QTLs for diabetic nephropathy

To identify QTL associated with diabetes-related kidney disease, we performed histological and gene expression studies on kidneys from a mouse cohort that segregated for obesity-induced diabetes. Approximately 500 F2 mice were generated from diabetes-resistant (B6) and diabetes-susceptible (BTBR) mice. All mice were made genetically obese in response to leptin-deficiency (*Leptin*$^{ob/ob}$), resulting in a wide range of diabetes-related traits. One kidney from each mouse was used for gene expression profiling, while the other was used for histological assessment for two traits associated with diabetic nephrophathy; mesangial matrix and glomerular volume.

Two QTL were identified from the histological evaluations (**Figure 1**). Mesangial matrix and glomerular volume both showed linkage to Chr 7 at ~28 Mb. We performed genome-wide permutations that indicated a LOD score of > 5.0 yields a false-discovery rate of 5% or less. We view the QTL on Chr 7 as our primary locus for diabetic nephropathy and are pursuing this locus by generating congenic mouse strains. These mice will enable us to use fine-mapping to identify the causal gene that underlies the linkage for the histological phenotypes.

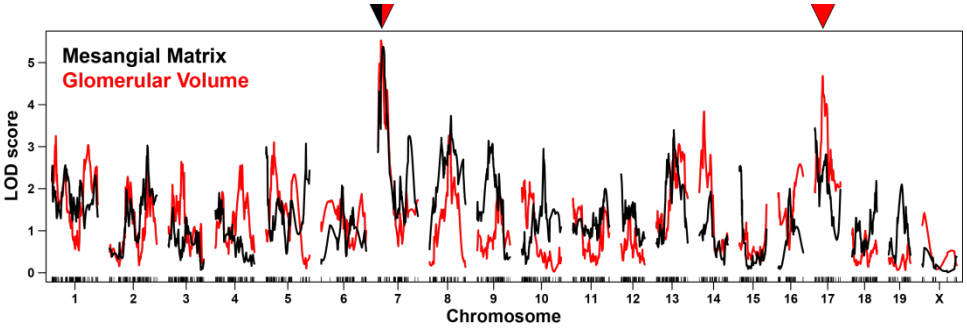

Figure 1: QTL for diabetes nephropathy.
Genome-wide LOD profile for mesangial matrix (black trace) and glomerular volume (red trace) in kidneys from obese B6:BTBR-F2 mice. Arrow heads indicate linkages that were identified on chromosomes 7 and 17.

While the congenic strains will be instrumental in defining the causal gene that underlies the Chr 7 QTL, we have identified one candidate gene based on genetic polymorphism between B6 and BTBR mice, as well as reported function. Nephrin (gene symbol, *Nphs1*) is located at ~30 Mb on Chr 7. This location is within the error of our QTL mapping for the loci identified for mesangial matrix and glomerular volume. Furthermore, we fully sequenced *Nphs1* in B6 and BTBR, and identified 8 coding SNPs that alter the amino acid within the protein. Several of these amino acids are highly conserved across multiple species indicating that they may be important for proper function of Nphs1 protein. Nphs1 has previously been linked to diabetic nephropathy in human genetic studies (Bonomo et al., 2014) giving us further confidence that Nphs1 may be the causal gene for the Chr 7 QTL in our study. Nephrin is a transmembrane protein that is an essential component of the filtration apparatus of the podocyte. Defects in nephrin have been linked to proteinuria, where large amounts of circulating protein are secreted into the urine.

In addition to the histological assessment described above, we quantified gene expression in the kidneys from the F2 mice. Analogous to histological scoring, measurements of gene expression provide a quantifiable metric that is amenable to QTL mapping. Expression QTL, or eQTL, identify loci that regulate the expression of the mapped gene. Gene expression can map locally, referred to as cis-regulation, or map to a distant location, referred to as trans-regulation. We identified a total of ~21,400 eQTL in kidney, ~80% of which were trans-eQTL (**Figure 2**).

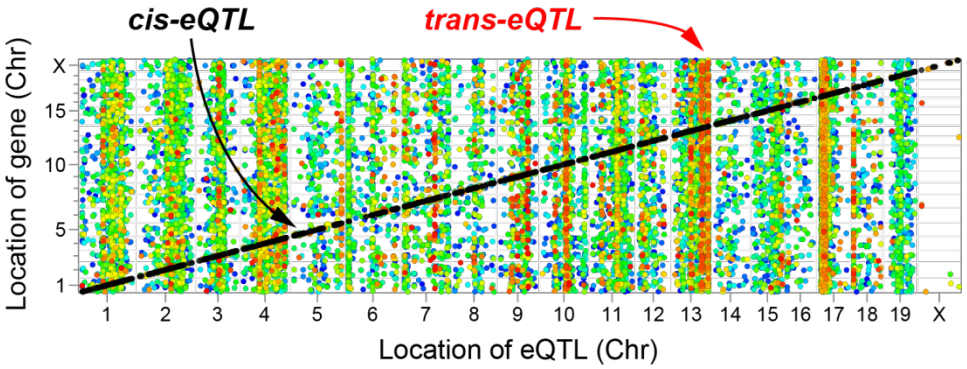

Figure 2: eQTL identified in kidney.
Genome-wide mapping of ~21,400 kidney eQTL. Black diagonal show cis-eQTL; colored vertical bands show trans-eQTL. eQTL hotspots where a large number of eQTL co-map were identified on several chromosome, including 4, 13, and 17.

Chromosome 17 was both an eQTL hotspot in kidney, and was a locus to which we observed linkage for glomerular volume, one of our histological markers for diabetic nephropathy. Co-mapping of expression and physiological traits to a common locus allows us to construct causal gene networks. For example:

QTL → Expression traits → Glomerular volume

We are using this type of network analysis to establish testable hypotheses for the locus at Chr 17.