Shared genetic effects between hepatic steatosis and fibrosis: A prospective twin study

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Abstract

Introduction—Nonalcoholic fatty liver disease (NAFLD) is associated with metabolic risk factors including hypertension and dyslipidemia, and may progress to liver fibrosis. Previous studies have shown that hepatic steatosis and fibrosis are heritable but whether they have a significant shared gene effect is unknown. This study aimed to examine the shared gene effects between hepatic steatosis, fibrosis, and their associations with metabolic risk factors.

Methods—This is a cross-sectional analysis of a prospective cohort of well-characterized, community-dwelling twins (45 monozygotic, 20 dizygotic twin pairs, 130 total subjects) from Southern California. Hepatic steatosis was assessed with MRI-proton density fat fraction (MRI-PDFF) and hepatic fibrosis was assessed with magnetic resonance elastography (MRE). A standard bivariate twin AE model was used to estimate the proportion of phenotypic variance between two phenotypes accounted for by additive genetic effects (A) and individual-specific environmental effects (E). Genetic correlations (rG) estimated from this model represent the degree to which the genetic determinants of two phenotypes overlap.

Results—The mean (±SD) age and BMI were 47.1 (±21.9) years and 26.9 (±6.5) kg/m^2, respectively. 20% (26/130) of the cohort had hepatic steatosis (MRI-PDFF ≥ 5%) and 8.2% (10/122) had hepatic fibrosis (MRE ≥ 3Kpa). Blood pressure (systolic and diastolic), triglycerides, glucose, homeostatic model assessment of insulin resistance (HOMA-IR), insulin, hemoglobin A1c (HbA1c), and low high-density lipoprotein (HDL) had significant shared gene effects with hepatic steatosis. Triglycerides, glucose, HOMA-IR, insulin, HbA1c, and low HDL had significant shared gene effects with hepatic fibrosis. Hepatic steatosis and fibrosis had a highly significant shared gene effect of 0.756 (95% CI: 0.716–1, p<0.0001).

Conclusions—Genes involved with steatosis pathogenesis may also be involved with fibrosis pathogenesis.

Keywords

NAFLD; steatosis; fibrosis; genetics; MRI

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) comprises of a spectrum of liver pathologies characterized by hepatic steatosis in patients with little to no history of alcohol use or secondary causes of hepatic steatosis.(1) Nonalcoholic steatohepatitis (NASH) is an advanced form of NAFLD and predisposes patients to the development of hepatic fibrosis, which is associated with increased risks of cirrhosis, mortality, and liver transplantation.(2–5) NAFLD, including its complications, is now a leading cause of liver disease in the United States and worldwide.(6–8) Due to the heavy disease burden of NAFLD and its associated morbidity and mortality, there is a great need to characterize the heritability of NAFLD to identify patients who may be at risk for the disease, improve the understanding of NAFLD pathogenesis, and identify potential targets for treatment.

Hepatic steatosis represents the initial step for the pathogenesis of NASH and hepatic fibrosis, and we have previously demonstrated in twin models that both hepatic steatosis and

fibrosis are heritable traits.(9) Various genes, including PNPLA-3 and TM6SF2, are associated with the development of hepatic steatosis and fibrosis, although variations in these genes do not account for all the variance seen in hepatic steatosis and fibrosis, and additional genes remain to be identified.(10–15) But while hepatic steatosis can progress to hepatic fibrosis, it is unknown if there are direct genetic links between these two traits. Hepatic fibrosis has been shown to be the most important predictor of mortality and liver transplantation in NAFLD patients,(5, 16) and an improved understanding of the shared heritability between hepatic steatosis and fibrosis may elucidate potential targets for NAFLD prevention and treatment. If steatosis and fibrosis gene regulation significantly overlaps then it is plausible that improvement in steatosis by common shared mechanistic pathway may eventually trigger improvement in fibrosis in the context of targeting specific nodal points in the mechanistic pathway. Additionally, previous studies have shown NAFLD to be associated with metabolic risk factors including obesity, hypertension, dyslipidemia, and insulin resistance,(17–20) and there is a genetic component to this association.(21) However, further studies are needed to characterize the genetic association between hepatic steatosis, fibrosis, and individual metabolic risk factors.

