Transcriptional Profiling of Type 2 Diabetic Mice Reveals Changes in Fat Metabolism

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Abstract

Approximately 16 million Americans have been diagnosed with Type 2 diabetes and approximately 3 new cases are diagnosed every minute. Diabetic neuropathy (DN) is a serious complication of diabetes resulting in loss of sensation in the limbs, cardiac complications, and is the leading cause of non-traumatic amputations in the United States. The BLKS-db/db mouse model develops severe Type 2 diabetes, and has symptoms of severe DN by 24 weeks of diabetes. We observe a significant increase in the concentration of oxidized lipid in both dorsal root ganglia (DRG) and the sciatic nerve (SCN) in the diabetic mice.

In order to identify the mechanisms of DN in these mice, we transcriptionally profiled DRG and SCN from mice following 24 weeks of diabetes and age matched controls. The NCIBI GenePattern pipeline was used to identify the significantly regulated genes in each tissue. 2505 genes were significantly regulated in the SCN and 1419 were regulated in DRG, but only a small fraction of these genes were co-regulated in both tissues. Chinese Restaurant Clustering was used to find clusters of co regulated genes, and clusters enriched for mitochondrial genes were isolated in each tissue. These clusters of co-regulated genes with similar function were analyzed for conserved promoter elements. A 3 element transcriptional module of two SP1 binding sites and a CTCF site was found that is shared between the tissues and is conserved across species.

One of the regulated genes with this three element module, Acsl1, is highly relevant to lipid metabolism. The expression of this gene in human disease has been confirmed by comparison with a human neuropathy microarray dataset. Because of its possible role in lipid mediated DN, it has been targeted for biological confirmation of the bioinformatics search. We confirmed the increase in gene expression by PCR and western blot analysis. The promoter of the gene has been confirmed to be functional by a luciferase assay.

Mouse Model – BKS db/db

An emerging hypothesis in the etiology of DN is the effect of dyslipidemia – an altered abundance and ratio of serum lipids. In order to explore this hypothesis, we first confirmed that our mouse model of diabetes develops both hyperglycemia and dyslipidemia.

Following 24 weeks of diabetes, the db/db mice had significantly elevated:
- Blood Glucose
- Body Weight
- Serum Cholesterol
- Serum Triglycerides
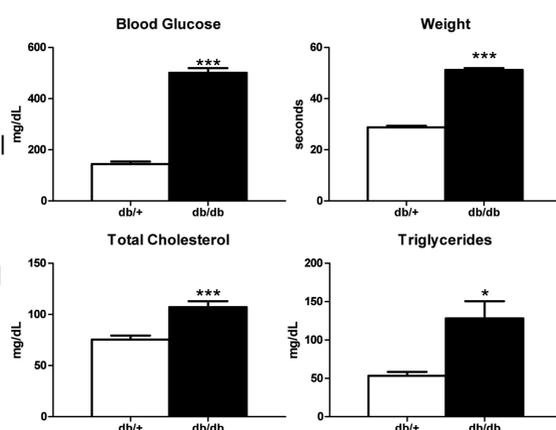
Oxidized Lipids

Dyslipidemia is not thought to act solely in isolation from the effects of hyperglycemia. Instead, they amplify the effects of the other. Hyperglycemia results in the production of oxidative stress, including oxidized lipids. Oxidized lipids are highly damaging to neurons, and their presence may accelerate DN progression.

Following 24 weeks of diabetes, the db/db mice have significantly elevated oxidized lipids (HODE) in both the dorsal root ganglion (DRG) of the nerve and in the sciatic nerve itself (SCN).
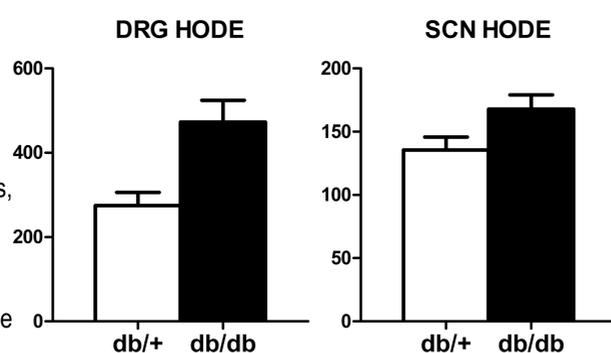
Microarray Methods

The SCN and DRG of the db/+ (n = 6) and db/db (n = 6) mice were excised following 24 weeks of diabetes (or a matched time point for db/+ mice). RNA was extracted and hybridized to Affymetrix Mouse 430 v2 chips at the University of Michigan Comprehensive Cancer Center (UMCCC) core facility. Microarray data was processed using the NCIBI GenePattern pipeline. The BrainArray CustomCDF version 10 annotation was used with RMA to normalize the expression data. Probesets with absolute expression less than 120% of the negative control probesets were excluded from the analysis. Differential expression was determined using a Student's t-test and CyberT false discovery rate.

Model Based Clustering

Significantly regulated genes were clustered using Chinese Restaurant Clustering (CRC), a Baysean model-based clustering method. CRC identifies small, discrete clusters of highly co-regulated genes. Both co-regulation and anti-correlation are considered by the algorithm.

