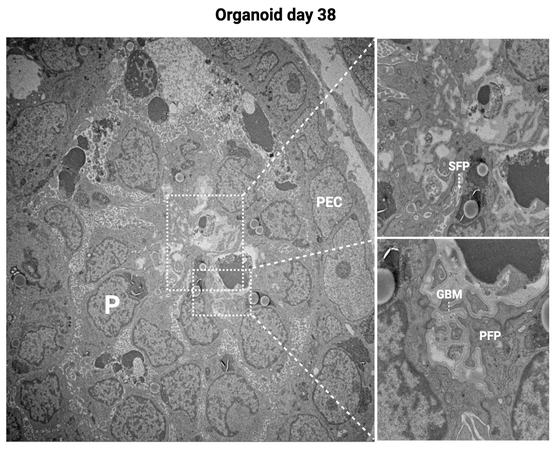


Boisson *et al*., Kidney International, 2023.

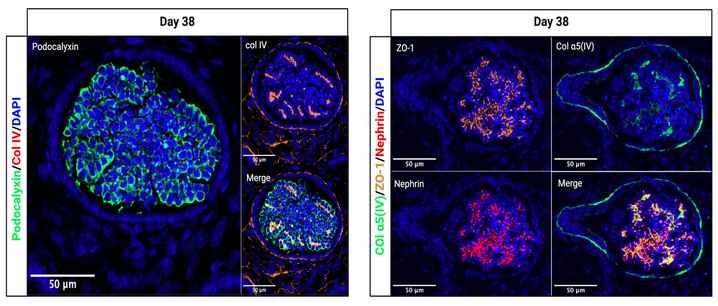
Collagen IV Switch in Developing Organoids

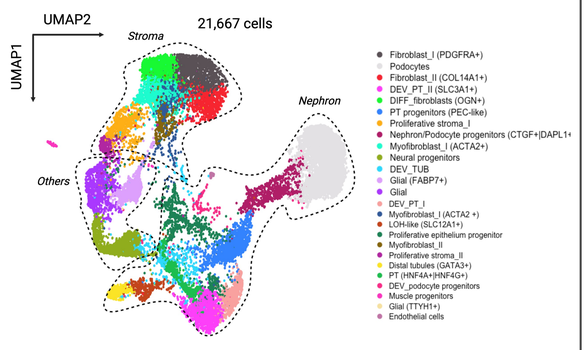
Kidney organoids recapitulate basement membrane assembly



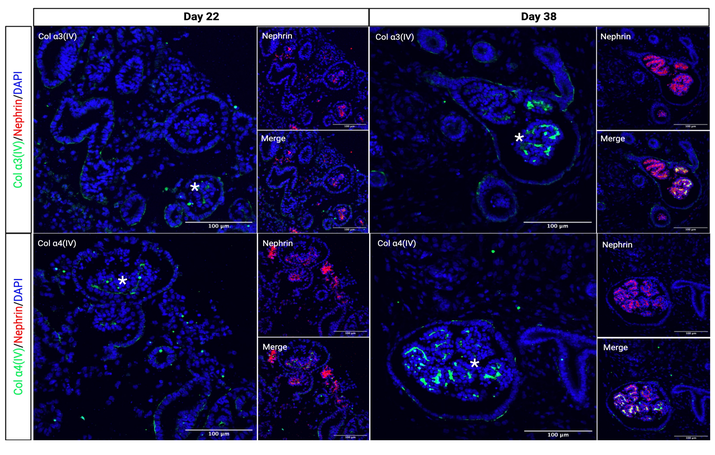


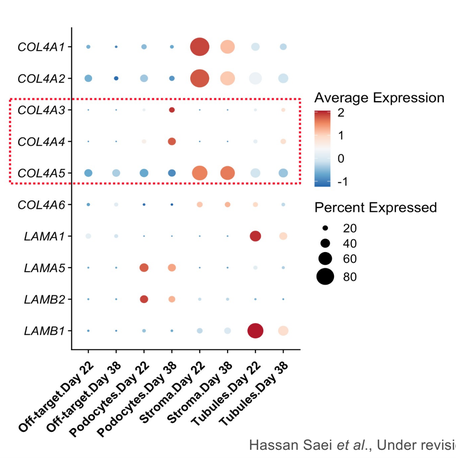
Podocytes in kidney organoids are polarized



