SUPPLEMENTAL MATERIAL

Inclusion criteria and exclusion criteria of the *derivation* biopsy-proven NAFLD cohort

Participants were included in the study if they were 18 years or older with suspected NAFLD and were willing and able to provide informed consent.

Participants were excluded if they met any of the following criteria: history of regular and excessive alcohol consumption within 2 years of recruitment (≥14 drinks/week for men or ≥7 drinks/week for women); use of hepatotoxic drugs or drugs known to cause hepatic steatosis; any evidence of secondary causes of hepatic steatosis, including viral hepatitis (detected with positive serum hepatitis B surface antigen or hepatitis C viral RNA), Wilson's disease, hemochromatosis, alpha-1 antitrypsin deficiency, autoimmune hepatitis, and cholestatic or vascular liver disease; evidence of decompensated liver disease (defined as Child-Pugh score > 7 points); clinical or laboratory evidence of chronic illnesses associated with hepatic steatosis, including human immunodeficiency virus infection (HIV), type 1 diabetes mellitus, celiac disease, cystic fibrosis, lipodystrophy, dysbetalipoproteinemia, and glycogen storage diseases; evidence of active substance abuse, significant systemic illnesses; contraindication(s) to MRI, pregnant or trying to become pregnant; or any other condition which, in the investigator's opinion, may affect the patient's competence or compliance in completing the study.

Clinical assessments and laboratory test

All participants underwent a standardized clinical research visit at the UCSD NAFLD Research Center. A detailed history was obtained from all participants. A physical exam, which included vital signs, height, weight, and anthropometric measurements, was performed by a trained clinical investigator. Body mass index was defined as the body weight (in kilograms) divided by height (in meters) squared. Alcohol consumption was documented outside clinical visits and confirmed in the research clinic using the Alcohol Use Disorders Identifications Test and the Skinner questionnaire. A detailed history of medications was

obtained and no patient took medications known or suspected to cause steatosis or steatohepatitis. Other causes of liver disease and secondary causes of hepatic steatosis were systemically ruled out using detailed history and laboratory data. After completion of the earlier described elements of the history and physical examination, participants had the following fasting laboratory work: complete blood count, screening etiologic tests (hepatitis B, surface antigen, hepatitis C antibody, and iron panel including serum ferritin), clinical chemistry (creatinine, total protein, blood urea nitrogen, uric acid), hepatic panel (total bilirubin, direct bilirubin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, g-glutamyltransferase, albumin, prothrombin time, and international normalized ratio), lipid profile, hemoglobin A1c, and glucose-insulin levels.

Inclusion and exclusion criteria of the *validation* Twin and Family Cohort

Patients were included if they were twins, siblings or parent-offspring at least 18 years old, willing and able to complete all research procedures and observations. For each twin pair, a detailed assessment of twinship status (ie, monozygotic (MZ) or dizygotic (DZ)) was obtained. The majority of twin-pairs (34) were diagnosed by their physician as either MZ or DZ by genetic testing. Furthermore, twin-ship status was confirmed by using a previously published questionnaire[1].

Participants were excluded from the study if they met any of the following criteria: significant alcohol intake (>10 g/day in females or >20 g/day in males) for at least 3 consecutive months over the previous 12 months or if the quantity of alcohol consumed could not be reliably ascertained; clinical or biochemical evidence of liver diseases other than NAFLD (eg, viral hepatitis, HIV, coeliac disease, cystic fibrosis, autoimmune hepatitis); metabolic and/or genetic liver disease (eg, Wilson's disease, haemochromatosis, polycystic liver disease, alpha-1-antitrypsin deficiency, dysbetalipoproteinaemia); clinical or laboratory evidence of systemic infection or any other clinical evidence of liver disease associated with hepatic steatosis; use of drugs known to cause hepatic steatosis (eg amiodarone, glucocorticoids, methotrexate,

L-asparaginase and valproic acid) for at least 3 months in the last past 6 months; history of bariatric surgery; presence of systemic infectious illnesses; females who were pregnant or nursing at the time of the study; contraindications to MRI (eg metal implants, severe claustrophobia, body circumference greater than the imaging chamber); any other condition(s) which, based on the principal investigator's opinion, may significantly affect the participant's compliance, competence, or ability to complete the study.

