Same Signal, Different Route: Lactate Transport Across Mouse Models

Mark Mubarak, Andrea Reyna-Neyra, Michael Caplan

Abstract

- Autosomal Dominant Polycystic Kidney Disease (ADPKD) is a progressive genetic disorder caused by mutations in genes encoding polycystin 1 (*Pkd1*) and polycystin 2 (*Pkd2*), characterized by massive cyst growth, altered cellular metabolism, and ultimately renal failure. Recent research from the Caplan Lab demonstrated that the Polycystin-1 (PC1) C-terminal tail, including the last 200 amino acids of PC1 (PC1-CTT), suppresses ADPKD progression via a pathway dependent on the mitochondrial enzyme NNT in a mouse model of ADPKD1.
- Transcriptomic profiling performed in mice kidney samples expressing NNT-*Pkd1* KO+CTT (here labeled as "N"), and in mice NNT-deficient-*Pkd1*-KO+CTT (here labeled as "J"), identified the *Slc5a8* gene (SMCT1), a transporter responsible for renal lactate reabsorption dependent on sodium, as a primary gene potentially involved in this protective axis.
- To investigate how SMCT1 kidney expression and lactate dynamics are modulated, we performed Western blots on kidney samples across cystic disease progression in both strains (N and J) at 10 weeks, and in N mice through 14 and 16 weeks of age.
- A densitometric analysis was conducted using NIH ImageJ software to quantify SMCT1 protein expression across different cystic stages.

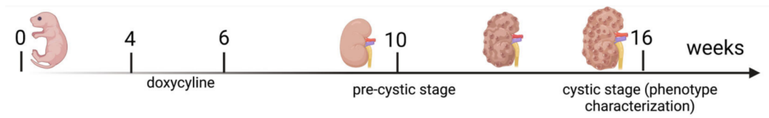


Figure 1. Experimental timeline for tracking disease progression. Doxycycline is administered from weeks 4 to 6 to induce Pkd1 gene knockout. Tissues are collected at week 10 during the early, pre-cystic stage and at week 16 to analyze the fully developed cystic disease.

NNT expression regulates the protective effect of CTT in *Pkd1*-KO mice.

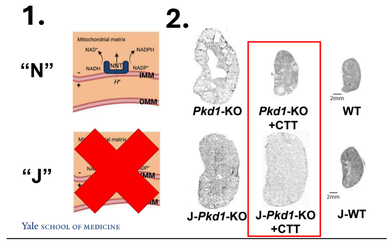


Figure 2. NNT localization and strain-dependent kidney phenotypes. (1) NNT is present in the inner mitochondrial membrane of N strain mice but deficient in J strain mice. **(2)** Representative kidney cross-sections demonstrate that the protective effect of CTT expression against Pkd1-KO cystogenesis occurs in N strain mice but is absent in J strain mice.

Cystic kidneys uptake significantly more metabolic fuel.

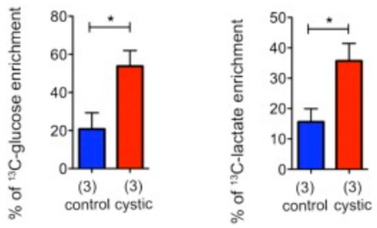


Figure 3. Enhanced glucose and lactate accumulation in ADPKD kidney tissue. Quantitative comparison showing significantly higher accumulation of 13C-glucose (left) and 13C-lactate (right) in cystic tissue relative to healthy controls in an 18-week slow-progression ADPKD mouse model.

Citations

- 1. Onuchic et al., (2023) The C-terminal tail of polycystin-1 suppresses cystic disease in a mitochondrial enzyme dependent fashion. Nature Communications | (2023) 14:1790
- 2. Chiaravalli et al. 2016. Journal of the American Society of Nephrology
- 3. Rai et al, 2026. C -terminal tail suppression of ADPKD produces mitochondrial-dependent gene expression changes. Manuscript in preparation.

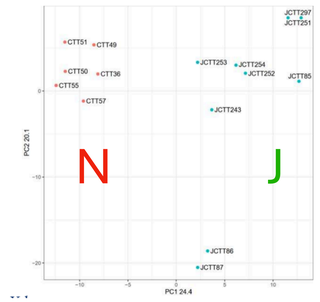
Connect on LinkedIn

Acknowledgements: We thank the members of the Caplan lab for their support and guidance. Special thanks to Dr. Victoria Rai for contributing the preliminary transcriptomic data comparing *N-Pkd1-KO+CTT* and *J-Pkd1-KO+CTT*mouse genotypes and for her helpful insights throughout the project.

Introduction

Autosomal Dominant Polycystic Kidney Disease (ADPKD) is a common genetic disorder where fluid-filled cysts balloon the kidneys from the size of a fist to the size of a football. While there is currently no cure, work from the Caplan Lab has identified a promising biological "brake" on this growth: a tiny protein fragment called PC1-CTT. When active, this fragment works alongside the cell's powerhouses, the mitochondria, to essentially reprogram diseased kidney cells, steering their behavior back toward a healthy, normal state.

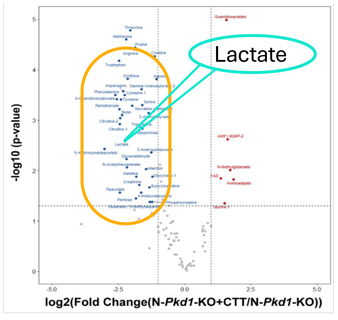
Principal components analysis (PCA) of RNA-sequencing data demonstrates distinct transcriptomic profiles between N-CTT and J-CTT mice.



