Supplemental Material

Supplemental Methods:

Definition of Co-morbid Conditions:

The presence or absence of co-morbid conditions in the Teen-LABS study was determined by trained study personnel following standard definitions using medical records, physical exam, participant interview and laboratory values.[12] Specifically, hypertension was defined as having systolic or diastolic blood pressure ≥ 95th percentile indexed to age, gender, and height during the baseline visit as measured using the standard Teen-LABS protocol, or current use of anti-hypertensive medication. The presence of dyslipidemia was defined as triglyceride value ≥ 130 mg/dL or LDL cholesterol ≥ 130 mg/dLor HDL cholesterol < 40 mg/dL or use of medication for dyslipidemia. Diabetes mellitus type 2 was defined by presence of laboratory findings (baseline HbA1c of ≥ 6.5%, fasting glucose ≥ 126 mg/dL, or confirmatory 2 hour oral glucose tolerance test within prior 6 months) or use of medications to treat diabetes.

Histological Analysis:

The well-validated NASH Clinical Research Network scoring system was used by a central study hepatopathologist (DEK) to grade features of NAFLD and stage fibrosis, with all clinical characteristics masked. The NAFLD activity score (NAS, range 0-8) was calculated by combining individual component scores for steatosis (range 0-3), lobular inflammation (0-3) and ballooning degeneration (0-2). Fibrosis was categorized as: stage 0 (none); stage 1 including mild (1a), moderate zone 3 perisinusoidal (1b) or portal/periportal only (1c); stage 2 (mild/moderate zone 3 perisinusoidal and periportal); stage 3 (bridging fibrosis); or stage 4 (cirrhosis). Although no single histologic feature is considered diagnostic of NASH, a typical set of minimum criteria for definite NASH would include steatosis (more than 5%), lobular inflammation and hepatocyte injury as manifested by ballooning degeneration. Borderline

NASH cases demonstrated a lesser degree of one or more findings and were further designated as zone 1 or zone 3 accentuated pattern of lesions.

Missing Data

All missing data were laboratory values, ranging from 3.4% (n=5) for white blood cell count to 18.9% for alkaline phosphatase (n=28). The SAS (version 9.4) MI procedure was used to generate 35 imputed data sets for use in multivariable modeling analyses. Four subjects with diabetes who were receiving exogenous insulin were excluded from insulin or HOMA-IR calculations (n=144), as the insulin levels in those subjects may not reflect endogenous insulin secretion, invalidating calculation of HOMA-IR.

Microarray Methodology:

Additional liver tissue, obtained at time of the intraoperative liver biopsy and immediately submerged into RNAlater® solution (Qiagen, Venlo, The Netherlands), was available in a subset of 67 participants from the analysis cohort. The quality of the total RNA samples was analyzed by running a RNA 6000 Nano assay with the Agilent 2100 bioanalyzer. Genome wide liver expression datasets for individual participants were generated using biotinylated cRNAs synthesized from 0.5 µg of total RNA isolated from frozen liver sample. The cRNA pools were hybridized to oligonucleotide-based Affymetrix Human Gene 1.0 ST Array (Affymetrix, Santa Clara, CA, USA) carrying over 700,000 unique oligonucleotide probes analyzing the expression level of 28,869 best characterized human genes. The hybridized arrays were scanned and monitored for specific signals with GeneChip® Operating Software. Affymetrix CEL files were imported into GeneSpring v 12.6.1 (Agilent Technologies, Santa Clara, CA, USA) and subjected to Robust Multichip Average 16 normalization. Using the GeneSpring platform, standard “per-gene” quantile normalization was performed for the entire gene expression dataset, prior to statistical analysis as outlined in the main manuscript. Additionally, we filtered genes with a requirement of an intensity value of 50 in 100% of all samples in at least one of the histological categories. Microarray data are available through the National Center for Biotechnology Information's Gene Expression Omnibus (GSE66676).

SUPPLEMENTAL TABLE LEGEND

(Tables uploaded separately)

Supplemental Table 1: Sample characteristics of Teen-LABS participants with and without intra-operative liver biopsy. Reported p-values are two-sided and considered statistically significant at ≤0.05.

