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Chronic Kidney Disease(CKD) affects about 10% of the world's population but there is currently no treatment targeting its causes. To identify potential novel drug targets for CKD, we performed high-throughput CRISPR KO screens in two disease-relevant cell models using phenotypic assay endpoints.

First, we established gene editing capabilities in 2 renal cell models (Human Glomerular Microvascular Endothelial Cells(HGMEC) and Renal Proximal Tubule Epithelial Cells(RPTEC)) using electroporation of gRNA/cas9 ribonucleoprotein.

Second, we established cytokine stress assays (IL1β and TNFα assays) that mimic renal cell injury and dysfunction seen in the loss of cell junction markers (CD31 in endothelial cells and ZO-1 in epithelial cells) which contributes to CKD pathogenesis. TNFα and IL1β stress assays were analysed by high content confocal imaging for the cell junction markers CD31 and ZO-1. The assays were optimised for automation workflows and coupled to gene editing to enable CRISPR KO screening in an arrayed format.

We screened a CKD related gene list (226 genes) in presence or absence of cytokines with the aim of identifying genes that are able to prevent or induce the disease-like phenotype. The imaging endpoints of CRISPR screen were analysed using deep learning-based image analysis to separate the healthy and stressed phenotypes and to detect the active KOs.

To overcome the possibility that single endpoint assays may not fully recapitulate the complex disease aetiology, an AI-outlier detection tool was implemented to detect targets that differ from the healthy phenotype even without resembling the cytokine stressed phenotype. This allowed us to identify KOs that induce different stressed phenotypes and so to unveil "Genotype-Phenotype" mechanism of action that could be relevant for the disease.

The screens showed excellent robustness and identified statistically significant genes of interest and among them, genes involved in the cytokine signalling pathways.

Disclosure: The authors A.S., U.K., N.M., L.E.I., C.W., D.G., A.C., S.N., H.S.L., C.Q., J.M.W., K.W., C.B., P.L.H. and L.B. declare the following competing interest: they are employees of, and shareholders in, AstraZeneca.