# Repurposing chemical chaperones to the rescue of mouse models of Alport syndrome

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## ABSTRACT

Alport Syndrome (AS) is a severe inherited glomerulopathy caused by mutations in the genes encoding the α-chains of type IV collagen [1,2], the most abundant component of the glomerular basement membrane (GBM). Alport patients lack effective therapies beyond blockade of the renin-angiotensin system.

Recently, we have characterized in detail the kidney phenotype of the knockin mouse [3], carrying the Col4a3-p.Gly1332Glu in homozygosity and of a compound heterozygous mouse model for AS, that carries the Col4a3-p.Gly1332Glu substitution in compound heterozygosity with a Col4a3 knocked-out allele. Both mice recapitulate essential features of AS, including shorten lifespan by 30–35%, increased proteinuria, increased serum urea and creatinine, pathognomonic alternate GBM thinning and thickening, and podocyte foot process effacement [4].

After a long-term treatment, with these two chaperones, we found that the GBM morphology and structure of the 4-PBA treated mice showed a considerable improvement compared with the control (placebo treated) group. Based on EM measurements there is a 43% reduction of lesions and a significant decline of the lesion's severity in the GBM of 4-PBA treated Alport mice. However, the measurements from TUDCA-treated AS mice did not differ from the placebo group. Additionally, the 4-PBA treatment could inhibit proteinuria and hematuria and fibrosis in AS mice when compared with control mice. Probably, the administration of 4-PBA can effectively stabilize the conformation of the mutated Col4a3, improve its folding, alleviating with this way the glomerular filtration barrier in the Alport mice.

## METHODS

**Treatment with:**

Chaperones:

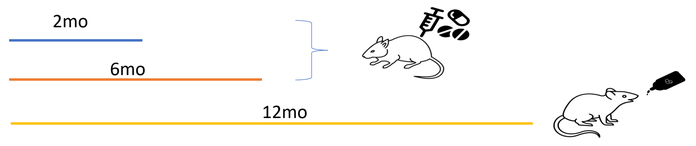
Sodium 4-phenylbutyrate **(4-PBA)**

Sodium tauroursodeoxycholate **(TUDCA)**

Vehicle:

Phosphate buffered saline **(PBS)**

**Duration and type of treatment:**




**Treated mice:**

1) Col4a3 knockin (**mut/mut**),
2) Col4a3 compound heterozygous (**mut/-**),
3) Wild type mice