Differential gene expression in N vs J mice expressing PC1-CTT

1. **Rps6ka1: Ras/Raf/MEK/ERK pathway**
2. Cry1
3. **Slc5a8: kidney lactate reabsorption**
4. **Atp12a: non gastric H/K ATPase**
5. **Ly6e: innate immune**
6. Rorc
7. **Hpd: enzyme that breaks down tyrosine**
8. **Cdh11: cell adhesion**

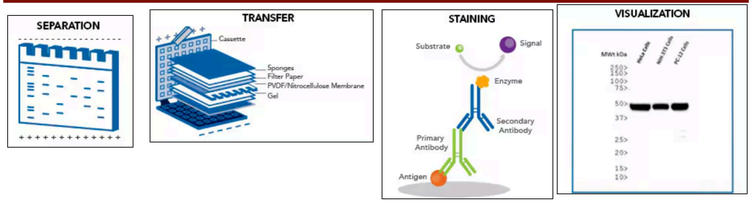
Figure 4. Differential transcriptomic profile of N-*Pkd1*KO+CTT vs J-*Pkd1*KO+CTT mice kidneys depicting the 8 most different genes found during early stages of ADPKD (10 weeks).



Lactate accumulation is significantly lower in N-*Pkd1*-KO+CTT in comparison to N-*Pkd1*-KO.

Figure 4. Identifying key metabolic changes driven by the CTT fragment. A volcano plot highlighting altered metabolites between N-*Pkd1*-KO+CTT and N-*Pkd1*-KO kidneys. The orange circle highlights a cluster of significantly downregulated amino acids and metabolic intermediates, including lactate (cyan circle).

Methodology



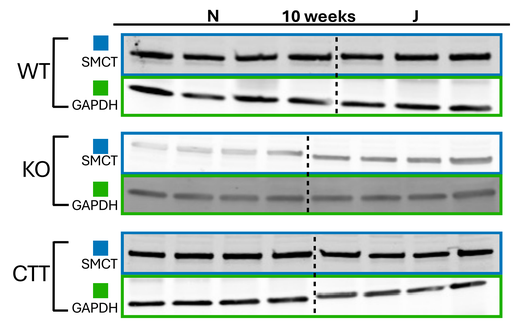
- **Protein Separation:** Protein samples were separated by molecular weight using SDS-PAGE
- **Membrane Transfer:** The resolved proteins were then electro-transferred onto a membrane, which was assembled into a cassette with filter papers and sponges
- **Immunodetection:** The membrane was first probed with primary antibodies (rabbit anti-SMCT1 and mouse anti-GAPDH), followed by appropriate secondary antibodies (goat anti-rabbit and goat anti-mouse) for detection
- **Visualization:** Protein bands were visualized by scanning the membrane on a LICOR imaging system

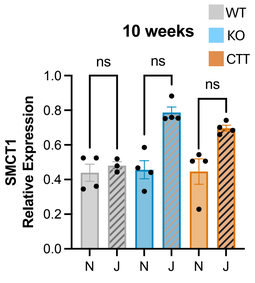
Hypothesis

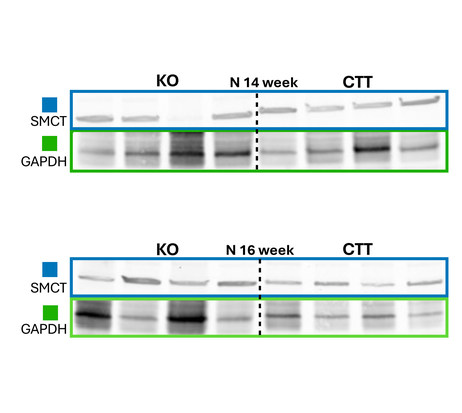
J-KO/CTT mice will have higher levels of SMCT expression in comparison to N-KO/CTT mice that have significantly lower accumulation levels of lactate.

Results

Western Blotting to Determine SMCT1 Expression







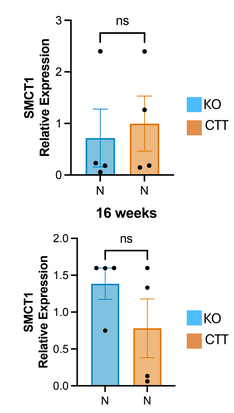


Figure 6: Longitudinal analysis of SMCT protein expression. Time-course evaluation (10, 14, and 16 weeks) of SMCT1 expression across multiple mouse strains and genotypes (WT, KO, CTT). Representative Western blots and quantitative densitometry are shown, with expression levels normalized to a GAPDH loading control. Data are expressed as mean±SEM. Multiple group comparison were performed followed by a t-test and brakets mark $p<0.05$ determined by Student t-test .

Conclusion

SMCT1 Expression Is Unchanged Across Genotypes: Western blot analysis revealed **no statistically significant differences** in SMCT1 protein levels between N and J strains or across WT, KO, and CTT-expressing mice at 10, 14, or 16 weeks of age, suggesting lower lactate levels observed in the N-CTT mice models are not due to increased expression of the lactate transporter SMCT1.

Future Directions

- We will investigate the molecular signaling connecting mitochondrial NNT activity to the transcriptional regulation of the *Slc5a8* (SMCT1) gene.
- To assess by immunofluorescence, the specific localization of SMCT1 in kidney sections of N and J mice at different stages of ADPKD development.
- To measure the concentration of lactate in kidney samples from J-Pkd1-KO vs J-Pkd1-KO+CTT to determine if there is a differential accumulation of lactate during different stages of ADPKD.