Core 1: Gene Targeting Core

SPECIFIC AIM 1: Targeted PKD associated genes with CRISPRs or TALENs in mice.

The Core will provide a unique gene targeting service to the PKD community, focused on disrupting genes thought to be involved in PKD progression or that encode interactors of the polycystin complex. Importantly, we can target genes in any mouse strain, including mice with complex PKD genotypes since there are no restrictions to strains or species using these approaches. TALENs can be designed, assembled and transfected into ES cells with a 2-week turnaround, and then injected into zygotes. The development of CRISPR reagents may be slower, but the development of CRISPR reagents will be faster, and we will work with Core users to develop strategies to point-pair the region of the gene to be targeted and the strain of mice to be used, including by mimicking the hypomorphic Pkd1R3277C/Pkd1R3277C, Pkd1R3277C/Pkd2R3277C mice, and the Pkd1R3277C and Pkd2R3277C mice. We can also make specific custom alterations (e.g. edits in the Pkd1 and Pkd2 genes in the rat).

SPECIFIC AIM 2: Targeted PKD associated genes with CRISPRs or TALENs in cell lines.

The Core will also provide a service dedicated to mutating genes implicated in PKD, using PC3:PKD1-interactors in cells. We can target cell lines (including ES cells) producing matched lines with mutations for physiological studies, including proliferation assays, cyst formation assays, electrophysiology and pathway analysis. Targeted affinity tags (TAP) can be introduced into the open reading frames of candidates by TALEN/CRISPR mediated homologous recombination, allowing the candidate protein and its interacting proteins to be isolated from the cells under physiological conditions, without overexpression. We will select these integrative events using a ‘push–pull’ (pax–tku (pax5- tku/uk)h selection method making it possible to alter the gene without leaving a selectable cassette in the genome.

The Core will provide a range of gene-editing services and the capability to establish at least five new animal models per year and multiple novel cell lines using the TALEN and CRISPR methods.

Core 2: Epigenetics Core

SPECIFIC AIM 1: Provide mouse lines and reagents to investigate epigenetic regulation of PKD associated genes and associated signaling pathways.

SPECIFIC AIM 2: Provide technical services to facilitate identification of aberrant epigenetic modifications on PKD associated genes, including performing assays in DNA methylation, protein-nucleic acid association (ChIP and ChIP-Seq analyses).

SPECIFIC AIM 3: Provide an educational outreach on epigenetics in PKD and epigenetic techniques that can be applied to PKD research.

Core 3: Biomarkers Core

SPECIFIC AIM 1: Provide biomarkers that can be used for drug development.

The Core will support investigators in the discovery and validation of biomarkers of early PKD. Currently, there is an unmet need for biomarkers for diagnosing and monitoring PKD progression beyond conventional indices for renal function, such as blood urea nitrogen (BUN), serum creatinine and urinary protein. The long-term goal of the Core is to establish a panel of blood and urine biomarkers that provide a non-invasive predictor of early PKD progression and to monitor treatment response. The Core will establish and maintain a repository of serum, plasma, urine and urinary exosomes from a cohort of well-characterized ADPKD patients, their unaffected family members and healthy volunteers in a longitudinal study for the use in biomarker research. Plasma, serum, urine and urinary exosomes can be isolated from DNA to be isolated for identification of the PKD gene mutation. MRI scans will be obtained every 2 years for TKV measurements. Clinical information on medical history, current medications, risk factors, family history, comorbidities, smoking history and caffeine and alcohol intake will be collected by a nurse coordinator.

The Core will provide a range of services related to the discovery and validation of biomarkers of early PKD.