Utilizing a prospective study design of community-dwelling monozygotic and dizygotic twins, we aimed to evaluate if study participants with genetic susceptibilities to hepatic steatosis also have genetic susceptibilities to hepatic fibrosis. We also aimed to evaluate the genetic susceptibilities of hepatic steatosis and fibrosis with metabolic risk factors, including blood pressure, cholesterol levels, triglycerides, insulin resistance, and diabetes. Mathematical models involved additive genetics and unique environment effects (called AE models) were constructed for the cohort to distinguish between the shared genetic versus environmental determination of individual traits. Magnetic resonance imaging – proton density fat fraction (MRI-PDFF) and magnetic resonance elastography (MRE), two novel, accurate, and non-invasive imaging biomarkers, were respectively used to assess for hepatic steatosis and fibrosis in this prospective study.

METHODS

Experimental Design

This was a cross-sectional analysis of a prospectively recruited cohort of monozygotic and dizygotic twin pairs living in Southern California. All twin pairs underwent clinical research assessments, including medical history, physical and anthropometric exams, and biochemical testing, at the University of California at San Diego (UCSD) NAFLD Research Center.(15, 21–23) Participants also underwent MRI-PDFF for hepatic steatosis and MRE for hepatic fibrosis at the UCSD MR3T Research Laboratory. Clinical and imaging visits were performed on the same day for each twin pair, and the study took place from 2012 to 2015. All participants provided written informed consent before enrolling in the study. The study protocol was approved by the UCSD Institutional Review Board.

Inclusion Criteria

Participants were included in the study if they were twins at least 18 years old who provided written informed consent. The zygosity of the majority of twin pairs as monozygotic (MZ)

or dizygotic (DZ) had been previously confirmed via genetic testing before the participants enrolled in the study. A previously published questionnaire, as described by Boyd et al,(24) was used to further confirm twinship status (see Supplementary Text for details).

Exclusion Criteria

Participants were excluded from the study if they met any of the following criteria: (1) significant alcohol intake (>10 grams/day in females or >20 grams/day in males) for at least three consecutive months over the previous 12 months, or if the quantity of alcohol consumption could not be reliably ascertained; (2) clinical or biochemical evidence of liver diseases other than NAFLD, including hepatitis B, hepatitis C, alpha-1 antitrypsin deficiency, hemochromatosis, Wilson's disease, autoimmune hepatitis, polycystic liver diseases, cholestatic liver diseases, and vascular liver diseases; (3) chronic illnesses associated with hepatic steatosis, including human immunodeficiency virus infection, type I diabetes mellitus, celiac disease, cystic fibrosis, lipodystrophy, dysbetalipoproteinemia, and glycogen storage diseases; (4) use of drugs known to cause hepatic steatosis, including amiodarone, glucocorticoids, methotrexate, L-asparaginase, and valproic acid for at least three out of the previous six months; (5) history of bariatric surgery, including roux-en-Y gastric bypass and gastroplasty; (6) presence of systemic infectious illnesses; (7) females who were pregnant or nursing at the time of the study; (8) contraindications to MRI, including metal implants, claustrophobia, and body circumference greater than that of the imaging chamber; (9) 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.

Definition of NAFLD

Participants were considered to have NAFLD if they had hepatic steatosis (MRI-PDFF 5%) and no secondary causes of hepatic steatosis due to factors including the use of steatogenic medications, other liver diseases, and significant alcohol intake (see Exclusion Criteria above for details).

Clinical Research Assessment

All participants underwent clinical research assessments at the UCSD NAFLD Research Center (See Supplementary Text for details).

