Characterising the effects of cystinuria causing missense mutations M467T, G458A and T471R on cystine transport system b(0,+) in a human proximal tubule cell line using automated fluorescent microscopy

**Background/objectives:** Cystinuria is a chronic cystine stone forming condition resulting almost entirely from mutations in SLC7A9 or SLC3A1 genes which code for the two heterodimeric subunit proteins, rBAT and b0,+AT respectively. These comprise the cystine amino acid transporter found in the proximal convoluted tubule. Mutations in either of these genes can result in failure to heterodimerise, translocate to the brush border membrane or function as a transporter at the membrane; all of which result in build-up and precipitation of extracellular cystine into renal calculi. This present study investigated the effects of SLC3A1 cystinuria causing mutations M467T, G458A and T471R, with the primary aim of elucidating the mechanism by which a functional heterodimer is prevented from being expressed at the plasma membrane and to develop an automated fluorescent microscopy assay to assess the effects of mutations on translocation to the membrane.

**Methods:** A previously developed conditionally immortalised human proximal tubule epithelial cell line that stably expressed FLAG tagged b0,+AT was transiently transfected with either an mCherry fluorescently tagged rBAT construct or one of three known mutants (M467T, G458A and T471R). The effect of each mutant on heterodimerisation, trafficking and transporter functionality was assessed via co-immunoprecipitation, biotinylation and radiolabelled cystine uptake assay respectively. Automated fluorescent microscopy was employed to further quantify localisation and corroborate findings.

**Results:** Our results indicate that human proximal tubule cells expressing M467T, G458A and T471R mutations fail to take up extracellular cystine due to a failure to traffic to the membrane which is in turn caused by a reduced ability to heterodimerise with the b0,+AT light subunits.

**Conclusion:** These findings build on experiments that have previously reported on the effects on trafficking of M467T in *Xenopus* oocytes and contribute to our understanding of the recently discovered G458A mutation and T471R mutations. Automated fluorescent microscopy represents a potential high through put method for identifying translocation disruption. Determining the mechanisms by which these mutations disrupt normal function will be important in informing future pharmacological therapies.

**Conflicts of interest**: none