Diabetic Complications Consortium

Application Title: Imaging tools and models to study the effect of preterm birth on diabetic kidney

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1. Project Accomplishments:

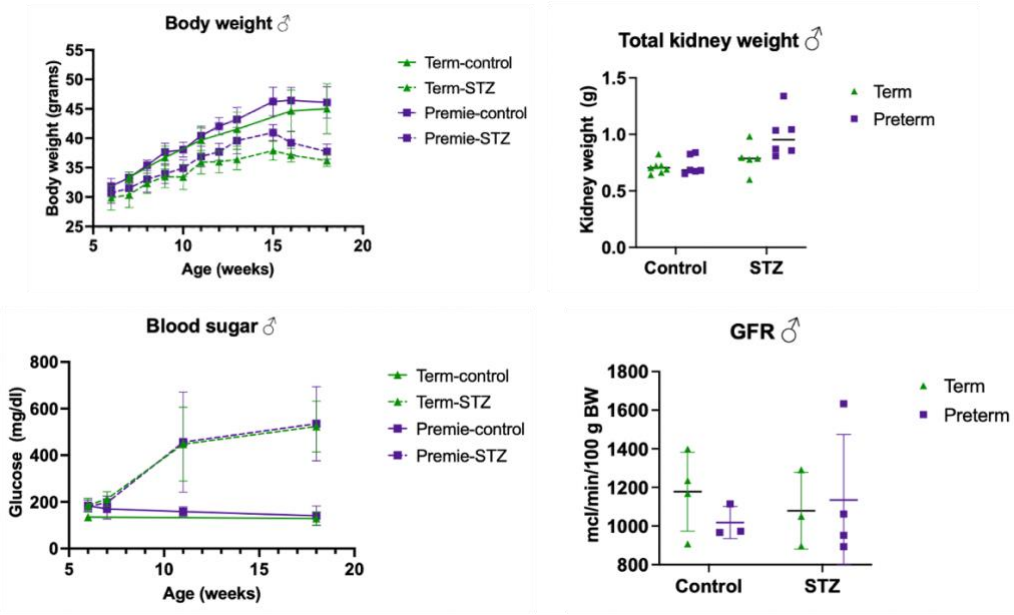
Over the past year, we have completed all of animal work for this project. We have been analyzing the single cell and bulk RNA-Seq data. We are optimizing the imaging protocol for the imaging portion of this project. Our work was accepted as a platform presentation at the 2023 Pediatric Academic Society annual meeting in April 2023.

Specific Aims:

Aim1: Characterize the structural phenotype of the kidney of a diabetic mouse model born preterm using CFE-MRI.

SA1 Results: We induced preterm birth at 19 days post conception (dpc) in CD-1 mice. Our control group was comprised of mice born full term (20 dpc). The preterm and term mice were assigned to the diabetes or no diabetes groups. Streptozocin was administered (50 mg/kg intraperitoneal for 5 consecutive days) to the half of the term and preterm mice with an equal distribution of males and females. Blood sugar was monitored. A second round of streptozocin was administered to those animals that did not become hyperglycemic with the first round.

In **Figure 1,** the male animals are included.



We have started imaging the kidneys of animals that received cationic ferritin. Using the 9T small animal imaging Bruker system at the University of Virginia, we can confirm that there is labeling of the glomeruli with cationic ferritin as seen in **Figure 2**. We have observed CF leakage and light labeling in both the diabetics and female. We are working to optimize the image quality.

