

Irbesartan but not Roflumilast Ameliorates Renal Injury in a *Col4a5* R471* Mutant Mouse Model of Alport Syndrome and CKD

Masami Yamamoto, Masashi Aoyama, Mitsuharu Matsumoto, Takumi Sugawara, Ryosuke Kobayashi, Ryotaro Hori and Yasunori Nio

Introduction and Objective

Alport syndrome (AS) is a hereditary form of chronic kidney disease (CKD) and is associated with progressive renal injury and anemia. Previous knockout models of *Col4a3* or *Col4a5* recapitulated renal disease but were less translatable to the human condition. To address this limitation, we previously developed a novel AS model carrying a patient-derived nonsense mutation (c.1411C>T, p.R471*) [1]. The phenotypic features of this model mirror those of human AS, including progressive proteinuria, glomerulosclerosis, and renal fibrosis; however, anemia has not yet been verified, yet. The most effective currently available treatments are angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs). However, no reports have demonstrated the renal ameliorative effects of ARBs in *Col4a3-5* mutation-based Alport syndrome mouse models. Therefore, we utilized the *Col4a5* R471* mutant mouse to evaluate the therapeutic efficacy of irbesartan, an ARB in AS, with particular focus on anemia, renal inflammation and injury. Beyond ARB, we previously reported phosphodiesterase (PDE)-4 inhibitor ameliorated diabetic nephropathy in mice [2]. Therefore, we hypothesized roflumilast, a PDE4 inhibitor, may attenuate renal fibrosis and disease progression. In this presentation, we report on the results of irbesartan and roflumilast on AS using *Col4a5* R471* mutant mouse.

Methods

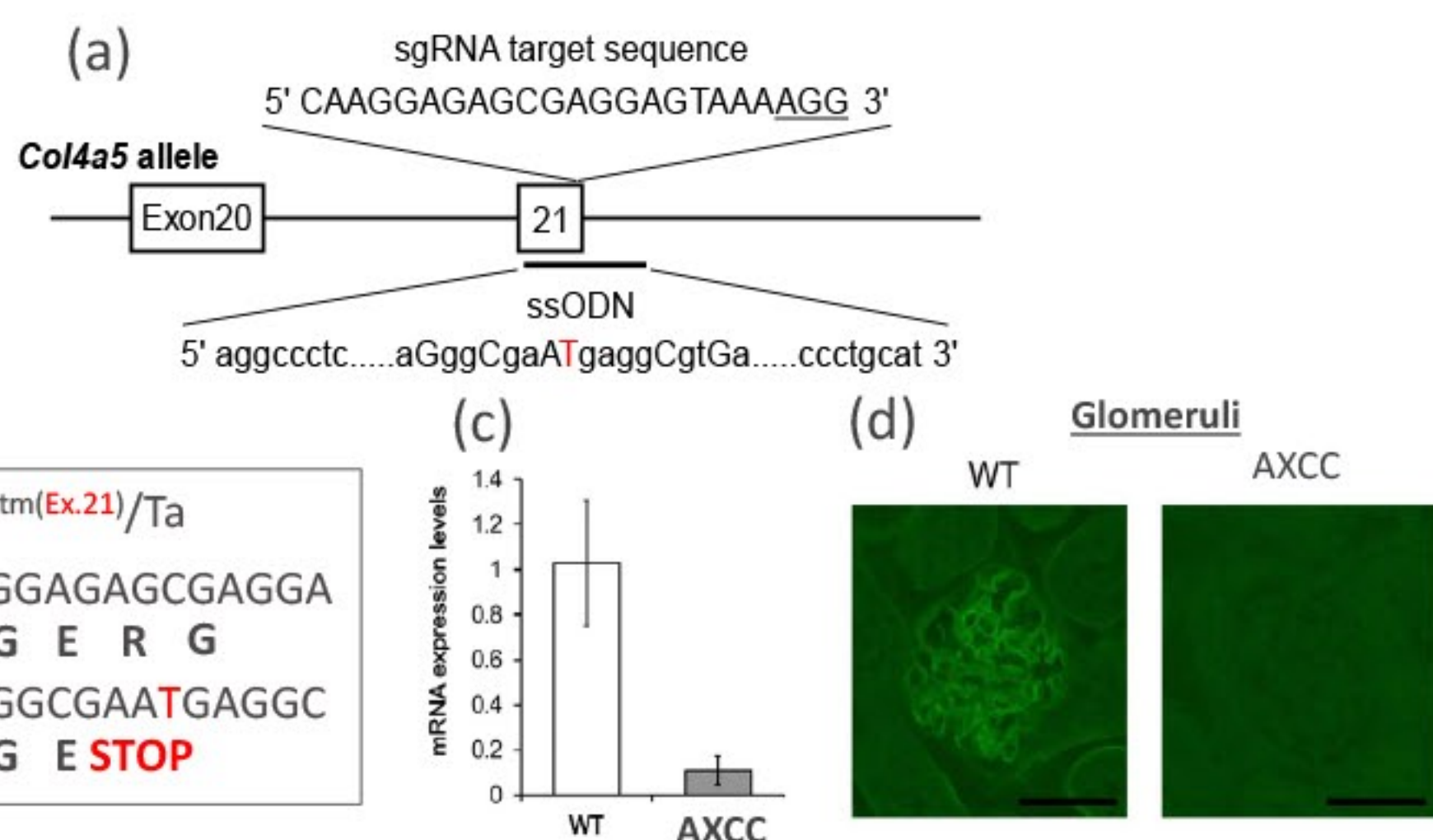
Efficacy of Irbesartan in *Col4a5* R471* mice

