BETAINE-DEPENDENT RE-METHYLATION ABNORMALITIES CONTRIBUTE TO HOMOCYSTEINE-MEDIATED ENDOTHELIAL DYSFUNCTION IN POLYCYSTIC KIDNEY DISEASE

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Background

Vascular abnormalities are the most significant non-cystic complications in PKD and contribute to renal disease progression. Endothelial dysfunction (ED), a systemic pathological state characterized by an imbalance between vasodilating and vasoconstricting substances, is the earliest detectable stage of vascular pathology. A major indicator of this state is decreased nitric oxide bioavailability. In PKD, Homocysteine (Hcy)-induced ED precedes vascular disease. However, the underlying mechanisms leading to increased Hcy in PKD remain unknown. **Study Aim:** To gain further insight into the molecular mechanisms implicated in Hcy-induced ED, we explored the Hcy pathway using a targeted metabolomics approach in a murine model of PKD.

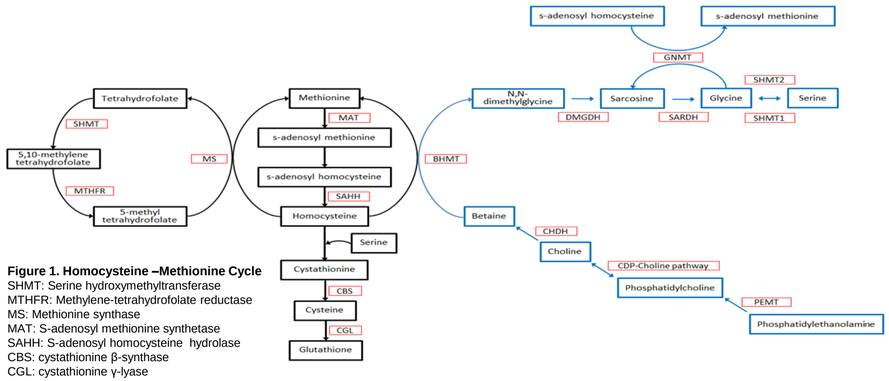

Figure 1. Homocysteine –Methionine Cycle
SHMT: Serine hydroxymethyltransferase
MTHFR: Methylene-tetrahydrofolate reductase
MS: Methionine synthase
MAT: S-adenosyl methionine synthetase
SAHH: S-adenosyl homocysteine hydrolase
CBS: cystathionine β-synthase
CGL: cystathionine γ-lyase
BHMT: Betaine-homocysteine methyltransferase

Methods

We included 4-week-old male and female, PKD (PCK) and control (SD) rats, (n=12 each). 24-hour urine and blood samples were collected for metabolomics analysis and related chemistries.

Animals were euthanized and the kidneys were either clamp-frozen for metabolomics or preserved in formalin for histological studies.

Hcy, folic acid, and vitamin B12 levels were determined by ELISA. Hcy-related metabolites were measured in blood, kidney tissue, and urine samples by ^{1}HNMR.

NMR spectra were acquired on a Bruker 600 MHz spectrometer. Spectra were phase and baseline corrected using the TopSpin software. A targeted profiling was accomplished using Chenomx 8.1 software, and compounds were identified by comparing to database Chenomx Version 10 and quantified based on internal standard (TSP-d4) peak integral.

Summary and Conclusions

- ED is shown by decreased eNOS immunoreactivity.
- Normal vitamin B12 and folic acid levels in PKD animals decreases the probability of a defect in the folate-dependent re-methylation pathway. Higher glutathione levels and normal methionine levels in PKD animals support the lack of an abnormality in the transsulfuration pathway.
- Increased urine betaine levels (betaine loss) and decreased betaine levels in the kidney tissue from PKD animals support a defect in the betaine-dependent re-methylation pathway.
- The correlation between urine betaine levels and plasma Hcy levels suggests that abnormalities in the betaine-dependent re-methylation pathway may be implicated in the Hcy-induced ED in early PKD.
- These findings provide novel insights into Hcy-induced ED in PKD and offer potential new targets for therapeutic interventions and novel biomarkers of vascular pathology and renal disease severity in early PKD.