Genotyping

DNA samples were extracted from whole blood samples collected during the clinical research visit. Genotyping was performed by Human Longevity Inc (San Diego, CA, USA).

Primary Outcome

The primary outcome was the presence of shared gene effect between hepatic steatosis and hepatic fibrosis.

Secondary Outcomes

The secondary outcome was the shared gene of hepatic steatosis and fibrosis with the following metabolic risk factors: systolic and diastolic blood pressures, total cholesterol, HDL, LDL, triglycerides, ferritin, glucose, HOMA-IR, insulin, and HbA1c.

Magnetic Resonance Imaging

MRI was performed at the UCSD MR3T Research Laboratory using the 3T research scanner (GE Signa EXCITE HDxt, GE Healthcare, Waukesha, WI, USA) with all participants in supine positions, on the same day as the clinical research visit to reduce potential confounding factors. MRI-PDFF was used to measure hepatic steatosis and MRE was used to measure hepatic fibrosis. MRI-PDFF has been previously shown to be a highly precise, accurate, and reproducible noninvasive biomarker to quantify hepatic fat content.(25, 26), correlates well with MR spectroscopy (r^2=0.99, p<0.001)(22, 23), and is superior to noninvasive imaging techniques such as ultrasound and computed tomography for measuring hepatic fat content (27) even in iron-overloaded livers that may coexist with NAFLD livers. (28) MRI-PDFF has also been shown to correlate well with histology from contemporaneous liver biopsies.(29, 30) MRE has been shown to be a highly accurate, noninvasive biomarker to estimate hepatic fibrosis quantified by liver stiffness values in units of kilopascals (kPa), (31) and has been shown to be more accurate than clinical prediction rules (32) and ultrasound-based acoustic radiation force impulse imaging (33) for quantifying hepatic fibrosis. Please see Supplementary Text for a description of the MR procedures.

Justification for not using liver biopsy to assess for hepatic steatosis and fibrosis

Due to the invasive nature of liver biopsies, it would be unethical to perform liver biopsies in study participants with no clinical indications for liver biopsies.(1) Therefore, we used noninvasive imaging techniques to quantify hepatic steatosis and fibrosis. MRI-PDFF have been previously shown to be accurate for estimating hepatic steatosis and more precise than liver biopsies.(25) MRE has also been previously shown to be accurate for estimating hepatic fibrosis.(31–33)

Statistical Analysis

Patients' demographic, anthropometric, clinical, and biochemical characteristics were summarized. Categorical variables were shown as counts and percentages and associations were tested using a Chi-square test or Fisher's exact test. Normally distributed continuous variables were shown as mean (± standard deviation) and differences between groups were analyzed using a two independent samples t-test or Wilcoxon– Mann–Whitney test. Odds ratios were derived from generalized estimating equations (PROC GENMOD) to account for intra-pair correlations within twinships. A two-tailed p-value < 0.05 was considered statistically significant. Statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, NC, USA).

AE models were used to estimate the shared genetic determination (rG) and shared environmental determination (rE) between twin pairs. In the classical twin study of sets of MZ and DZ twins, four latent factors can account for the variance of any phenotype: additive

genetic effects (A); non-additive genetic effects, including dominance (D); common or shared environmental effects (C); and non-shared or individual-specific environmental effects (E) (34). Because MZ twins are presumed to be genetically identical, they correlate perfectly ($r = 1.0$) with respect to both additive and non-additive genetic effects. DZ twins share, on average, 50% of their genes, resulting in correlations of 0.50 for additive genetic effects and 0.25 for non-additive genetic effects. The C term is defined as environmental factors that make twins similar; hence, common environmental factors correlate 1.0 across twin pairs, regardless of zygosity. The E term represents environmental factors that lead to differences between twins. Because these are individual-specific factors, they are assumed to be uncorrelated across twins. Error is assumed to be random across individuals, so measurement error forms part of the estimate of E in these analyses. These latent factors comprise what are referred to as the univariate ACE or ADE models; due to model under-identification, an ACDE model cannot be tested in the classical twin design (34).

