Gene expression profiling of PBMCs in alcoholic and non-alcoholic liver diseases

Trina M. Norden-Krichmar[1], Stanislav Listopad[1], Noora Siddiqui[1], Christophe Magnan[1], Zhang-Xu Liu[2], Jon Jacobs[3], Timothy Morgan[4], and the Southern California Alcoholic Hepatitis Consortium (SCAHC)

[1]University of California, Irvine, CA, USA; [2]University of Southern California, Los Angeles, CA, USA; [3]Pacific Northwest National Laboratory, Richland, WA, USA; [4]VA Long Beach Healthcare System, Long Beach, CA, USA

Introduction

- Alcoholic Hepatitis (AH) is the most severe alcohol-induced inflammatory liver disease, with high mortality and morbidity rates.
- The underlying genomic factors that distinguish AH from other liver diseases are not well understood.
- This study used RNA sequencing for gene expression profiling of human peripheral blood mononuclear cells (PBMCs) from patients with Alcoholic Hepatitis (AH), Alcoholic Cirrhosis (AC), Non-Alcoholic Fatty Liver Disease (NAFLD), and healthy controls.

Methods

- **Sample ascertainment.**
  - The blood samples in this study were collected at baseline by the Southern California Alcoholic Hepatitis Consortium (SCAHC) from patients with Alcoholic Hepatitis (AH, n=38), abstinent Alcoholic Cirrhosis (AA, n=19), recently drinking Alcoholic Cirrhosis (DA, n=20), Non-Alcoholic Fatty Liver Disease (NF, n=20), and from normal healthy controls (C, n=20).
- **RNA sequencing.**
  - PBMCs were isolated by the Liu lab (USC) from the blood samples into cell pellets. RNA was extracted from the cell pellets.
  - RNA was sequenced at 2x100 paired-end on an Illumina HiSeq.
  - The RNAseq data was aligned to the human genome (hg19) using Tophat2 alignment software (Kim et al., Genome Biol. 2013).
- **Differential gene expression analysis.**
  - Differential expression (DE) analysis was performed with the Cufflinks software (Trapnell et al., Nature Methods, 2012), using upper quartile normalization between the data files.
  - Normalized DE between the groups was filtered for FPKM>=2, abs(log2(fold change)) >= 1.0, and that were significant at FDR-adjusted p-value <= 0.05.
  - Heatmaps and expression plots were constructed with cummeRbund (Goff et al., 2012) to display a subset of the differentially expressed genes that met the thresholds listed above.
- **Proteomics**
  - Proteomics data was provided by the Jacobs lab (PNNL) from a high mass accuracy LC-MS/MS platform. Data was filtered by abs(fold change) >= 0.5.
- **Pathway Analysis.**
  - Ingenuity Pathway Analysis IPA software (QIAGEN) was used to determine the top canonical pathways and for comparison to proteomics data.

Acknowledgements

- The **Southern California Alcoholic Hepatitis Consortium (SCAHC)** is comprised of researchers at the following locations: Veterans Administration Long Beach (VALB), Long Beach, CA; University of California, Los Angeles (UCLA), Los Angeles, CA; University of Southern California (USC), Los Angeles, CA; Pacific Northwest National Laboratory (PNNL), Richland, WA; University of California, Irvine (UCI), Irvine, CA
- Funding for this study was provided to the researchers in the SCAHC by the National Institute on Alcohol Abuse and Alcoholism (NIAAA) award numbers: U01AA021838(Norden-Krichmar), U01AA021886 (Morgan), U01AA021884 (Morgan), U01AA021918 (Jacobs), U01AA021898 (French), and U01AA021857 (Liu).
- The authors would also like to thank and acknowledge the members of the UC Irvine Genomics High-Throughput Facility (GHTF) for their role in the RNA extraction and sequencing of the samples.