## RESULTS

### 1. Treatment with 4-PBA restores the GBM thickness in AS mice

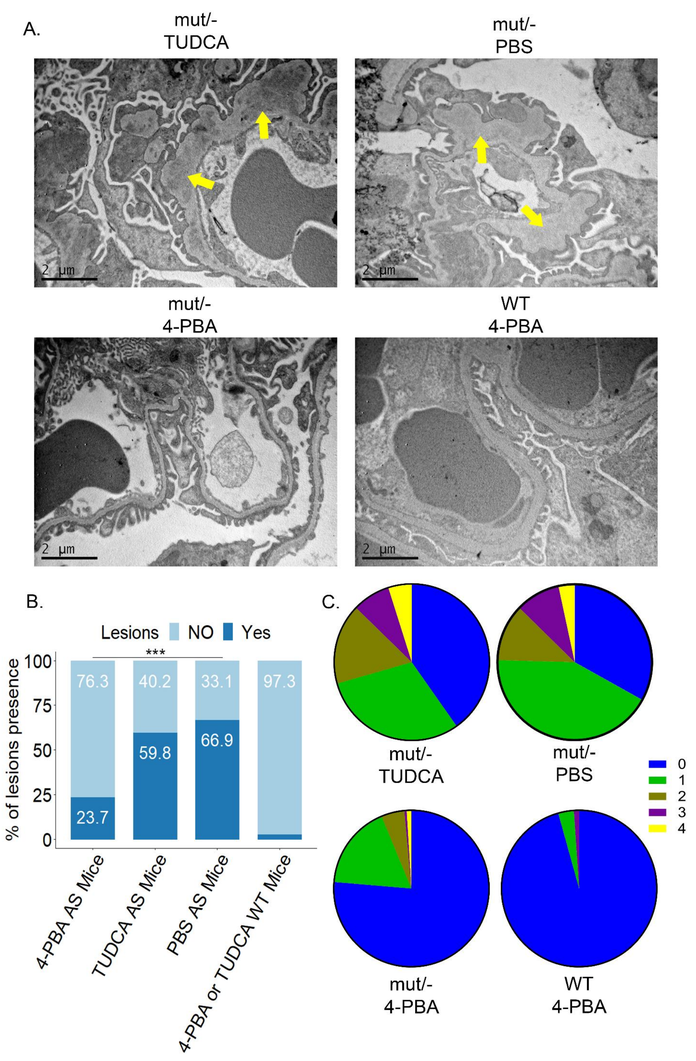



Figure 1: **Administration with 4-PBA can restore the GBM thickness in compound heterozygous Col4a3 mut/− and homozygous Col4a3 mut/mut mice**.
**(A)** The PBS-treated control group of Col4a3 mut/− mice, demonstrate thin GBMs with areas of severe irregular thickening (yellow arrows), consistent with AS nephritis. The same irregular morphology was noticed in the GBM of the TUDCA treated Col4a3 mut/− mice. However, the 4-PBA treated Col4a3 mut/− mice showed a restored and more regular GBM compared to control groups. Importantly, the treatment of wild type mice with these two chaperones had no adverse effects on the GBM morphology. **(B)** Diagram showing the percentage of lesions (dark blue color) that are present in 539 examined loops in the GBM from all groups. Note the small percentage (23,7%, n=207) of lesions in the 4-PBA treated mutant mice compared to the PBS (66,9%, n=118, p-value <0.0001) and TUDCA (59,8%, n=102, p-value= 0.2719 NS) group. Results were analysed using chi-square with Tukey post-testing.
**(C)** Diagram showing the degree of lesions severity in the GBM of each treated group. The 4-PBA treated mutant mice had less severe or milder lesions compared to the PBS or TUDCA treated mutant mice. Samples that followed 4-PBA treatment have statistical differences with P<0.001 compared with non-treated samples. Results were analysed using one-way ANOVA with Tukey post-testing.

