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## Supplemental information

# Defining the serum proteomic signature of hepatic steatosis, inflammation, ballooning and fibrosis in non-alcoholic fatty liver disease

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**Supplementary methods**

**Number of univariate proteins (Table S2).** Using the training set, univariate t-tests were used to assess associations of analytes with each histological parameter. Multiple testing correction was completed using the Benjamini-Hochberg procedure for the false discovery rate. As an initial feature selection step, analytes were filtered based on the minimal false discovery rate (FDR)-corrected p-values using an alpha of 0.1. Table S2 lists the number of significant proteins at a range of FDR cutoffs.

**Performance by study, treatment arm, and time point.** DeLong's test was used to test for differences in AUC with a two-sided alternative. Differences in AUC were calculated on unrounded values and are reported as "End-of-Treatment AUC" – "Baseline AUC."

**Evaluation of model specificity.** Each model was applied to the matched and mis-matched histology data. Accuracy metrics, and odds ratios from Fisher's exact test, were obtained from applying the protein models to their intended biopsy results using the predefined dichotomies and also applying them to deliberately mis-matched biopsy results using all the samples from FLINT and PIVENS with biopsy results. Similarly, accuracy metrics were obtained for histology results by applying the biopsy results from one component to deliberately mis-matched components in order to characterize the inherent correlations in disease severity.

**Fig. S1.**

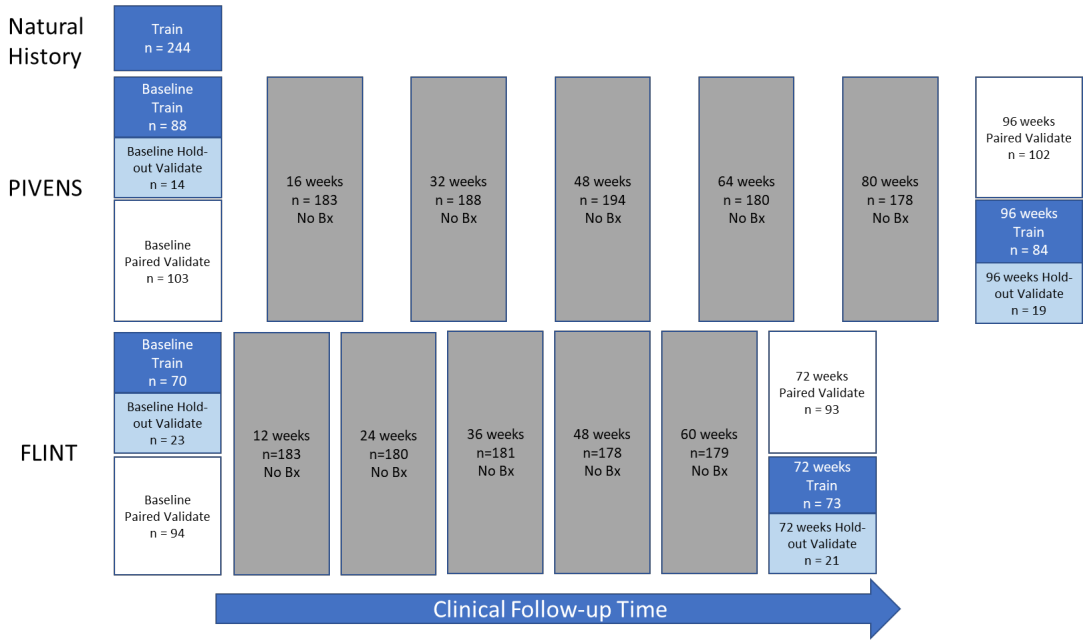


**Fig. S1. Design of the training and validation studies for study aims 1 and 2.** Numbers of evaluable serum samples used for training vs. biopsy [Bx] are shown in dark blue, primary validation in paired samples from same participants at different timepoints in white, and hold-out independent validation in light blue. Samples used for longitudinal monitoring without biopsy are shown in gray**.**

**Table S1: Serum and biopsy sampling schedule for the three studies included in training and validation**

| Study Cohort | Design | Serum Collection (weeks) | Liver Biopsy Timing | # Participants | # Samples |
|---|---|---|---|---|---|
| Natural History | Observational, cross sectional, broad spectrum of NAFLD severity | Baseline | Baseline | 244 | 244 |
| PIVENS | 96-week parallel group: pioglitazone, vitamin E, placebo, non-diabetics with probable non-cirrhotic NASH | Baseline, 16, 32, 48, 64, 80, 96 | Baseline, end of therapy | 205 | 1333 |
| FLINT | 72-week parallel group: obeticholic acid, placebo, diabetics and non-diabetics with probable non-cirrhotic NASH | Baseline, 12, 24, 36, 48, 60, 72 | Baseline, end of therapy | 187 | 1275 |
| Total | | | | 636 | 2852 |