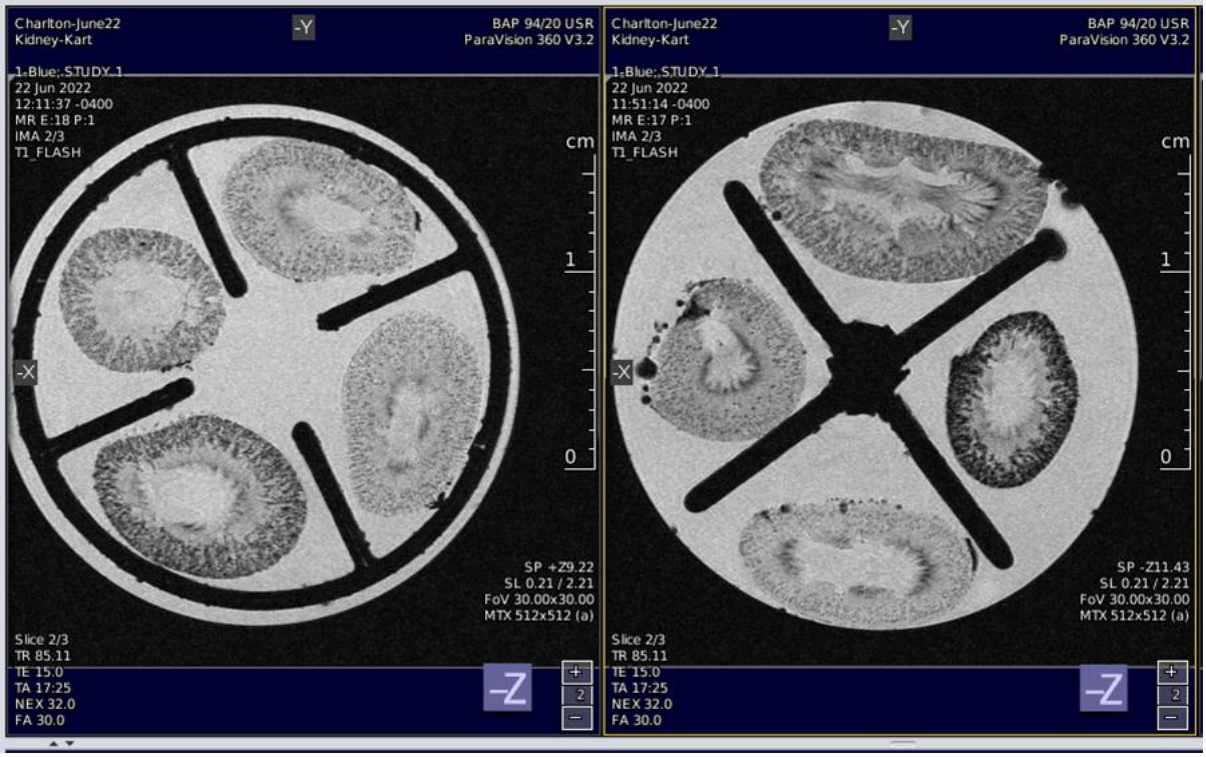


Figure 2. Cationic ferritin enhanced-MR images. 2D images obtained on the 9T Bruker MRI. A combination of term-nondiabetic, term-diabetic, preterm-nondiabetic and preterm-diabetic were scanned to ensure CF labelling in all of th experimental conditions.

Aim2: Compare the alterations of the kidney gene expression profiles in the diabetic preterm mouse to the diabetic term mouse.

SA2 Results: At 18 weeks of age, we euthanized the animals and dissected kidneys. A kidney from the following groups were processed for single cell RNA-Seq (scRNA-Seq): term-no diabetes, preterm-no diabetes, term-diabetes, preterm-no diabetes. A gentle dissociation of the whole kidney using a cold-temperature protease protocol[2] was used and scRNA-seq libraries were generated using 10xGenomics Chromium Single Cell 3' Reagent Kit (n=1 per group). The cell viability after the dissociation was >95% in both samples. We detected 3025 (term-no diabetes), 3931 (preterm-no diabetes), 2249 (term-diabetes), and 2777 (preterm-diabetes) cells from the sequencing results, respectively. Based on the gene expression profile similarities, we identified 28 clusters in both preterm and term born kidneys (**Figure 3A**).

