**Generation of *CTNS* and *MFSD*12 double knockout induced pluripotent stem cell lines to test if *MFSD*12 is a candidate therapeutic target for a new class of cystinosis drugs**

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**Lay abstract**

Cystinosis is a rare disease that results in the build-up of cystine in all cells of the body. Cystine is a protein building block and normally the excess is moved out of cells. In cystinosis the transporter for cystine does not work and cystine builds up inside the cells and forms crystals which cause damage to all organs and muscles. There is, as yet, no cure for cystinosis and there is only one medication, called cysteamine. However, cysteamine is not completely effective and it does not reverse the damage caused to the kidney in particular. Thus new treatments are needed. We have developed cystinosis stem cells using gene editing technology called CRISPR/Cas9. These cells behave like the cells of cystinosis patients, most importantly, these cells accumulate large amounts of cystine compared to controls. A recent study shows that when a gene that is involved in skin pigmentation is missing in cystinotic cells there is no accumulation of cystine. Importantly, loss of this skin pigmentation gene leads to no other harmful effects. This exciting discovery implies that if we can disrupt this gene in cystinosis patients using drugs that are already available for other treatments, then we can reduce the need for the drug cysteamine. We are testing this in our cystinosis stem cell lines and hope to show that when the skin pigmentation gene is taken out that we get the same reduction in cystine. This work would pave the way for the development of drugs or treatments.

**Scientific abstract**

Cystinosis causes widespread cystine accumulation resulting in a multisystemic disease that also leads to other symptoms including growth retardation, photophobia due to cystine crystals in the cornea, hypothyroidism, muscle wastage and neurological deterioration. In spite of its therapeutic benefit, cysteamine therapy has many disadvantages such as a strict dose regimen, sweating odours, and severe gastrointestinal effects. Therefore, there is pressing need for better therapies to reduce cystine loading. We have previously generated a human induced pluripotent stem cell (iPSC) model of cystinosis (*CTNS*-knockout (KO) iPSCs) and found that compared to isogenic controls these cells accumulated 54-fold higher levels of cystine (Hollywood et al., 2020). Using our cystinotic iPSC model we will investigate whether *MFSD12* is a candidate therapeutic target to treat cystinosis. *MFSD12* encodes a transmembrane protein that imports cysteine into melanosomes and lysosomes, and we hypothesise that disruption of this gene in our cystinotic iPSC lines will prevent the accumulation of cystine in these cells. This is based on the study by the Sabatini group who showed that short-term knockdown of the *MFSD12* gene can reduce cystine levels in the lysosomes of cystinotic cells (Adelmann et al., 2020). We will test this by generating *CTNS*-*MFSD12* double-KO iPSCs and comparing the cystine levels in these cells to control and *CTNS*-KO iPSC lines with and without cysteamine treatment. As a proof-of-concept we first transfected our cells with small interfering RNA (siRNA) to knockdown *MFSD12* in control and *CTNS*-KO iPSCs and achieved 70% knockdown of gene expression. If successful, siRNA will be the quickest route to the clinic for cystinotic patients.

Adelmann, C.H., Traunbauer, A.K., Chen, B. *et al.* MFSD12 mediates the import of cysteine into melanosomes and lysosomes. *Nature* 588,699–704 **2020**

Hollywood JA, Przepiorski A, D’Souza R, Sreebhaven S, Wolvetang, EJ, Harrison, PT, Davidson, AJ, Holm, TM. Use of human iPSCs and kidney organoids to develop a cysteamine/mTOR inhibition combination therapy to treat cystinosis. *J Am Soc Nephrol,* **31**(5):962-982 **2020**