Diabetic Complications Consortium

Application Title: Defining the Influence of the Gut Microbiome on Diabetic Renal Disease

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1. Project Accomplishments:

Provide broad overview of the accomplishments of this project:

We have pursued these Aims with two approaches, as described below: first, we have carried out studies to compare diabetic renal disease in male and female mice with and without gut microbes. Based on a comment from Reviewer #2, we revised our planned model and used antibiotic-treatment to drastically and dramatically suppress gut microbes instead of germ-free mice.

Second, we have performed RNASeq to evaluate host gene expression in male and female mice with and without gut microbiota (germ-free vs conventionalized). We have also performed 16S sequencing of gut microbes. Moving forward, we hope to also perform these studies in diabetic mice.

2. Specific Aims:

Specific Aim 1:

Determine how the gut microbiota alters diabetic renal disease in males and females. Studies were performed on 8 groups of mice: Male conventional control diet, Male conventional high fat diet (HFD), male antibiotic-treated control diet, male antibiotic-treated HFD, female conventional control diet, female conventional HFD, female antibiotic-treated control diet, and female antibiotic-treated HFD. Using antibiotic treatment also allowed us to make serial measurements of glomerular filtration rate and plasma glucose, which would not have been possible in a germ-free model. At 6 weeks of age, mice were put on either a control or a high fat diet and were given drinking water with or without antibiotics. Mice were weighed weekly for 9 weeks. Non-fasting glucose and insulin, and glomerular filtration rate (GFR, measured by transcutaneous fluorescence following FITC-sinistrin bolus) were measured at weeks 0, 5 and 9. At week 9, glucose tolerance (GTT) and insulin tolerance tests (ITT) were performed, and then mice were sacrificed.

Antibiotic-treatment did not alter body weight, GTT or ITT (although HFD, of course, did). However, we found that GFR increases with both HFD (hyperfiltration – as we reported previously in males[1]) and also with antibiotic treatment (**Figure 1**) in males and females. We have seen this in two different cohorts and in both males and females. In females it is apparent on both control diet and on HFD; in males, antibiotics do not further elevate GFR above that of the HFD. We are currently working to (a) measure GFR in germ-free mice to confirm if GFR is elevated, and (b) uncover the mechanism. We think there are three possibilities: (a) altered blood pressure could elevate GFR, but, we previously published[2] only ~mild blood pressure changes with this same antibiotic treatment (~10mmHg) and this should be well within the range of autoregulation. Thus, this possibility is unlikely. (b) Because oncontic pressure in the glomerular capillaries opposes filtration, changes in oncontic pressure (proteinuria?) could drive

