**Gene surgery as a potential treatment option for nephropathic cystinosis**

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Cystinosis is a rare, monogenetic disease caused by mutations in the *CTNS* gene. In this project we aim to develop a novel gene repair strategy for the most predominant 57kb deletion of *CTNS* using CRISPR-Cas9 gene-editing technology. In particular, we use the homology-independent targeted integration (HITI) approach which allows for the delivery of a large *CTNS* repair template into a specific location in the genome. For this study we used conditionally immortalized proximal tubule epithelial cells (ciPTEC) lines which lack functional *CTNS*. The *CTNS-/-* cells have been derived from a healthy ciPTEC line in which CTNS was knocked out using CRISPR/Cas9. For the delivery of the Cas9-guideRNA ribonucleoprotein (RNP) complex we used a novel non-viral peptide-mediated delivery system. The repair construct for *CTNS* consists of a double-stranded DNA sequence of 3.2kb containing the *CTNS* promotor and the first 10 exons of the *CTNS* gene and a fluorescent reporter gene (mCherry). After transfection of the repair construct in the cells we achieved a ~5% insertion efficiency in ciPTEC *CTNS*-/-, indicating these cells had successfully taken up and inserted the repair template into their genomic DNA. Further analysis of these cells showed a >80% reduction of cystine levels, indicating that in a majority of the cells the *CTNS* function was restored.

In conclusion, these preliminary data show that the *CTNS* repair template can be precisely inserted into the genome, leading to the translation of a functional cystinosin transporter, which consequently restores the lysosomal cystine accumulation. Although the efficiency should be improved, eventually this gene repair system may offer a potential curative therapy for cystinosis, as well as a system for the *in vitro* restoration of several other genes involved in monogenic diseases.

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