

Cystinosis Research Foundation

Third interim report

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Project Title: Impact of diet composition on renal function and bone disease of *Ctns*^{-/-} mice

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In the first year of the project, we have tested the impact of methionine on the kidney function of female *Ctns*^{-/-} rats based on prior data showing that commercial diets enriched with methionine protect kidney function in *Ctns*^{-/-} mice. We have compared the effects of a commercial diet that elicit the Fanconi syndrome in mice (Envigo 2018, Teklad Global) with the same diet supplemented with 0.3% D,L-methionine. Animals were monitored until 8 months of age. Methionine is a sulfur-containing amino acid involved in the synthesis of cysteine through the trans-sulfuration pathway. Since cysteine is a key metabolite for the synthesis of two anti-oxidant agents, namely taurine and GSH, whose expression levels are altered in cystinosis, we have hypothesized that a methionine-enriched diet could exert an anti-oxidant protective effect.

We observed no effects of D,L-methionine on growth and health of wild type and *Ctns*^{-/-} animals. As expected, methionine levels measured in blood and urine were significantly higher in animals fed the methionine-supplemented diet. Based on a recent metabolomic study that has identified biomarkers of the disease in the plasma of patients with cystinosis, including metabolites of trans-sulfuration pathway (cysteine, taurine, GSH, serine) (Nemutlu E, et al, 2023), we have investigated if a similar profile was present in *Ctns*^{-/-} rats. Similarly to patients with cystinosis, we observed lower levels of taurine and serine in 8-month-old female *Ctns*^{-/-} rats, compared to wild type animals. Moreover, the methionine supplemented diet restored taurine plasma values in *Ctns*^{-/-} animals. We are now investigating if GSH levels are also improved.

From the renal standpoint, we had observed an initial trend towards a protective effect of methionine on kidney function at the age of 6 months in *Ctns*^{-/-} rats (glycosuria, low molecular weight proteinuria, albuminuria and polyuria). This was however, no longer observed at 8 months. Nonetheless, Masson's trichrome staining showed increased interstitial fibrosis in kidney sections of *Ctns*^{-/-} rats fed a standard diet, but not of *Ctns*^{-/-} rats supplemented with methionine. Additional analyses are underway.