Results and Discussion

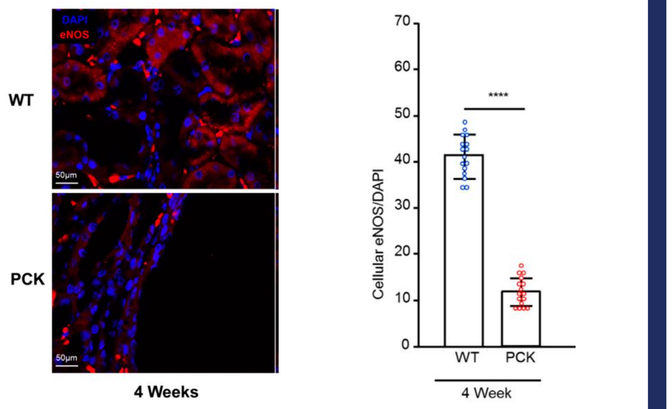

Figure 2. eNOS: Renal eNOS immunoreactivity is downregulated in PKD. Representative IF staining for eNOS (red) in WT and PCK rats at 4 weeks (above left) and its quantification (above right) showing decreased eNOS immunoreactivity in PCK animals at 4 weeks.**** $p < 0.0001$; eNOS was quantified as % stained area and adjusted to DAPI-stained % area.

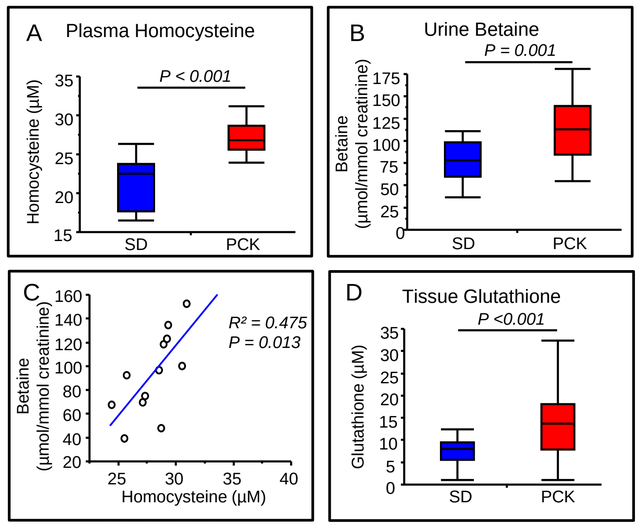

Figure 3. Metabolite Quantification: Circulating homocysteine (Hcy) levels were elevated in PKD (PCK) rats compared to SD controls (A), as well as urine levels of betaine (B), and both correlated directly (C). Tissue glutathione levels were elevated in PKD rats compared to SD controls (D), The top and bottom of the boxes are the estimated 75th and 25th percentiles, respectively. The vertical lines extend from the 75th percentile to the highest and from the 25th percentile to the lowest data points.

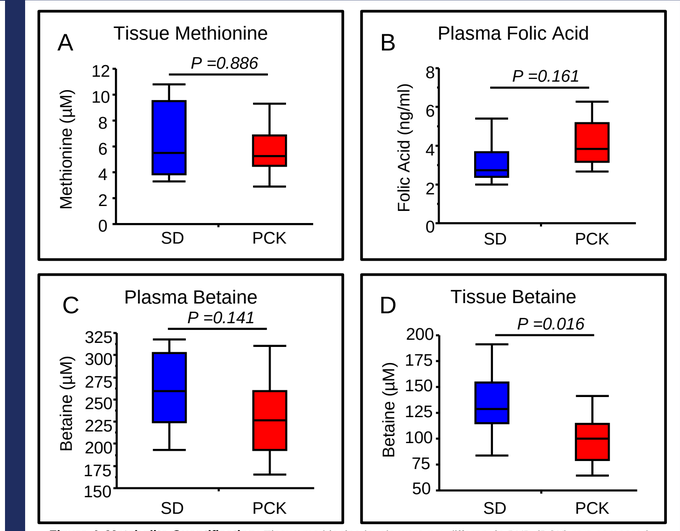

Figure 4. Metabolite Quantification: Tissue methionine levels were not different in PKD (PCK) rats compared to SD controls (A), as well as plasma levels of folic acid (B), and plasma levels of betaine (C). On the other hand, tissue betaine levels were lower in PKD rats compared to SD controls (D), The top and bottom of the boxes are the estimated 75th and 25th percentiles, respectively. The vertical lines extend from the 75th percentile to the highest and from the 25th percentile to the lowest data points.

References

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