**Table S2 Characteristics of the adult study population with Nonalcoholic Fatty Liver Disease used for modeling the proteomic risk prediction models**

| Characteristics | Baseline (N=636) | Analysis Model* Training (N=559) | Paired Validation (N=392) | Hold-out Validation (N=77) |
|---|---|---|---|---|
| **Sample characteristics** | | | | |
| Study of sample, n (%) | | | | |
| NAFLD Adult Database | 84 (13%) | 84 (15%) | 0 (0%) | 0 (0%) |
| Adult Database 2 | 150 (24%) | 150 (27%) | 0 (0%) | 0 (0%) |
| PIVENS trial | 215 (34%) | 182 (33%) | 205 (52%) | 33 (43%) |
| FLINT trial | 187 (29%) | 143 (26%) | 187 (48%) | 44 (57%) |
| Visit of sample, n (%) | | | | |
| Baseline | 636 (100%) | 402 (72%) | 197 (50%) | 37 (48%) |
| Follow-up: end of treatment[†] | *n/a* | 157 (28%) | 195 (50%) | 40 (52%) |
| Days between plasma collection and biopsy date[‡] (mean (SD) [minimum, maximum] | -37.0 (47.0) [-189, 124] | -20.7 (42.4) [-189, 124] | -20.0 (45.6) [-187, 171] | -18.4 (42.1) [-154, 83] |
| **Demographics**, n (%) | | | | |
| Age (years) | 48.6 (12.1) | 48.5 (12.0) | 48.9 (11.7) | 48.9 (13.1) |
| Male | 234 (37%) | 200 (36%) | 148 (38%) | 34 (44%) |
| Race | | | | |
| White | 514 (84%) | 446 (83%) | 323 (85%) | 68 (88%) |
| Black or African-American | 18 (3%) | 17 (3%) | 7 (2%) | 1 (1%) |
| Asian | 39 (6%) | 33 (6%) | 22 (6%) | 6 (8%) |
| American Indian/Alaska Native | 14 (2%) | 14 (3%) | 10 (3%) | 0 (0%) |
| Native Hawaiian/Pacific Islander | 4 (1%) | 4 (1%) | 4 (1%) | 0 (0%) |
| Any other/More than one race | 24 (4%) | 23 (4%) | 14 (4%) | 1 (1%) |
| Refusal/not stated | 23 (4%) | 22 (4%) | 12 (3%) | 1 (1%) |
| Hispanic ethnicity | 85 (13%) | 80 (14%) | 50 (13%) | 5 (6%) |
| **Liver enzymes & chemistries,** mean (SD) | | | | |
| Alanine aminotransferase (U/L) | 75.5 (47.7) | 66.9 (45.7) | 68.0 (46.8) | 60.8 (35.1) |
| Aspartate aminotransferase (U/L) | 53.1 (32.0) | 48.1 (30.2) | 51.0 (31.9) | 45.8 (24.9) |
| Alkaline phosphatase (U/L) | 84.3 (51.8) | 83.7 (54.1) | 80.3 (31.0) | 82.7 (32.5) |
| γ-glutamyl transferase (U/L) | 72.4 (128.1) | 69.0 (132.9) | 56.9 (58.0) | 56.4 (65.7) |
| Total bilirubin (mg/dL) | 0.7 (0.4) | 0.7 (0.4) | 0.7 (0.4) | 0.7 (0.4) |
| Direct bilirubin (mg/dL) | 0.1 (0.1) | 0.2 (0.1) | 0.1 (0.1) | 0.1 (0.1) |
| Creatinine (mg/dL) | 0.8 (0.2) | 0.8 (0.2) | 0.8 (0.2) | 0.8 (0.2) |
| Albumin (g/dL) | 4.2 (0.4) | 4.2 (0.4) | 4.2 (0.4) | 4.3 (0.4) |
| **Lipids,** mean (SD) | | | | |