### 2. Decreased interstitial fibrosis, segmental and global glomerulosclerosis in the 4-PBA treated AS mice

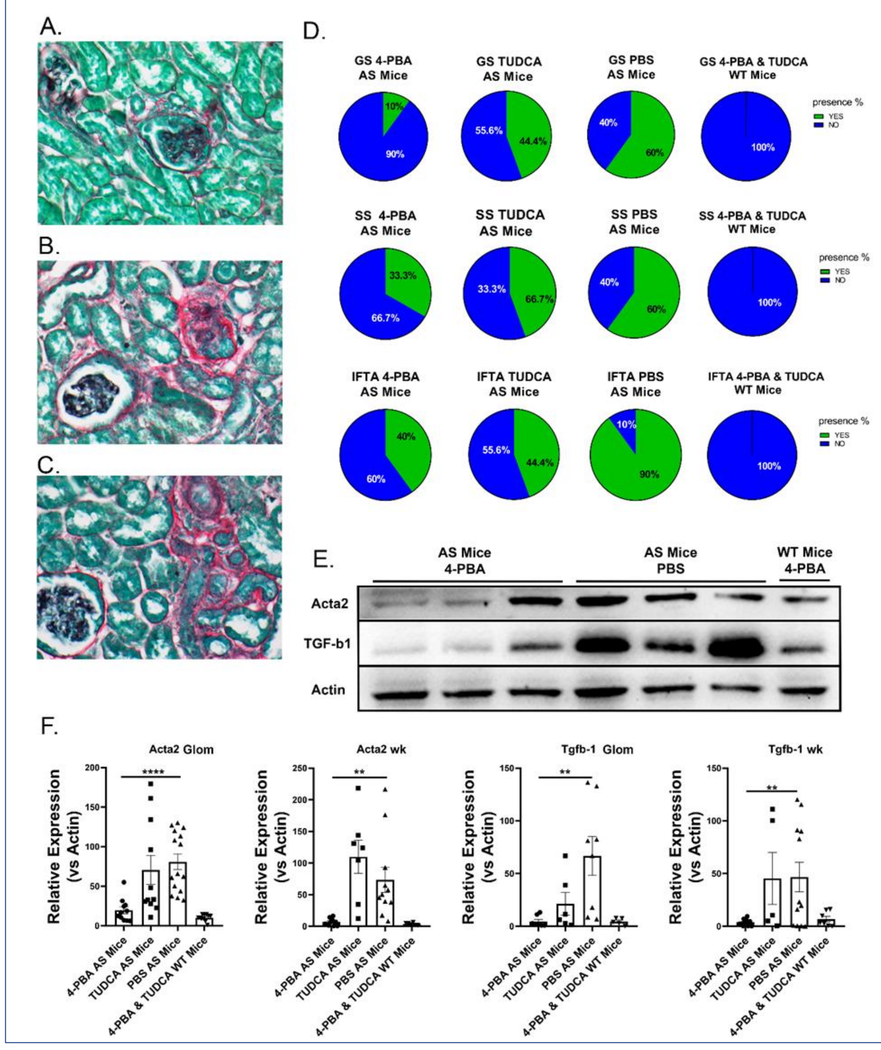



Figure3: **Reduced interstitial fibrosis, segmental and global glomerulosclerosis in the 4-PBA treated AS mice**

(**A-C**) Sirius-red staining on kidney sections of 4-PBA treated mice (A) and PBS-treated mutant mice (B-C) to assess renal fibrosis. (D) Diagram showing the percentage of (green colour) of glomeruli with segmental sclerosis (SS), global glomerulosclerosis (GS) and interstitial fibrosis and tubular atrophy (IFTA) that are present in treated and non-treated mice. (E-F) Western blot analysis of profibrotic markers TGF-β1 and Acta2 displaying decreased levels in glomeruli isolates of 4-PBA treated AS mice. (F) Quantification of representative blots for the expression levels of Acta2 and Tgfb1 in either glomerular lysates (left graph) or whole kidney lysates (right graph) in treated and non-treated AS mice normalized to Actin levels after the long-term treatment. AS mice: Col4a3 mut/mut and Col4a3 mut/− mice; Acta2, alpha-smooth muscle actin.

