Abstract No: **WCN26-AB-2518**

Inducible Exon Skipping After Disease Onset as a Therapeutic Strategy for CKD: Evaluation in a Patient-Derived Mouse Model of Alport Syndrome

Kentarou Hashikami, Ryosuke Kobayashi, Ryotaro Hori, Kenta Danbayashi, Yasunori Nio

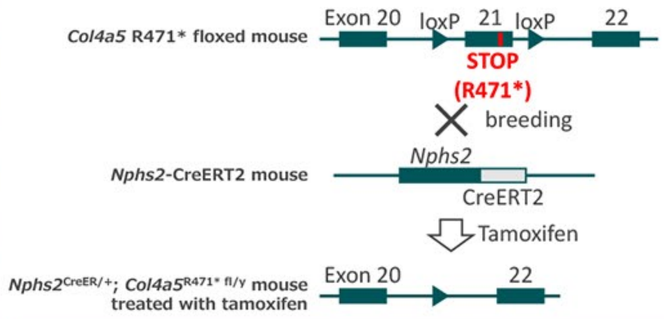
Introduction

Alport syndrome (AS) is a progressive hereditary nephropathy caused by mutations in COL4A3, COL4A4, or COL4A5, which encode the α3, α4, and α5 chains of type IV collagen. These mutations disrupt the glomerular basement membrane (GBM), leading to proteinuria, and eventual renal failure. Although angiotensin II receptor blockers (ARBs) can delay progression, no curative therapies currently exist. Given the monogenic nature of AS, exon skipping therapy offers a promising mutation-specific strategy to restore functional protein expression. This study aimed to evaluate the therapeutic potential of post-onset exon skipping using a tamoxifen-inducible mouse model harboring a patient-derived Col4a5 nonsense mutation.

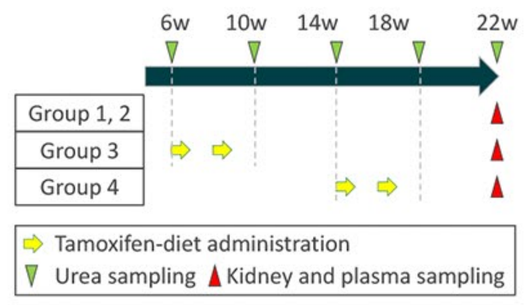
Methods

All figures adapted from our own publication: Hashikami K. et al., Scientific Reports, 2025;15:41766. (CC BY 4.0)

A. Exon skipping strategy



B. Protocol of Tamoxifen administration



Group	Genotype	Tamoxifen	Administration period	N
1	WT	-	-	6
2	CreER; flox	-	-	6
3	CreER; flox	+	6-10w	6
4	CreER; flox	+	14-18w	6

CreER : *Nphs2*$^{CreER/+}$, flox : *Col4a5*$^{R471*\ fl/y}$
The tamoxifen was administered via diet at 0.025% (w/w)

(A) The Col4a5 R471* floxed mouse carried a nonsense mutation of exon 21 (c. 1411 C > T, p. R471*) and two loxP sequences flanking exon 21 in *Col4a5* allele. The *Nphs2*-CreERT2 mouse carried the CreERT2 sequence linked to the *Nphs2* gene via a T2A sequence. In *Nphs2*$^{CreER/+}$; *Col4a5*$^{R471*\ fl/y}$ mice, tamoxifen administration induced glomerular-specific deletion of the mutated *Col4a5* exon21. **(B)** Starting at 6 or 14 weeks of age, tamoxifen-containing diet was administrated in 2 cycles of 1 week on and 1 week off for a total of 4 weeks. Urine sampling was performed every 4 weeks, and kidney and plasma samples were collected at 22 weeks of age. Phenotypic analyses included measurements of urinary and blood biochemical parameters, immunostaining, histopathology, and transmission electron microscopy to assess the effects of tamoxifen-induced exon skipping.

