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Developing and characterizing X-linked Alport Syndrome kidney organoid model with deep-intronic variation for drug discovery

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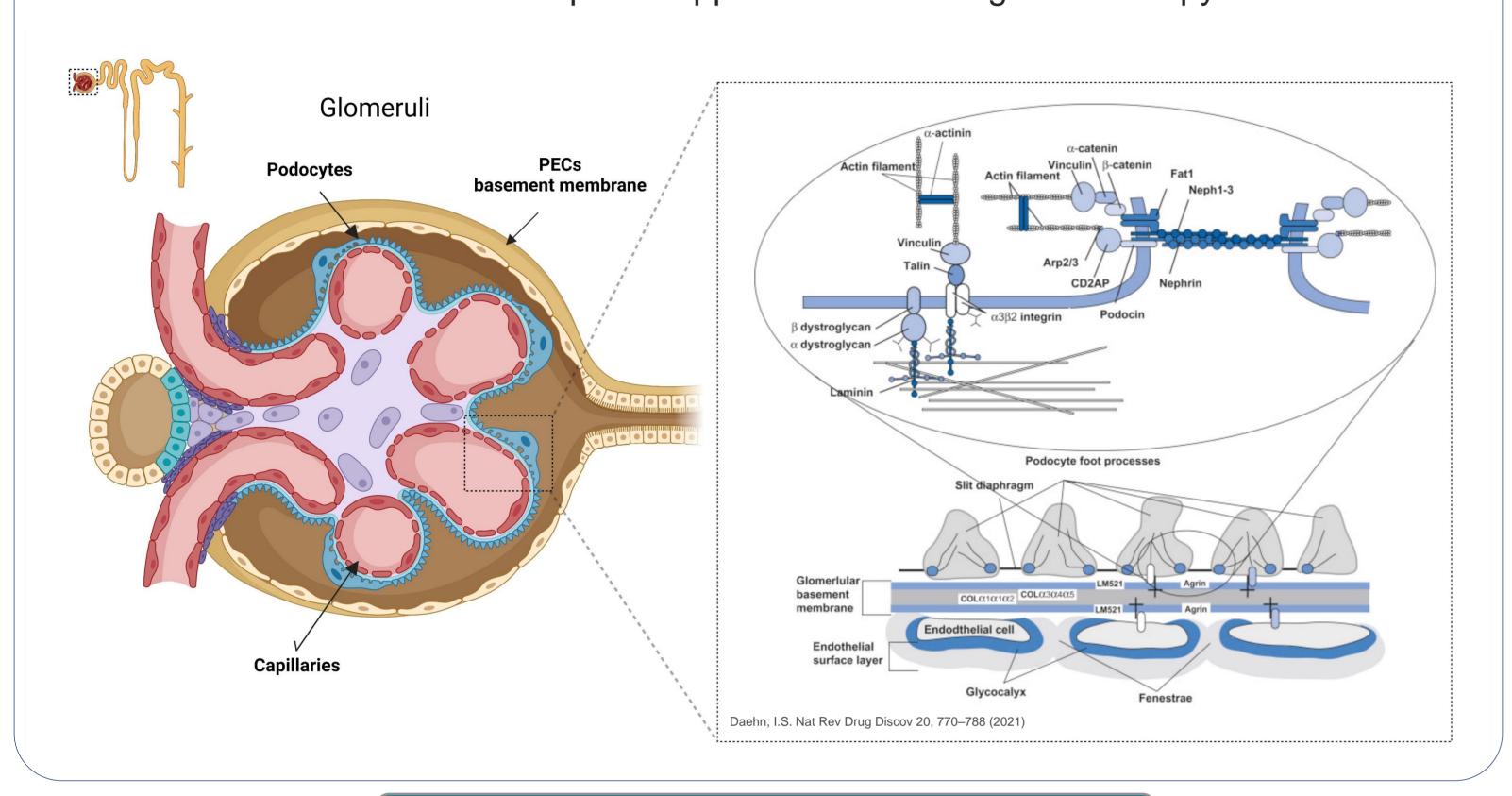
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INTRODUCTION AND OBJECTIVE

X-linked Alport syndrome (XLAS) is a hereditary glomerulopathy arising from genetic mutations in the COL4A5 gene, encoding the $\alpha 5$ chain of the collagen IV [$\alpha 5(IV)$] in the glomerular basement membrane (GBM).