Supplemental Table 2: Crude and adjusted odds ratios of increasing NAFLD severity by clinical characteristic among participants with NAFLD (excluding those with Not-NAFLD). A cumulative logit model was used to evaluate predictors of increasing liver disease severity only among participants with NAFLD, ordinally defined as 1) NAFL (not NASH), 2) Borderline NASH and 3).Definite NASH. The model simultaneously evaluated two separate comparisons: 1) all NASH (Definite NASH or Borderline NASH) vs. NAFL; 2) Definite NASH vs. [Borderline NASH or NAFL]. The final model met the proportional odds assumption ($p = 0.63$), confirming no difference in estimates between the two comparisons. All demographic and clinical variables listed in Table 2 were considered for inclusion in the final models. ALT elevation was categorized as normal (<22 U/L for females, < 26 U/L for males), mild (22-39 U/L for females, 26-39 U/L for males) or high (≥ 40 U/L). Reported p-values are two-sided and considered statistically significant at ≤0.05.

Supplemental Table 3: Crude and adjusted prevalence ratios for presence of any degree of NAFLD (all NAFLD subgroups combined) versus not NAFLD. Modified poisson regression with robust estimates (SAS Proc GENMOD) was used to evaluate predictors of any degree of NAFLD (NAFL, Borderline and Definite NASH combined) vs. No NAFLD. All demographic and clinical variables listed in Table 2 were considered for inclusion in the final models. Reported p-values are two-sided and considered statistically significant at ≤0.05.

Supplemental Table 4: Top up and down-regulated genes identified in comparisons of Borderline NASH, Definite NASH and Not NAFLD. The top 15 up- and 15 down-regulated genes identified in each comparison of 1) Borderline NASH vs. Not NAFLD, 2) Definite NASH vs. Not NAFLD and 3) Borderline vs. Definite NASH are shown in alphabetical order of gene symbol, including gene description, fold change in the respective comparison(s), and attributed function. Some genes were differentially expressed in more than one comparison.

Supplemental Table 5: Key biological processes/pathways enriched in definite NASH relative to borderline NASH. Associated genes were uncovered through ontological analysis of genes differentially regulated between definite NASH and borderline NASH. Genes in italics have been previously reported to be correlated with severely fibrotic (stage 3 or 4) NAFLD in in adults.[23]

Supplemental Table 1: Sample Characteristics of Teen- LABS Participants with Liver Biopsy vs. Participants without Liver Biopsies (of total Teen-LABS cohort n=242)

	Teen-LABS with baseline intra-operative Liver Biopsy (n=165)	**Teen-LABS without baseline intra-operative Liver Biopsy (n=77)**	**P value**
Male	45 (27%)	14 (18%)	0.13
Age in y, mean ± SD (min, max)	16.9 ± 1.55 (13.3, 19.9)	17.5 ± 1.51(13.5, 20.0)	< 0.01
White race (vs. non-white)	113 (68%)	61 (79%)	0.08
Hispanic ethnicity	13 (8%)	4 (5%)	0.44
BMI in kg/m^2, median (min, max)	51.6 (34.0, 87.7)	48.0 (38.7, 74.3)	< 0.01
Pre-op weight loss during preparation for bariatric surgery			
No weight loss	78 (47%)	41 (53%)	0.44
Weight loss of <5%	69 (42%)	26 (34%)	
Weight loss of 6-10%	16 (10%)	10 (13%)	
Weight loss >10%	2 (1%)	0 (0%)	
ALT (U/L), median (IQR)	27 (20, 38) (n=136)	34 (23, 47) (n=63)	0.14
Triglycerides, mg/dL, median (IQR)	116.0 (83, 167.5)	103.5 (76, 154)	0.21
Dyslipidemia, n (%)	130 (79%)	50 (65%)	0.02
Diabetes, n (%)	23 (14%)	10 (13%)	0.84

Supplemental Table 2. Crude and Adjusted Odds Ratios of Increasing NAFLD Severity by Clinical Characteristic Among Participants with NAFLD (excluding Not NAFLD category)