Results

Recovery of COL4A5 expression after exon 21 skipping

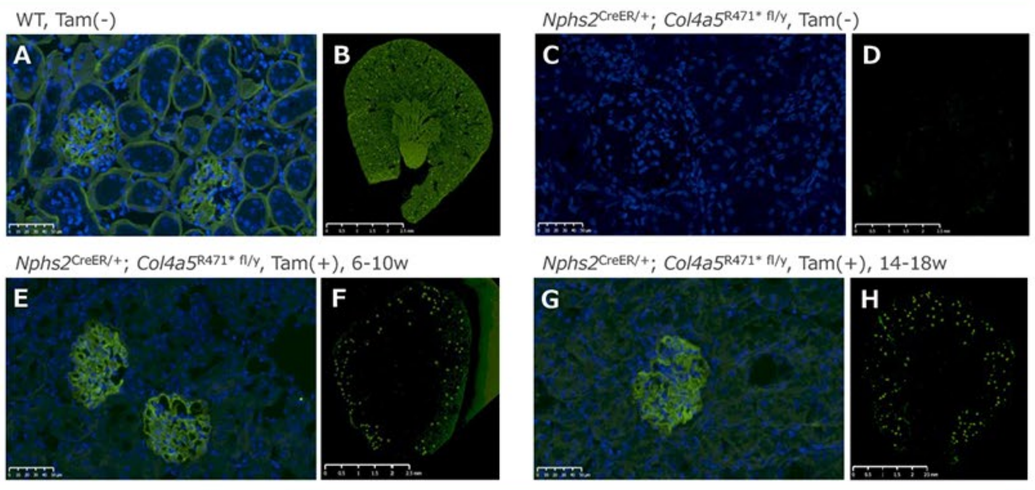


Fig. 1 Restoration of COL4A5 expression by tamoxifen-induced exon 21 skipping.
(A, B) WT mice showed COL4A5 staining along glomerular and tubular basement membranes. (C, D) In untreated Alport mice, COL4A5 was completely negative. (E-H) Early (6-10w) and late (14-18w) tamoxifen treatments restored COL4A5 expression in glomeruli despite ongoing disease progression. Kidney sections were stained with FITC-conjugated anti-COL4A5 antibody (H53; Shigei Medical Research Institute, Okayama, Japan); representative images at 22 weeks of age. Green: COL4A5, Blue: nuclei.

Improvement in body weight, albuminuria, and plasma biomarkers after exon skipping

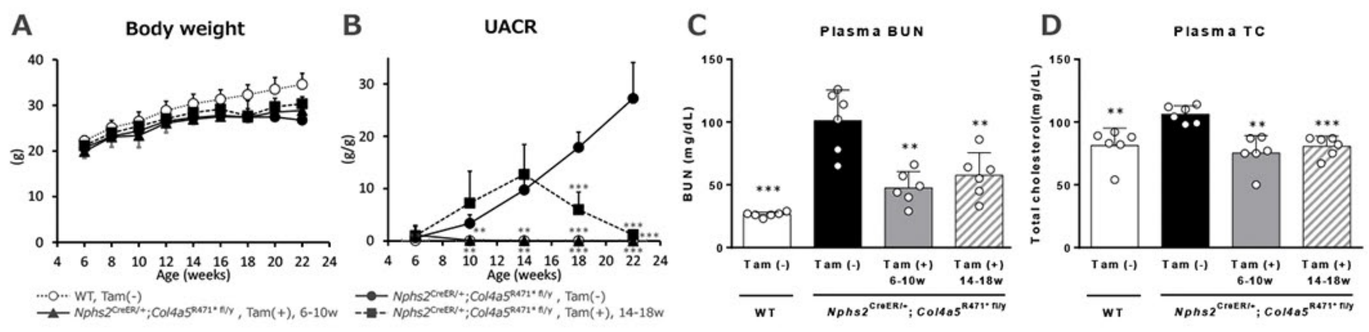


Fig. 2 Tamoxifen-induced Col4a5 exon 21 skipping improves growth, albuminuria, and plasma biomarkers in AS model mice.
(A) Tamoxifen treatment, initiated either early or late, partially restored body weight compared with untreated AS mice.(B) Exon skipping significantly reduced elevated UACR to near wild-type levels in both treatment windows. (C) At 22 weeks, increased plasma BUN in untreated AS mice was ameliorated following tamoxifen administration. (D) At 22 weeks, tamoxifen treatment suppressed the increase in TC associated with the nephrotic condition in both early and late treatment groups. Data are shown as mean ± SD. Statistical analysis was performed using the Aspin-Welch t-test. **: $p < 0.01$, ***: $p < 0.001$ for *Nphs2*$^{CreER/+}$; *Col4a5*$^{R471*\ fl/y}$, Tam(-) group vs. other groups. UACR: urinary albumin to creatinine ratio, BUN: blood urea nitrogen, TC: Total cholesterol.

