

## Cystinosis Research Foundation Progress Report

Title: **Cysteamine effects on extracellular matrix accumulation in chronic kidney disease.**

Grant number: 412560090101

Investigators: Allison Eddy M.D. and Daryl Okamura M.D.

Funding Period: July 1, 2008 to June 30, 2011

Progress Report: July 1, 2009 to December 31, 2009

### OVERVIEW

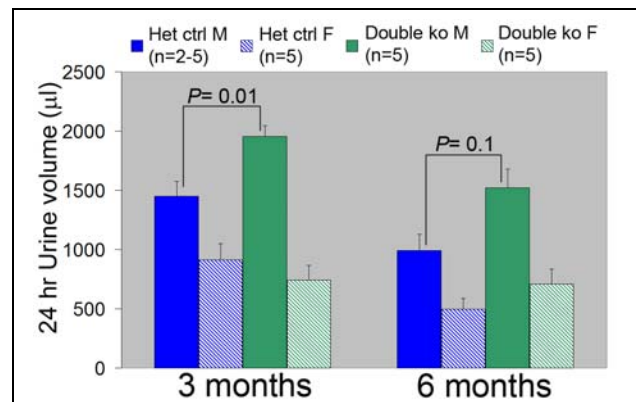
The project is progressing according to the original research plan. The three mouse lines are all actively breeding (*Ctns*<sup>-/-</sup>, *vanin*<sup>-/-</sup> and *Ctns*<sup>-/-</sup>*vanin*<sup>-/-</sup> double knockouts). The animal work for the first cysteamine *in vivo* study has been completed and tissue analyses continue. We now have a cohort of 10 *Ctns*<sup>-/-</sup>*vanin*<sup>-/-</sup> double knockout mice who are 6 months of age. Although data are still preliminary, it appears that the males are polyuric compared to the *Ctns*<sup>+/-</sup>*vanin*<sup>+/-</sup> double heterozygous sibling controls.

1) **Aim #1:** To perform preliminary studies to develop an optimal model and drug delivery strategy to investigate the effect of cysteamine in a mouse chronic kidney disease model.

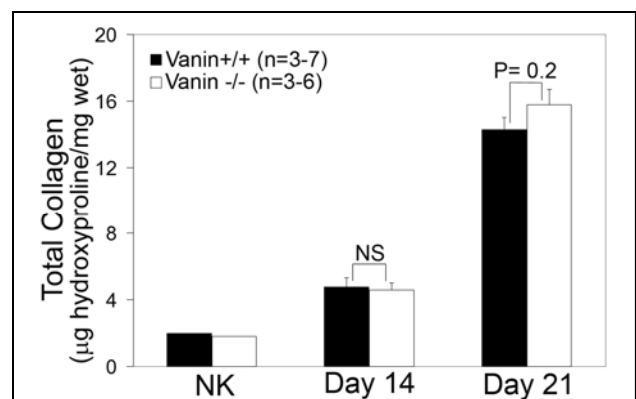
#### a) **Searching for a mouse model of cystinosis-associated nephropathy.**

Based on our hypothesis that *Ctns*<sup>-/-</sup> mice have an essentially normal phenotype due to the expression of endogenous cysteamine synthesized via an enzymatic pathway that is encoded by the *vanin* gene, we have generated a colony of *Ctns*<sup>-/-</sup>*vanin*<sup>-/-</sup> double knock-out mice. We are currently following 26 double knockouts and 16 heterozygous sibling controls. Initial findings based on 24h urine samples collected in individual mouse metabolic cages (n = 2-5 mice per time-point) suggest that the double knockout mice have higher urine volumes, especially in the males (Figure 1). We plan to collect 24h urines every 3 months. As the sample sizes increase, the findings will hopefully reach statistical significant (P values for males at 3 months is already 0.01). Serum BUN levels will be measured at 9 months. At this point we plan to follow the mice until at least 1 year of age before they are sacrificed to examine kidney histology. Two cages of double heterozygous mice are actively breeding to expand the number of mice in this study.

