Development and characterization of a rat model of cystinosis (extension)

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CRISPR/Cas9 based generation of a new rat model for cystinosis

Cystinosis is a lysosomal storage disease (LSD) caused by recessive, inactivating mutations in the *CTNS* gene coding for the proton-driven transporter cystinosin that exports cystine out of lysosomes. The loss of cystinosin results in the accumulation of cystine in lysosomes within all organs, leading to proximal tubular dysfunction and kidney failure, diabetes, hypothyroidism, myopathy, and central nervous system deterioration. The most commonly used model to study cystinosis has been the *Ctns* knockout (*Ctns*$^{-/-}$) mouse. However, the *Ctns* KO mice present with a late-onset, variable and globally less severe phenotype, including incomplete kidney dysfunction, compared to cystinosis patients.

To overcome these limitations, effort has been made to create a new disease model for cystinosis using the rat. As described in our previous report, the CRISPR/Cas9 mediated deletion of the *Ctns* gene resulted in cystine accumulation within various tissues including brain, eye, kidney, liver, heart, muscle and spleen. The *Ctns* KO rats showed growth retardation in both genders, as well as very early manifestations of PT dysfunction (6 weeks). The latter include inappropriate loss of glucose, low-molecular weigth (LMW) proteins, albumin, phosphate and calcium, indicating the development of a complete renal Fanconi syndrome. Histological analysis of rat *Ctns*$^{-/-}$ kidneys revealed the progressive development of fibrotic lesions, including the characteristic swan neck tubules. Similar to patients with cystinosis, the *Ctns*$^{-/-}$ rats develop glomerular lesions with age, outranking the *Ctns* mice which only show tubulopathy. The severe kidney damage detected in *Ctns*$^{-/-}$ rats over time was correlating with increased levels of urea nitrogen and creatinine in the blood, indicating kidney failure, and was associated with decreased survival.

As previously described, we were able to associate PT dysfunction with the loss of endocytic receptors such as megalin (LRP2) and increased levels of regulatory proteins involved in proliferation and cell cycle (PCNA and KI67) in *Ctns*$^{-/-}$ rat kidneys. The underlying force driving this phenotype switch is an impaired lysosomal homeostasis. Using confocal microscopy, we were able to demonstrate increased amount of LAMP1+ vesicles in *Ctns*$^{-/-}$ rat PTs. By following the *in vivo* processing of Cy5 labeled β-lactoglobulin, we showed that enlarged lysosomes in *Ctns*$^{-/-}$ rats lost their degradative capacity. Electron microscopy exposed the presence of cystine crystals already at 3 months of age in *Ctns*$^{-/-}$ rats, significantly earlier compared to the 6 months reported in *Ctns*$^{-/-}$ mice. Crystals were also detected in other organs such as liver and thyroid. Cystine crystals were also detected in the eyes of the *Ctns*$^{-/-}$ rats using an OCT-scanner and a slit lamp as well as severe alterations of the bones using micro-CT.

Beyond the complete phenotypical characterization of the *Ctns* rat model and the development of a new primary PT cell culture system described in our previous report, we performed a head-to-head comparison of the cystinosis rat and mouse models. In summary, the *Ctns*$^{-/-}$ rat displays earlier accumulation of cystine, growth retardation and signs of PT dysfunction compared to the *Ctns*$^{-/-}$ mouse, better reflecting the pathophysiology of human cystinosis. We performed comparative omics analyses between the rat and mouse models, in an effort to gain a comprehensive overview of genes and hence pathways that may differ between the species and be relevant for disease progression. We plan to use a drug–disease network–based computational modeling approach based on these profiles to drive our research towards the discovery of new possible drugs for cystinosis.

Summary: We generate a novel *Ctns*$^{-/-}$ rat model which faithfully recapitulates the clinical symptoms of human nephropathic cystinosis, including early onset of PT dysfunction and progressive evolution towards kidney failure and extra-renal manifestations. Complemented with the derived primary PT cell system and other resources, this *Ctns* rat model will be an invaluable tool for further cystinosis research.