| | | | | |
|---|---|---|---|---|
| Total cholesterol (mg/dL) | 193.1 (42.7) | 191.3 (42.3) | 191.4 (42.7) | 191.6 (44.9) |
| HDL cholesterol (mg/dL) | 44.2 (12.8) | 44.6 (13.6) | 43.3 (11.7) | 40.8 (10.5) |
| LDL cholesterol (mg/dL) | 117.4 (37.3) | 116.3 (37.3) | 116.6 (37.9) | 114.6 (36.8) |
| Triglycerides (mg/dL) | 177.7 (167.1) | 169.9 (129.8) | 178.2 (182.7) | 188.5 (110.5) |
| **Metabolic factors,** mean (SD) | | | | |
| Fasting serum glucose (mg/dL) | 104.5 (34.0) | 105.2 (36.6) | 105.9 (31.6) | 106.9 (34.1) |
| Insulin (µU/mL) | 23.7 (24.9) | 23.7 (26.3) | 26.6 (28.8) | 23.9 (21.7) |
| HOMA-IR† (glucose [mmol/L] x insulin[µU/mL]/22.5)§ | 6.4 (7.9) | 6.6 (8.5) | 7.4 (9.2) | 6.7 (7.4) |
| HbA1c (%) | 6.1 (1.1) | 6.1 (1.1) | 6.1 (1.0) | 6.1 (0.9) |
| Waist circumference (cm) | 108.7 (15.0) | 108.5 (15.1) | 109.1 (15.4) | 110.5 (14.2) |
| Weight (kg) | 96.9 (22.0) | 96.6 (22.3) | 97.4 (21.1) | 100.6 (22.4) |
| Body-mass index (kg/m$^2$) | 34.2 (6.6) | 34.1 (6.6) | 34.4 (6.4) | 34.8 (7.1) |
| Systolic blood pressure (mm Hg) | 131.2 (14.9) | 130.1 (14.7) | 131.3 (14.3) | 131.2 (15.9) |
| Diastolic blood pressure (mm Hg) | 76.7 (10.4) | 76.3 (10.1) | 77.8 (9.9) | 77.4 (10.3) |
| **Comorbidities and treatment,** n (%) | | | | |
| Ever doctor-diagnosed: | | | | |
| Diabetes | 172 (27%) | 156 (28%) | 109 (28%) | 26 (34%) |
| Hyperlipidemia | 373 (59%) | 336 (60%) | 234 (60%) | 51 (66%) |
| Hypertension | 323 (51%) | 295 (53%) | 212 (54%) | 42 (55%) |
| Cardiovascular disease§ | 28 (4%) | 27 (5%) | 15 (4%) | 4 (5%) |
| Kidney disease | 20 (3%) | 20 (4%) | 7 (2%) | 3 (4%) |
| Treatment group assignment | | | | |
| Placebo | 164 (26%) | 134 (24%) | 159 (41%) | 30 (39%) |
| Pioglitazone | 65 (10%) | 54 (10%) | 63 (16%) | 11 (14%) |
| Vitamin E | 76 (12%) | 66 (12%) | 73 (19%) | 10 (13%) |
| Obeticholic acid | 97 (15%) | 71 (13%) | 97 (25%) | 26 (34%) |
| No treatment assignment | 234 (37%) | 234 (42%) | 0 (0%) | 0 (0%) |
| Antidiabetic medication use | 181 (28%) | 153 (27%) | 114 (29%) | 27 (35%) |
| Antilipidemic medication use | 242 (38%) | 225 (40%) | 166 (42%) | 35 (45%) |
| Use of statins | 176 (28%) | 167 (30%) | 119 (30%) | 28 (36%) |
| Use of fibrates | 35 (6%) | 71 (13%) | 74 (19%) | 12 (16%) |
| Antihypertensive medication use | 347 (55%) | 311 (56%) | 226 (58%) | 46 (60%) |
| Use of any Vitamin E (OTC) | 132 (21%) | 97 (17%) | 71 (18%) | 10 (13%) |
| **Liver histology findings,** n (%) | | | | |
| Steatohepatitis (NASH)§ | | | | |
| Not NAFLD | 34 (5%) | 46 (8%) | 9 (2%) | 2 (3%) |
| NAFLD, not NASH | 169 (27%) | 176 (31%) | 64 (16%) | 11 (14%) |
| Borderline pattern | 52 (8%) | 50 (9%) | 67 (17%) | 13 (17%) |
| Definite | 381 (60%) | 287 (51%) | 252 (64%) | 51 (66%) |
| Fibrosis stage§ | | | | |
| 0 – None | 187 (30%) | 196 (35%) | 76 (19%) | 15 (19%) |
| 1 – Mild (Z3 perisinusoidal/ Moderate (Z3 perisinusoidal/ Portal/periportal) | 166 (26%) | 58 (10%) | 122 (31%) | 22 (29%) |

