Diabetic Complications Consortium

Application Title: Genetic susceptibility to mitochondrial dysfunction as a risk factor for diabetic kidney disease

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1. Project Accomplishments:

In this proposal we have used a novel genetic platform, the Collaborative Cross Gene Mine, to investigate genetic susceptibility to diabetic kidney disease (DKD). Specifically we suggested that inherited factors which predisposed kidney power stations, the mitochondria to failure in the context of diabetes may be important risk factors for DKD. The gene mine is a sophisticated mouse population which allows for rapid genetic mapping of major effect genes and complex genetic traits. It may be considered as a vast family tree of hundreds of "cousins" that are inbred homozygous strains descended from eight of the most genetically diverse mouse strains. These eight "founder" strains represent mice with varying susceptibility to diabetes such as NODLt mice (which develop T1D). The cousin strains show enormous variability for specific traits, providing a bell curve of distribution for a particular trait of interest, representative of a "general" population. We have obtained renal functional data as well as collecting tissue for determination of kidney ATP content. This will allow for us to map the genes which could contribute to mitochondrial dysfunction in DKD.

Specific Aims:

Hypothesis: Genetic susceptibility to mitochondrial dysfunction affects the development of kidney disease in Type 1 diabetes.

Aim: To examine if genetic susceptibility to mitochondrial dysfunction contributes to nephropathy in type 1 diabetes using the collaborative cross gene mine.

Results:

1. Assessment of kidney function assessed as GFR using the Collaborative Cross

We have collected serum from 70 strains of mice encompassing the genetic variation within the collaborative cross. Comprising each of the 70 different strain medians there are at least n=3 mice that were measured for GFR by assessing serum cystatin C. Both genders were represented in all strains tested. The mouse serum samples were collected from week 6 to 10 of life (adolescence/early adulthood). Figure 1 (below) shows the distribution of GFR across the strains, ranging from 400-780ng/ml of cystatin C. From left to right (Figure 1), the data represent decreasing GFR as the serum concentration of cystatin C increases.

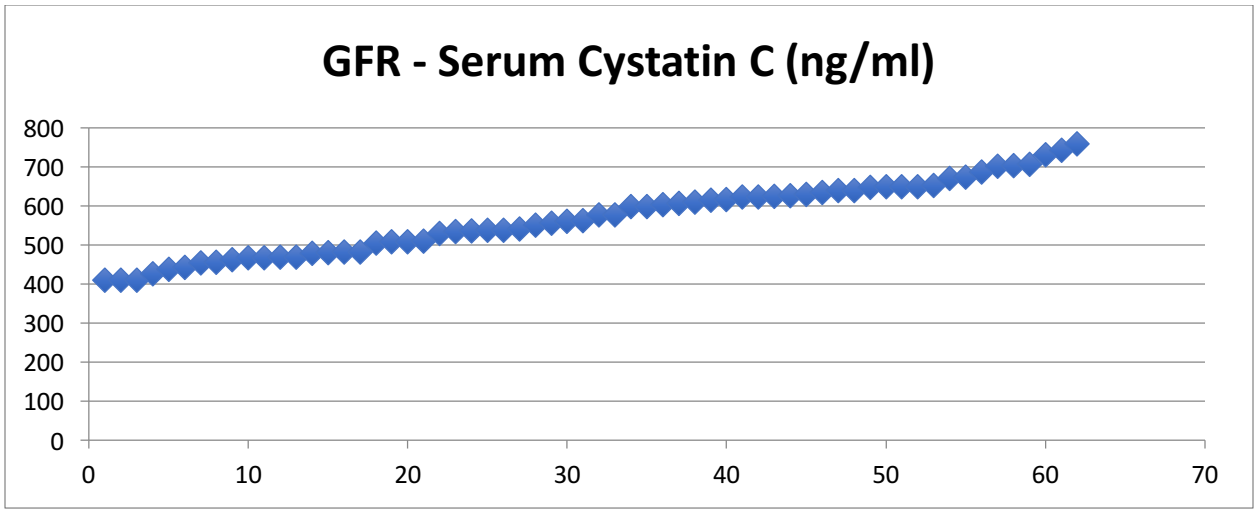

Figure 1: A) Distribution of serum cystatin C (GFR) in 70 different Collaborative Cross (CC) strains (at least n=3 mice/strain) and one founder, C57BL/6 (red asterisk). The strains show variation over a three-fold range.