Characteristic	**Unadjusted**		**Adjusted**	
(Groups)	OR (95% CI)	p-value	OR (95% CI)	p-value
Sex		0.05		
Female	1.00			
Male	2.46 (0.99, 6.11)			
Age Categories (yrs)		0.43		
13 – 15	1.00			
16 – 17	2.00 (0.66, 6.12)			
18 +	1.25 (0.37, 4.19)			
Race		0.39		
White	1.00			
Black/Other	1.52 (0.58, 3.95)			
BMI (kg/m^2)	1.00 (0.96, 1.05)	0.90		
ALT Elevation		0.26		
Normal	1.00			
Mild	0.97 (0.28, 3.44)			
High	2.28 (0.61, 8.66)			
ALK	1.00 (0.99, 1.02)	0.34	1.01 (1.00, 1.03)	0.07
Glucose		<.02		
< 100	1.00			
100-125	1.49 (0.50, 4.44)			

≥ 126	8.20 (2.46, 27.33)			
HOMA-IR	1.03 (0.96, 1.11)	0.41		
Triglycerides	1.00 (1.00, 1.01)	0.34		
Albumin	0.40 (0.08, 2.06)	0.27		
White Blood Cell	1.10 (0.94, 1.28)	0.23		
Diabetes		<.01		<.01
No	1.00		1.00	
Yes	8.51 (2.70, 26.80)		12.01 (3.48,41.40)	
Hypertension		0.05		
No	1.00			
Yes	2.49 (0.99, 6.24)			

Supplemental Table 3: Crude and Adjusted Prevalence Ratios for Presence of Any NAFLD vs. Not NAFLD

Characteristic (Groups)	**Unadjusted**		**Adjusted**	
	PR (95% CI)	P-Value	PR (95% CI)	P-Value
Sex		0.19		
Female	1.00			
Male	1.35 (0.86, 2.11)			
Age Categories (yrs)		0.49		
13-15	1.00			
16-17	1.06 (0.63, 1.79)			
18-19	1.23 (0.70, 2.14)			
Race		0.44		
White	1.00			
Black/Other	0.83 (0.51, 1.33)			
BMI (kg/m^2)	1.01 (0.99, 1.03)	0.22		
ALT Elevation		<.01		<.01
Normal	1.00		1.00	
Mild	1.63 (0.91, 2.94)		1.63 (0.91, 2.94)	
High	2.09 (1.11, 3.93)		2.09 (1.11, 3.93)	
ALK	1.00 (0.99, 1.00)	0.60		
Glucose		0.06		
< 100	1.00			
100-125	1.14 (0.68, 1.90)			
≥ 126	1.52 (0.84, 2.76)			

HOMA-IR	1.02 (0.99, 1.05)	0.15
Triglycerides	1.00 (1.00, 1.01)	0.05
Albumin	1.33 (0.67, 2.64)	0.42
White Blood Cell	1.04 (0.96, 1.12)	0.37
Diabetes		0.21
No	1.00	
Yes	1.42 (0.82, 2.47)	
Hypertension		0.11
No	1.00	
Yes	1.42 (0.93, 2.17)	

Supplemental Table 4. Top up- and down-regulated genes identified in comparisons of Definite NASH, Borderline NASH and Not NAFLD.

