**Using antisense oligonucleotides to target mutant uromodulin: therapeutic proof-of-principle.**

**Background:** Mutations in *UMOD*, which encodes uromodulin (UMOD) or Tamm-Horsfall protein, cause Autosomal Dominant Tubuloniterstitial Kidney Disease (ADTKD). This renal disorder causes progressive and irreversible CKD with interstitial fibrosis, and there are no known disease-modifying treatments. The mechanism involves intracellular accumulation of uromodulin in the cells where it is made, in the loop of Henle. As it is a dominant condition, the mutant (misfolded or aggregated) protein is thought to exert a dominant negative retentive effect on the normal allele. We hypothesised that therapeutic benefit might be gained from silencing the mutant allele such that protein produced by the normal allele could be secreted without hindrance. A mutation commonly found in our patient population is an Indel, which we chose for this study.

**Methods:** To express UMOD in mammalian cells, constructs containing two copies of wild-type and/or Indel mutant *UMOD* cDNA (WT/Indel, or Indel/Indel) were generated in the inducible pTRE3G-BI vector, transfected into HEK293 cells and stable lines selected. Control of inducibility and UMOD expression efficiency of the system were examined by Western blot analysis prior to use. Antisense oligonucleotides designed to capture the mutation-containing region were used to knock down transcription of Indel UMOD in the HEK-UMOD-expressing cells. Non-target oligos were applied simultaneously as a negative control. ER stress of the oligo-applied cells was examined by measuring Grp94 protein levels in these cells.

**Results:** Cells transfected with either *UMOD* construct showed that expression of UMOD was tightly controlled and at good levels. 13 of 15 antisense oligonucleotides designed across the mutated region of *Indel UMOD* were able to deplete UMOD in the stable HEK Indel/Indel cells, to 10-40% of control levels. The six best-performing oligos were then applied to cells expressing WT and Indel UMOD (heterozygous); four retained significant knock-down capacity, by 20-40% less compared to their effect on Indel/Indel cells. In addition, the active oligos applied to heterozygous cells led to significant reduction in Grp94 protein compared to control oligos, indicating a reduction in ER stress of these cells.

**Conclusions:** We have provided proof-of-principle that knocking down a mutant transcript can be safely achieved *in vitro*, opening the way to further studies to translate this strategy into an animal model. This represents the potential for a novel therapy to preserve renal function in ADTKD.