### 3. Less albuminuria and hematuria in 4-PBA treated AS mice

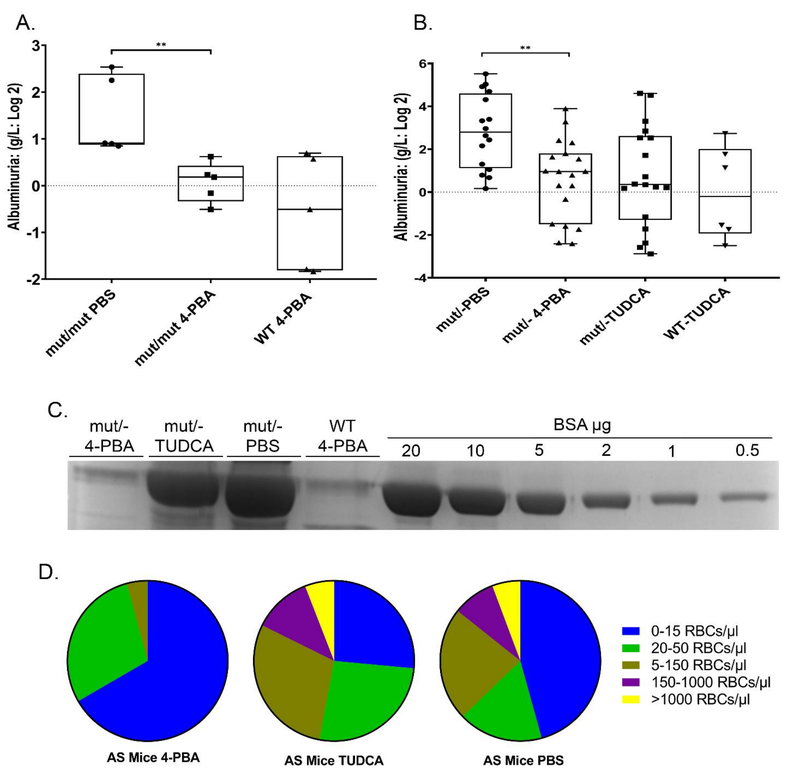



Figure 2: **Reduced albuminuria and hematuria in Col4a3 mutant mice after the long-term treatment with 4-PBA chaperone.** (A,B) Graph of urine albumin concentration (g/L) in chaperone treated and non-treated mut/mut (left graph) and mut/- (right graph) mice after the long-term treatment. Results were analysed using one-way ANOVA with Tukey post-testing. Statistically significant differences (*P*<0.01) were noted in 4-PBA treated mice compared with non-treated samples.
(C) An illustrative example of urine fractionation by SDS-PAGE. 20μL of urine from mut/mut mice, that were treated either with 4-PBA (1st lane) or with PBS (2nd lane), were fractionated together with standard concentrations of Bovine serum albumin on a 7.5% SDS-PAGE and stained with Coomassie blue. D) Hematuria in chaperone treated mutant mice. Diagram represents the percentages of AS Mice that have different levels of hematuria (number of red blood cells per μl of urine) after the long-term treatment compared to control mice.

### 4. Reduction of Col4a3 in glomeruli of 4-PBA treated AS mice

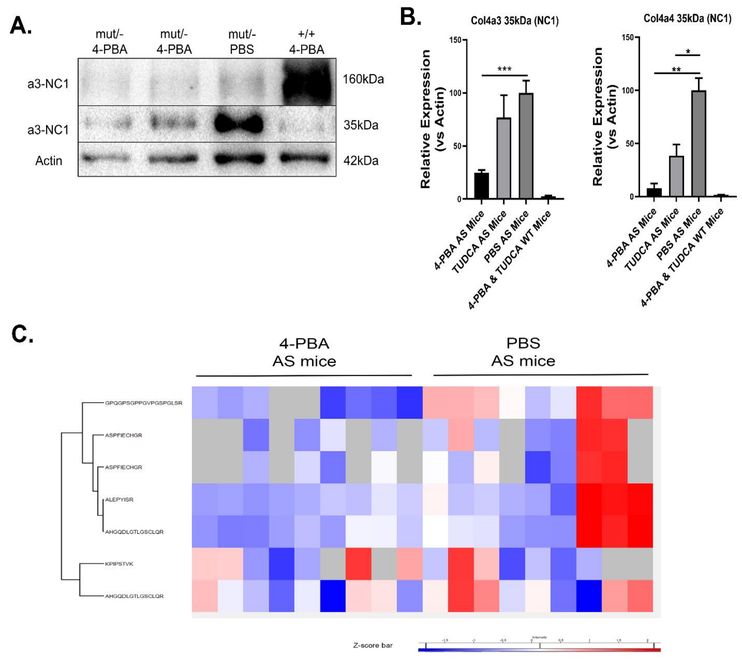



Figure 4: **The 35-kDa Col4a3 fragment is downregulated in glomerular isolates of 4-PBA treated AS mutants (mut/mut and mut/-)**. (**A**) Representative WB to demonstrate Col4a3 expression level change in glomeruli lysates after 4-PBA treatment. A specific antibody that recognizes the NC1 domain of Col4a3 chains was used to identify the α3 expression. (**B**) Quantification of representative blots as shown on the graphs where the expression levels of α3 and α4 chains (in glomerular lysates) were normalized to Actin levels after long-term chaperone treatment. Data are means ± SEM (n≥3). Results were analyzed using one-way ANOVA with Tukey post- testing (*P<0.05, **P<0.01, *** P<0.001). (**C**) Mass spectrometry analysis from glomeruli lysates, validating the reduction of Col4a3 peptides that correspond to the 35kDa fragment next to p.1332, in 4-PBA treated AS mice compared to PBS treated AS mutants. Heatmap illustrating specific Col4a3 peptides downregulated in glomeruli isolates which are reduced after 4-PBA treatment of AS mice. High expression is in red and low expression is in blue.

## CONCLUSIONS

- The long-term treatment with **4-PBA** proved beneficial for the two AS mouse models: the Col4a3 knockin and the compound heterozygous mouse, that both carry the ***Col4a3***-p.Gly1332Glu substitution
- The chaperone 4-PBA could effectively restore to a sufficient degree the morphology and thickness of GBM in both mutant mice
- The chaperone 4-PBA could prevent the increase of proteinuria or hematuria and inhibit the emergence of fibrosis

## ACKNOWLEDGEMENTS

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## REFERENCES

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[3] Pieri M, et al. (2014) *J Am Soc Nephrol* [4] Odiatis C, Savva I, et al. (2020) *Matrix Biol Plus*