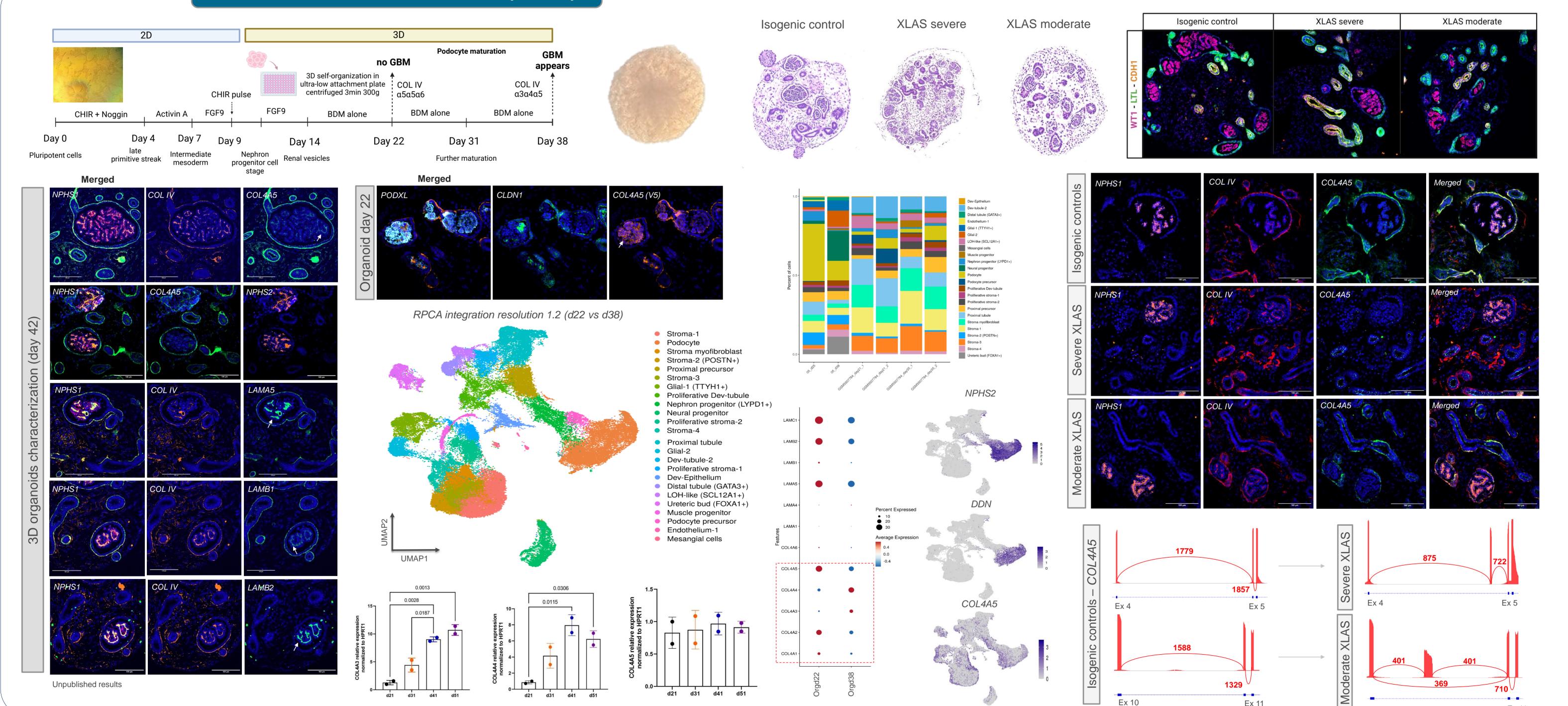
In a study on a cohort of 19 patients with clinically proven XLAS, we identified deep-intronic variants responsible for the aberrant splicing events (17/19) using a targeted RNA sequencing approach.