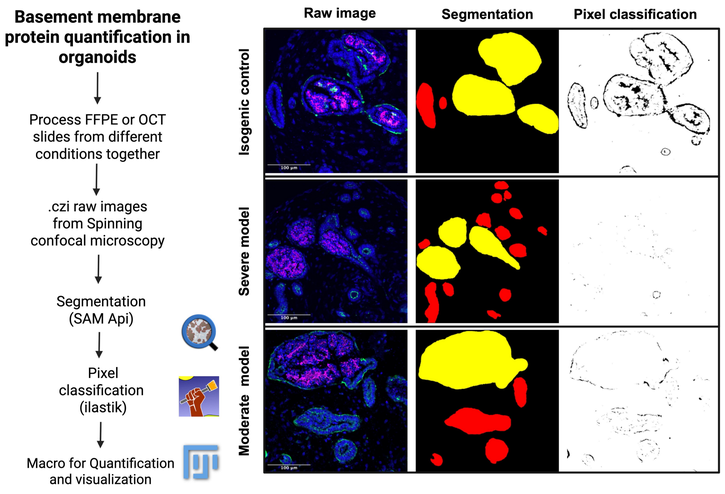


Prolonged organoid culture is essential for maturation of the GBM collagen network





Hassan Saei *et al*., Under revision. 2025



BMQuant: A FIJI macro to quantify basement membrane proteins

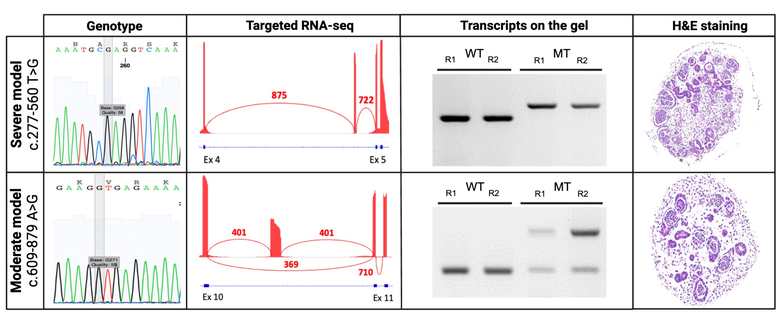
This workflow begins with segmentation of glomeruli and tubule using QuPath, followed by pixel classification with ilastik. SAM API was used for accurate identification of object border and ROIs were saved. The channel of interest is being uploaded to ilastik for random forest model-based pixel classification. The FIJI macro generates csv files that contains measurements such as the full border area, mean channel intensity in the border and inside each object.