Magnetic Resonance Imaging protocols

Proton density fat fraction (PDFF)

The MRI proton-density fat fraction protocol has been previously described and validated as a standardised and objective measure of liver fat content.[2, 3] Patients were scanned in a supine position using a 3T MR scanner (SIGNA Excite HDxt; GE Medical Systems, Milwaukee, WI, USA), with an 8-channel torso-phased array surface coil centred over the liver. Images were obtained once at baseline, and again at post-treatment. Noncontrast axial-magnitude MR images were obtained of the whole liver using a 2-dimensional spoiled gradient-recalled-echo sequence. A low flip-angle (10°) was used at a repetition time of more than 100 ms to minimise T1 effects. Six fractional echo magnitude images were obtained at serial opposed-phase and in-phase echo times 1.15, 2.3, 3.45, 4.6, 5.75 and 6.9 ms in a single breathhold (12–24 s). Other imaging parameters included: 8–10-mm slice thickness, 14–26 slices covering the whole liver, 0-mm-slice gaps, 192 × 192 base matrix, 1 signal average and rectangular field of view adjusted to the body habitus and breath-hold capacity.

By using a custom open-source software plug-in for Osirix (Pixmeo Co., Geneva, Switzerland) that corrects for exponential T2* decay and that incorporates a multipeak fat spectral model, MRI proton-density fat fraction parametric maps were reconstructed offline from the source MR images. Circular regions of

interest with a 1-cm radius were placed in each of the four right liver lobe segments (segments 5–8) on the proton-density fat fraction maps. Proton-density fat fraction values were recorded for each region of interest/segment, and a final right-lobe MRI proton-density fat fraction value for each participant was obtained by averaging the values of the four corresponding regions of interest.

Untargeted Metabolome profiling

Sample Preparation: Samples were prepared using the automated MicroLab STAR® system from Hamilton Company. Recovery standards were added prior to the first step in the extraction process for QC purposes. Sample preparation was conducted using a methanol extraction to remove the protein fraction while allowing maximum recovery of small molecules. Samples were placed briefly on a TurboVap® (Zymark) under nitrogen to remove the organic solvent. For LC, the samples were stored under nitrogen overnight.

QA/QC: Several types of controls were analyzed in concert with the experimental samples: a pooled matrix sample generated by taking a small volume of each experimental sample (or alternatively, use of a pool of well-characterized human plasma) served as a technical replicate throughout the data set; extracted water samples served as process blanks; and a cocktail of QC standards that were carefully chosen not to interfere with the measurement of endogenous compounds were spiked into every analyzed sample, allowed instrument performance monitoring and aided chromatographic alignment. Instrument variability was determined by calculating the median relative standard deviation (RSD) for the standards that were added to each sample prior to injection into the mass spectrometers. Overall process variability was determined by calculating the median RSD for all endogenous metabolites (i.e., non-instrument standards) present in 100% of the pooled matrix samples. Experimental samples were randomized across the platform run with QC samples spaced evenly among the injections.

Ultrahigh Performance Liquid Chromatography-Tandem Mass Spectroscopy (UPLC-MS/MS): The LC/MS portion of the platform was based on a Waters ACQUITY ultra-performance liquid chromatography (UPLC) and a Thermo Scientific Q-Exactive high resolution/accurate mass spectrometer interfaced with a heated electrospray ionization (HESI-II) source and Orbitrap mass analyzer operated at 35,000 mass resolution. The sample extract was dried then reconstituted in acidic or basic LC-compatible solvents, each of which contained 8 or more injection standards at fixed concentrations to ensure injection and chromatographic consistency. One aliquot was analyzed using acidic positive ion optimized conditions and the other using basic negative ion optimized conditions in two independent injections using separate dedicated columns (Waters UPLC BEH C18-2.1x100 mm, 1.7 µm). Extracts reconstituted in acidic conditions were gradient eluted from a C18 column using water and methanol containing 0.1% formic acid. The basic extracts were similarly eluted from C18 using methanol and water, however with 6.5mM Ammonium Bicarbonate. The third aliquot was analyzed via negative ionization following elution from a HILIC column (Waters UPLC BEH Amide 2.1x150 mm, 1.7 µm) using a gradient consisting of water and acetonitrile with 10mM Ammonium Formate. The MS analysis alternated between MS and data-dependent MS2 scans using dynamic exclusion, and the scan range was from 80-1000 m/z. Raw data files are archived and extracted as described below.

