Project Details	
Project Code	CMD27Br Saleem
Title	Targeted gene integration therapy for cystinosis
Research Theme	Cardiometabolic Disease
Project Type	Wet lab
Summary	Cystinosis is a rare condition caused by mutations in the CTNS gene. This leads to cystine build-up in cells which damages many organs, especially the kidneys. The current treatment, cysteamine, slows the disease but many patients still develop serious complications. This project aims to develop a new, one-time gene therapy that provides a lasting treatment for cystinosis, by integrating a healthy copy of the CTNS gene into cells. We will test whether this reduces the harmful cystine build-up. If successful, this work could lead to a single treatment that prevents serious multi-organ complications and improves quality of life in cystinosis.
Description	Cystinosis is a rare inherited condition affecting ~1 in 150,000 people. It is caused by pathogenic variants in CTNS, resulting in lysosomal cystine accumulation and multi-organ disease. Kidney proximal tubular epithelial cells (PTECs) are affected earliest, leading to Fanconi syndrome with urinary loss of water, electrolytes and low-molecular-weight proteins, followed by progression to kidney failure in childhood. Although cystinosis can now be diagnosed early through genetic testing or blood tests, there is still no cure. The current treatment, cysteamine, reduces cystine accumulation and slows the disease, but must be taken every six hours for life and can cause stomach problems, bad breath and body odour. Many patients still develop serious complications despite treatment. In addition, cysteamine does not restore cystinosin-dependent cellular functions beyond lysosomal cystine transport, including regulation of mTOR signalling, autophagy, mitophagy and apoptosis. Gene addition offers a rational therapeutic strategy. However, conventional Adeno Associated Virus (AAV)-mediated gene addition is unlikely to provide durable benefit in proliferating PTECs because episomal transgenes are progressively diluted during cell turnover. Stable genomic integration is therefore required to achieve sustained therapeutic expression. Current CRISPR-mediated homology-independent targeted integration (HITI) of transgenes is a highly promising method of integrating genes into genomic DNA, but this results in ~10% integration of viral backbone. We have optimised HITI for high integration efficiency and minimal undesirable integration. To enable translation, this will need to be delivered with a dual-AAV platform. Building on recently described AAVLINK technology for highly efficient reconstitution of large genetic cargoes, we have engineered vectors with threefold greater recombination efficiency and identified AAV serotypes with enhanced PTEC transduction. Together with our optimised HITI architecture, which achieves 30–40% targeted integration in PTECs in vitro, these advances provide a strong foundation for therapeutic development. This project will evaluate targeted integration of a functional CTNS cassette in human and rat cystinosis models in vitro. Therapeutic efficacy

	will be assessed by restoration of CTNS expression and reduction of lysosomal cystine accumulation. By combining advances in AAV delivery with durable CRISPR-mediated genome integration, this work aims to establish a disease-modifying therapy for cystinosis. Aim: Develop an AAVLINK-HITI platform for targeted CTNS integration and phenotypic rescue in in vitro cystinosis models Work Package 1 Optimising HITI-mediated CTNS integration (0-18 months) Using our optimised HITI with high efficiency and reduced backbone integration, the student will engineer guide-RNAs (gRNAs) targeting the CTNS locus. The gRNAs will direct the Cas9 nuclease to the correct location within genomic DNA for HITI-mediated integration. At least three gRNA/donor combinations will be tested in human and rat PTECs (Wild type and CTNS knockout) to identify optimal integration strategy. The rat represents the most robust model of cystinosis, recapitulating the disease well. Editing will be assessed by targeted amplicon deep-sequencing, flow cytometry and microscopy and CTNS expression. Functional rescue will be determined by cystine quantification. The most efficient gRNA/ donor combinations will be taken forward to work package 3. By the end of the first 6-12 months, the student will have acquired significant training and experience in molecular biology, genomics and cell culture, as well as a good basis of knowledge to be able to take ownership and steer the project. Work Package 2 Enhancing AAVLINK recombination efficiency (12-24 months) We have improved on the published AAVLINK design by using a recombination enzyme with superior specificity and recombination efficiency, with preliminary results demonstrating a threefold improvement. The student will further improve AAVLINK efficiency by adopting novel superior engineered variants of this recombination enzyme, together with systemic assessment of orthogonal cut sites to maximise recombination efficiency. The reconstitution efficiency will be measured using a fluorescent protein in vitro. Recombination efficiency will be quantified by microscopy, flow cytometry and junction-specific qPCR comparing full-length and partial recombination products. The enhanced version of AAVLINK will be progressed to work package 3 Work Package 3: AAVLINK-HITI correction of cystinosis (24-48 months) Optimised HITI from WP1 will be incorporated into enhanced AAVLINK from work package 2. AAVLINK-HITI will be used to integrate HA-tagged CTNS to CTNS knockout human and rat PTECs. Editing efficiency and phenotypic rescue will be assessed as per work package 1. Finally, AAVLINK-HITI integrating CTNS will then be applied to CTNS knockout human kidney organoids. The same editing efficiency and phenotypic rescue readouts will be measured. In addition, wild type human kidney organoids, CTNS knockout human kidney organoids and treated CTNS knockout human kidney organoids will be sent for single

	cell RNA sequencing, to characterise whether the diverse functions of cystinosin are rescued effectively in human kidney cells. This project will establish the feasibility of targeted CTNS integration for cystinosis and provide a broadly applicable platform for durable treatment of monogenic and non-monogenic kidney diseases. This will prevent progression to renal failure and development of cardio-renal-metabolic disease.
Can be completed part time?	Yes
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