| | | | | |
|---|---|---|---|---|
| 2 - Zone 3 and periportal, any combination | 141 (22%) | 116 (21%) | 89 (23%) | 18 (23%) |
| 3 - Bridging | 119 (19%) | 83 (15%) | 94 (24%) | 21 (27%) |
| 4 - Cirrhosis | 21 (3%) | 27 (5%) | 10 (3%) | 1 (1%) |
| Fibrosis stage¶ (mean, SD) | 1.40 (1.19) | 1.30 (1.23) | 1.59 (1.13) | 1.62 (1.12) |
| Hepatocellular ballooning score (0-2) | | | | |
| None (0) | 226 (36%) | 242 (43%) | 107 (27%) | 23 (30%) |
| Few (1) | 162 (25%) | 135 (24%) | 118 (30%) | 19 (25%) |
| Many (2) | 248 (39%) | 182 (33%) | 167 (43%) | 35 (45%) |
| Steatosis grade (0-3) | | | | |
| < 5% (0) | 57 (9%) | 77 (14%) | 19 (5%) | 3 (4%) |
| 5-33% (1) | 181 (28%) | 181 (32%) | 160 (41%) | 31 (40%) |
| 34-66% (2) | 220 (35%) | 172 (31%) | 126 (32%) | 33 (43%) |
| ≥ 66% (3) | 178 (28%) | 129 (23%) | 87 (22%) | 10 (13%) |
| Lobular inflammation under 20X magnification (amount) (0-3) | | | | |
| 0 (0) | 12 (2%) | 17 (3%) | 0 (0%) | 0 (0%) |
| < 2 (1) | 330 (52%) | 344 (62%) | 213 (54%) | 40 (52%) |
| 2-4 (2) | 217 (34%) | 157 (28%) | 125 (32%) | 28 (36%) |
| > 4 (3) | 77 (12%) | 41 (7%) | 54 (14%) | 9 (12%) |
| Portal inflammation score (0-2) | | | | |
| None (0) | 113 (18%) | 99 (18%) | 52 (13%) | 6 (8%) |
| Mild (1) | 402 (63%) | 347 (62%) | 247 (63%) | 51 (66%) |
| More than mild (2) | 121 (19%) | 113 (20%) | 93 (24%) | 20 (26%) |
| NAFLD activity score¶, mean (SD) | 4.42 (1.83) | 3.92 (1.86) | 4.46 (1.68) | 4.40 (1.66) |
| NAFLD activity score > 4, n (%) | 313 (49%) | 212 (38%) | 194 (49%) | 37 (48%) |

*Data are n (%) or mean (SD). Ten participants from the Natural History study previously participated in PIVENS and their characteristics are included in the PIVENS baseline category. Data splits are shown for the inflammation model.

†If a sample was collected at follow-up (end-of-treatment), then the characteristics of the participant were those at the follow-up visit. For comorbidities reported, the participant's value represents both the baseline report, along with any incident cases over follow-up.

‡Days between the biopsy and sample date calculated as sample collection date subtracted from the biopsy collection date

§HOMA-IR is homeostasis model assessment-estimated insulin resistance; Cardiovascular disease (diagnosed with cerebrovascular or coronary artery disease or congestive heart failure); *n/a* denotes not applicable to the model

§Steatohepatitis Diagnosis Borderline Zone 3/Borderline Zone 1 was collapsed to all Borderline (Baseline: 2 (<1%); Training: 1 (<1%); Paired Validation: 3 (1%); Hold-out Validation: 0 patients classified as Zone 1. Fibrosis stage assessed on a scale of 0-4.

¶ Mean fibrosis stage assessed on a scale of 0-4, with higher scores showing more severe fibrosis.

NAFLD activity score was assessed on a scale of 0-8, with higher scores showing more severe disease (the components of this measure are steatosis [assessed on a scale of 0-3], lobular inflammation [assessed on a scale of 0-3], and hepatocellular ballooning [assessed on a scale of 0-2]).

**Table S3: Numbers of proteins used in the univariate list for pathway enrichment analysis.** Using the training set, univariate t-tests were used to assess associations of analytes with each histological parameter. Multiple testing correction was completed using the Benjamini-Hochberg procedure for the false discovery rate (FDR). The number of significant proteins at a range of FDR cutoffs are compared to the number of proteins in the final model, which are a subset of the proteins with an FDR < 0.1.

| Model | Final Model | Univariate List (FDR< 0.01) | Univariate List (FDR< 0.05) | Univariate List (FDR< 0.1) |
|---|---|---|---|---|
| **Steatosis** | 12 | 254 | 397 | 532 |
| **Ballooning** | 5 | 827 | 1,174 | 1,408 |
| **Lobular Inflammation** | 14 | 415 | 632 | 809 |
| **Fibrosis** | 8 | 1,360 | 1,883 | 2,201 |