Bioinformatics: The informatics system consisted of four major components, the Laboratory Information Management System (LIMS), the data extraction and peak-identification software, data processing tools for QC and compound identification, and a collection of information interpretation and visualization tools for use by data analysts. The hardware and software foundations for these informatics components were the LAN backbone, and a database server running Oracle 10.2.0.1 Enterprise Edition.

Supplementary References

1. Boyd NF, Dite GS, Stone J, et al. Heritability of mammographic density, a risk factor for breast cancer. N Engl J Med 2002;347:886-94.

2. Noureddin M, Lam J, Peterson MR, et al. Utility of magnetic resonance imaging versus histology for quantifying changes in liver fat in nonalcoholic fatty liver disease trials. Hepatology 2013;58:1930-40.
3. Reeder SB. Emerging quantitative magnetic resonance imaging biomarkers of hepatic steatosis. Hepatology 2013;58:1877-80.

Supplemental Table 1 STARD Checklist

Section & Topic	No	Item	Reported on page #
TITLE OR ABSTRACT			
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	3
ABSTRACT			
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)	3
INTRODUCTION			
	3	Scientific and clinical background, including the intended use and clinical role of the index test	5
	4	Study objectives and hypotheses	6
METHODS			
Study design	**5**	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	6
Participants	**6**	Eligibility criteria	6-7
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	6-7
	8	Where and when potentially eligible participants were identified (setting, location and dates)	6-7
	9	Whether participants formed a consecutive, random or convenience series	6-7
Test methods	**10a**	Index test, in sufficient detail to allow replication	8&
	10b	Reference standard, in sufficient detail to allow replication	8 and supplemental material
	11	Rationale for choosing the reference standard (if alternatives exist)	5
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory	8
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory	10
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test	NA
	13b	Whether clinical information and index test results were available to the assessors of the reference standard	NA
Analysis	**14**	Methods for estimating or comparing measures of diagnostic accuracy	10
	15	How indeterminate index test or reference standard results were handled	NA
	16	How missing data on the index test and reference standard were handled	9
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	13
	18	Intended sample size and how it was determined	10
RESULTS			
Participants	**19**	Flow of participants, using a diagram	Supplemental Fig 1
	20	Baseline demographic and clinical characteristics of participants	11 and Table 1
	21a	Distribution of severity of disease in those with the target condition	Table 1
	21b	Distribution of alternative diagnoses in those without the target condition	Table 3

	22	Time interval and any clinical interventions between index test and reference standard	
Test results	**23**	Cross tabulation of the index test results (or their distribution) by the results of the reference standard	Sup fig 1
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	Table 2
	25	Any adverse events from performing the index test or the reference standard	NA
DISCUSSION			
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	16
	27	Implications for practice, including the intended use and clinical role of the index test	13
OTHER INFORMATION			
	28	Registration number and name of registry	6-7
	29	Where the full study protocol can be accessed	6-7
	30	Sources of funding and other support; role of funders	1

Supplement Table 2. Serum metabolites significantly associated with advanced fibrosis