Male *Col4a5* R471* mutant mice at 7 weeks of age were divided into experimental groups based on Urine Albumin-Creatinine Ratio (UACR) and body weight. Irbesartan was administered orally at a dose of 50 mg/kg once daily for 17 weeks. At 23 weeks of age, urinary albumin and neutrophil gelatinase-associated lipocalin (NGAL) concentrations were determined using ELISA Kit, while creatinine was measured using the Clinical Analyzer 7180 (Hitachi High-Technologies, Tokyo, Japan). UACR was then calculated. After 17 weeks of treatment, mice were anesthetized with 3–5% isoflurane, and whole blood was collected from the abdominal vena cava. Anemia related factors were measured using the ADVIA 120 Hematology System (Siemens Healthcare Diagnostics, Japan). Kidneys were also harvested for gene expression analysis.

Comparison between Irbesartan and Roflumilast in *Col4a5* R471* mice

Male *Col4a5* R471* mutant mice at 11 weeks of age and age-matched wild-type C57BL/6J mice were used. Mice were orally administered either irbesartan (50 mg/kg) or roflumilast (3mg/kg) for 8 weeks. Urine was collected and blood was obtained via a small incision at the distal tail under unanesthetized conditions. At the end of the study, animals were sacrificed under deep anesthesia. Both kidneys were excised and weighed. Plasma and urinary biochemical parameters (albumin, creatinine, BUN, etc.) were measured using the Clinical Analyzer 7180 or ELISA. Kidneys were also harvested for cAMP measured by ELISA.