Gene Symbol	**Gene Description**	**Comparison(s) and Fold Change**	**Gene Function**
ACOT11	Acyl-CoA thioesterase 11	Borderline NASH vs Not NAFLD: FC = -1.59 Definite NASH vs Not NAFLD: FC = -2.13	Catalyst for the conversion of activated fatty acids to non-esterified fatty acid and coenzyme A
ACSL4	Acyl-CoA synthetase long-chain family member 4	Borderline NASH vs Not NAFLD: FC = 1.81	Converts free long-chain fatty acids into fatty acyl-CoA esters
ASPG	Asparaginase	Borderline NASH vs Not NAFLD: FC = -1.55	Exhibits lysophospholipase, transacylase, PAF acetylhydrolase and asparaginase activities
C5orf27	Chromosome 5 open reading frame 27	Definite NASH vs Not NAFLD: FC = -2.549 Definite NASH vs Borderline NASH: FC = -2.11	Uncharacterized
CD5L	CD5 molecule-like	Definite NASH vs Borderline NASH: FC = -1.92	Apoptosis inhibitor
CFHR1	Complement factor H-related 1	Definite NASH vs Borderline NASH: FC = -2.09	Complement regulation and lipid metabolism
CFHR2	Complement factor H-related 2	Definite NASH vs Borderline NASH: FC = -1.95	Complement regulation and lipid metabolism
CFHR3	Complement factor H-related 3	Borderline NASH vs Not NAFLD: FC = 2.21	Complement regulation
CHI3L1	Chitinase 3-like 1 (cartilage glycoprotein-39)	Borderline NASH vs Not NAFLD: FC = 2.145 Definite NASH vs Not NAFLD: FC = 2.26	Pathogen defense and tissue remodeling

CLGN	Calmegin	Definite NASH vs Borderline NASH: FC = -1.95	Unfolded protein binding and calcium ion binding
COL1A1	Collagen, type I, alpha 1	Definite NASH vs Not NAFLD: FC = 2.80 Definite NASH vs Borderline NASH: FC = 3.20	Platelet-derived growth factor binding and identical protein binding
COL1A2	Collagen, type I, alpha 2	Definite NASH vs Not NAFLD: FC = 2.645 Definite NASH vs Borderline NASH: FC = 2.64	Protein binding, bridging, and identical protein binding
CYP7A1	Cytochrome P450, family 7, subfamily A, polypeptide 1	Borderline NASH vs Not NAFLD: FC = 2.50 Definite NASH vs Not NAFLD: FC = 3.13	Cholesterol catabolism and bile acid biosynthesis
DUSP1	Dual specificity phosphatase 1	Definite NASH vs Not NAFLD: FC = -2.04 Definite NASH vs Borderline NASH: FC = -1.94	Dephosphorylates MAP kinase, regulating activity during meiosis
FABP5	Fatty acid binding protein 5	Definite NASH vs Not NAFLD: FC = 2.85 Definite NASH vs Borderline NASH: FC = 2.63	Fatty acid uptake, transport, and metabolism
GPAM	Glycerol-3-phosphate acyltransferase, mitochondrial	Borderline NASH vs Not NAFLD: FC = 1.79 Definite NASH vs Borderline NASH: FC = -2.55	Glycerolipid biosynthesis
GPNMB	Glycoprotein (transmembrane) nmb	Definite NASH vs Not NAFLD: FC = 2.56 Definite NASH vs Borderline NASH: FC = 1.87	Growth delay and reduction of metastatic potential
GSTA2	Glutathione S-transferase alpha 2	Borderline NASH vs Not NAFLD: FC = 1.734 Definite NASH vs Borderline NASH: FC = -1.95	Detoxification of electrophilic compounds by glutathione conjugation
GSTM1	Glutathione S-transferase mu 1	Borderline NASH vs Not NAFLD: FC = -2.30 Definite NASH vs Not NAFLD: FC = -4.17	Detoxification of electrophilic compounds by glutathione conjugation

