BCAT1 is Associated with Clinical Decompensation in NAFLD: A Pilot Study

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***BCAT1*, which initiates the catabolism of essential branched chain amino acids, was upregulated in NAFLD patients with clinical decompensation. Our results suggest that perturbations in hepatic metabolism are associated with poor outcomes in NAFLD patients, and points toward the need for further investigation of such targets.**

Background

- Nonalcoholic fatty liver disease (NAFLD) causes substantial morbidity and mortality, but genetic factors predicting poor outcomes are incompletely understood.[1,2,3]
- Recently, a 64 gene expression profile differentiated mild and severe NAFLD liver injury fibrosis in humans, independent of clinical factors.[4]

Objective

- To evaluate the association between gene expression and clinical decompensation and death with the goal of identifying molecular pathways linked to morbidity and mortality in NAFLD.

Methods

- Patients: retrospective analysis of NAFLD patients previously identified from the Duke NAFLD Biorepository with available gene expression (GEx) results from percutaneous liver biopsy specimens. Patients previously stratified based on liver fibrosis stage: mild NAFLD (i.e., Metavir Stage F0-1, n=47) and advanced NAFLD (i.e., Metavir Stage F3-4, n=39). Initial demographic data and laboratory studies were obtained on all patients within 6 months of liver biopsy. Biorepository approved by the Duke University Institutional Review Board.
- Gene expression analyses: previously described[4]. Hepatic DNA and RNA were isolated using the AllPrep Micro Kit (QIAGEN). GEx and genomic data were generated using Illumina Infinium HM450 bead chips and Affymetrix HG U133 Plus 2 arrays.
- Statistical Analyses: patients evaluated from liver biopsy (2007-2009) until death, liver transplantation, or July 1, 2015. Outcomes included death, hepatic decompensation (i.e., ascites, hepatic encephalopathy, hepatocellular carcinoma, or variceal bleeding) and a composite outcome of stroke, myocardial infarction and death. Associations between the outcomes and gene expression were quantified using generalized linear models controlling for age, body mass index, diabetes mellitus and fibrosis stage. Principal component analysis and hierarchical clustering were also performed. All analyses were performed in R with Bioconductor packages.

Results

Table 1: Baseline characteristics (n=86)

	Mild NAFLD (n=47) N (%)		Advanced NAFLD (n=39) N (%)		p-value
Fibrosis Stage	F0 (n=17)	F1 (n=30)	F3 (n=30)	F4 (n=9)	
Gender (%female)	64.7	60.0	73.3	77.8	0.63
Age, mean (STD)	51.1 (9.1)	50.6 (10.7)	48.3 (10.8)	59.7 (8.7)	0.04
Race					0.64
White (%)	88.2	90.0	90.0	88.9	
Black (%)	11.8	3.3	6.7	11.1	
Body Mass Index (BMI) (kg/m2)	35.4 (8.3)	35.8 (8.9)	36.9 (7.9)	37.4 (13.2)	0.91
Diabetes Mellitus (%DM)	23.5	23.3	60.0	77.8	0.001
Hyperlipidemia (%yes)	82.3	63.3	60.0	44.4	0.24
Hypertension (%yes)	64.7	53.3	63.3	88.9	0.28
Alcohol (%yes)	28.6	62.6	54.2	0	0.008
Smoking (%yes)	11.8	3.8	11.5	0	0.52
Hypercholesterolemia (%yes)	41.1	50.0	13.3	0	0.002
NAFLD Activity Score (NAS) >= 5	11.8	36.7	63.3	22.2	0.003

- Outcomes: Four patients, all white women, experienced clinical outcomes (two with hepatic decompensation, two with strokes). Three of these had fibrosis F3-F4 and three had diabetes.

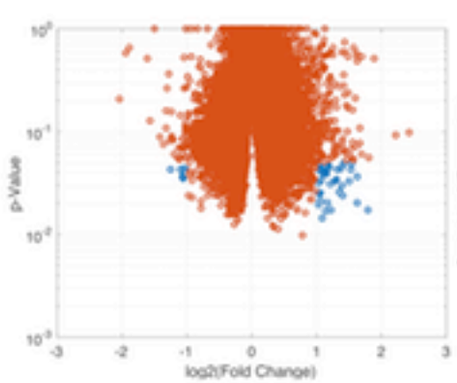

Figure 1: Forty-two genes showed significant differential expression ($p<0.05$) and a two-fold change in expression between patients with and without the composite outcome.

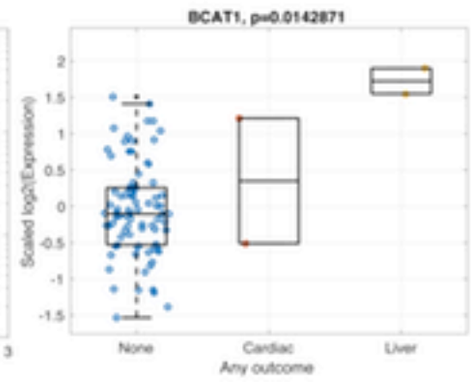

Figure 2: *BCAT1* is upregulated in patients with outcomes, particularly those with hepatic decompensation.

Table 2: Selected differentially expressed genes ($p < 0.05$) in patients with and without outcomes

Gene	GO Function	Fold Change
BCAT1 (2 probes)	Branched chain amino acid metabolism	2.13, 2.20
ADAMDEC1	Metalloendopeptidase activity, Immune response	3.47
PF4V1	Chemokine activity	3.08
BCL2A1	Apoptosis signaling	2.24
ITGAM	Integrin, Cell adhesion	2.33
MTHFD2	One carbon metabolism	2.13

Conclusions

- ***BCAT1* initiates the catabolism of essential branched chain amino acids and here we found it to be upregulated in NAFLD patients with clinical decompensation.**
- **Its upregulation has previously been associated with high liver fat content and poor prognosis in hepatocellular carcinoma.**
- **Our results suggest that perturbations in hepatic metabolism are associated with poor outcomes in NAFLD patients, and points toward the need for further investigation of such targets.**

References

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