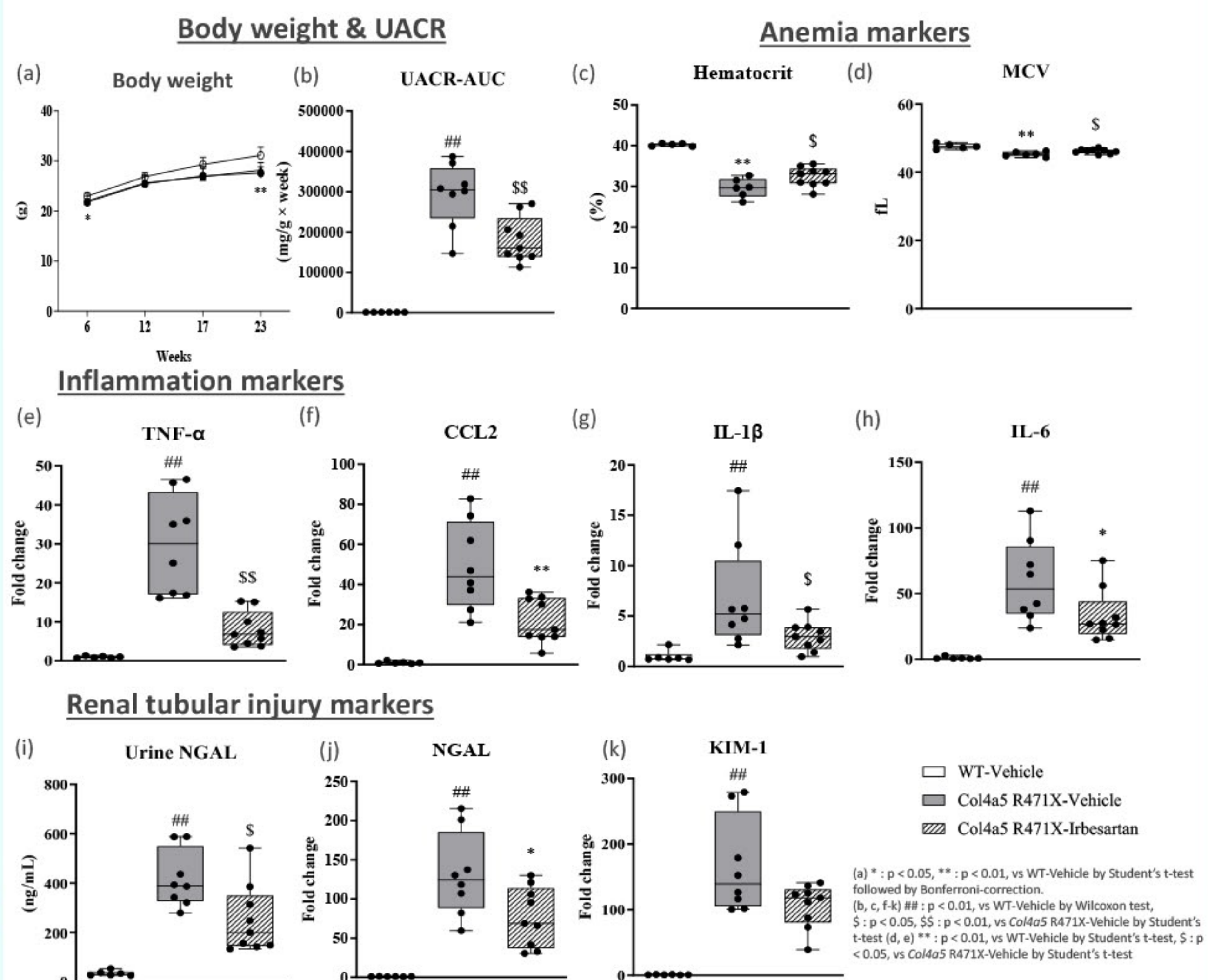
Introduction of the *Col4a5* R471* mutant mouse



Targeting strategy, sequencing analysis, and expression assay for *Col4a5* mutant mice.

(a) Target sequence of sgRNA is capitalized and NGG protospacer adjacent motif sequence is underlined. (b) Sequencing results of wild-type (WT) and mutant (R471*) alleles. (c) *Col4a5* mRNA level was measured in WT and R471* male mice at 6 weeks of age (n = 5). Values are normalized by the expression level of WT and presented as the mean ± SD. (d) Representative images of immunostaining for *Col4a5* in the kidneys of WT and R471* male mice [1].

Figure 1. Efficacy of Irbesartan treatment on kidney injury in *Col4a5* R471* mice



(a) Body weight over the study duration. (b) AUC of UACR over the study duration. (c, d) Hematocrit and MCV (Mean Corpuscular Volume) values in blood. (e-h) Pro-inflammatory gene expressions, (i-k) renal injury markers, urinary NGAL, renal NGAL and KIM-1 gene expressions in kidney of *Col4a5* R471* mice. Data is shown mean ± standard deviation (SD).

