Characterization of the Intrarenal Renin Angiotensin System in Experimental Alport's Syndrome

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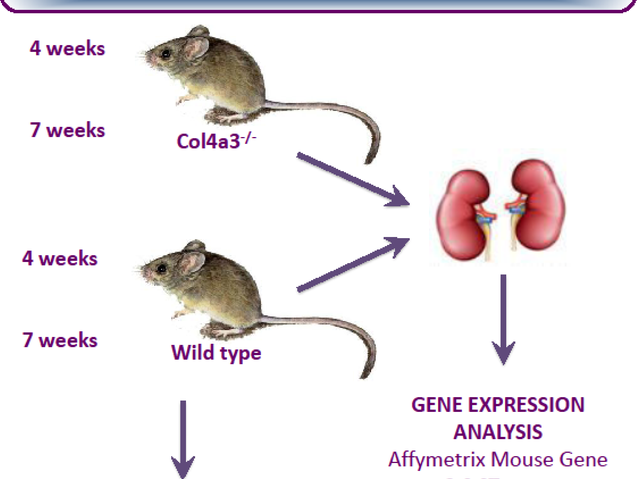
ABSTRACT

Alport syndrome (AS) is a hereditary nephropathy caused by mutations in type-IV collagen genes. Although studies have shown that renin-angiotensin system (RAS) blockade attenuates both experimental and clinical AS-induced nephropathy, the impact of AS on the intrarenal RAS has not been elucidated. Accordingly, we evaluated RAS in 4 and 7-week-old Col4A3$^{-/-}$ and wild-type (WT) mice. Both, angiotensinogen and renin expression were increased in Col4A3$^{-/-}$ compared to WT mice. Although angiotensin converting enzyme (ACE) expression was decreased, kidney angiotensin II (AngII) levels tended to be higher in the Col4A3$^{-/-}$ mice. Renal ACE2 expression and activity was decreased in Col4A3$^{-/-}$ mice, and the urinary excretion rate of ACE2 paralleled the decline in tissue expression. These changes in ACE2 were associated with a decline in the tissue Ang-(1-7) levels and a corresponding marked increase in the AngII/Ang-(1-7) ratio. Microarray analysis showed that renal expression of putative markers of RAS activity, including heme oxygenase-1 (HO-1), was up-regulated in the kidneys of 7-week-old Col4A3$^{-/-}$compared to WT mice. HO-1 kidney protein expression and urine excretion rate were also increased in Col4A3$^{-/-}$ mice compared to WT mice. In conclusion, progressive kidney injury in AS is associated with marked changes in expression of intrarenal RAS components. These changes associate with altered angiotensin peptide levels and an increase in kidney RAS bioactivity.

METHODS



RESULTS

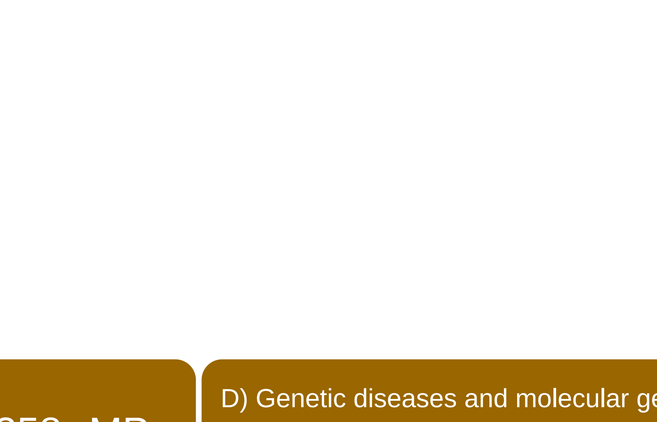


Figure 1.PCR genotyping for the mice utilized in the study(A) and the heat map for collagen IV α1-α5 gene expression in kidney tissue derived was from the microarray analysis of 4- and 7-week-old WT and Col4A3$^{-/-}$mice (KO) (B). Urinary albumin excretion was significantly increased from the 4 week point in the Col4A3$^{-/-}$(KO) mice (C), while serum creatinine was increased at 7 weeks without statistical significance (D). Histopathologic injury in the kidney is evident in the periodic acid–Schiff (PAS) (E) and Masson's trichrome-stained sections (F). Tubule dilatation and tubulointerstitial scarring was particularly prominent in the Col4A3$^{-/-}$ mice at 7 weeks of age compared to the WT mice. Each column represents mean ± SE. *p< 0.05 compared with the WT. Magnification, ×100