Metabolites	Super pathway	Sub pathway	Fold change AF/no AF	p- value	q-value
3β-diol monosulfate	Lipid	Steroid	0.38	**< 0.001**	**0.008**
fucose	Carbohydrate	Pentose Metabolism	1.56	**< 0.001**	**0.008**
pregnanediol-3-glucuronide	Lipid	Steroid	0.25	**< 0.001**	**0.009**
androsterone sulfate	Lipid	Steroid	0.40	**< 0.001**	**0.009**
epiandrosterone sulfate	Lipid	Steroid	0.39	**< 0.001**	**0.009**
palmitoleate (16:1n7)	Lipid	Long Chain Fatty Acid	1.61	**< 0.001**	**0.009**
DHEA-S	Lipid	Steroid	0.40	**< 0.001**	**0.012**
taurine	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism	0.77	**< 0.001**	**0.012**
3β-diol disulfate	Lipid	Steroid	0.46	**< 0.001**	**0.013**
glycocholate	Lipid	Primary Bile Acid Metabolism	3.83	**< 0.001**	**0.013**
glycochenodeoxycholate	Lipid	Primary Bile Acid Metabolism	4.07	**< 0.001**	**0.013**
5alpha-pregnan-3beta,20alpha-diol monosulfate (2)	Lipid	Steroid	0.14	**< 0.001**	**0.016**
etiocholanolone glucuronide	Lipid	Steroid	0.42	**< 0.001**	**0.017**
4-androsten-3beta,17beta-diol monosulfate (1)	Lipid	Steroid	0.39	**< 0.001**	**0.022**
7-HOCA	Lipid	Sterol	1.42	**0.001**	**0.025**
myristoleate (14:1n5)	Lipid	Long Chain Fatty Acid	1.68	**0.001**	**0.025**
glycyltryptophan	Peptide	Dipeptide	0.56	**0.001**	**0.025**
xanthurenate	Amino Acid	Tryptophan Metabolism	0.39	**0.001**	**0.025**

taurocholate	Lipid	Primary Bile Acid Metabolism	7.27	**0.001**	**0.025**
alpha-hydroxyisovalerate	Amino Acid	Leucine, Isoleucine and Valine Metabolism	1.42	**0.001**	**0.025**
4-androsten-3alpha,17alpha-diol monosulfate (3)	Lipid	Steroid	0.51	**0.001**	**0.025**
N-palmitoyl glycine	Lipid	Fatty Acid Metabolism(Acyl Glycine)	1.44	**0.001**	**0.025**
5alpha-androstan-3beta,17alpha-diol disulfate	Lipid	Steroid	0.67	**0.001**	**0.025**
ADSGEGDFXAEGGGVR*	Peptide	Fibrinogen Cleavage Peptide	1.44	**0.001**	**0.026**
5,6-dihydrothymine	Nucleotide	Pyrimidine Metabolism, Thymine containing	1.53	**0.001**	**0.026**
16-hydroxypalmitate	Lipid	Fatty Acid, Monohydroxy	1.31	**0.001**	**0.030**
tryptophylleucine	Peptide	Dipeptide	0.46	**0.001**	**0.034**
10-heptadecenoate (17:1n7)	Lipid	Long Chain Fatty Acid	1.36	**0.002**	**0.042**
serine	Amino Acid	Glycine, Serine and Threonine Metabolism	1.17	**0.002**	**0.042**
pyridoxate	Cofactors and Vitamins	Vitamin B6 Metabolism	0.36	**0.002**	**0.043**
phenylalanylvaline	Peptide	Dipeptide	0.37	**0.002**	**0.043**
gamma-glutamylleucine	Peptide	Gamma-glutamyl Amino Acid	0.81	**0.002**	**0.0458**

Abbreviation: DHEA-S: dehydroisoandrosterone sulfate; 3β-diol: 5alpha-androstan-3beta,17beta-diol; AF: advanced fibrosis (stage 3-4).