Contact Information

Trina Norden-Krichmar: tnordenk@uci.edu

Results

- **Gene expression profiles of differentially expressed (DE) genes for PBMCs at baseline across all liver disease groups:**

LEGEND:

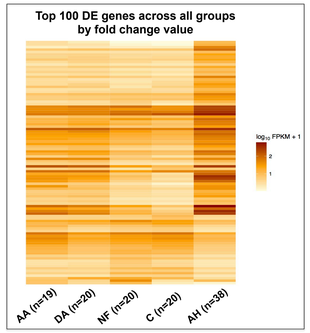
AA = **A**bstinent **A**lcoholic Cirrhosis

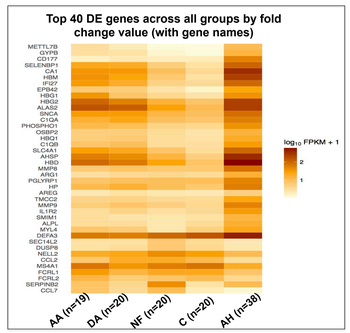
DA = Recently **D**rinking **A**lcoholic Cirrhosis

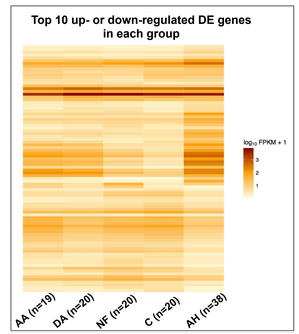
NF = **N**A**F**LD

C = Healthy **C**ontrol

AH = **A**lcoholic **H**epatitis



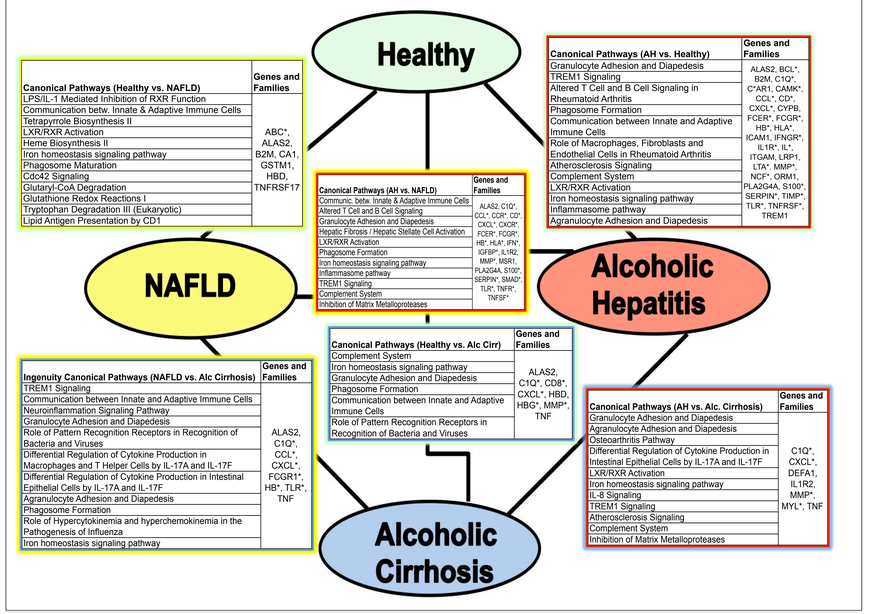




- The first two heatmaps were generated using the top differentially expressed genes across all groups by fold change value.
- The Abstinent and Recently Drinking Alcoholic Cirrhosis groups were very similar to each other.
- The NAFLD and Healthy Controls were very similar to each other.
- The Alcoholic Hepatitis (AH) group showed the most significant differences in gene expression compared to the other groups.
- In the rightmost heatmap, the top 10 up- and down-regulated genes were selected for each group in order to accentuate the subtle differences between the groups.

- **Most significant canonical pathways between liver diseases:**

TRANSCRIPTOMICS



Canonical Pathways (Healthy vs. NAFLD)	Genes and Families
LPS/IL-1 Mediated Inhibition of RXR Function	ABC*, ALAS2, B2M, CA1, GSTM1, HBD, TNFRSF17
Communication betw. Innate & Adaptive Immune Cells	
Tetrapyrrole Biosynthesis II	
LXR/RXR Activation	
Heme Biosynthesis II	
Iron homeostasis signaling pathway	
Phagosome Maturation	
Cdc42 Signaling	
Glutaryl-CoA Degradation	
Glutathione Redox Reactions I	
Tryptophan Degradation III (Eukaryotic)	
Lipid Antigen Presentation by CD1	

Canonical Pathways (AH vs. NAFLD)	Genes and Families
Communic. betw. Innate & Adaptive Immune Cells	ALAS2, C1Q*, CCL*, CCR*, CD*, CXCL*, CXCR*, FCER*, FCGR*, HB*, HLA*, IFN*, IGFBP*, IL1R2, MMP*, MSR1, PLA2G4A, S100*, SERPIN*, SMAD*, TLR*, TNFR*, TNFSF*
Altered T Cell and B Cell Signaling	
Granulocyte Adhesion and Diapedesis	
Hepatic Fibrosis / Hepatic Stellate Cell Activation	
LXR/RXR Activation	
Phagosome Formation	
Iron homeostasis signaling pathway	
Inflammasome pathway	
TREM1 Signaling	
Complement System	
Inhibition of Matrix Metalloproteases	