GSTT1	Glutathione S-transferase theta 1	Borderline NASH vs Not NAFLD: FC = 1.92	Detoxification of electrophilic compounds by glutathione conjugation
H19	H19, imprinted maternally expressed transcript	Definite NASH vs Not NAFLD: FC = -2.17	Tumor suppressor
HGFAC	HGF activator	Borderline NASH vs Not NAFLD: FC = -1.70	Conversion of hepatocyte growth factor to the active form
HLA-DQA1	Major histocompatibility complex, class II, DQ alpha 1	Borderline NASH vs Not NAFLD: FC = -1.64	Presents peptides derived from extracellular proteins for immune system function
HSD17B14	Hydroxysteroid (17-beta) dehydrogenase 14	Definite NASH vs Borderline NASH: FC = 2.14	Steroid, fatty acid, prostaglandin, and xenobiotic metabolism
IGJ	Immunoglobulin J polypeptide	Definite NASH vs Not NAFLD: FC = 2.10	Antigen binding
LIPC	Lipase, hepatic	Definite NASH vs Borderline NASH: FC = -2.01	Triglyceride hydrolase, receptor-mediated lipoprotein uptake
LMF2	Lipase maturation factor 2	Borderline NASH vs Not NAFLD: FC = -1.53	Maturation of endoplasmic reticulum proteins
LOC100293539	NA	Borderline NASH vs Not NAFLD: FC = 6.60 Definite NASH vs Not NAFLD: FC = 7.36	Uncharacterized
LPL	Lipoprotein lipase	Definite NASH vs Borderline NASH: FC = 2.10	Receptor-mediated lipoprotein uptake
LUM	Lumican	Definite NASH vs Not NAFLD: FC = 2.36	Extracellular matrix structural constituent and collagen binding

MALAT1	Metastasis associated lung adenocarcinoma transcript 1	Borderline NASH vs Not NAFLD: FC = 1.81	Cell motility regulation
MARCO	Macrophage receptor with collagenous structure	Definite NASH vs Borderline NASH: FC = -2.06	Pattern recognition receptor that binds Gram-positive and Gram-negative bacteria
MLIP	Muscle-enriched A-type laminin interacting protein	Borderline NASH vs Not NAFLD: FC = 1.97	Uncharacterized
MPLKIP	M-phase specific PLK1 interacting protein	Borderline NASH vs Not NAFLD: FC = 1.78	Mitosis and cytokinesis regulator
MT1E	Metallothionein 1E	Definite NASH vs Not NAFLD: FC = -1.79	Heavy metal binding
MT1M	Metallothionein 1M	Definite NASH vs Not NAFLD: FC = -2.16	Heavy metal binding
NR0B2	Nuclear receptor subfamily 0, group B, member 2	Borderline NASH vs Not NAFLD: FC = -2.32 Definite NASH vs Not NAFLD: FC = -3.23	Negative regulator of receptor-dependent signaling pathways
NRG1	Neuregulin 1	Definite NASH vs Borderline NASH: FC = 2.05	Direct ligand for ERBB3 and ERBB4 tyrosine kinase receptors
PLA2G7	Phospholipase A2, group VII (platelet-activating factor acetylhydrolase, plasma)	Definite NASH vs Not NAFLD: FC = 2.41 Definite NASH vs Borderline NASH: FC = 2.11	Diverse roles in inflammation, cell growth, signaling, and death, and maintenance of membrane phospholipids
PZP	Pregnancy-zone protein	Borderline NASH vs Not NAFLD: FC = -1.58 Definite NASH vs Not NAFLD: FC = -3.88 Definite NASH vs Borderline NASH: FC = -3.52	Endopeptidase inihibitor
RFC1	Replication factor C	Borderline NASH vs Not NAFLD: FC = 1.94	DNA replication and repair

	(activator 1) 1, 145kDa		
RMRP	RNA component of mitochondrial RNA processing endoribonuclease	Borderline NASH vs Not NAFLD: FC = -2.32 Definite NASH vs Not NAFLD: FC = -1.71	Mitochondrial DNA repair
RN7SL1	RNA, 7SL, cytoplasmic 1	Borderline NASH vs Not NAFLD: FC = -1.71	Mediation of cotranslational insertion of secretory proteins into the lumen of the endoplasmic reticulum
RPPH1	Ribonuclease P RNA component H1	Borderline NASH vs Not NAFLD: FC = -3.34 Definite NASH vs Not NAFLD: FC = -2.33	tRNA maturation
RPS26P11	Ribosomal protein S26 pseudogene 11	Borderline NASH vs Not NAFLD: FC = 1.73	Uncharacterized
SCARNA10	Small Cajal body-specific RNA 10	Borderline NASH vs Not NAFLD: FC = -2.23 Definite NASH vs Not NAFLD: FC = -2.10	RNA processing
SCARNA5	Small Cajal body-specific RNA 5	Borderline NASH vs Not NAFLD: FC = -1.56	RNA processing
SLC12A8	Solute carrier family 12, member 8	Definite NASH vs Not NAFLD: FC = -1.95	Cation/chloride cotransporter
SLC1A2	Solute carrier family 1, member 2	Definite NASH vs Not NAFLD: FC = 2.458 Definite NASH vs Borderline NASH: FC = 2.48	Sodium- and potassium-dependent glutamate transporter
SLC2A9	Solute carrier family 2, member 9	Definite NASH vs Borderline NASH: FC = 1.92	Urate and fructose transporter
SLCO4C1	Solute carrier organic anion transporter family,	Definite NASH vs Not NAFLD: FC = -1.713 Definite NASH vs Borderline NASH: FC = -2.03	Membrane transport of bile acids, conjugatd steroids,