The ACE and ADE models are easily extended to the multivariate case (34). In addition to genetic and environmental sources of variance, sources of covariance can also be examined in the bivariate model. In the present study, we used bivariate models to compute genetic correlations between two phenotypes. A phenotypic correlation measures shared variance; a genetic correlation measures shared genetic variance. More specifically, a phenotypic correlation is defined as the total covariance (genetic plus environmental) of two variables divided by the square root of the product of the total variance of variable 1 and the total variance of variable 2. After decomposing the sources of variance in the bivariate model, we computed genetic correlations. These are defined as the *genetic* covariance divided by the square root of the product of the *genetic* variance of variable 1 and the *genetic* variance of variable 2. The analyses were performed using OpenMx, a structural equation modeling software package for genetically informative data (http://openmx.psyc.virginia.edu). Prior to the model fitting, the measures were adjusted for controlling age, gender and ethnicity. Overall, AE models tended to provide the best fits to the data. Consequently, the genetic effects estimated in these AE models refer to broad-sense heritability, reflecting the proportion of phenotypic variance accounted for by the combined effect of all genetic influences (A+D).

Sample Size Estimation

In previous studies, the heritability of hepatic steatosis ranged from 0.37, when hepatic steatosis was assessed using ultrasound and serum ALT levels,(35) to almost 1.0, when hepatic steatosis was assessed using MRI in obese Hispanic probands and their relatives.(36) We have also previously estimated the heritability of hepatic steatosis and fibrosis to be approximately 0.5.(9) Based on these numbers, we anticipated that the heritability of hepatic steatosis and fibrosis with one another should also be approximately 0.5. It has previously been shown that, to detect an additive genetic component of 0.4 to 0.8 in an ACE model, approximately 36–74 twin pairs are needed to produce a power of 0.95 with an alpha value of 0.05.(37) Therefore, the 65 twin pairs in this study should provide adequate sample size to assess the heritability of steatosis and fibrosis in our population.

RESULTS

Baseline Characteristics

130 participants (45 monozygotic twin pairs, 25 dizygotic twin pairs) who underwent clinical research assessments and imaging with MRI-PDFF and MRE were included in this study. 438 participants were initially assessed for eligibility, 152 provided informed consent, and 130 was included in the final analysis (see Supplementary Figure 1 for details). The mean (±SD) age was 47.1 (±21.9) years and the mean (±SD) BMI was 26.2 (±5.8) kg/m^2. 26/130 (20%) of the cohort had hepatic steatosis (MRI-PDFF   5%) and 10/122 (8.2%) of the cohort had hepatic fibrosis (MRE   3 kPa). Compared to twins without NAFLD, twins with NAFLD were significantly older (54.9 ± 17.3 years vs. 45.2 ± 22.5 years, p = 0.04) and had higher BMI (31.5 ± 4.8 kg/m^2 vs. 24.8 ± 5.1 kg/m^2, p < 0.0001). As expected, twins with NAFLD also had significantly higher measurements of hepatic steatosis via MRI-PDFF (10.7 ± 5.1 vs. 2.4 ± 0.9, p < 0.0001) and hepatic fibrosis via MRE (3.0 ± 1.2 vs. 2.2 ± 0.4, p < 0.0001). Detailed demographic, biochemical, and imaging data of the cohort, stratified by the presence or absence of NAFLD, are summarized in Table 1.

Shared Gene Effect

Using AE models, the shared gene effect rG between hepatic steatosis, fibrosis, and metabolic risk factors are summarized below.

