The Col4A3 KO mouse:
A New Model of Experimental Diabetic Kidney Disease

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BACKGROUND
Attenuating GFR loss is the primary outcome in clinical trials of kidney disease. Current treatments for diabetic kidney disease (DKD) are essentially the same as in non-diabetic kidney disease (ACEI, SGLT2i, MRAs). The dearth of mouse models of DKD that develop a progressive GFR decline and the similar response to treatment in DKD and non-DKD in humans suggest that non-diabetic mouse models may be useful in testing the efficacy of additive non-glycemic interventions in both DKD and non-DKD.

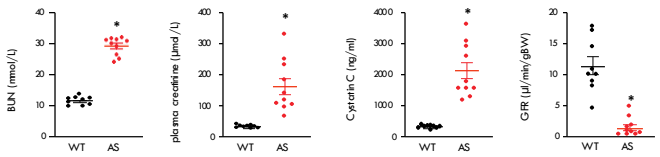
AIMS
We sought to: (1) find a model of kidney disease with progressive loss of GFR, (2) to then find a treatment regimen dose that recapitulates human disease where treatment halves the rate of GFR loss (the human equivalent treatment regimen or HETR), and (3) to determine if such a model was suitable for studying novel approaches to kidney disease in combination with an ACE inhibitor.

ALPORT MOUSE
Like DKD, Alport Syndrome (AS) is also a fibrotic kidney disease that leads to a decline in kidney function where the progressive reduction in the glomerular filtration rate (GFR) determines disease outcome with end-stage kidney disease, requiring dialysis, defined as an eGFR of <15 ml/min/1.73 m^2. Accordingly, the ability of a drug to reduce GFR loss is the primary outcome for studying therapeutic effectiveness in patients. As such, GFR should also be the primary outcome in preclinical studies of CKD including DKD. However, unlike humans, standard dose ACE inhibition (ramipril 10 mg/kg) almost completely normalizes kidney function (GFR, ACR) and structure (glomerulosclerosis, tubulointerstitial fibrosis and podocyte loss) in the kidneys of the commonly used Col4a3 KO mouse model. This near-complete normalization with ramipril precludes investigators being able to examine the effect of newer agents added on top of standard of care.

METHODS
We sought to establish an Alport Syndrome/CKD/DKD mouse model with more similarity to its human counterpart by:
(1) Using GFR as the primary outcome to compare the reliability of different GFR methods given the potential influence of food intake and muscle mass in Alport mice that typically eat less and have lower body weights than wild type mice, and
(2) Again, using GFR as the primary outcome we sought to establish a model for testing agents on top of ACE inhibition by examining the dose-response relationship to ramipril treatment aiming to find a dose that more closely approximated the response to ACE inhibition in humans ~50% improvement).
Comparing the dynamic range of commonly used indices of kidney injury, including the assessment GFR & proteinuria, glomerulosclerosis, and podocyte loss as key structural outcomes.

TWO STUDIES
To satisfy the above aims we conducted two studies:
Study 1: GFR was measured (mGFR) in wild type and Col4a3 KO mice comparing the gold standard of fluorescein-labelled sinistrin with plasma creatinine, BUN and cystatin C.
Study 2: Having established cystatin C as a preferred method, we assessed the ACE inhibitor dose-response relationship, aiming for an approximate 50% improvement in GFR.

STUDY 1: RESULTS
An 11-fold difference in WT *v* AS mice was evident for sinistrin-GFR, in comparison with ~5 fold for plasma creatinine, ~3 fold for BUN and 7 fold for cystatin C. All comparisons p <0.0001. Unlike BUN that is influenced by food and creatinine that is affected by muscle mass (AS mice being notably smaller), cystatin C is not and may provide a more reliable, easily accessible method for assessing GFR in Col4a3 KO (AS) mice.



