Investigation of *Lad1* as a Candidate Modifier and Early Target of Polycystic Kidney Disease Signaling

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Introduction: Polycystic Kidney Disease (PKD) is a monogenic disease caused by mutations in either *PKD1* or *PKD2*, which encode polycystin-1 (PC1) and polycystin-2 (PC2), respectively. One proposed function for PC1 is to regulate cell migration and cell-cell interaction, possibly through modulation of the cytoskeleton. In prior transcriptomic studies of *Pkd1* mutant kidneys, we had identified *Lad1* as one of the few genes whose expression was dysregulated pre-cystogenesis. *Lad1* is a largely uncharacterized protein that may interact with actin. The goal of this study is to investigate the function of Lad1 and its roles in mediating/modifying PKD.

Methods: We used CRISPR/Cas9 technology to generate two *Lad1* mutant mouse lines that have large deletions spanning most of the coding region of *Lad1* and characterized the phenotype of homozygous mutants. *Lad1* mutants were crossed with *Pkd1*$^{cond/cond}$ mice with Ksp-Cre and tamoxifen-Cre to test for genetic interaction in early and late onset PKD models. Lastly, *Lad1* expression was quantified by quantitative polymerase chain reaction (qPCR) and western blot analysis in kidney epithelial cell lines and tissues of *Pkd1* conditional mutant mice.

Results: Mice with homozygous deletion of *Lad1* exons 3 to 8 were born at normal mendelian ratios and lacked obvious histopathological abnormalities up to 1 year of age. *Pkd1/Lad1* double mutants have inconclusive differences in their genetic distributions. Kidney-body weight ratio studies of double mutants are ongoing, but thus far are statistically indistinguishable. *Lad1* expression levels were confirmed to be lower in *Pkd1* mutant kidney tissue and epithelial cells.

Conclusions: *Lad1* was one of the earliest target genes found to be transcriptionally different in *Pkd1* mutant kidneys. *Lad1* CRISPR knock-out mice lack any obvious phenotypes. Genetic interaction studies are ongoing, but early data suggests very mild differences. Transcriptional studies of *Lad1* are currently being pursued to understand potential pathway mediated transcriptional changes.

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