**Table S4: Biologic functions of proteins in the models and any known relation to NAFLD or NASH.**

## Steatosis

| EntrezGene Symbol | Target Full Name | UniProt | Association with NASH or NAFLD |
|---|---|---|---|
| *INSL5* | insulin-like peptide insl5 | Q9Y5Q6 | • Insl5 expression is regulated by the gut microbiota and energy availability. INSL5 is a hormone that could play a role in promoting hepatic glucose production during periods of energy deprivation.<br>• Highly expressed in rectum with lower levels in uterus and ascending and descending colon. |
| *FABP12* | fatty acid-binding protein 12 | A6NFH5 | • FBP12 present in human retinoblastoma cell lines, rodent retina and testis.<br>• FBP1 and 4 present in liver and adipocyte, respectively. |
| *RECQL* | atp-dependent dna helicase q1 | P46063 | • High expression in heart, lung, skeletal muscle and kidney, low expression in brain. |
| *GUSB* | beta-glucuronidase | P08236 | • β-Glucuronidase inhibitors are suggested as potential hepatoprotective agents. |
| *INHBC* | inhibin beta c chain | P55103 | • Expressed in benign prostatic hyperplasia. |
| *HEXB* | beta-hexosaminidase subunit beta | P07686 | • 2750 genes/proteins associated with the disease Non-alcoholic Fatty Liver Disease, HEXB is one of them (https://amp.pharm.mssm.edu/Harmonizome/gene_set/Non-alcoholic+Fatty+Liver+Disease/CTD+Gene-Disease+Associations). |
| *CNDP1* | beta-ala-his dipeptidase | Q96KN2 | • Found in serum and adult nervous central system.<br>• Absent in serum from patients with homocarnosinosis. |
| *GH2* | growth hormone variant | P01242 | • Growth hormone deficiency and nonalcoholic fatty liver disease with insights from humans and animals: pediatric implications. Metab Syndr Relat Disord. 2018.<br>• Growth Hormone is different from Growth Hormone Variant. |
| *PTGR1* | prostaglandin reductase 1 | Q14914 | • Experimental Nonalcoholic Steatohepatitis and Liver Fibrosis Are Ameliorated by |

| | | | |
|---|---|---|---|
| | | | Pharmacologic Activation of Nrf2 (NF-E2 p45-Related Factor 2).<br>• Prostaglandin reductase 1 is regulated by Nrf2. (Namani A, et al. NRF2-regulated metabolic gene signature as a prognostic biomarker in non-small cell lung cancer. Oncotarget. 2017;8(41):69847-62.) |
| *BPIFB1* | bpi fold-containing family b member 1 | Q8TDL5 | • Detected in duodenum mucosal crypts of cholera patients.<br>• Detected in trachea, nasal septal epithelium and lung.<br>• Alternative names: Long palate, lung and nasal epithelium carcinoma-associated protein 1; von Ebner minor salivary gland protein |
| *GRID2* | glutamate receptor ionotropic; delta-2 | O43424 | • Highest expression level in cerebellar vermis.<br>• Involved in the disease of Spinocerebellar ataxia, autosomal recessive, 18 (SCAR18). |
| *ERN1* | serine/threonine-protein kinase/endoribonuclease ire1 | O75460 | • Ubiquitously expressed. High levels observed in pancreatic tissue.<br>• IRE1, also called ERN1.<br>• The expression levels of IRE1 were decreased in the livers of human obese patients with NASH, compared to those of obese patients without NASH (Shamsa EH, Zhang K. The primary unfolded protein response transducer endoplasmic reticulum-to-nucleus signaling 1 is downregulated in livers of human nonalcoholic steatohepatitis patients. Environmental Disease. 2018; 3(4): 80-2.). |

## Inflammation

| Target | Target Full Name | UniProt | Association with NASH or NAFLD |
|---|---|---|---|
| *ACY1* | aminoacylase-1 | Q03154 | • Expression is highest in kidney, strong in brain and weaker in placenta and spleen.<br>• It is a zinc-binding protein that catalyzes the hydrolysis of N-acetyl amino acids into free aliphatic amino acids and acetic acid. |

| | | | |
|---|---|---|---|
| | | | • It was increased in hepatic lipid droplets of mice subjected to caloric restriction suggesting a role in the metabolic adjustments to the overfed state in NAFLD. (Wood GC, et al. A multi-component classifier for nonalcoholic fatty liver disease (NAFLD) based on genomic, proteomic and phenomic data domains. Sci Rep. 2017; 7; 43238.) |
| *RPN1* | dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 1 | P04843 | • Expressed in all tissues tested.<br>• Glycosyltransferases have roles in the pathogenesis of NAFLD (Zhan YT, et al. Glycosyltransferases and non-alcoholic fatty liver disease. World J Gastroenterol. 2016;22(8):2483-93.).<br>• No data specifically for RPN1 with NAFLD. |
| *C1orf198* | uncharacterized protein c1orf198 | Q9H425 | • Expressed in 204 organ(s), highest expression level in C1 segment of cervical spinal cord. |
| *CTCF* | transcriptional repressor ctcf | P49711 | • The protein CTCF plays a role in repressing the insulin-like growth factor 2 (IGF2) gene.<br>• IGF2 was associated with fibrosis in NAFLD. |
| *SAA2* | serum amyloid a-2 protein | P0DJI9 | • Serum amyloid A (SAA) is a family of apolipoproteins mainly synthesized in mammalian liver.<br>• There are constitutive family members (SAA4) and acute-phase members (SAA1 and SAA2) that respond to tissue damage and inflammation.<br>• SAA1 is a mediator to reduce fat deposition (Tai CC, et al. Docosahexaenoic acid enhances hepatic serum amyloid A expression via protein kinase A dependent mechanism. J Biol Chem. 2009;284(47):32239-47.).<br>• The sequence homology in the proximal promoter regions is high between human SAA1 and SAA2 and their promoter activities in response to cytokine treatments are also similar. |
| *FCGR3B* | low affinity immunoglobulin gamma fc region receptor iii-b | O75015 | • Expressed specifically by polymorphonuclear leukocytes (neutrophils). Also expressed by stimulated eosinophils.<br>• Gerhard GS, et al. Transcriptomic profiling of obesity-related nonalcoholic steatohepatitis reveals a core set of fibrosis-specific genes. J Endocr Soc. 2018; 2 (7): 710-26. |
| *ADIPOQ* | adiponectin | Q15848 | • Synthesized exclusively by adipocytes and secreted into plasma.<br>• Important adipokine involved in the control of fat metabolism and insulin sensitivity, with direct |