Table 1. Comparison between Irbesartan and Roflumilast in *Col4a5* R471* mice

| Mice | WT (wild type) | <i>Col4a5</i> R471* Mutant | | |
|---|-----------------|----------------------------|----------------------------------|-----------------------------|
| Treatment | Vehicle (n = 6) | Vehicle (n = 7) | Irbesartan (n = 7) | Roflumilast (n = 7) |
| UACR (mg/g) | | | | |
| after 8 weeks | 64.30 ± 5.80 | 31899.66 ± 11265.97** | 16772.05 ± 10125.26 [§] | 34260.46 ± 11917.53 |
| Urinary KIM-1 (creatinine correction) (pg / mL) | | | | |
| after 8 weeks | 7.2 ± 2.1 | 142.2 ± 50.7** | 102.5 ± 39.7 | 159.8 ± 23.8 |
| Kidney cAMP (pmol/mg) | | | | |
| after 8 weeks | 0.256 ± 0.019 | 0.268 ± 0.015 | 0.270 ± 0.030 | 0.352 ± 0.045 ^{††} |
| Plasma cAMP (μM) | | | | |
| after 8 weeks | 0.019 ± 0.013 | 0.024 ± 0.005 | 0.031 ± 0.008 | 0.067 ± 0.016 ^{††} |
| PDEs gene expression | | | | |
| <i>Pde4a</i> | 0.55 ± 0.06 | 1.00 ± 0.28** | 0.90 ± 0.11 | 0.84 ± 0.23 |
| <i>Pde4b</i> | 0.64 ± 0.07 | 1.00 ± 0.20** | 0.92 ± 0.11 | 1.06 ± 0.13 |
| <i>Pde4c</i> | 1.11 ± 0.11 | 1.00 ± 0.13 | 0.97 ± 0.12 | 0.92 ± 0.07 |
| <i>Pde4d</i> | 0.87 ± 0.13 | 1.00 ± 0.08 [§] | 1.01 ± 0.08 | 1.17 ± 0.10 |
| Fibrosis-related gene expression | | | | |
| α -SMA | 0.32 ± 0.04 | 1.00 ± 0.23** | 0.95 ± 0.30 | 1.07 ± 0.22 |
| COL3A1 | 0.08 ± 0.01 | 1.00 ± 0.59** | 0.79 ± 0.22 | 0.56 ± 0.12 |
| COL1A1 | 0.06 ± 0.01 | 1.00 ± 0.51** | 0.79 ± 0.25 | 0.66 ± 0.15 |

Fold-changes of UACR, urinary KIM-1, kidney and plasma cAMP, PDEs and pro-fibrotic gene expressions in kidney were measured and calculated. Data are presented as the mean ± standard deviation (SD).

§§ : p < 0.01, § : p < 0.05, vs WT Vehicle by Student's t-test, ** : p < 0.01 vs WT Vehicle by Wilcoxon-test, # : p < 0.05, vs *Col4a5* R471* Mutant Vehicle by Student's t-test, †† : p < 0.01 vs *Col4a5* R471* Mutant Vehicle by Wilcoxon-test

Summary

- Col4a5* R471* mutant mice exhibited significant increases in UACR, renal injury and pro-inflammatory gene expressions and decreased anemia-related factors.
- We investigated that Irbesartan ameliorated UACR, anemia, renal injury and inflammation markers in AS mice with a patient-derived mutation, for the first time.
- While roflumilast tended to reduce renal pro-fibrotic gene expressions, was insufficient to ameliorate AS physiology.

Conclusion

- These findings underscore the importance of targeting renal vascular dynamics and inflammation in the management of AS, and provide a foundation for future therapeutic strategies.

References

- Biochemistry and Biophysics Reports 17 (2019) 81–86
- Eur J Pharmacol. 2021 Mar 5:894:173852.

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