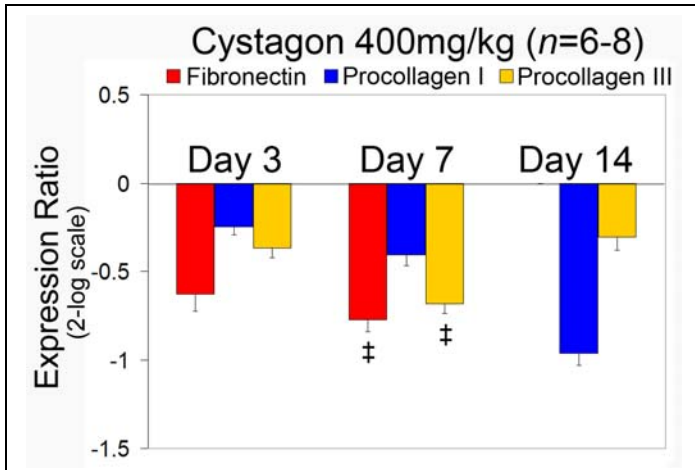
Findings from a study of the unilateral ureteral obstruction (UJO) model in 8-week old male mice, comparing the degree of fibrosis between wild-type (*vanin*<sup>+/+</sup>) and *vanin*<sup>-/-</sup> mice, suggest that *vanin*-deficiency alone does not alter the degree of kidney fibrosis (Figure 2). Since the last progress report the number of 21 day UJO mice has been increased.



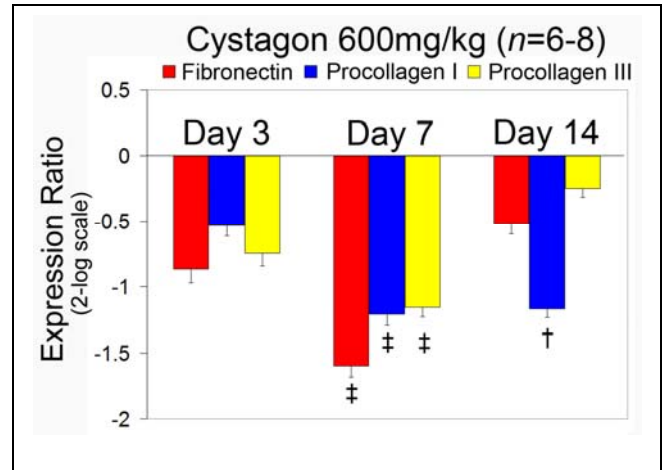
**Figure 1.** Initial data on 24h urine volumes show larger volumes in male (M) *Ctns*<sup>-/-</sup>*vanin*<sup>-/-</sup> double knockouts (Double ko) compared to *Ctns*<sup>+/-</sup>*vanin*<sup>+/-</sup> heterozygous controls (Het ctrl). Additional mice will be entered into this study.



**Figure 2.** Total kidney collagen levels between *vanin*<sup>+/+</sup> and *vanin*<sup>-/-</sup> mice 14 and 21 days after UJO shows no significant differences.



**Figure 3. Cystagon 400 mg/kg group.** Kidney extracellular matrix protein mRNA levels measured by real-time RT-PCR are significantly lower in Cystagon®-treated mice 7 days after UUU. Data are shown as means of the relative expression ratios (treated versus untreated) (n=8/group).



**Figure 4. Cystagon 600 mg/kg group.** Kidney extracellular matrix protein mRNA levels measured by real-time RT-PCR are significantly lower after Cystagon®-treated mice 7 days after UUU. Data are shown as means of the relative expression ratios (treated versus untreated) (n=8/group).

