Cystinosis Research Foundation

First and second reports

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

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In preliminary experiments included in our grant application, we have tested in *Ctns*$^{-/-}$ mice the impact of four commercial diets on the severity of the Fanconi syndrome. These diets provide a similar amount of calories/dry weight and have a similar content of proteins, fats and carbohydrates to guarantee a balanced nutritional allowance to rodents of all ages. As detailed in the application, we observed a significant prevention of glycosuria, proteinuria, aminoaciduria, polyuria and kidney tissue damage in *Ctns*$^{-/-}$ mice that were fed with the D10001 diet from OpenSource.

The aim of this project is to investigate which dietary component of the D10001 diet is protective against the development of Fanconi syndrome in *Ctns*$^{-/-}$ mice, or alternatively, which component of the other three diets is toxic to proximal tubules of *Ctns*$^{-/-}$ mice.

Our first approach was based on a simple comparison of the data sheet illustrating the composition of different diets, to identify components that are only present in the D10001 diet. Of note, diet compositions provided by different vendors are not uniform, and are incomplete. Nonetheless, a first difference was in D,L-methionine content, which was only supplemented in the D10001 diet. In theory, D,L-methionine supplements could provide an alternative source of cytosolic cysteine to cystinotic cells, in which lysosomal cystine efflux is impaired. Since cysteine is the limiting factor for the synthesis of glutathione, methionine supplementation could help cystinotic cells defending against oxidative stress. To support this hypothesis we have performed *in vitro* experiments in which *CTNS*$^{-/-}$ cells were grown in culture media supplemented with 100 or 300 μM D,L-methionine and have shown improved cell responses against oxidative stimuli.

On these bases, we have fed wild type and *Ctns*$^{-/-}$ female rats with the E18 diet from Teklad Global, which causes a severe Fanconi syndrome. Food pellets were supplemented with 0.3% D,L-methionine in half of the animals, to match the concentration of D,L-methionine of the D10001 diet. Food palatability is being checked by verifying daily food intake, which seems to be unaffected by the methionine supplementation. 24h-urine samples are being collected every 8 weeks. Results of these experiments will be available in the coming months.