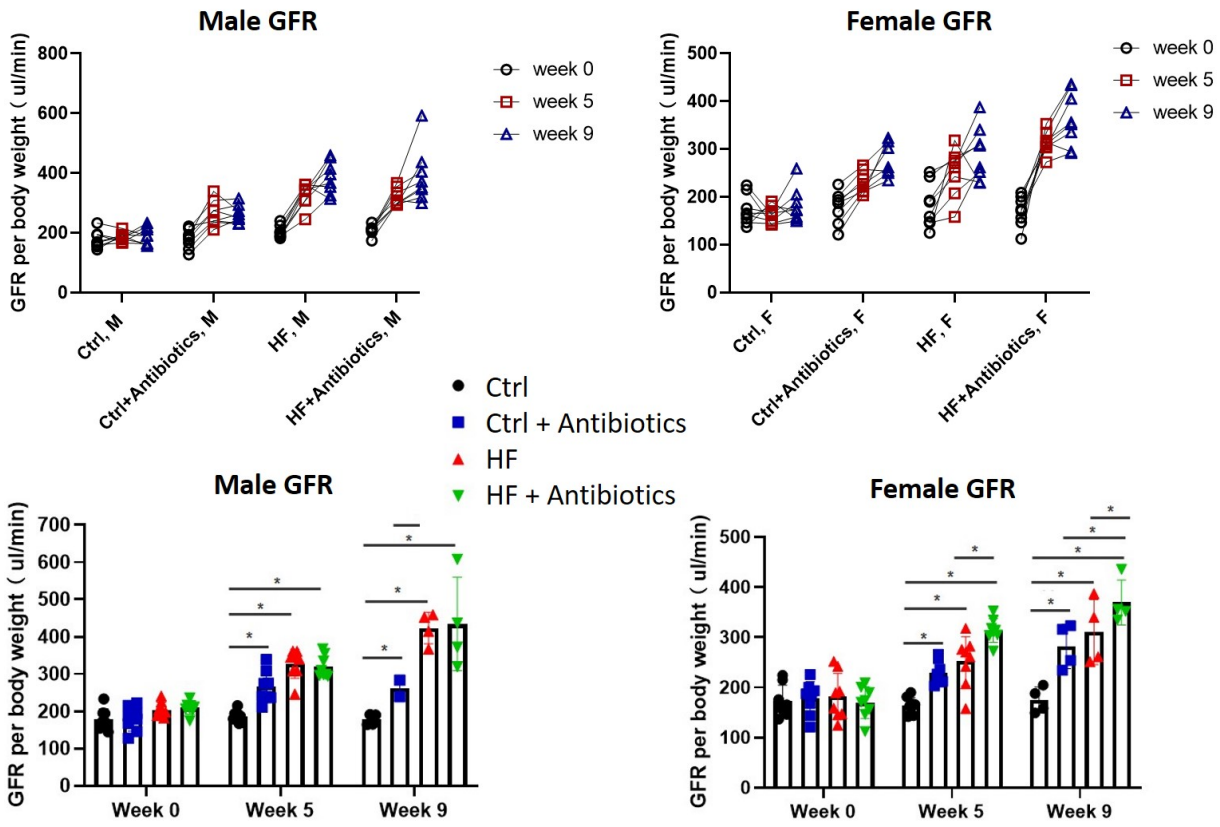

Figure 1 – GFR data plotted in two different ways for males (left) and females (right). In the top graphs, each individual animal is shown separately. The lower graphs show summary data with statistical analysis. In males, both antibiotics and HFD increase GFR, but the effects are not additive (i.e., HF and HF+antibiotics have similar GFR at weeks 5 and 9). In females, both antibiotics and HFD increase GFR, and the effects are additive (i.e., at weeks 5 and 9, HF+antibiotics has a higher GFR than either HF or antibiotics alone, but all three groups are elevated over control diet).

an elevation in GFR – although this is possible in principle, to our knowledge it has not been reported in the literature and thus seems unlikely. (c) Altered sodium delivery to the macula densa could elevate GFR – perhaps this the lack of gut microbes alters expression of sodium transporters proximal to the macula densa? We are now measuring sodium transporter expression as well as screening for proteinuria from existing urine samples.

Specific Aim 2:

Determine how the gut microbiota alters host gene expression in diabetic renal disease. For this study, we have performed whole-kidney RNASeq on kidneys from female mice (germ

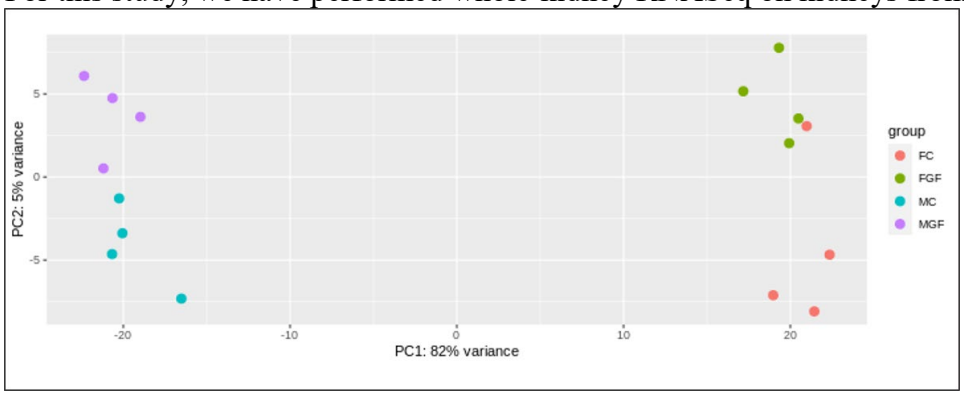

Figure 2 –PCA of RNASeq data from kidney. Both sex (PC1 axis) and microbiome status (PC2 axis) influence renal gene expression.

free and conventional) and male mice (germ free and conventional). Microbiome status did not influence body weight, blood electrolytes, blood creatinine, blood glucose, hematocrit or hemoglobin. We find that the largest variance in gene expression is due to sex (Fig 2, PC1 axis), but there is also significant separation based on microbiome status (PC2 axis). We have identified a number of genes which are differentially expressed in both males and females based on microbiome status, as well as several signaling pathways based on pathway analysis. We are still working to identify specific changes that we believe are most impactful on renal function. Although we have not yet performed this analysis for HFD-fed mice, we still hope that these studies can help us to better understand and interpret the studies in Aim 1.

3. Publications:

None at this time.

References

1 Halperin Kuhns, V. L. & Pluznick, J. L. Novel Differences in Renal Gene Expression in a Diet Induced Obesity Model. *Am J Physiol Renal Physiol*, ajprenal003452017, doi:10.1152/ajprenal.00345.2017 (2017).

2 Pluznick, J. L. *et al.* Olfactory receptor responding to gut microbiota-derived signals plays a role in renin secretion and blood pressure regulation. *Proc. Natl. Acad. Sci U. S. A* **110**, 4410-4415, doi:1215927110 [pii];10.1073/pnas.1215927110 [doi] (2013).