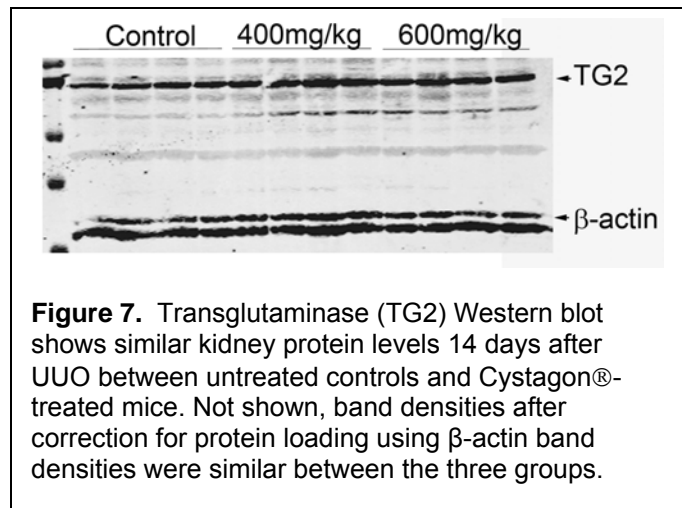
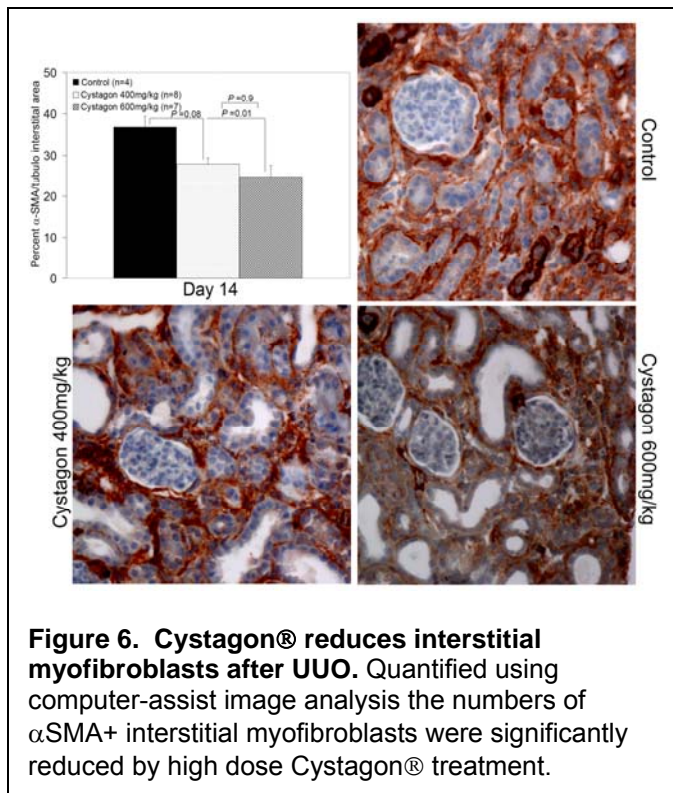
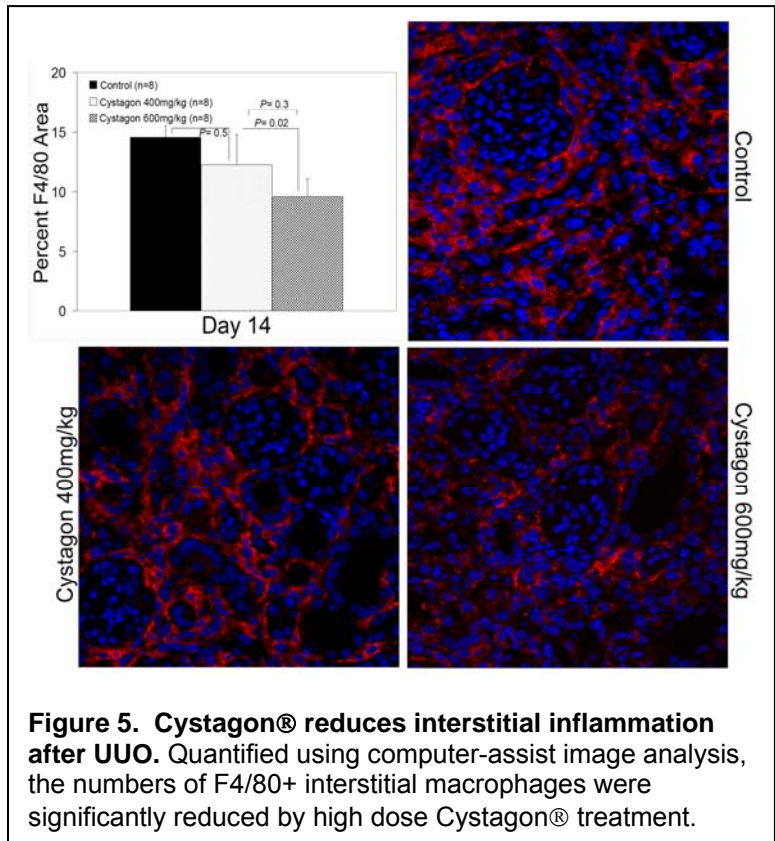
2) **Aim #2:** To investigate the efficacy of cysteamine therapy for interstitial renal matrix protein reduction in chronic kidney disease and to determine its mechanism of anti-fibrotic action.