| | | | |
|---|---|---|---|
| | | | anti-diabetic, anti-atherogenic and anti-inflammatory activities.<br>• Polyzos SA, et al. Adiponectin as a target for the treatment of nonalcoholic steatohepatitis with thiazolidinediones: a systematic review. Metabolism. 2016; 65 (9): 1297-306. |
| *TXNRD 1* | thioredoxin reductase 1; cytoplasmic | Q16881 | • Oxidative stress is a core abnormality responsible for disease progression in nonalcoholic steatohepatitis (NASH).<br>• Thioredoxin reductase 1 and 2 were significantly underexpressed in NASH livers of mice (Gornicka A, et al. Transcriptional profile of genes involved in oxidative stress and antioxidant defense in a dietary murine model of steatohepatitis. Antixoid Redox Signal. 2011; 15(2):437-45.). |
| *GSTZ1* | maleylacetoacetate isomerase | O43708 | • Mostly expressed in liver followed by kidney, skeletal muscle and brain. |
| *TACSTD2* | tumor-associated calcium signal transducer 2 | P09758 | • Expressed in placenta, pancreatic carcinoma cell lines.<br>• Belonging to Epithelial Cell Adhesion Molecular (EPCAM) family and may function as a growth factor receptor. |
| *PYY* | peptide yy | P10082 | • Mokhtari Z, et al. Nonalcoholic fatty liver disease, the gut microbiome, and diet. Adv Nutr. 2017; 8(2): 240-52. |
| *CCL23* | c-c motif chemokine 23 | P55773 | • Hart KM, et al. Type 2 immunity is protective in metabolic disease but exacerbates NAFLD collaboratively with TGF-β. Sci Transl Med. 2017; 9(396): pii: eaa13694. |
| *PCOLCE2* | procollagen c-endopeptidase enhancer 2 | Q9UKZ9 | • Highly expressed in the heart, trabecular meshwork, pituitary gland, bladder, mammary gland, trachea and placenta.<br>• PCPE2 plays a critical role in maintaining scavenger receptor class B type 1(SR-BI) conformation, which, in turn, controls adipocyte maturation (https://onlinelibrary.wiley.com/doi/full/10.1111/eci.12748). |
| *ACP1* | low molecular weight phosphotyrosine protein phosphatase | P24666 | • PPAC functions as an acid phosphatase and a protein tyrosine phosphatase.<br>• Low molecular weight phosphotyrosine protein phosphatase was confirmed to be one of the targets, which can be suppressed by upregulated miR-576-5p in NAFLD liver. (Soronen J, et al. Novel hepatic microRNAs upregulated in human |

| | | | |
|---|---|---|---|
| | | | nonalcoholic fatty liver disease. Physiol Rep. 2016;4(1). Pii:e12661.) |

## Cell Ballooning

| Target | Target Full Name | UniProt | Association with NASH or NAFLD |
|---|---|---|---|
| *AKR1B10* | aldo-keto reductase family 1 member b10 | O60218 | • Arendt BM, et al. Altered hepatic gene expression in nonalcoholic fatty liver disease is associated with lower hepatic n-3 and n-6 polyunsaturated fatty acids. Hepatology. 2015; 61(5): 1565-78. |
| *PTGR1* | prostaglandin reductase 1 | Q14914 | • Experimental Nonalcoholic Steatohepatitis and Liver Fibrosis Are Ameliorated by Pharmacologic Activation of Nrf2 (NF-E2 p45-Related Factor 2).<br>• Prostaglandin reductase 1 is regulated by Nrf2. (Namani A, et al. NRF2-regulated metabolic gene signature as a prognostic biomarker in non-small cell lung cancer. Oncotarget. 2017;8(41):69847-62.) |
| *ADAMTSL2* | adamts-like protein 2 | Q86TH1 | • ATL2 is related to an autosomal recessive disorder, Geleophysic Dysplasia 1. |
| *CTLA4* | cytotoxic t-lymphocyte protein 4 | P16410 | • Inhibitory receptor acting as a major negative regulator of T-cell responses. The affinity of CTLA4 for its natural B7 family ligands, CD80 and CD86, is considerably stronger than the affinity of their cognate stimulatory coreceptor CD28. |
| *CNN2* | calponin-2 | Q99439 | • Higher CNN2 expression was seen from the higher liver fibrosis group (Bracht T, et al. Analysis of disease-associated protein expression using quantitative proteomics-fibulin-5 is expressed in association with hepatic fibrosis. J Proteome Res. 2015; 14(5): 2278-86.). |