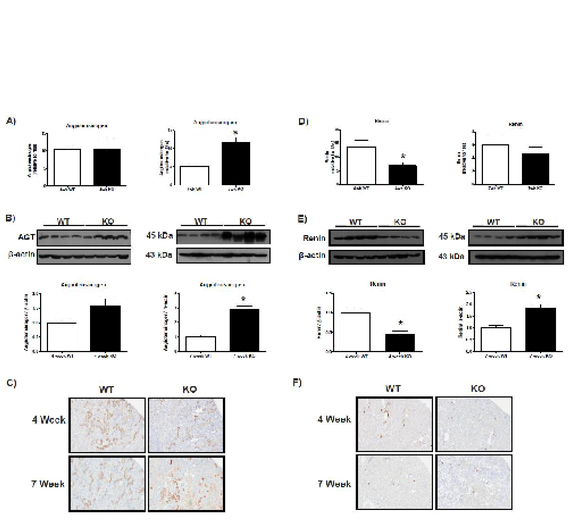


Figure 2. Angiotensinogen (AGT) and renin expression in kidneys of KO and WT mice. AGT mRNA levels increased in 7 week old KO mice compared to age-matched WT mice, while the expression was similar in 4 week old KO and WT mice (A). AGT protein expression in the kidney was significantly higher in 7week old KO mice compared with 7week old WT (B).Immunostainingfor AGT.Angiotensinogen positive area was markedly increased in 7week old KO mice compared to 7week old (C).Renin mRNA was decreased in 4 week old KO mice compared to age-matched WT mice, while there was no difference between 7 week old WT and KO mice (D). Renal renin protein expression was also significantly higher in 7week old KO mice compared with 7-week-old WT mice, while renin protein expression was decreased in 4 week old KO mice compared to age-matched WT mice (E).Immunostaining for renin demonstrates predominant staining in juxtaglomerular cells, with most abundant staining visible in 4 week old WT and 7 week old KO mice (F). Each column represents mean ± SE. *p< 0.05 compared with the WT. Magnification, ×100

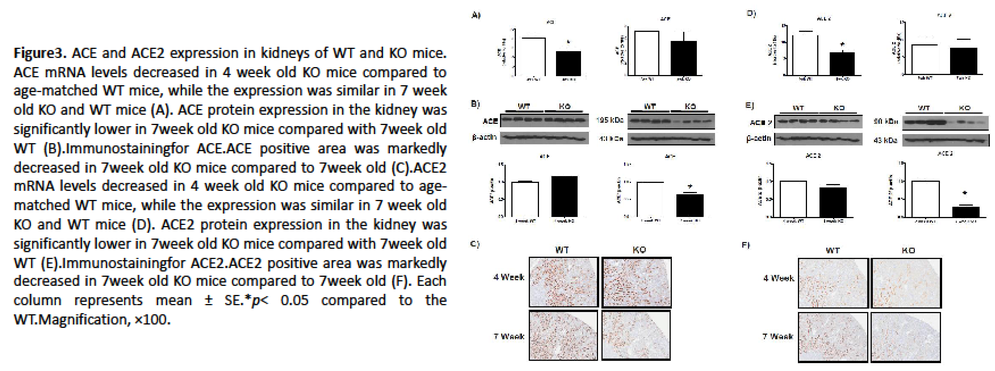


Figure3. ACE and ACE2 expression in kidneys of WT and KO mice. ACE mRNA levels decreased in 4 week old KO mice compared to age-matched WT mice, while the expression was similar in 7 week old KO and WT mice (A). ACE protein expression in the kidney was significantly lower in 7week old KO mice compared with 7week old WT (B).Immunostainingfor ACE.ACE positive area was markedly decreased in 7week old KO mice compared to 7week old (C).ACE2 mRNA levels decreased in 4 week old KO mice compared to age-matched WT mice, while the expression was similar in 7 week old KO and WT mice (D). ACE2 protein expression in the kidney was significantly lower in 7week old KO mice compared with 7week old WT (E).Immunostainingfor ACE2.ACE2 positive area was markedly decreased in 7week old KO mice compared to 7week old (F). Each column represents mean ± SE.*p< 0.05 compared to the WT.Magnification, ×100.