Histological improvement following tamoxifen-induced COL4A5 recovery

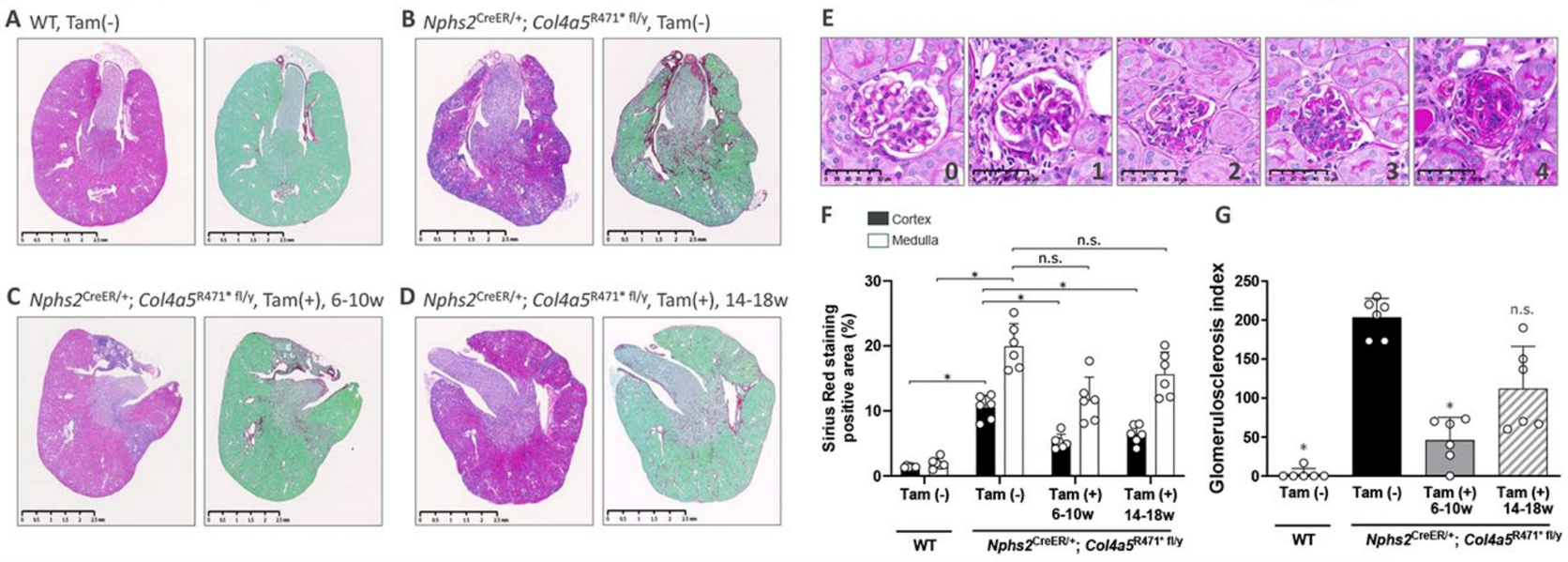


Fig. 3 Exon 21 skipping reduces renal fibrosis and glomerulosclerosis in AS model mice.
(A–D) Representative HE and SR staining images. Untreated AS mice showed marked glomerular and tubulointerstitial lesions with increased fibrosis, whereas WT kidneys exhibited normal morphology. Tamoxifen-treated mice (early and late treatment groups) showed decreased lesion size and decreased SR-positive fibrotic areas. (E) Representative PAS-stained glomeruli illustrating glomerulosclerosis grades (0–4). (F, G) Quantification demonstrated significant reductions in fibrotic area and glomerulosclerosis index at 22 weeks of age after treatment . Data are shown as mean ± SD. Statistical analysis was performed using the Steel–Dwass multiple comparison test. *: $p < 0.05$, *Nphs2*$^{CreER/+}$; *Col4a5*$^{R471*\ fl/y}$, Tam(-) group vs. other groups.

Exon 21 skipping partially restores GBM structure and podocyte morphology

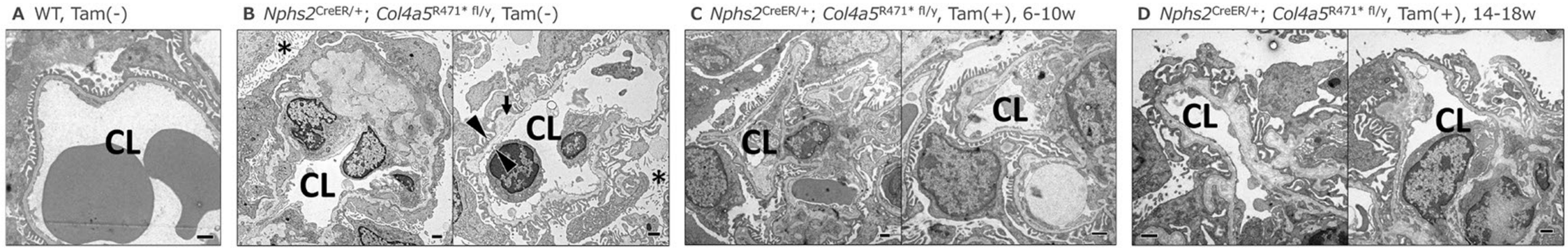


Fig. 4. Exon 21 skipping improves ultrastructural GBM integrity in AS model mice.
(A)WT mice showed normal GBM architecture. (B)Untreated AS mice exhibited severe GBM defects, including irregular thickening or thinning, splitting (arrowheads), electron-dense deposits (arrow), lucent areas, foot process effacement, and increased podocyte microvilli (asterisk). (C, D)Tamoxifen-induced exon skipping (early and late treatment) partially restored GBM structure and reduced foot process effacement, indicating improved podocyte integrity and filtration function. Scale bar = 1 μm; CL: glomerular capillary lumen.

Conclusion

Our inducible exon-skipping approach in the AS mouse model provides a robust platform for evaluating mutation-targeted therapies.
The results demonstrate that exon skipping is a feasible and effective therapeutic strategy for AS, even when initiated after disease onset.
These findings highlight the potential of post-onset genetic correction for monogenic kidney diseases and support the future clinical translation of exon-skipping therapies.

COI disclosure information: We have no financial relationship to disclose for our presentation contents.

Axcelead Drug Discovery Partners, Inc.
https://www.axcelead-us.com/

WEB LinkedIn