## Fibrosis

| Target | Target Full Name | UniProt | Associated with NASH or NAFLD (Y/N) |
|---|---|---|---|
| *ADAMTSL2* | adamts-like protein 2 | Q86TH1 | • ATL2 is related to an autosomal recessive disorder, Geleophysic Dysplasia 1. |

| | | | |
|---|---|---|---|
| *C7* | complement component c7 | P10643 | • Bell LN, et al. Serum proteomics and biomarker discovery across the spectrum of nonalcoholic fatty liver disease. Hepatology. 2010; 51(1):111-20. |
| *NFASC* | neurofascin | O94856 | • Cell adhesion, ankyrin-binding protein which may be involved in neurite extension, axonal guidance, synaptogenesis, myelination and neuron-glial cell interactions. |
| *COLEC11* | collectin-11 | Q9BWP8 | • Detected in adrenal gland, kidney, liver, ovaries and testis.<br>• Collectin 11 was one of the proteins in the panel to discriminate early and advanced fibrosis in NASH patients using SomaScan assay platform. (file:///C:/Users/yjia/Downloads/file.pdf) |
| *KDR* | vascular endothelial growth factor receptor | P35968 | • Widely expressed.<br>• Tyrosine-protein kinase that acts as a cell-surface receptor for VEGFA, VEGFC and VEGFD. Plays an essential role in the regulation of angiogenesis, vascular development, vascular permeability, and embryonic hematopoiesis. Promotes proliferation, survival, migration and differentiation of endothelial cells. Promotes reorganization of the actin cytoskeleton.<br>• Coulon S, et al. Role of vascular endothelial growth factor in the pathophysiology of nonalcoholic steatohepatitis in two rodent models. Hepatology. 2013; 57(5): 1793-805. |
| *WNT5A* | protein wnt-5 | P41221 | • Expression is increased in differentiated thyroid carcinomas compared to normal thyroid tissue and anaplastic thyroid tumors where expression is low or undetectable. Expression is found in thyrocytes but not in stromal cells.<br>• Ligand for members of the frizzled family of seven transmembrane receptors. Can activate or inhibit canonical Wnt signaling, depending on receptor context.<br>• Tian F, et al. Celecoxib ameliorates non-alcoholic steatohepatitis in type 2 diabetic rats via suppression of the non-canonical Wnt signaling pathway |

| | | | |
|---|---|---|---|
| | | | expression. PLoS One. 2014; 9(1): e83819. |
| *PLOD3* | procollagen-lysine;2-oxoglutarate 5-dioxygenase 3 | O60568 | • Detected in heart, placenta and pancreas and at lower levels in lung, liver and skeletal muscle.<br>• Marcolin E, et al. Quercetin treatment ameliorates inflammation and fibrosis in mice with nonalcoholic steatohepatitis. J Nutr. 2012; 142(10): 1821-8. |
| *FCRL3* | fc receptor-like protein 3 | Q96P31 | • Promotes TLR9-induced B-cell proliferation, activation and survival but inhibits antibody production and suppresses plasma cell differentiation. Enhances activation of NF-kappa-B and MAPK signaling pathways in TLR9 stimulated B-cells. |

**Table S5. Performance by study, treatment arm, and time point**. Based on DeLong's test* for differences in AUC with a two-sided alternative, there were no statistically significant differences in AUC between baseline and end-of-treatment for any subgroup at a significance level of 0.05. Differences in AUC were calculated on unrounded values and are reported as "End-of-Treatment AUC" – "Baseline AUC."

**Steatosis**

| Study | Treatment Arm | N | Baseline AUC | End-of-Treatment AUC | Diff | DeLong's Test p-value |
|---|---|---|---|---|---|---|
| FLINT | Obeticholic Acid | 97 | NA[1] | 0.81 | NA[1] | NA[2] |
| FLINT | Placebo | 90 | NA[1] | 0.74 | NA[1] | |
| PIVENS | Pioglitazone | 63 | NA[1] | 0.91 | NA[1] | NA[2] |
| PIVENS | Vitamin E | 73 | NA[1] | 0.83 | NA[1] | |
| PIVENS | Placebo | 69 | NA[1] | 0.65 | NA[1] | NA[2] |

[1]Number of non-events too small ($N \leq 2$) to calculate AUC.
[2]Number of non-events too small to apply DeLong's test for differences in AUC.