Based on the findings from a pilot study, the effect of cysteamine (administered as Cystagon® added to the drinking water that was freshly made every 24h) on the degree of renal fibrosis was investigated using two doses: 400 and 600 mg/kg/day. Groups of mice (n = 8 at each time-point) were studied 3, 7 and 14 days after the onset of chronic injury induced by UUU. Both doses were shown to significantly reduce kidney collagen levels by day 14 by ~20% compared to the untreated group (shown on last process report). We plan to extend these observations to a 21-day UUU study.

Kidney tissue studies are now focusing on elucidating the mechanism by which Cystagon® reduces kidney fibrosis. One potential pathway may be its ability to reduce collagen synthesis rates. Since the last progress report matrix gene mRNA levels have now been measured by real-time RT-PCR (qRT-PCR) in all mice 3, 7 and 14 days after UUU (Figures 3 and 4). The peak effect of Cystagon® was observed at 7 days at both treatment doses. Additional mechanisms must also contribute to the anti-fibrotic actions of Cystagon®. Computed-assisted image analysis of immunohistochemically stained kidney sections at day 14 UUU show significantly less interstitial inflammation as measured by the number of F4/80+ interstitial macrophages and significantly fewer interstitial myofibroblasts as measured by the number of  $\alpha$ SMA+ cells, in the 600 mg/kg treatment groups (Figures 5 and 6 respectively). Additional studies are ongoing.

3) **Aim #3:** To investigate the effect of cysteamine on apoptosis of renal tubular epithelial cells, oxidant stress, and other novel target pathways of chronic kidney disease. As originally proposed, the effects on apoptosis, glutathione activity and novel cysteamine targets will be the primary focus of these studies. We have begun to investigate possible effects on transglutaminase (TG2) expression and activity. By Western blotting total kidney TG2 protein levels were unaffected by Cystagon® treatment (Figure 7). Assessment of TG2 activity is planned next.

In summary, early data from the first Cystagon® treatment study establishes its significant anti-fibrotic effects. Ongoing studies are planned to identify how this effect is achieved and to determine if it is sustained when chronic kidney damage persists for longer periods of time. Over the next 6 months we will be able to determine if the early observations on the renal phenotype of the *Ctns*<sup>-/-</sup>*vanin*<sup>-/-</sup> double knock-out mice (polyuria) are sustained as the mice age.



## BUDGET EXPENDITURES

July 1, 2009 to December 31, 2009

Category	Award *	Prior expenditures **	Expenditures this period *** (01/01/09 – 06/30/09)	Anticipated expenditures ****	Total expenditures this period	Total expenditures to date	Remaining Balance
Personnel	\$88,802	\$46,355	\$21,167	\$4,108	\$25,274	\$71,629	\$17,173
Animals/ Maintenance	\$8,333	\$7,108	\$2,330	\$1,109	\$3,439	\$10,546	\$(2,214)
Supplies	\$19,695	\$19,414	\$4,730	\$1,390	\$6,120	\$25,534	\$(5,839)
Travel	\$2,273	-	-	-	-	-	\$2,273
Subtotal	\$119,102	\$72,877	\$28,226	\$6,606	\$34,832	\$107,709	\$11,393
IDC	\$11,911	\$7,288	\$2,823	\$661	\$3,483	\$10,771	\$1,139
Total	\$131,013	\$80,165	\$31,049	\$7,267	\$38,316	\$118,481	\$12,532

\* Award amount for 07/01/08-06/30/09

\*\* Prior expenditures: 07/01/08-06/30/09

\*\*\* Expenditures this period: 07/01/09-12/14/09

\*\*\*\* Anticipated expenditures: 12/15/09-12/31/09

10% budget remaining