**042**

## AN *IN VITRO* MODEL TO STUDY CYSTINURIA

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**Aim of the Study:** Cystinuria is an inherited renal stone disease caused by mutations in the amino acid exchanger system $b^{0,+}$ found on the proximal tubular epithelial cells (PTEC) of the kidney. Defects in either subunit of the transporter, rBAT or $b^{0,+}$AT, lead to the formation of recurrent cystine stones and significant renal impairment. There are no effective treatments available, and no recent therapeutic advances. Mutation analysis has recently been made available in the UK. This work aims to establish a functional *in vitro* model which can be used to investigate the cellular effects of mutations in rBAT and $b^{0,+}$AT to enable the identification of new therapeutic targets at the molecular level.

**Methods:** Epitope-tagged rBAT and $b^{0,+}$AT were expressed and characterised in human conditionally immortalised PTEC, and cystine uptake was quantified using radio-labelled cystine assays. Disintegrations per minute (DPM) were measured per microgram of protein following 60 second incubation of transfected and untransfected cells with 0.5mM cystine plus 0.5mCi [14C]-cystine and compared with one-way ANOVA with Tukey's multiple comparison post hoc test. Ethics approval 12/SC/0456.

**Main Results:** Immunoprecipitation studies confirmed that expressed rBAT and $b^{0,+}$AT formed dimers in PTEC *in vitro*. Biotinylation studies confirmed correct trafficking of the dimerised transporter to the plasma membrane. Radiolabelled cystine uptake more than doubled in cells co-expressing both rBAT and $b^{0,+}$AT compared to untransfected PTEC, or PTEC expressing rBAT or $b^{0,+}$AT alone ($p<0.0001$, n=3 performed in triplicate**)**, see Figure.

**Conclusion:** We have established a functional *in vitro* model that can be used to further interrogate system $b^{0,+}$ in the cell type specifically affected in cystinuria. This model can be manipulated to investigate known and discovered cystinuria mutations with the aim of facilitating the development of novel disease therapies.