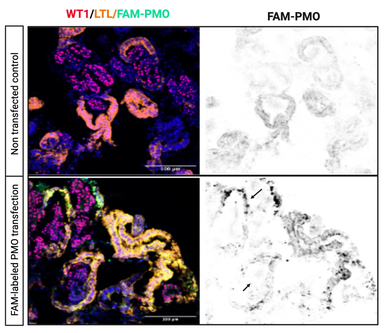
https://github.com/hassansaei/BMQuant

Schindelin J, *et al*., 2012; Bankhead P, *et al*., 2017; Berg S, *et al*., 2019

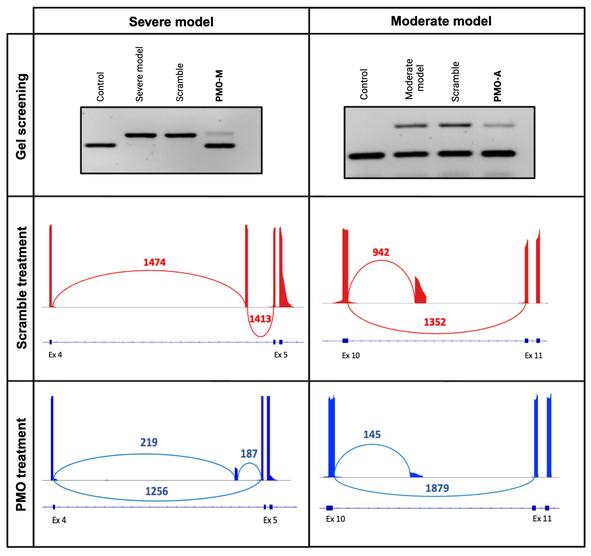
ASO Treatment in XLAS Organoids with Deep Intronic Variants

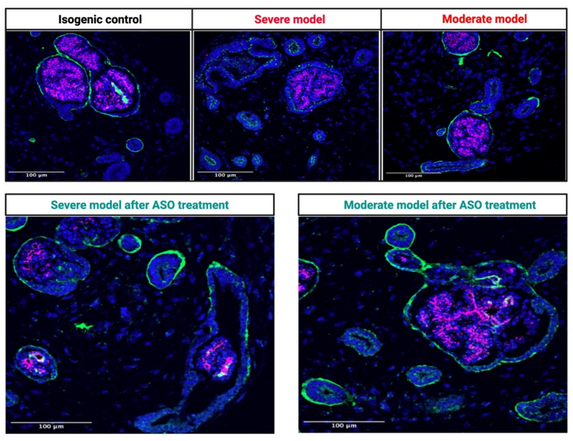
Kidney organoids carrying patient variant showed aberrant *COL4A5* splicing

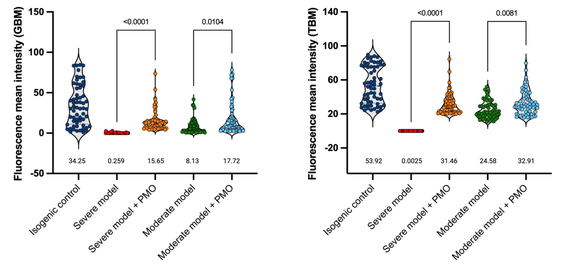




ASO treatment reinstates collagen IV assembly in organoid models of XLAS



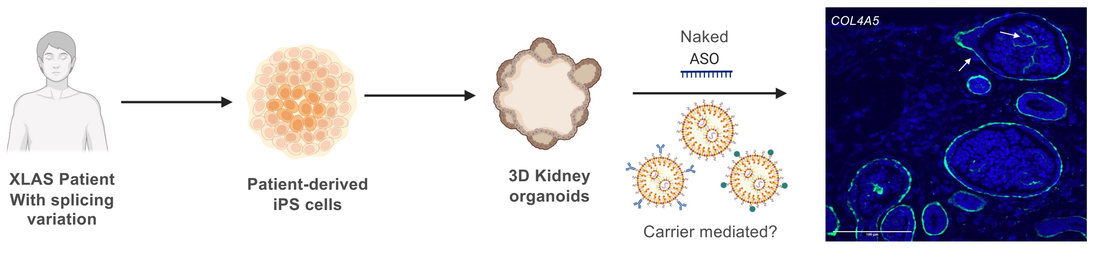




The severe model showed a complete absence of wild-type *COL4A5* transcript, which was significantly restored at both mRNA and protein levels following ASO transfection in organoids

Hassan Saei *et al*., Under revision. 2025

Organoid Enable Scalable Development of Personalized Therapies



- We optimized antisense oligonucleotide (ASO) treatment in a kidney organoid model to develop splice modulation therapy
- Kidney organoids serve as a scalable platform to develop and test personalized therapies
- Despite being an invaluable readout model, these organoids lack a fully developed glomerular filtration barrier, and key mechanistic readouts are still missing

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