**Inflammation**

| Study | Treatment Arm | N | Baseline AUC | End-of-Treatment AUC | Diff | DeLong's Test p-value |
|---|---|---|---|---|---|---|
| FLINT | Obeticholic Acid | 97 | 0.71 | 0.80 | 0.09 | 0.27 |
| FLINT | Placebo | 90 | 0.71 | 0.82 | 0.11 | 0.13 |
| PIVENS | Pioglitazone | 63 | 0.62 | 0.62 | 0.01 | 0.94 |
| PIVENS | Vitamin E | 73 | 0.71 | 0.81 | 0.10 | 0.36 |
| PIVENS | Placebo | 69 | 0.72 | 0.79 | 0.07 | 0.42 |

**Hepatocyte Ballooning**

| Study | Treatment Arm | N | Baseline AUC | End-of-Treatment AUC | Diff | DeLong's Test p-value |
|---|---|---|---|---|---|---|
| FLINT | Obeticholic Acid | 97 | 0.84 | 0.80 | -0.05 | 0.49 |
| FLINT | Placebo | 90 | 0.78 | 0.83 | 0.04 | 0.62 |
| PIVENS | Pioglitazone | 63 | 0.80 | 0.81 | 0.00 | 0.98 |
| PIVENS | Vitamin E | 73 | 0.79 | 0.71 | -0.08 | 0.35 |
| PIVENS | Placebo | 69 | 0.80 | 0.84 | 0.04 | 0.65 |

**Fibrosis**

| Study | Treatment Arm | N | Baseline AUC | End-of-Treatment AUC | Diff | DeLong's Test p-value |
|---|---|---|---|---|---|---|
| FLINT | Obeticholic Acid | 97 | 0.83 | 0.84 | 0.02 | 0.74 |
| FLINT | Placebo | 90 | 0.89 | 0.89 | 0.00 | 1.00 |
| PIVENS | Pioglitazone | 63 | 0.79 | 0.89 | 0.11 | 0.16 |

| Study | Treatment Arm | N | Baseline AUC | End-of-Treatment AUC | Diff | DeLong's Test p-value |
|---|---|---|---|---|---|---|
| PIVENS | Vitamin E | 72 (73) baseline (EoT) | 0.88 | 0.94 | 0.06 | 0.25 |
| PIVENS | Placebo | 69 | 0.86 | 0.81 | -0.05 | 0.48 |

* DeLong, E. R., DeLong, D. M., & Clarke-Pearson, D. L. (1988). Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics, 837-845.

**Table S6: A post-hoc evaluation of model specificity by applying each model to the matched and mis-matched histology data.** Upper panel: accuracy metrics, and odds ratios from Fisher's exact test, obtained from applying the protein models to their intended biopsy results using the predefined dichotomies and also applying them to deliberately mis-matched biopsy results using all the samples from FLINT and PIVENS with biopsy results. Lower panel: accuracy metrics from applying the biopsy results from one component to deliberately mis-matched components to characterize the inherent correlations in disease severity.

| | **Accuracy and odds ratios from Fisher's exact test of protein model prediction when applied to biopsy results of:** | | | | **Average Reduction in Accuracy from Mismatch** | **Average % Reduction in Accuracy from Mismatch** |
|---|---|---|---|---|---|---|
| **Model prediction applied:** | **Ballooning** | **Fibrosis** | **Inflammation** | **Steatosis** | | |
| **Ballooning** | **0.751**<br>**9.7** | 0.727<br>8.3 | 0.666<br>4.7 | 0.633<br>4.9 | 0.076 | 10.1% |
| **Fibrosis** | 0.656<br>5.8 | **0.782**<br>**12.7** | 0.606<br>2.4 | 0.524<br>3.0 | 0.187 | 23.9% |
| **Inflammation** | 0.662<br>6.4 | 0.645<br>3.3 | **0.709**<br>**6.0** | 0.541<br>9.0 | 0.093 | 13.15% |
| **Steatosis** | 0.718<br>3.1 | 0.544<br>2.0 | 0.533<br>2.2 | **0.847**<br>**12.8** | 0.249 | 29.4% |
| | **Accuracy of histology biopsy result when applied to biopsy results of:** | | | | **Average Reduction in Accuracy from Mismatch** | **Average % Reduction in Accuracy from Mismatch** |
| **Biopsy result applied:** | **Ballooning** | **Fibrosis** | **Inflammation** | **Steatosis** | | |
| **Ballooning** | **1** | 0.653 | 0.631 | 0.739 | 0.326 | 32.6% |
| **Fibrosis** | 0.653 | **1** | 0.605 | 0.508 | 0.411 | 41.1% |
| **Inflammation** | 0.631 | 0.605 | **1** | 0.510 | 0.418 | 41.8% |
| **Steatosis** | 0.739 | 0.508 | 0.510 | **1** | 0.414 | 41.4% |