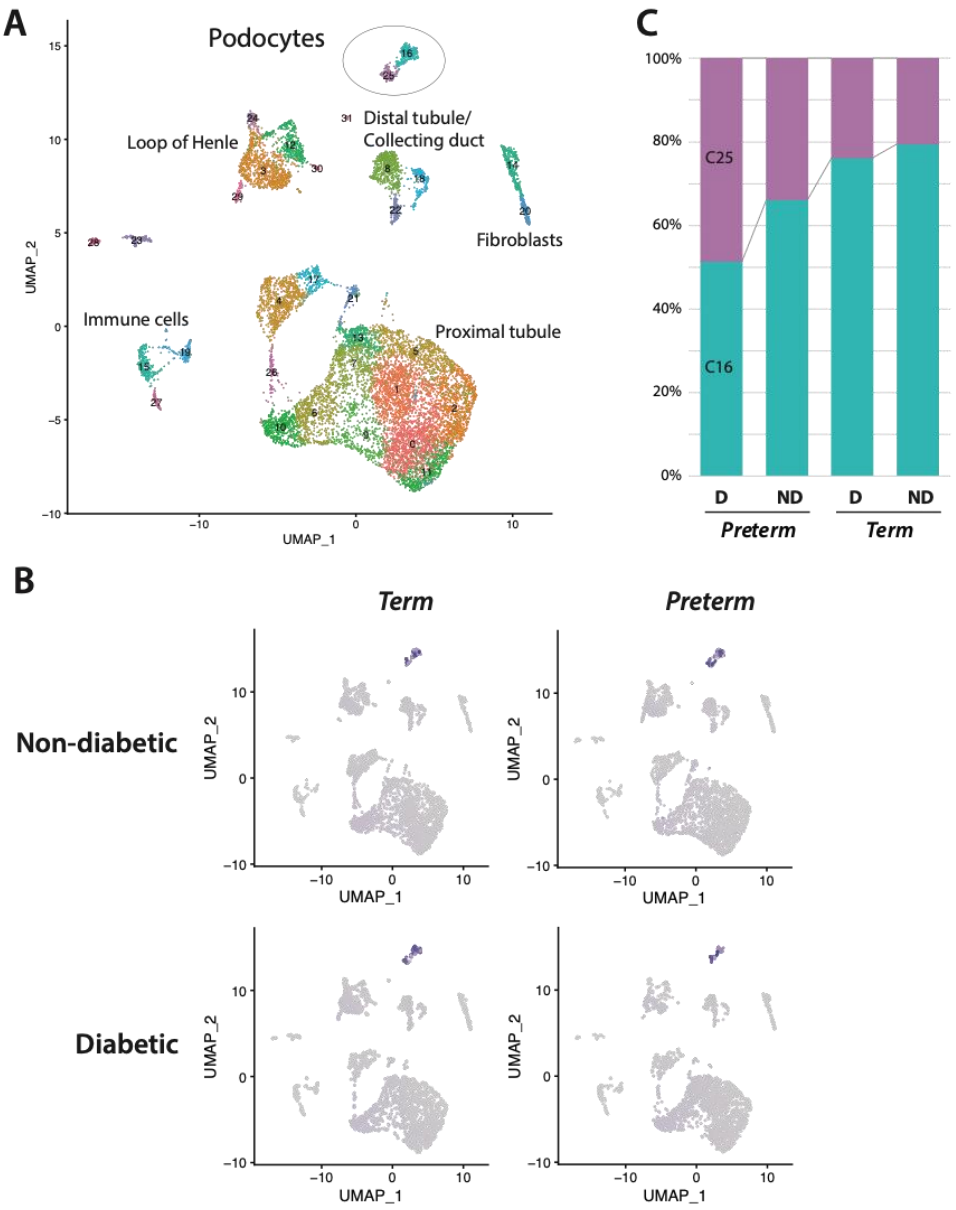
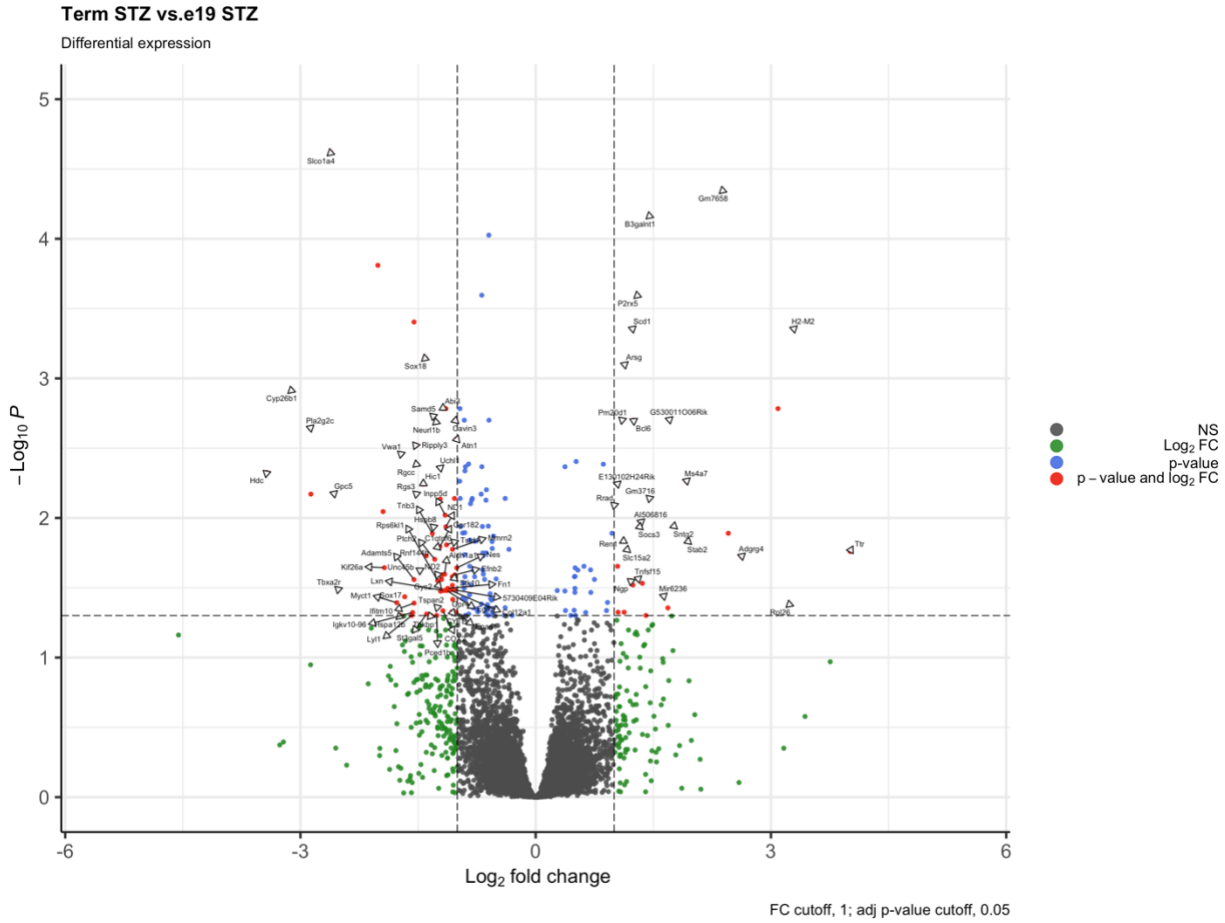