Supplemental Table 3 Serum metabolites significantly associated with NASH

Metabolites	Super pathway	Sub pathway	Fold change NASH/no NASH	p- value	q-value
isoleucylglycine	Peptide	Dipeptide	0.50	**<0.001**	**<0.001**
hypoxanthine	Nucleotide	Purine Metabolism, (Hypo)Xanthine/Inosine containing	0.79	**<0.001**	**0.001**
isoleucylvaline	Peptide	Dipeptide	0.69	**<0.001**	**0.001**
N-acetylmethionine	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism	0.77	**<0.001**	**0.001**
caprate (10:0)	Lipid	Medium Chain Fatty Acid	1.29	**<0.001**	**0.007**
leucylglycine	Peptide	Dipeptide	0.65	**<0.001**	**0.008**
3-hydroxybutyrate (BHBA)	Lipid	Ketone Bodies	1.98	**<0.001**	**0.010**
hydroxybutyrylcarnitine*	Lipid	Fatty Acid Metabolism(Acyl Carnitine)	1.62	**<0.001**	**0.012**
3-hydroxyisobutyrate	Amino Acid	Leucine, Isoleucine and Valine Metabolism	1.32	**<0.001**	**0.012**
threonylphenylalanine	Peptide	Dipeptide	0.66	**<0.001**	**0.012**
valylglycine	Peptide	Dipeptide	0.51	**<0.001**	**0.012**
scyllo-inositol	Lipid	Inositol Metabolism	0.76	**<0.001**	**0.012**
2'-deoxyuridine	Nucleotide	Pyrimidine Metabolism, Uracil containing	0.87	**<0.001**	**0.016**
16-hydroxypalmitate	Lipid	Fatty Acid, Monohydroxy	1.25	**<0.001**	**0.017**
taurine	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism	0.84	**<0.001**	**0.021**
2-oleoyl-GPC* (18:1)*	Lipid	Lysolipid	0.81	**<0.001**	**0.021**
TL16:0 (palmitic acid)	Lipid	Total Fatty Acid	1.06	**<0.001**	**0.021**
leucylleucine	Peptide	Dipeptide	0.70	**<0.001**	**0.024**

carnitine	Lipid	Carnitine Metabolism	0.90	**<0.001**	**0.024**
tryptophylleucine	Peptide	Dipeptide	0.67	**<0.001**	**0.025**
gamma-glutamylhistidine	Peptide	Gamma-glutamyl Amino Acid	1.78	**<0.001**	**0.025**
alpha-hydroxyisovalerate	Amino Acid	Leucine, Isoleucine and Valine Metabolism	1.30	**<0.001**	**0.025**
cyclo(L-phe-L-pro)	Peptide	Dipeptide	2.32	**<0.001**	**0.028**
valylleucine	Peptide	Dipeptide	0.71	**<0.001**	**0.034**
inosine	Nucleotide	Purine Metabolism, (Hypo)Xanthine/Inosine containing	0.62	**<0.001**	**0.043**
caprylate (8:0)	Lipid	Medium Chain Fatty Acid	1.23	**<0.001**	**0.043**
malonate (propanedioate)	Lipid	Fatty Acid Synthesis	1.23	**<0.001**	**0.043**
docosatrienoate (22:3n3)	Lipid	Polyunsaturated Fatty Acid (n3 and n6)	1.35	**<0.001**	**0.044**
5alpha-pregnan-3beta,20alpha-diol monosulfate (2)	Lipid	Steroid	0.14	**<0.001**	**0.044**
guanosine	Nucleotide	Purine Metabolism, Guanine containing	0.56	**<0.001**	**0.045**
5-methyluridine (ribothymidine)	Nucleotide	Pyrimidine Metabolism, Uracil containing	0.92	**<0.001**	**0.045**

Supplemental Table 4. Baseline characteristics of the validation biopsy-proven NAFLD Cohort