Each cluster generated by CRC was tested for functional enrichment using the Database for Annotation, Visualization and Integrated Discovery (DAVID). DAVID combines many different sources of annotation into a complete, non-redundant set of functional categories.

A cluster of genes was found in both the SCN (cluster A) and DRG (cluster B) that DAVID annotated as being mitochondrially associated. However, none of the same genes were present in both clusters.

The cluster of genes regulated in SCN contained three genes associated with lipid metabolism: Acsl1, Acox1 and Acaa2. These genes all have increased expression in the diabetic condition, and may be associated with the increase in oxidized lipids in the nerve.
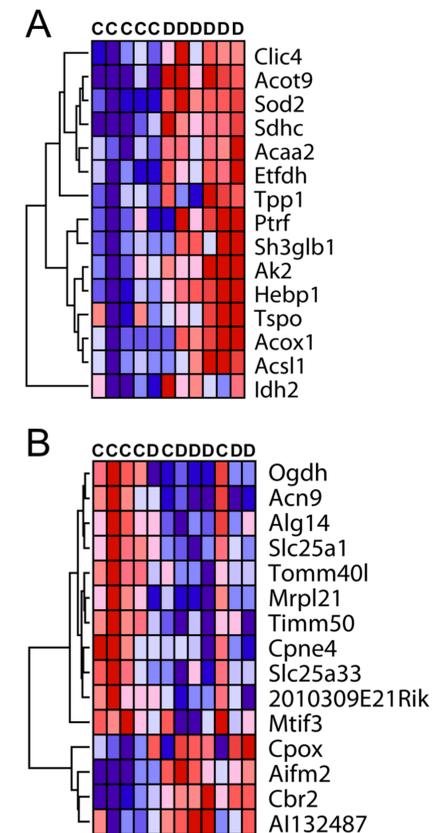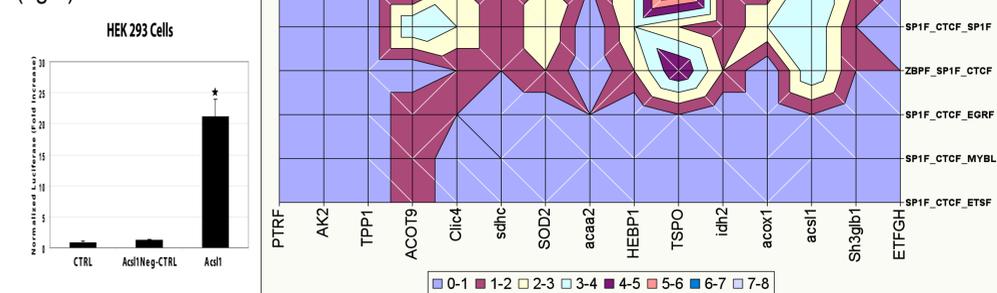

Biological Confirmation

Acsl1 was prioritized for biological confirmation because it displays cross-species conservation of regulation in diabetes (data not shown) and has the greatest fold change in expression of the lipid metabolism genes identified. A technical replicate of the gene expression data was run using RT-PCR (left). Because transcript regulation does not always result in changes in the abundance of protein, protein expression was confirmed using Western blotting (middle). Having confirmed that protein expression is modified by diabetes, the protein was localized to the Schwann cell cytoplasm using immunohistochemistry (right).
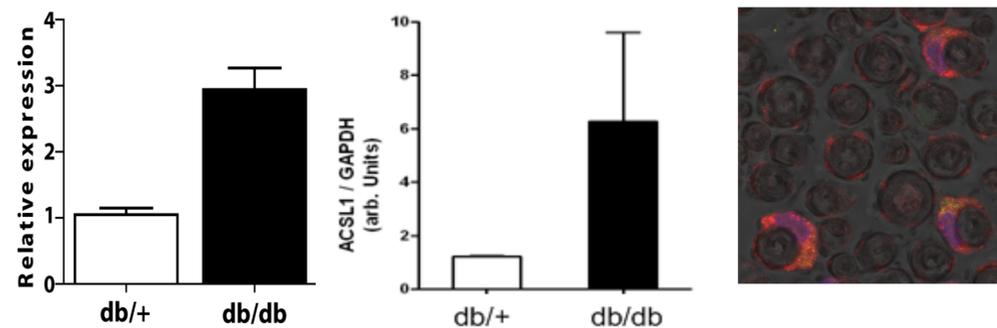
Regulatory Mechanism

Three genes with concordant expression, similar function (lipid metabolism) and annotated localization were identified by clustering. The expression of one of these genes was confirmed on the protein level. We attempt to identify any common regulatory elements in these genes using a combination of in vitro and in silico approaches. We confirmed the ability of the Acsl1 cis-promoter to drive gene expression using a luciferase reporter system in HEK 293 cells (left). Genomatix FrameWorker was used to identify binding site configurations that are conserved among the co-regulated genes. These configurations are charted with their cross-species conservation below (right).

Acknowledgements