Figure 3. Single cell RNA-seq results identifying kidney cell types including podocytes. A. A total of 28 clusters was identified and displayed on this UMAP. Using marker genes, cells were categorized into the following cell types: podocytes, proximal tubule, loop of Henle, distal tubules, collecting duct, and immune cells. B. Podocytes (clusters 16 and 25) were identified by the expression of *Podxl* gene and two subtypes can be seen in the UMAP. C. The proportions of two podocyte clusters are different

We identified the cell types of each clusters using reported marker gene expression status. As expected, proximal tubule cells were the main subtype of kidney[3]. Importantly, we detected podocytes (**Figure 3B**) without enrichment for these rare cells. Interestingly, we found that the proportions of two podocyte cell clusters were different between preterm and term groups (**Figure 3C**).

We now have bulk RNA Sequencing on both sexes in each cohort. We have begun to analyze this data. **Figure 4** is a volcano plot showing the differential expression of the genes between the preterm and term animals each exposed to STZ, hyperglycemia.

Figure 4. Volcano plot of term-STZ versus preterm-STZ.



These data support our hypothesis that preterm birth may alter cell subtype compositions in the adult tissue.

2. <u>Publications:</u>

None to date.

Oral abstract:

Cwiek, A, Suzuki, M, deRonde, K, Reidy, K, Charlton, JR. Preterm birth increases susceptibility to hyperglycemia-induced glomerular alterations. Pediatric Academic Society, April 2023, Washington DC.