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Medical and molecular therapeutic approaches for cystinuria

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Cystinuria is an autosomal recessive disease characterized by an increased urinary excretion of the amino acids cystine, lysine, arginine and ornithine. Due to the low solubility of cystine in urine, the increased excretion results in the formation of cystine stones. Recurrent cystine stone formation necessitates multiple invasive treatments during the life time which leads to a risk of renal insufficiency and reduction in quality of life.

Medical treatment for cystinuria aims to decrease the concentration of cystine in the urine, increase its solubility and therefore prevent stone formation. Ascorbic acid (AA; Vitamin C) and captopril have been recommended as alternatives to thiol drugs which are effective in reducing cystine but have severe side effects. However, a beneficial effect of AA or captopril on cystine stone formation is controversial and has only been demonstrated in small case series. The first cystinuria mouse model, *Pebbles*, that mimics human cystinuria type I provides an ideal tool for evaluation of stone preventive measures. The characterization of *pebbles* mice revealed that the heterozygous and wild type males have similar metabolic and physiological characteristics and no cystinuria specific metabolic pattern has been observed. All homozygous *pebbles* male mice developed urinary cystine stones during the first year of life. No reduction in the urinary cystine concentration was seen in homozygous *pebbles* mice fed with AA or captopril supplemented diet. An insignificant reduction of cystine stone mass was seen in mice supplemented with AA. Though the stone mass did vary largely in the study and a beneficial effect of AA in some of the animals is possible, an overall statistical relevance was not seen. Captopril supplemented diet did not result in any reduction in the cystine stone mass. The urinary indices in mice on captopril supplemented diet showed that the renal function though was deteriorating in all the mice, the mice on captopril supplemented diet had a better renal function compared to the control homozygous mice. This study could not confirm that AA and captopril are effective reducing agents against cystine as previously thought.

rBAT- $b^{0,+}$AT heterodimer mediates the transport of cystine from the extra-cellular space into the cells. Cystinuria type I results due to a defect in the SLC3A1 (rBAT) gene and being a monogenic disorder, it is ideal for a gene therapeutic approach. A simple, inexpensive and a sensitive assay based on the uptake of radiolabeled cystine and leucine into the cells was designed to analyze the cystine transport constitutively expressed in a human proximal tubular cell line. This functional assay is used to analyze the expression of rBAT in cultured cells in order to asses the efficacy of gene therapy *in vitro*. A significant reduction in cystine uptake when the rBAT subunit was downregulated by antisense oligonucleotides confirmed that the cystine uptake in HK-2 cells was only due to the rBAT- $b^{0,+}$AT heterodimer. Murine leukaemia viral (MLV) vectors were used to generate an rBAT knockdown in HK-2 cells as an *in vitro* model for cystinuria type I. In order to establish a gene therapy for cystinuria type I, the human and murine SLC3A1 genes were cloned into the mammalian expression vectors. Feline immunodeficiency virus (FIV) vectors have been selected as the vehicle to deliver genes to the kidney. FIV vectors carrying both the human and murine SLC3A1 genes (HA-tag) independently were produced. The vector particles are replication deficient but capable of infecting and integrating into the cells.

In summary, we demonstrated that *pebbles* serves as an ideal model for evaluation of treatment approaches to cystinuria. New drugs for effective stone prevention are required and could be evaluated safely in our model in a standardized environment. Gene therapeutic approaches have the potential to cure the disease and to overcome the vicious cycle of recurrent stone formation and surgical treatments leading to renal insufficiency and impaired quality of life. Gene transfer by lentiviral vectors offers a promising method that will now be established in our *in vivo* model.