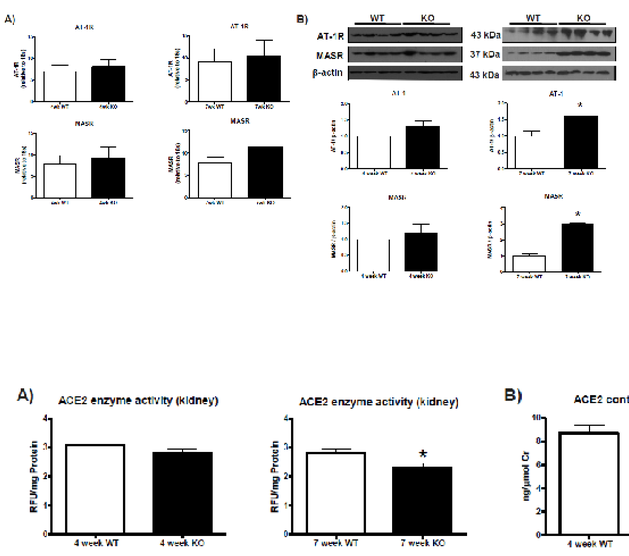


Figure4. AT-1R and MasR expression in kidneys of WT and KO mice. RT-PCR analyses demonstrate that AT-1R and MasR are not differentially regulated at the level of transcription in WT compared to KO mice (A).In contrast, AT-1R and MasRprotein expression in the kidney are significantly higher in 7week old KO compared with 7week old WT mice, while there are no differences between 4 week old WT and KO mice (B).Each column represents mean ± SE.*p< 0.05 compared with the WT.

Figure5. ACE2 enzyme activity in 4 and 7 week old WT and KO kidney tissue. ACE2 enzyme activity (determined by ELISA and normalized to total protein) in kidney tissues of 7 week old KO mice is significantly decreased compared to 7 week old WT mice (A). ACE2 protein excretion in urine is also significantly lower in 7 week old KO mice compared to 7 week old WT mice (B). Each column represents mean ± SE.*p< 0.05 compared with the WT.

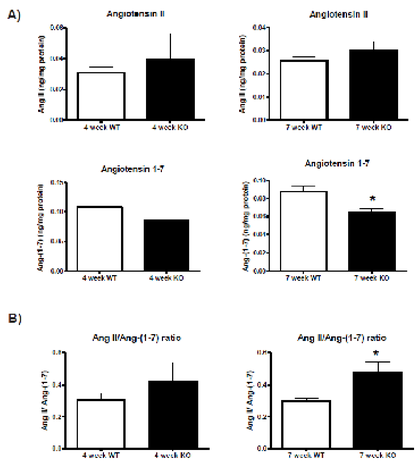


Figure 6. Angiotensin (Ang) II and Ang-(1-7) peptides in kidneys of 4 and 7 week old WT and KO mice. Peptide levels were determined by ELISA and normalized to total protein. Ang II expression is not significantly different between WT and KO mice at 4 and 7 weeks of age, while Ang-(1-7) peptide is significantly lower in 7 week old KO compared to WT mice. Ang-(1-7) renal expression is not different between 4 week old WT and KO mice (A). Ang II/Ang-(1-7) ratio is significantly increased in 7 week old KO mice compared to 7 week old WT mice (B). Each column represents mean ± SE. *p< 0.05 compared with the WT.