Shared Gene Effects between Hepatic Steatosis and Metabolic Risk Factors

There were significant shared gene effects between hepatic steatosis, as measured by MRI-PDFF, and BMI at 0.534 (95% CI: 0.305, 0.713), p=3.19e-5; systolic blood pressure at 0.360 (95% CI: 0.052, 0.636), p=0.023; diastolic blood pressure at 0.444 (95% CI: 0.444, 0.742), p=0.0071; HDL cholesterol at −0.451 (95% CI: −0.643, −0.216), p=3.57e-4; triglycerides at 0.678 (95% CI: 0.585, 0.830), p=4.69e-8; glucose at 0.716 (95% CI: 0.716, 1), p=1.64e-4; HOMA-IR at 0.490 (95% CI: 0.212, 0.739), p=8.71e-4; insulin at 0.289 (95% CI: 0.017, 0.531), p=0.038; and HbA1c at 0.588 (95% CI: 0.588, 1), p=9.83e-4. There were no significant shared gene effects between hepatic steatosis and total cholesterol, LDL cholesterol, and ferritin (Table 2). Significant shared gene effects between hepatic steatosis and metabolic risk factors are shown in Figure 1a.

Shared Gene Effects between Hepatic Fibrosis and Metabolic Risk Factors

There were significant shared gene effects between hepatic fibrosis, as measured by MRE, and BMI at 0.493 (95% CI: 0.493, 0.845), p=0.00649; HDL cholesterol at −0.614 (95% CI: −0.890, −0.614), p=5.74e-4; triglycerides at 0.657 (95% CI: 0.657, 1), p=3.44e-4; glucose at 0.746 (95% CI: 0.746, 1), p=0.0029; HOMA-IR at 0.610 (95% CI: 0.218, 1), p=0.0025; insulin at 0.429 (95% CI: 0.167, 0.735), p=0.023; and HbA1c at 0.566 (95% CI: 0.566, 1), p=0.015. There were no significant shared gene effects between hepatic fibrosis and systolic blood pressure, diastolic blood pressure, total cholesterol, LDL cholesterol, and ferritin (Table 2). Significant shared gene effects between hepatic fibrosis and metabolic risk factors are shown in Figure 1b.

Shared Gene Effect between Hepatic Steatosis and Fibrosis

There was a significant shared gene effect between hepatic steatosis and fibrosis at 0.756 (95% CI: 0.716, 1), p=2.54e-5 (Table 2, Figure 2). Figure 3 depicts MRI-PDFF images and MRE elastograms of a representative pair of 60 year old male twins with both hepatic steatosis and fibrosis traits.

Shared Environmental Effect

Using AE models, the shared environmental effect rE between hepatic steatosis, fibrosis, and metabolic risk factors are summarized below.

Shared Environmental Effect between Hepatic Steatosis and Metabolic Risk Factors

There was a significant shared environmental effect between hepatic steatosis, as measured by MRI-PDFF, and ferritin at 0.307 (95% CI: 0.019, 0.544), p=0.037. There were no other significant shared environmental effects between hepatic steatosis and other metabolic risk factors (Table 3).

Shared Environmental Effect between Hepatic Fibrosis and Metabolic Risk Factors

There were no significant shared environmental effects between hepatic fibrosis, as measured by MRE, and metabolic risk factors (Table 3).

Predictors of NAFLD in Overall Cohort

In our overall cohort of twins, generalized estimating equations were used to estimate the odds ratios of demographic, anthropometric, and laboratory variables for predicting NAFLD. Significant odds ratios as predictors of NAFLD included weight at 1.07 (95% CI: 1.04, 1.11), p<0.0001; BMI at 1.26 (95% CI: 1.12, 1.43), p=0.0002; BMI > 30 at 6.42 (95% CI: 2.40, 17.16), p=0.0002; systolic blood pressure at 1.03 (95% CI: 1.01, 1.04), p=0.0108; waist circumference at 1.10 (95% CI: 1.05, 1.15), p=0.0002; hip circumference at 1.09 (95% CI: 1.04, 1.14), p=0.0002; glucose at 1.03 (95% CI: 1.01, 1.05), p=0.0132; HbA1c at 4.31 (95% CI: 1.75, 10.65), p=0.0015; HOMA-IR at 1.63 (95% CI: 1.08, 2.44), p=0.0189; ALT at 1.03 (95% CI: 1.01, 1.06), p=0.0104; HDL cholesterol at 0.95 (95% CI: 0.92, 0.97), p=0.0002; triglycerides at 1.03 (95% CI: 1.01, 1.04), p<0.0001; white blood cells at 1.74 (95% CI: 1.26, 2.40), p=0.0008; and ferritin at 1.00 (95% CI: 1.00, 1.00), p=0.0479 (Table 4).