Characteristics	All (n=59)	No Advanced Fibrosis (stage 0-2) (n=44)	Advanced Fibrosis (stage 3-4) (n=15)	p-value*
Demographics				
Age, years	56.5 ± 9.4	55.7 ± 9.4	60.4 ± 9.7	**0.023**
Male, n (%)	18 (30.5)	15 (34.1)	3 (20.0)	0.343
White, n (%)	56 (94.9)	41 (93.2)	15 (100.0)	0.564
African American, n (%)	3 (5.1)	3 (6.8)	0 (0.0)	0.564
BMI, kg/m^2	33.6 ± 4.8	33.4 ± 5.2	32.6 ± 5.9	0.883
Clinical				
Type 2 Diabetes, n (%)	28 (47.5)	18 (40.9)	10 (66.7)	0.134
Biological data				
AST (U/L)	43.9 ± 36.5	36.2 ± 16.9	43.7 ± 16.1	0.918
ALT (U/L)	52.5 ± 28.2	52.0 ± 26.3	54.3 ± 24.5	0.874
Alk P (U/L)	81.5 ± 27.6	80.6 ± 35.2	92.0 ± 17.9	0.298
Total Bilirubin (mg/dL)	0.69 ± 0.42	0.66 ± 0.43	0.94 ± 0.56	0.173
Albumin (g/dL)	4.50± 0.32	4.50 ± 0.36	4.44 ± 0.32	0.594
Glucose (mg/dl)	119.3 ± 47.3	116.6 ± 45.1	157.1 ± 71.5	**0.019**
Hemoglobin A1c	6.4 ± 1.5	6.2 ± 1.1	7.4 ± 2.6	0.085
HOMA-IR	8.0 ± 5.9	7.2 ± 5.2	11.1 ± 7.5	0.096
Insulin (U/ml)	24.4± 13.7	23.9 ± 13.5	28.3 ± 17.4	0.395
Triglycerides (mg/dL)	162.6 ± 107.9	168.6 ± 127.1	148.3 ± 51.3	0.714
Total cholesterol (mg/dL)	184.6 ± 37.2	185.4 ± 36.8	168.4 ± 46.7	0.331
HDL-cholesterol (mg/dL)	48.2± 12.6	50.2 ± 13.0	42.6 ± 10.4	0.397
LDL-cholesterol (mg/dL)	119.2 ± 55.8	124.1 ± 65.6	107.0 ± 53.6	0.435
Platelet count ($10^3/\mu L$)	255.4 ± 67.7	271.8 ± 60.6	171.7 ± 45.9	0.087
Clinical Prediction Rules				
FIB-4	1.56 ± 1.97	1.13 ± 0.87	2.65 ± 1.97	0.616
NAFLD Fibrosis Score	-1.14 ± 1.58	-2.160± 1.437	-0.032 ± 1.755	**0.031**
Histology				
Fibrosis n (%)				**<0.001**
0	16 (27.1)	16 (36.4)	0 (0.0)	

1	19 (32.2)	19 (43.2)	0 (0.0)	
2	9 (15.3)	9 (20.5)	0 (0.0)	
3	13 (22.0)	0 (0.0)	13 (86.7)	
4	2 (3.4)	0 (0.0)	2 (13.3)	
Steatosis n (%)				0.484
0	4 (6.8)	3 (6.8)	1 (7.7)	
1	21(35.6)	18 (40.9)	3 (23.1)	
2	17 (28.8)	11 (25.0)	6 (46.2)	
3	15 (25.4)	12 (27.3)	3 (23.1)	
Lobular inflammation n (%)				0.983
0	5 (8.5)	4 (9.1)	1 (9.1)	
1	41 (69.5)	33 (75.0)	8 (72.7)	
2	9 (15.3)	7 (15.9)	2 (18.2)	
3	0 (0.0)	0 (0.0)	0 (0.0)	
Ballooning n (%)				**0.024**
0	20 (33.9)	19 (45.2)	1 (7.7)	
1	27 (45.8)	19 (45.2)	8 (61.5)	
2	8 (13.6)	4 (9.5)	4 (9.5)	
3	0 (0.0)	0 (0.0)	0 (0.0)	
NASH, n (%)				**0.005**
NAFLD, no NASH	23 (39.0)	22 (50.0)	1 (6.7)	
NASH	36 (61.0)	22 (50.0)	14 (93.3)	
NAS, Median (IQR)	3 (1)	3 (2)	4 (2)	0.602

Mean values are provided ± standard deviation, unless otherwise noted as n (%) or median and interquartile range (IQR). BMI: body mass index, ALT: alanine aminotransferase, AST: aspartate aminotransferase, Alk P: Alkaline Phosphatase, GGT: Gamma-Glutamyl Transferase, HbA1c: glycated hemoglobin, HDL: High Density Lipoprotein, LDL: Low Density Lipoprotein, INR: International Normalized Ratio, APRI: AST to platelet ratio, HOMA: Homeostasis Model Assessment, NAS: NAFLD Activity Score,

