Xenopus Animal Models for Autosomal Polycystic Kidney Disease with Automated Deep Learning Analysis of Cystogenesis

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Autosomal dominant polycystic kidney disease (ADPKD) has an unmet need for new drugs and therapeutic targets. Ideally, these are identified in clinically relevant vertebrate disease models amenable to screening efforts. Here we employ a combination of CRISPR/Cas9, advanced microscopy and deep learning to establish a screenable animal model for polycystic kidney disease in the diploid amphibian animal model *Xenopus tropicalis*.

We employ targeted and unilateral CRISPR/Cas9 editing in order to inactivate *pkd1* or *pkd2* in the *Xenopus* developing vertebrate kidney. Cystogenesis is then visualized by whole-mount immunostaining, allowing direct comparison between targeted kidney and untargeted kidney in the *Xenopus* embryo. Next, we use mesoSPIM light-sheet microscopy and advanced U-Net deep learning image processing for automated, unbiased and rapid scoring of kidney pathological states in two and three dimensions.

CRISPR/Cas9 genome engineering in *pkd1* and *pkd2* elicited cystic malformations in developing renal tubules two-days post-fertilization ($p<0.001$). Interestingly, as editing can be restricted unilaterally, one kidney remained wild-type while the other developed cystic kidney disease. We observed cystogenesis across different developmental stages by leveraging an image processing pipeline for automated scoring of ADPKD in *Xenopus* embryos using deep learning approaches. Using a combination of segmentation and classification deep learning architectures allowed for automated size measurement of kidneys, as well as a qualitative analysis of cystic hallmarks. Our models correlated well with an independent expert on test data ($n_{test}=120$; $r=0.96$; $P<0.001$). Next, using tissue clearing and light-sheet microscopy approaches, we extended to three-dimensional analysis of cystogenesis. Here we showed that three-dimensional assessment of cystogenesis can be achieved.

By combining light-sheet microscopy and deep learning we provide a framework for higher-throughput and in-depth characterization of novel *Xenopus* models for autosomal polycystic kidney disease (DOI: 10.1242/dev.199664).

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