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.

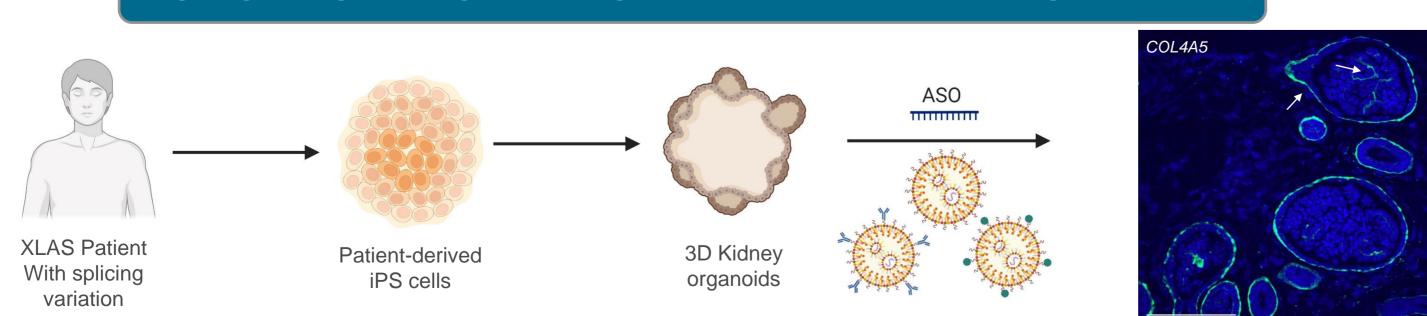


GENETIC DIAGNOSIS – SPLICING VARIANTS Patient 1: Severe XLAS (No WT transcript) ISE creation c.277-560T>G 19 patients __//__ Exon 4 **Clinically proven AS** No pathogenic variant 15 controls mRNA extraction from cultured fibroblasts expressing COL4A5 Bulk **Targeted** RNA-seq RNA-seq 19 patients 11 patients 15 controls 3 controls Patient 2: Moderate XLAS (Residual WT transcript) Donor site creation c.609+879A>G COL4A5 —//— Exon 10 Exon 11 —//— Ex 21 Boisson et al., Kidney International, 2023. Unpublished results

KIDNEY ORGANOID MODEL (XLAS)



ORGANOID MODEL FOR THERAPY DEVELOPMENT



- > We are optimizing the antisense-oligo (ASO) treatment in the kidney organoid model to restore splicing.
- There are still some challenges regarding drug delivery in *in vitro* models due to the lack of proper vascularization. However, if the ASO could penetrate and restore splicing back to normal with promising efficiency and minimal side effects, we can expedite the transition to clinical trials.

HIGHLIGHTS AND CONCLUSION

- ➤ We improved the genetic diagnosis of patients with XLAS with targeted RNA sequencing on patient-derived fibroblasts. Different independent patients have identified with the same intronic variation, which supports the importance of analyzing intronic sequences in the *COL4A5* gene.
- > We developed the XLAS organoid model for variant characterization and testing different therapeutic approaches including ASO therapy.
- ➤ Single-cell RNA sequencing identified different cell types and validated kidney organoids as a robust in vitro model, despite not being fully mature.
- ➤ Single-cell RNA sequencing on 3D organoids at two time points (d22 vs d38) confirmed podocyte maturation and the need for prolonged culture to detect "mature" glomerular basement membrane.