*P-value determined by comparing characteristics of individuals with advanced fibrosis (fibrosis stage 3-4) and without advanced fibrosis (fibrosis stage 0-2) were evaluated using an independent samples t-test

or Wilcoxon-Mann-Whitney Test. Chi-square test or Fisher's Exact Test, when appropriate, was used to compare categorical variables. Bold indicates significant P values <0.05.

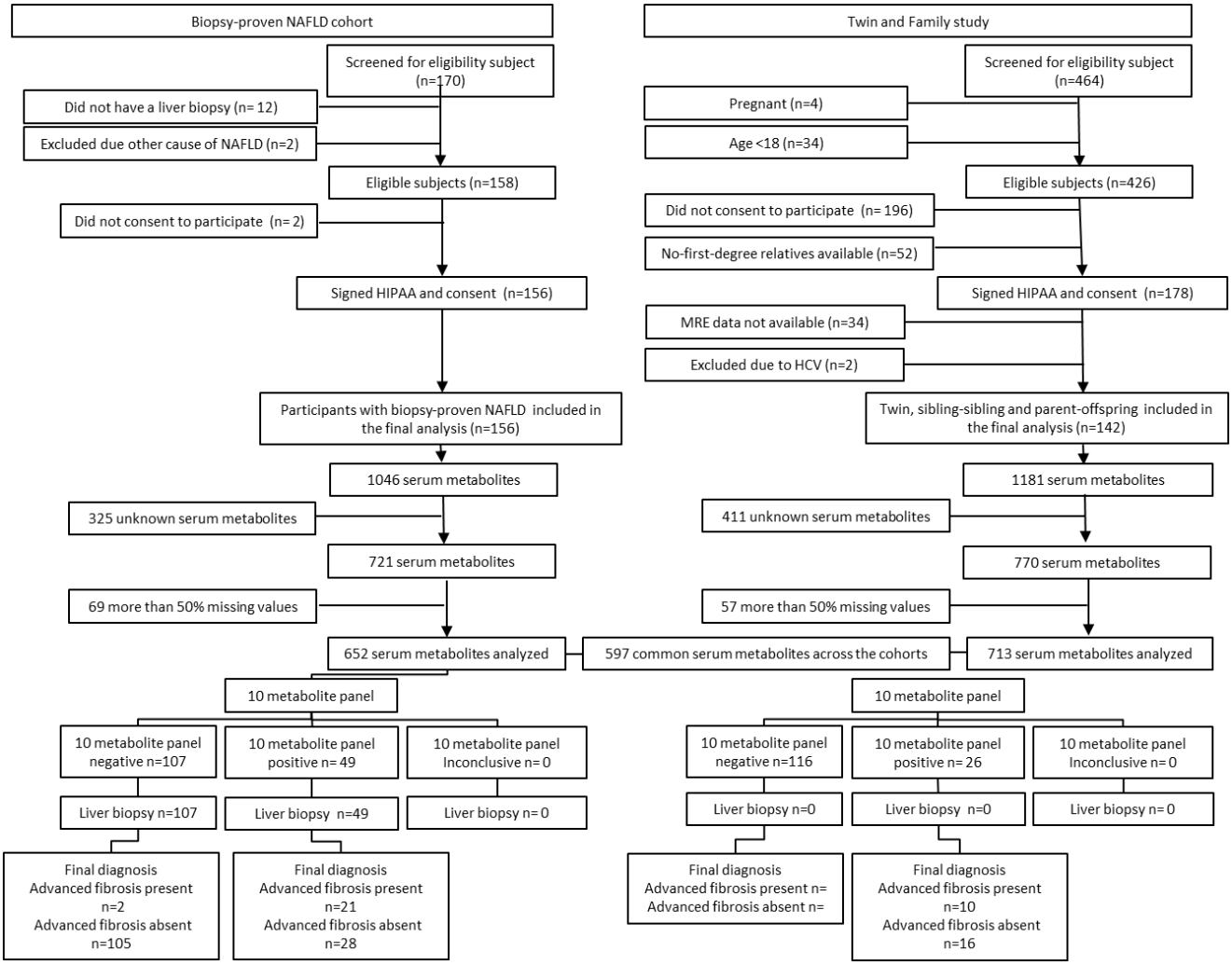
Supplemental Table 5 PNPLA3 genotype and serum metabolites level