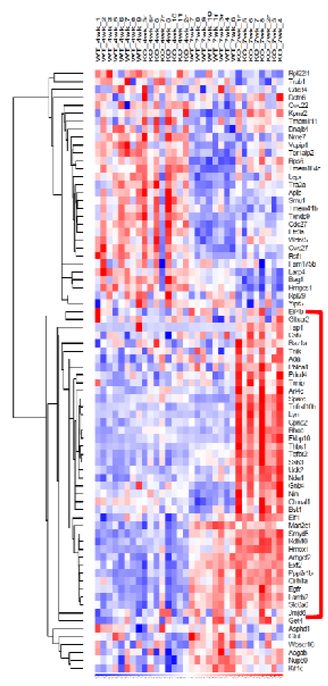


Figure 7. Heatmap of mRNA expression of Angiotensin II-regulated proteins in kidneys of 4 and 7weekold WT and KO mice. Unsupervised hierarchical cluster analysis of kidney expression of 76 Ang II-regulated genes in WT and KO animals. Gene expression was performed byAffymetrixGeneChip Mouse Gene 2.0 ST arrays (n=8/group and at each time point). Columns represent kidney samples, and rows represent different genes. Red colour indicates increased expression relative to mean (white colour), while blue colour indicates decreased expression relative to mean. Genes marked by red parenthesis are upregulated in 7weekold KO mice compared to age-matched WT mice.

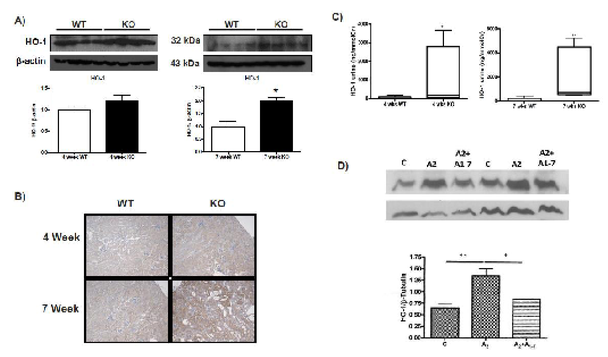
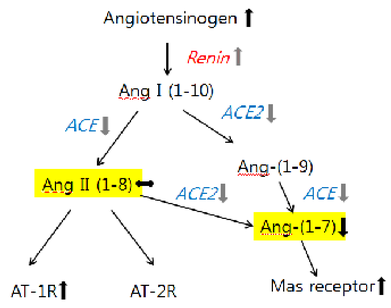


Figure 8. HO-1 analyses *in vivo* and *in vitro*.HO-1 protein expression in kidneys of WT and KO mice by Western blot analysis.HO-1 protein expression in the kidney was significantly higher in 7week old KO mice compared with age-matched WT or 4 week old mice (A).Immunostaining for HO-1 demonstrates that HO-1expression is increased in 7week old KO mice compared with age-matched WT or 4 week old mice (Magnification, ×100). HO-1 appears expressed in proximal tubules (B). Box-and-whiskers plot of HO-1 urine excretion in WT and KO mice. HO-1 urine excretion adjusted for creatinine in 4 week and 7weekold mice. There were 6 mice in the KO and 5 in the WT groups. (C). HO-1 expression in PTECs stimulated with Ang II and Ang-(1-7) *in vitro*. Representative Western blot and densitometry of PTECs stimulated with control, Ang II (A2), or Ang II + Ang-(1-7) (A2+A1-7) for 8 hours (D). β-tubulin is used as loading control. Each bar is representative of 3 experiments. Each column represents mean ± SE.*p<0.05, **p<0.01 compared to WT.

SUMMARY

Schematic diagram of RAS component expression



CONCLUSIONS

In conclusion, the early phase of kidney injury in mice with experimental AS is associated with marked changes in intrarenal RAS component expression. Reduced ACE2 expression and activity contributes to altered angiotensin peptide levels, in particular, a decrease in kidney Ang-(1-7) levels. This imbalance in angiotensin peptides is associated with an increase in Ang-II bioactivity, which is also reflected in decreased urinary excretion of ACE2 and HO-1. ACE2 and HO-1 may serve as markers of kidney injury and treatment, and administration of ACE2 and/or Ang-(1-7) may represent novel therapeutic approaches in AS.

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