Predictors of Fibrosis in Overall Cohort

Using generalized estimating equations, significant odds ratios as predictors of fibrosis in our overall cohort included male gender at 4.65 (95% CI: 1.09, 19.87), p=0.0379; weight at 1.05 (95% CI: 1.02, 1.08), p=0.0008; BMI at 1.21 (95% CI: 1.10, 1.32), p<0.0001; BMI > 30 at 7.72 (95% CI: 1.65, 27.39), p=0.0079; SBP at 1.04 (95% CI: 1.01, 1.08), p=0.0172; DBP at 1.04 (95% CI: 1.00, 1.08), p=0.0548; waist circumference at 1.08 (95% CI: 1.03, 1.13), p=0.0018; hip circumference at 1.04 (1.00, 1.08), p=0.0274; HbA1c at 3.48 (95% CI: 1.04, 11.61), p=0.0426; HOMA-IR at 1.70 (95% CI: 1.04, 2.79), p=0.0359; AST at 1.04 (95% CI: 1.00, 1.09), p=0.0427; ALT at 1.03 (95% CI: 1.01, 1.05), p=0.0046; GGT at 1.03 (95% CI: 1.01, 1.06), p=0.0175; HDL cholesterol at 0.90 (95% CI: 0.85, 0.95), p=0.0002;

triglycerides at 1.02 (95% CI: 1.01, 1.03), p<0.0001; and INR at 10.34 (95% CI: 2.03, 52.69), p=0.0049 (Table 4).

DISCUSSION

Main findings

In this study, we utilized a well-characterized, prospective, community-dwelling twin cohort study design to demonstrate that hepatic steatosis and hepatic fibrosis have statistically and clinically significant shared gene effect. This builds on our previous findings that both hepatic steatosis and fibrosis are each individually heritable traits.(9) We also demonstrated significant shared genetic effects between hepatic steatosis and fibrosis and a wide number of metabolic risk factors, including HDL, triglycerides, insulin resistance, and HbA1c. These results suggest a genetic basis underlying the pathogenesis of both hepatic steatosis and fibrosis, and also with metabolic risk factors. This is a paradigm changing finding, as most expert believe that hepatic steatosis is inconsequential and only hepatic fibrosis is associated with worse outcomes, including mortality and liver transplantation.(5) The shared gene effects between hepatic steatosis and hepatic fibrosis suggests that development of hepatic steatosis may itself portend a worse outcome. However, the time horizon for hepatic steatosis to reach these adverse outcomes may be long, and studies with 10 to 20 years of follow-up may be needed to assess these outcomes. It also has implications in developing targeted therapies for the treatment of NASH. It provides a biologic plausibility that reduction of hepatic steatosis over a sustained period of time may also influence the expression of genes associated with fibrosis progression/regression and may be viable target for the treatment of NASH.

In context of published literature

Previous studies have shown that both hepatic steatosis (9, 35, 36) and fibrosis are heritable traits.(9) We build on the results of these previous studies to show additional heritabilities between hepatic steatosis and fibrosis. Additionally, NAFLD has been shown to be associated with metabolic risk factors, (17–20) although it is unknown from these studies the relative contributions of genetic versus environmental factors to these associations. While we have previously demonstrated genetic covariance between NAFLD and metabolic risk factors in a prospective twin study design, gamma-glutamyl transferase was used as a marker of hepatic steatosis, and liver fat content was not measured directly.(21) Additionally, no previous studies have demonstrated genetic covariance between hepatic fibrosis and metabolic risk factors. This is the first study to demonstrate genetic covariance between metabolic risk factors and both hepatic steatosis and fibrosis in a community-dwelling cohort of twins, with accurate quantification of steatosis and fibrosis throughout the liver achieved through the use of non-invasive MRI-based imaging techniques.