	member 4C1		eicosanoids, peptides, and drugs
SPARCL1	SPARC-like 1 (hevin)	Definite NASH vs Borderline NASH: FC = -2.13	Calcium ion binding
SPP1	Secreted phosphoprotein 1	Borderline NASH vs Not NAFLD: FC = 1.91 Definite NASH vs Not NAFLD: FC = 5.70 Definite NASH vs Borderline NASH: FC = 4.61	Upregulation of interferon-gamma and interleukin-12
THBS2	Thrombospondin 2	Definite NASH vs Borderline NASH: FC = 1.88	Tumor growth and angiogenesis inhibition
TIMP1	TIMP metallopeptidase inhibitor 1	Definite NASH vs Not NAFLD: FC = 2.08 Definite NASH vs Borderline NASH: FC = 2.03	Matrix metalloproteinase inhibitor
TM4SF19	Transmembrane 4 L six family member 19	Definite NASH vs Not NAFLD: FC = 2.11 Definite NASH vs Borderline NASH: FC = 2.14	Uncharacterized
TSKU	Tsukushi small leucine rich proteoglycan homolog	Borderline NASH vs Not NAFLD: FC = -1.56	Signaling molecule inhibition
VIL1	Villin 1	Borderline NASH vs Not NAFLD: FC = -1.59 Definite NASH vs Not NAFLD: FC = -1.79	Brush border cytoskeleton component that functions in capping, severing, and bundling of actin filaments

Supplemental Table 5: Pathways and biological processes enriched in definite NASH relative to borderline NASH

Biological Process	Direction	Genes
Extracellular matrix organization (N=17)	Up	TGFB1,*COL3A1*,*COL4A1*,*COL6A3*,MFAP4,THBS1,*EFEMP1*,TIMP1,SPP1,ANXA2,*VCAN*,*LUM*,MMP9,CTSK,CASK,*COL1A1*,*COL1A2*
Cell Adhesion (N=22)	Up	TGFB1,*COL3A1*,S100A10,NRG1,*COL6A3*,*ITGBL1*,MFAP4,THBS1,CLDN11,*THBS2*,FAT1,THY1,MGP,AEBP1,SPP1,*VCAN*,*CD24*,*ANTXR1*,ROBO1,GPNMB,CASK,*COL1A1*
Protein targeting to membrane (N=9)	Down	RPL23A,RPL26,RPLP0,RPS3A,PEX3,SPCS2,RPS27A,RTP4,RPL7
Glutathione Transferase Activity	Down	GSTM2,GSTM4
Antibacterial humoral response (N=3)	Up	IGHA2,IGHM,IGJ
Carbohydrate transport (N=5)	Up	POM121,SLC2A9,SLC23A2,SLC1A2,FABP5
Lipid metabolic process (N=14)	Down	AADAC,ANG,ASAH2,G0S2,ALDH8A1,GPAM,FABP1,CROT,LIPC,MSMO1,ACOT11,PLD1,FGL1,NR0B2
Defense response (N=12)	Down	CASP1,CASP4,GPAM,RPS27A,KLRC4,CD163,CD5L,PLD1,MARCO,IL33,VCAM1,HIST1H2BK
Xenobiotic metabolic process (N=3)	Down	GSTA2,GSTM1,UGP2