Arterial Spin Labeling MRI as a Sensitive Imaging Marker of Congenital Hepatic Fibrosis in Autosomal Recessive Polycystic Kidney Disease (ARPKD)

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Introduction: Autosomal Recessive Polycystic Kidney Disease (ARPKD) is a potentially lethal multi-organ disease characterized by both polycystic kidneys and congenital hepatic fibrosis (CHF).[1] Unfortunately, there are currently no non-invasive methods to monitor CHF in ARPKD patients, which severely limits the study of potential therapeutic interventions. MR Elastography and ultrasound (i.e., Fibroscan) have been used to assess liver fibrosis, but either require additional hardware or offer limited soft tissue contrast, respectively. Delayed contrast-enhanced MRI has also been used to detect fibrotic scarring in liver and other organs, but is contraindicated for studies in pediatric ARPKD patients with advanced kidney disease. Herein, we evaluate Arterial Spin Labeling (ASL) MRI as a potential imaging biomarker to quantitatively assess CHF progression in the PCK rat model of ARPKD.

Methods: The ASL data were acquired using an ASL – Fast Imaging with Steady-state free Precession (ASL-FISP) technique.[2] Briefly, this method combines a conventional Flow-sensitive Alternating Inversion Recovery (FAIR) preparation (slice-selective or non-slice selective inversion) with a centrically-encoded FISP readout.[3] The ASL-FISP parameters were: TR/TE = 3.7/1.85 ms, inversion time = 1400 ms, FOV = 6 cm × 6 cm, matrix = 256 × 256, imaging slice thickness = 1.5 mm, FISP imaging flip angle = 60 degrees, 80 averages. In addition, the slice-selective inversion was applied with a thickness three times larger than the imaging slice to ensure uniform inversion over the entire imaging slice.[4] Longitudinal liver perfusion results were obtained for five PCK rats at 2 months and 3 months of age on a Bruker Biospec 7.0 T MRI scanner (Bruker Inc., Billerica, MA). Six 3-month-old Sprague-Dawley (SD) rats were scanned as controls. Paired and unpaired 2-tailed Student's t-tests were used to compare the liver perfusion results between 2-month and 3-month PCK rats and between 3-month PCK and SD rats, respectively. A Pearson correlation coefficient was used to compare the liver perfusion findings from the 3-month old PCK and SD rats with Masson's Trichome and hydroxyproline histological assessments of hepatic fibrosis.

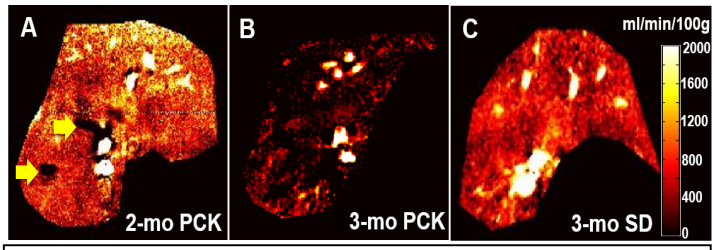

Fig.1: ASL-FISP hepatic perfusion maps from a single PCK rat at **(A)** 2 months of age and **(B)** 3 months of age and **(C)** corresponding 3-month-old Sprague-Dawley (SD) control rat. Note the extensive regions of reduced perfusion in the PCK rat liver.

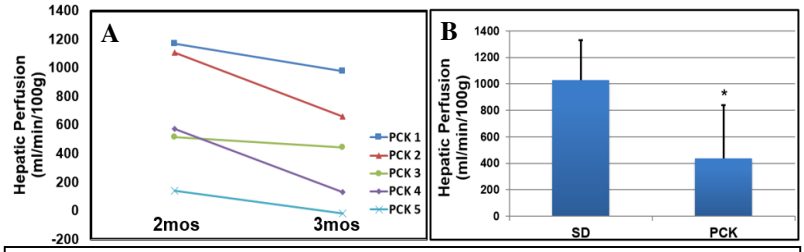

Fig.2: (A) Plot of mean liver perfusion value for individual PCK rats at 2-months and 3-months of age ($p < 0.05$). Note the variation in the individual rate of progression consistent with the variation observed in human ARPKD liver disease progression. **(B)** Plot of mean liver perfusion values from the ASL-FISP MRI acquisition for SD (n=6) and PCK (n=5) rats at 3 months of age (*$p < 0.05$).