There are currently few effective medical therapies to manage NAFLD and its complications. Vitamin E and thiazolidinediones have been shown to improve hepatic steatosis in NAFLD patients.(38–40) However, few treatments have been shown to be effective in reversing NAFLD-associated hepatic fibrosis. The genes PNPLA3 (10, 41) and TM6SF2,(42, 43) have been shown to modify the risks of hepatic steatosis and fibrosis, and

other genetic pathways associated with steatosis, fibrosis, and metabolic traits remain to be elucidated. Future identification of these genetic pathways may lead to individualized, targeted therapies that may prevent and/or reverse hepatic steatosis and fibrosis.

Strengths and Limitations

The strength of this study lies in its use of a twin study design that allows for the evaluation of the heritability of steatosis, fibrosis, and metabolic risk factors. The cohort consisted of well-characterized, community-dwelling twins in which twins with conditions such as excessive alcohol use, use of steatogenic medications, viral hepatitis, and secondary causes of steatosis were systemically excluded. The use of MRI-PDFF allowed for detailed mapping and steatosis quantification throughout the entire liver, and the use of MRE allowed for an accurate, non-invasive way to quantify hepatic fibrosis.

However, this study is limited by the lack of biopsy, which remains the gold standard for diagnosing liver steatosis and fibrosis. While biopsies are limited by their interobserver variability and sampling bias, they allow for the diagnosis of lobular inflammation, hepatocyte ballooning, and NASH that cannot be diagnosed non-invasively. However, because it is unethical to perform liver biopsies in normal control patients with no suspicion of NAFLD, a study involving liver biopsies can only be performed if at least one twin has suspected NAFLD. Our use of noninvasive biomarkers instead of liver biopsy to assess hepatic steatosis and fibrosis allowed us to utilize a community-dwelling cohort of patients, rather than pre-selected patients with increased risk of NAFLD. Although MRI-PDFF has been shown to have high inter-reader reproducibility in non-twin studies,(44) and the interobserver variability of MR readings in our study was minimized with only one analyst performing all the image analysis, the general interobserver variability of MR readings in similar twin study designs remain unknown. Additionally, MRI-PDFF has been shown to be highly accurate for mapping hepatic steatosis throughout the entire liver without the sampling variability associated with liver biopsies, and MRE has also been shown to be highly accurate for the diagnosis of hepatic fibrosis, so we believe our noninvasive diagnostic tests can reliably measure steatosis and fibrosis.(31–33)

Implication for future study

In this study, we demonstrate in a prospective, community-dwelling cohort of twins that patients with genetic susceptibility to hepatic steatosis also have genetic susceptibility to hepatic fibrosis. We also demonstrate that both hepatic steatosis and fibrosis have shared genetic effects with metabolic risk factors. Additional studies with larger sample sizes will be needed to identify individual genes or pathways that may be implicated in hepatic steatogenesis and/or fibrogenesis. The identification of these genes may allow for further individualized, targeted therapy that may prevent and even reverse hepatic steatosis and fibrosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Abbreviations

NAFLD	nonalcoholic fatty liver disease
NASH	nonalcoholic steatohepatitis
MRI-PDFF	magnetic resonance imaging – proton density fat fraction
MRE	magnetic resonance elastography
UCSD	University of California at San Diego
MZ	monozygotic
DZ	dizygotic
kPa	kilopascals
r_G	shared genetic determination
rE	shared environmental determination