2. Mapping of genes associated with GFR using the collaborative cross

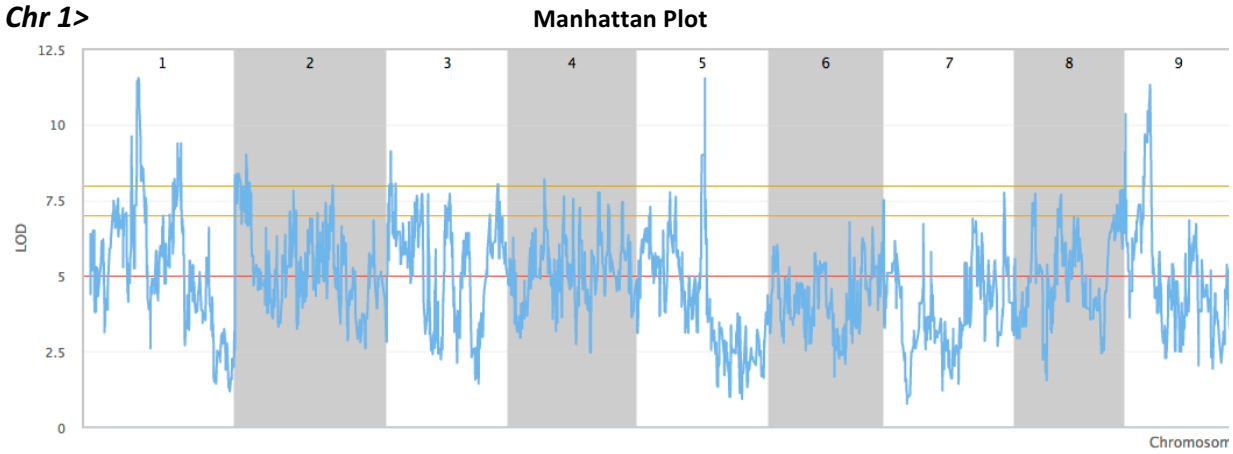

Figure 2: Genome linkage scan of serum cystatin C serum levels in CC strains. This Manhattan Plot shows evidence of linkage on a logarithmic scale (Y axis) at 600,000 genetic markers distributed across the genome (X axis). The upper yellow line (>LOD Score of =8) indicates the 99% confidence interval, as determined from permutations of the data. There are significant peaks shown on chromosomes 1, 5 and 9. There were no peaks which crossed the 99% confidence level seen at other chromosome loci (>10).

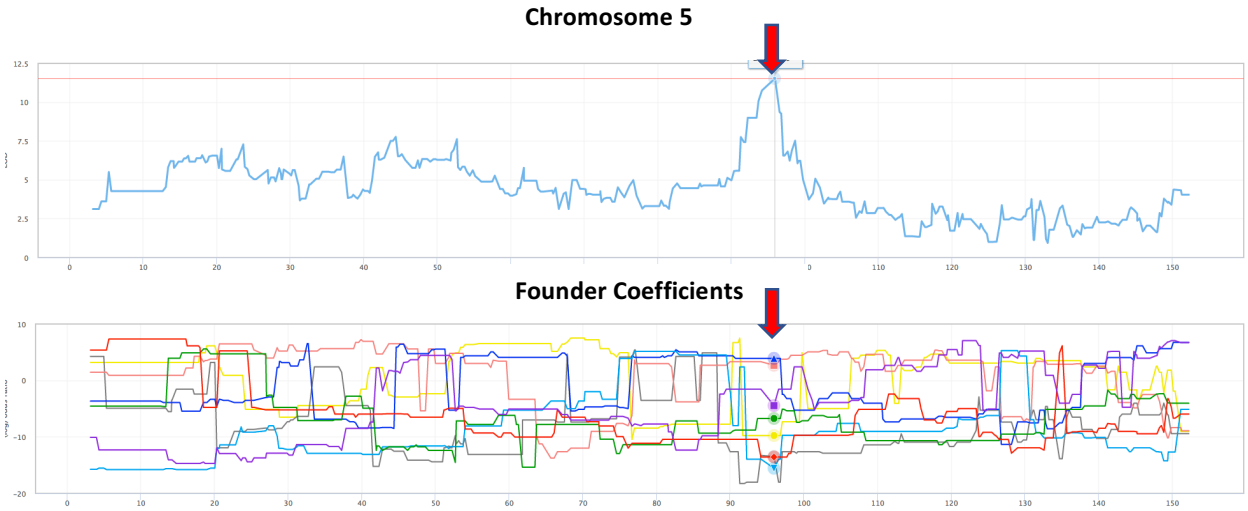

Figure 3: The top panel shows the linkage peak on chromosome 5 with a LOD Score = 11.5 at position 95.4 Mbp (red arrow). The bottom panel is the contribution of haplotypes inherited from the eight "founder" strains. These are color-coded and the PWK, NZO and C57Bl/6 founders (red, aqua and grey lines, red arrow) are significantly different from the NODLt at this locus (blue) on Chr 10 (Red arrow).

The Quantitative trait loci mapping performed to date has identified two linkage peaks on chromosome 5 and chromosome 9 which are associated with GFR as assessed using serum cystatin C. The linkage peak on chromosome 9 encompasses about 2000 Mbp and the linkage peak on chromosome 5 is about 2700 Mbp. The linkage peak in chromosome 5 contains a promising candidate gene, specifically Shroom 3. Intronic SNPs in Shroom 3 have been identified in a number of GWAS for chronic kidney disease (CKD) susceptibility and there are murine publications linking this gene to CKD. We will shortly complete analysis of Shroom3 gene expression in the rat model of type 1 diabetes, the STZ rat to check these findings.

For ATP determination, we have collected kidney cortical tissue which represent 41 different strains across the CC. We will collect up to 70 strains and then will collate the renal functional data with ATP concentrations and Seahorse analyses of ATP generation in these mice.

3. Publications:

None to date. However, preliminary data from this proposal was presented as a mini oral presentation at the Asia Pacific Congress of Nephrology in 2017.