	PNPLA3 CG or GG Mean (±SD) scaled intensity	**PNPLA3 CC Mean (±SD) scaled intensity**	**p-value***
Top 10 metabolites associated with advanced fibrosis			
3β-diol monosulfate	1.37 (1.16)	1.24 (1.03)	0.614
fucose	1.10 (0.50)	1.08 (0.73)	0.246
pregnanediol-3-glucuronide	2.60 (5.12)	2.68 (6.29)	0.992
androsterone sulfate	1.79 (1.55)	1.21 (0.81)	0.126
epiandrosterone sulfate	1.38 (1.05)	1.18 (0.83)	0.432
palmitoleate (16:1n7)	1.07 (0.76)	1.07 (0.54)	0.519
DHEA-S	1.36 (0.87)	1.29 (0.96)	0.483
taurine	0.97 (0.22)	0.99 (0.22)	0.614
3β-diol disulfate	1.29 (1.07)	1.28 (1.31)	0.893
glycocholate	1.75 (2.55)	1.83 (1.12)	0.264
Top 10 metabolites associated with NASH			
isoleucylglycine	1.13 (0.44)	1.07 (0.45)	0.392
hypoxanthine	1.03 (0.35)	1.07 (0.99)	0.766
isoleucylvaline	1.29 (1.52)	1.07 (0.42)	0.984
N-acetylmethionine	1.04 (0.23)	1.02 (0.20)	0.325
caprate (10:0)	1.11 (0.45)	1.19 (0.69)	0.725
leucylglycine	1.38 (1.54)	1.31 (1.24)	0.983
3-hydroxybutyrate (BHBA)	1.87 (2.50)	2.10 (2.07)	0.174
hydroxybutyrylcarnitine*	1.48 (1.50)	1.35 (0.94)	0.843
3-hydroxyisobutyrate	1.11 (0.49)	1.01 (0.38)	0.325
threonylphenylalanine	1.24 (1.00)	1.25 (0.83)	0.449

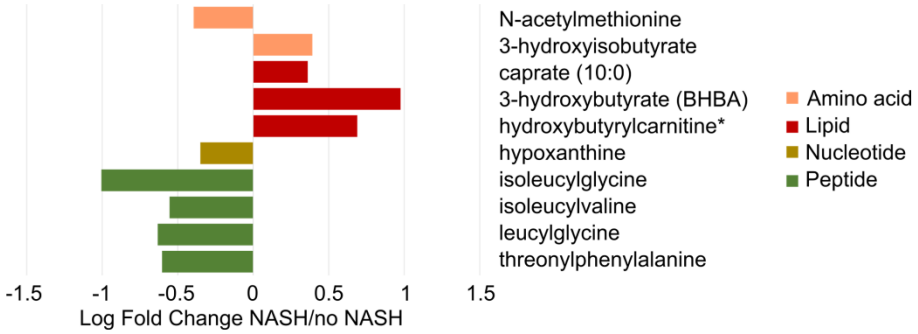
Serum metabolites levels are shown in scaled intensity obtained by re-scaling raw data to have median equal to 1. *p-value determined using Welsh t test.

Abbreviation: DHEA-S: dehydroisoandrosterone sulfate; 3β-diol: 5alpha-androstan-3beta, 17beta-diol;

Supplemental Figure 1 Study flow-chart



Supplemental Figure 2. Fold change of the top 10 serum metabolites associated with NASH



Supplemental Figure 3. Top 31 metabolites and 10 metabolites panel AUROC for the detection of NASH