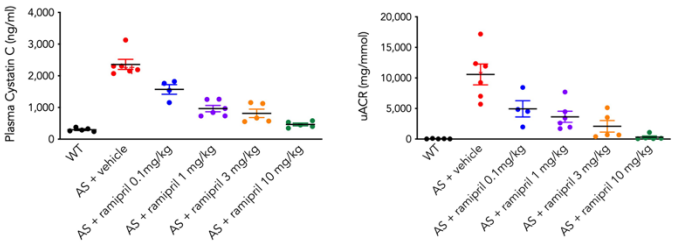
STUDY 2: HYPOTHESIS
Unlike humans, standard dose ACE inhibition (ramipril 10 mg/kg) almost completely normalizes the kidney in the Col4a3 KO (AS) mouse. We sought to establish a model with more similarity to its human counterpart by examining the ACE inhibitor dose-response relationship, aiming to get an approximate 50% improvement in GFR.

METHODS
Accordingly, Col4a3 KO (AS) mice were randomized to receive:
1. Plain reverse osmosis (RO) water
2. Ramipril 0.1 mg/kg/day in RO water
3. Ramipril 1 mg/kg/day in RO water
4. Ramipril 3 mg/kg/day in RO water
5. Ramipril 10 mg/kg/day in RO water

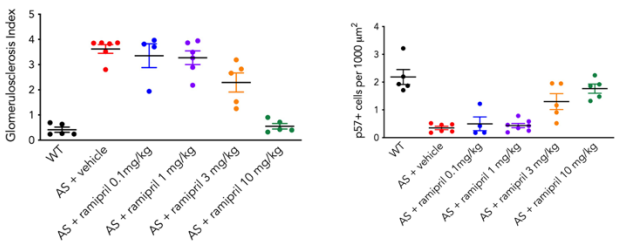
METHODS CONT
Function and structure were assessed as:
a. Cystatin C for GFR
b. Urinary albumin: creatinine ratio (UACR)
c. Glomerulosclerosis index (GSI) on PAS-stained sections
d. Podocyte count by p57 immunohistochemistry

STUDY 2 RESULTS
Ramipril induced a dose-dependent improvement in cystatin C and albuminuria with 1-3 mg/kg/day leading to an ~50% improvement. Standard, high dose therapy (ramipril 10 mg/kg/day) completely abrogated the loss of GFR and ↑ACR in the AS mouse.
Kidney structural changes were also dose-dependent but with a dose-response relationship that was different from the functional changes observed above. Standard, high dose therapy (ramipril 10 mg/kg/day) reversed the extent of glomerulosclerosis and podocyte loss in this model with ramipril 3 mg/kg/day having an intermediate effect. Unlike the effects seen at lower doses on CysC and UACR, ramipril at 0.1 and 1 mg/kg/day did not attenuate the glomerular structural abnormalities in the AS mouse.

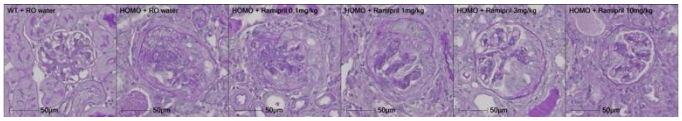
KIDNEY FUNCTION



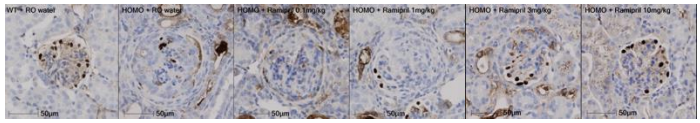
KIDNEY STRUCTURE



HISTOPATHOLOGY: GLOMERULOSCLEROSIS (PAS)



HISTOPATHOLOGY: PODOCYTE LOSS (P57)



KIDNEY FUNCTION
Based on the following characteristics the col4A3 KO Alport mouse serves as an excellent model of progressive kidney disease that recapitulates the features of human CKD including DKD whereby:
- mice develop a steadily progressing loss of GFR with albuminuria
- plasma cystatin C provides an easily quantifiable and robust measure of GFR
- mice develop progressive and severe glomerulosclerosis akin to human DKD/CKD
- like human DKD/CKD with proteinuria, podocyte loss is also a key feature of this model
- dose-dependent effects of ACEI provide the ability to assess the efficacy of new primarily non-glycemic therapies used in addition to standard of care with ACE inhibition