Canonical Pathways (AH vs. Healthy)	Genes and Families
Granulocyte Adhesion and Diapedesis	ALAS2, BCL*, B2M, C1Q*, C*AR1, CAMK*, CCL*, CD*, CXCL*, CYPB, FCER*, FCGR*, HB*, HLA*, ICAM1, IFNGR*, IL1R*, IL*, ITGAM, LRP1, LTA*, MMP*, NCF*, ORM1, PLA2G4A, S100*, SERPIN*, TIMP*, TLR*, TNFRSF*, TREM1
TREM1 Signaling	
Altered T Cell and B Cell Signaling in Rheumatoid Arthritis	
Phagosome Formation	
Communication between Innate and Adaptive Immune Cells	
Role of Macrophages, Fibroblasts and Endothelial Cells in Rheumatoid Arthritis	
Atherosclerosis Signaling	
Complement System	
LXR/RXR Activation	
Iron homeostasis signaling pathway	
Inflammasome pathway	
Agranulocyte Adhesion and Diapedesis	

Canonical Pathways (Healthy vs. Alc Cirr)	Genes and Families
Complement System	ALAS2, C1Q*, CD8*, CXCL*, HBD, HBG*, MMP*, TNF
Iron homeostasis signaling pathway	
Granulocyte Adhesion and Diapedesis	
Phagosome Formation	
Communication between Innate and Adaptive Immune Cells	
Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses	

Ingenuity Canonical Pathways (NAFLD vs. Alc Cirrhosis)	Genes and Families
TREM1 Signaling	ALAS2, C1Q*, CCL*, CXCL*, FCGR1*, HB*, TLR*, TNF
Communication between Innate and Adaptive Immune Cells	
Neuroinflammation Signaling Pathway	
Granulocyte Adhesion and Diapedesis	
Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses	
Differential Regulation of Cytokine Production in Macrophages and T Helper Cells by IL-17A and IL-17F	
Differential Regulation of Cytokine Production in Intestinal Epithelial Cells by IL-17A and IL-17F	
Agranulocyte Adhesion and Diapedesis	
Phagosome Formation	
Role of Hypercytokinemia and hyperchemokinemia in the Pathogenesis of Influenza	
Iron homeostasis signaling pathway	

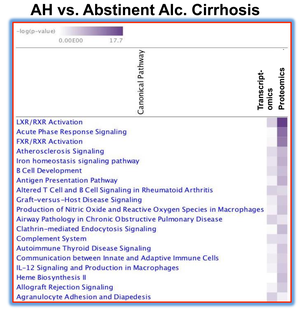
Canonical Pathways (AH vs. Alc. Cirrhosis)	Genes and Families
Granulocyte Adhesion and Diapedesis	C1Q*, CXCL*, DEFA1, IL1R2, MMP*, MYL*, TNF
Agranulocyte Adhesion and Diapedesis	
Osteoarthritis Pathway	
Differential Regulation of Cytokine Production in Intestinal Epithelial Cells by IL-17A and IL-17F	
LXR/RXR Activation	
Iron homeostasis signaling pathway	
IL-8 Signaling	
TREM1 Signaling	
Atherosclerosis Signaling	
Complement System	
Inhibition of Matrix Metalloproteases	

Similar top canonical pathways in TRANSCRIPTOMICS and PROTEOMICS

AH vs. Healthy

AH vs. NAFLD

AH vs. Abstinent Alc. Cirrhosis



AH vs. Drinking Alc. Cirrhosis

Conclusions

- Differences in gene expression were observed between the AH samples and those from the other liver diseases and the normal healthy controls, with the most significantly enriched pathways related to immune and inflammatory function. Comparison with proteomics data confirmed enrichment in these pathways.
- Differences were also found between NAFLD and the other groups, with enriched pathways involved in iron homeostasis, in addition to inflammatory pathways.
- The ability to detect differentially expressed genes by PBMCs in the blood may pave the way for a liquid biopsy approach for diagnosis, disease progression monitoring, treatment response, and drug target discovery for alcoholic hepatitis, as well as other liver diseases.