Validation of the ob/ob mouse model for NASH progression by transcriptional profiling of mouse liver using RNA-Seq

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1 ABSTRACT

Non-alcoholic steatohepatitis (NASH) is associated with metabolic dysfunction. Multiple pathways are affected during NASH progression. In this work, ob/ob mice were fed a high-fat, high-fructose and cholesterol diet to induce NASH and RNA-Seq was performed on liver to uncover pathways affected during NASH progression.

Unbiased clustering of the RNA-seq data by principal component analysis showed clear separation of the control (chow) and two diet-fed groups (12 and 18 weeks). Differentially expressed (DE) genes were identified by filtering on adjusted p-value < 0.01 and absolute fold change ≥ 2. Pathway analysis of the DE genes revealed that in both cohorts, the diet induced metabolic changes associated with NASH progression, such as downregulation of cholesterol biosynthesis. Inflammatory pathways as well as matrix metalloproteinases (MMPs) and collagens were upregulated, indicative of a fibrotic response. Both cohorts on high-fat diet had elevated liver lipids (steatosis), immune cell infiltration and higher collagen expression.

Conclusion: Liver transcriptional profiling of ob/ob mice on high-fat high-fructose diet resulted in enrichment of pathways associated with metabolic dysfunction, inflammation and fibrosis. Biochemical assays and histopathology independently confirmed advanced liver disease in both cohorts. These data support the validity of ob/ob mice as a model to study NASH progression and shed light on the genes in the involved pathways.

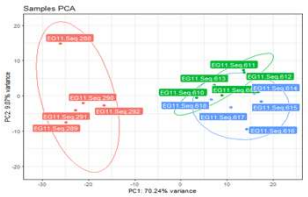
2 EXPERIMENTAL

- Six-week old B6.Cg-Lepob/J (ob/ob) mice were fed 40% fat (18% trans-fat), 40% carbohydrate (20% fructose) and 2% cholesterol diet (Research Diets D09100301) for 18 weeks.
- One cohort was sacrificed after 12 weeks and the other after 18 weeks. One cohort was maintained on chow diet for 18 weeks, that served as control. Each cohort had 5 animals.
- Livers were excised from the three cohorts, RNA was extracted and prepared for sequencing using a TruSeq library prep kit. RNA sequencing was done on a NextSeq system with read length at 2x75bp and sequencing depth of 30 million reads.
- RNA-seq reads were quality filtered, aligned with HiSAT2, assembled into transcripts using StringTie and differential expression analysis was performed using DESeq2.
- Pathway analysis on DE genes with WikiPathways was performed using the statistics module in PathVisio.
- Liver lipids (steatosis) were estimated by biochemical assay, inflammation and fibrosis was assessed by histopathology (haemotoxylin and eosin (H&E), collagen staining).

3 RESULTS

Differential Gene Expression

Raw RNA-seq data was used in a Principal Component Analysis (PCA) to investigate the association between the treatment groups. This unbiased approach revealed a clear separation between chow and diet-fed mice (Figure 1). The PCA also showed a high degree of overlap between 12 weeks and 18 weeks of high fat diet. Based on these results, differential expression analysis was performed on three comparisons: chow vs diet (combination of 12 and 18 weeks diet cohorts), chow vs 12 weeks diet, and chow vs 18 weeks diet. Differentially expressed genes were used for hierarchical clustering, which revealed clear changes in expression patterns in diet-fed mice (Figure 2). Pathway analysis was performed on the DE genes and consistently resulted in the down- and upregulation of pathways associated with NASH, for each comparison (Table 1).



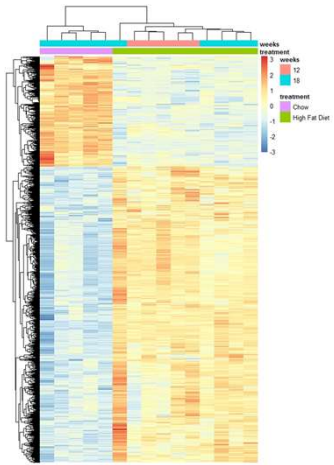


Figure 1 (above): PCA analysis of liver RNA-seq data. Chow diet depicted in red, 12 weeks diet in green and 18 weeks diet in blue.

Figure 2 (right): Heatmap showing hierarchical clustering of the differentially expressed genes (adjusted p-value < 0.01 and a ≥ 2 -old up- or down-regulation, approximately 1350 genes).

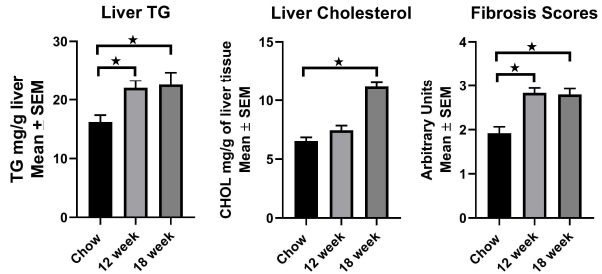
Table 1 (below): Significantly down- and upregulated pathways identified in each comparison associated with NASH progression. Permuted p-value is reported.

	Control vs 12 weeks	Control vs 18 weeks	Control vs Diet
Chemokine signaling pathway	<0.0001	<0.0001	<0.0001
Macrophage markers	<0.0001	<0.0001	<0.0001
Estrogen metabolism	0.006	0.079	0.005
Nuclear receptors in lipid metabolism and toxicity	<0.0001	0.413	<0.0001
Lung fibrosis	0.068	<0.0001	0.01
PPAR signaling pathway	0.001	0.363	0.015
Matrix Metalloproteinases	0.066	0.005	0.034

3 RESULTS

Steatosis and fibrosis

Liver lipid accumulation (steatosis) was measured using a biochemical assay. Fibrosis were assessed histopathology (collagen staining) and scored by a board certified veterinary pathologist.



★p< 0.05 Vs. chow

4 CONCLUSIONS

- Transcriptional profiling of liver of ob/ob mice on high-fat high-fructose diet resulted in enrichment of pathways associated with metabolic dysfunction, inflammation and fibrosis.
- Biochemical assays and histopathology independently confirmed advanced liver disease in both diet cohorts.
- These data support the validity of ob/ob mice as a model to study NASH progression and shed light on the individual genes from various pathways in disease progression.

5 ACKNOWLEDGEMENTS

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