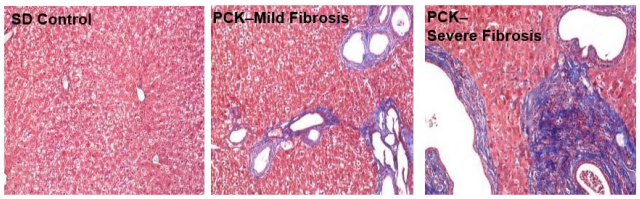

Fig.3: Photomicrographs of PCK and SD rat liver specimens stained with Masson's Trichrome to assess collagenic fibrosis (in blue). Note the visible regions of hepatic fibrosis in the PCK rats consistent with ARPKD liver disease progression.

month *in vivo* liver perfusion findings correlated significantly with both Masson's trichrome ($R = 0.79$, $p < 0.005$, **Fig. 4A**) and hydroxyproline content ($R = 0.68$, $p < 0.05$, **Fig. 4B**).

Discussion: We have performed an initial preclinical study to demonstrate that ASL MRI is sensitive to progressive liver fibrosis in the orthologous PCK rat model of ARPKD. We have shown that the ASL-FISP MRI technique can 1) sensitively identify altered liver perfusion in PCK rats in comparison to SD controls; and 2) longitudinally assess hepatic perfusion changes as a measure of ARPKD liver disease progression. In addition, we have demonstrated that these ASL-based liver perfusion assessments significantly correlated with two gold-standard histopathologic assessments of hepatic fibrosis. Therefore, liver perfusion as measured by ASL MRI may provide a sensitive and non-invasive imaging biomarker to safely detect and monitor ARPKD liver disease.

References: 1. Dell KM, ACKD, 2011. 2. Gao Y, et al, NBM 2014. 3. Kim SG, MRM, 1995. 4. Lu L, et al, MRM 2012.

Results: Representative ASL-FISP hepatic perfusion maps from a single PCK rat at 2 months of age (**Fig. 1A**) and 3 months of age (**Fig. 1B**) as well as a corresponding 3-month SD control rat (**Fig. 1C**) are shown in **Figure 1**. Note the visible focal regions of reduced hepatic perfusion in the 2-month perfusion map (yellow arrows) while greatly expanded region in the 3-month perfusion map indicative of progressive ARPKD liver disease. PCK rats exhibited a significant decrease in liver perfusion between 2 months and 3 months of age (mean ± standard deviation = 700 ± 434 vs. 439 ± 401 ml/min/100g, $p < 0.05$, **Fig. 2A**). The 3-month-old PCK rats also had significantly lower liver perfusion compared to age-matched SD control rats (439 ± 401 vs. 1030 ± 299 ml/min/100g, $p < 0.05$, **Fig. 2B**). Photomicrographs of Masson's Trichrome stained liver sections from a 3-month-old SD rat and two 3-month-old PCK rats are shown in **Figure 3**. Note the increased regions of fibrosis (blue regions staining collagen) in PCK rats liver. The 3-

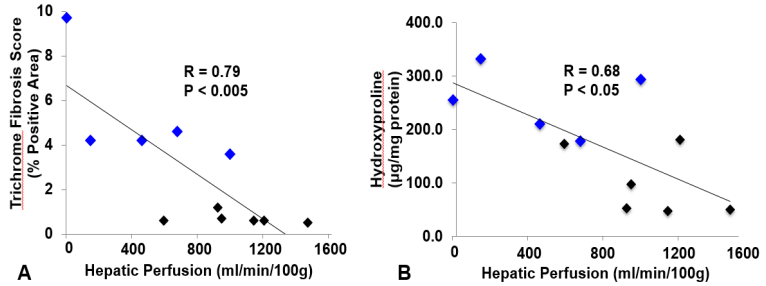

Fig.4: *In vivo* ASL-FISP MRI assessments of hepatic perfusion for 3-month-old PCK (blue diamonds) and SD (black diamonds) rats in comparison to **(A)** blinded Masson's Trichrome scores and **(B)** biochemical assessments of hepatic hydroxyproline content. Both trichrome scores ($p < 0.005$) and hydroxyproline ($p < 0.05$) assessments resulted in significant Pearson correlations with liver perfusion.