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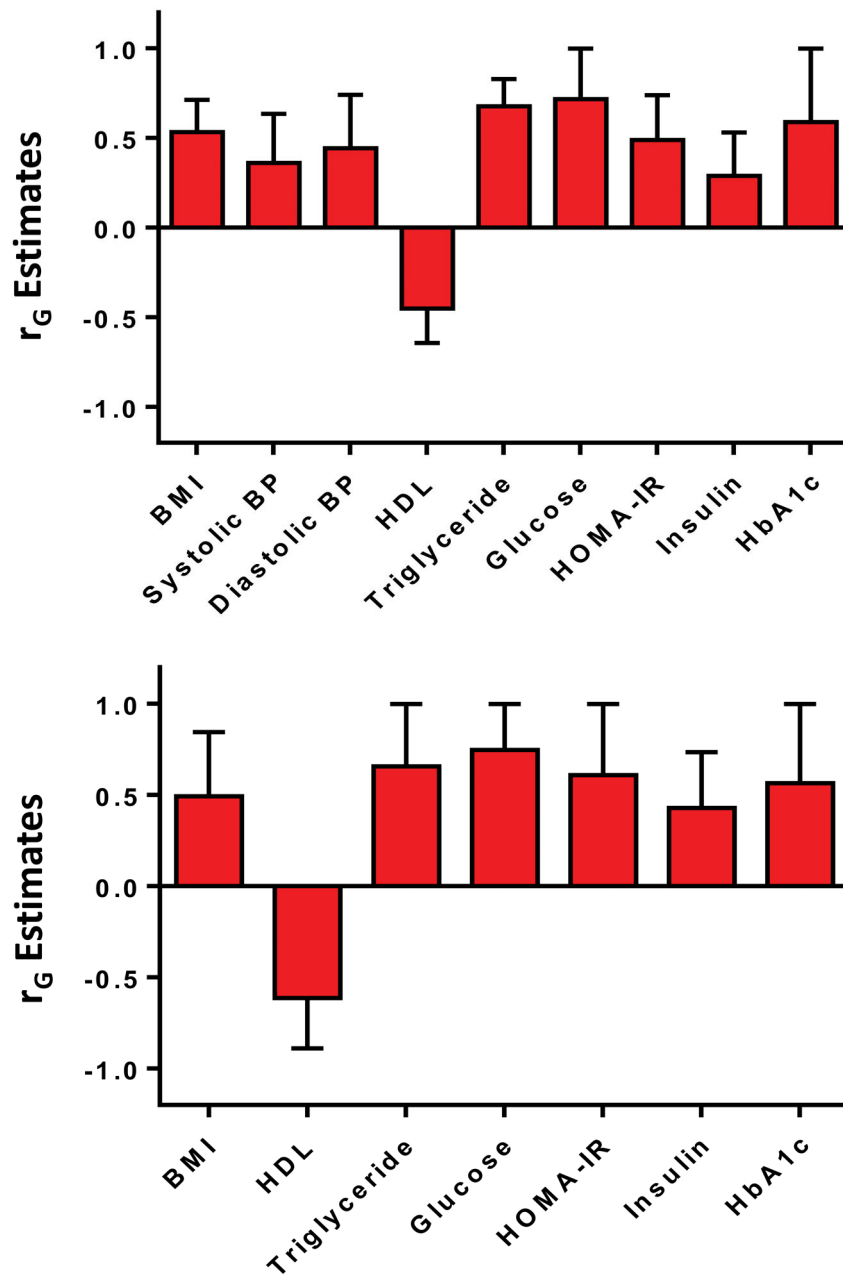
Figure 1.

Figure 1a–1b: The significant shared gene effects rG of hepatic steatosis and hepatic fibrosis with metabolic risk factors. Hepatic steatosis (Figure 1a) had significant shared gene effects rG with BMI, systolic blood pressure, diastolic blood pressure, HDL, triglycerides, glucose, HOMA-IR, insulin, and HbA1c. Hepatic fibrosis (Figure 1b) had significant shared gene effects rG with BMI, HDL, triglycerides, glucose, HOMA-IR, insulin, and HbA1c.
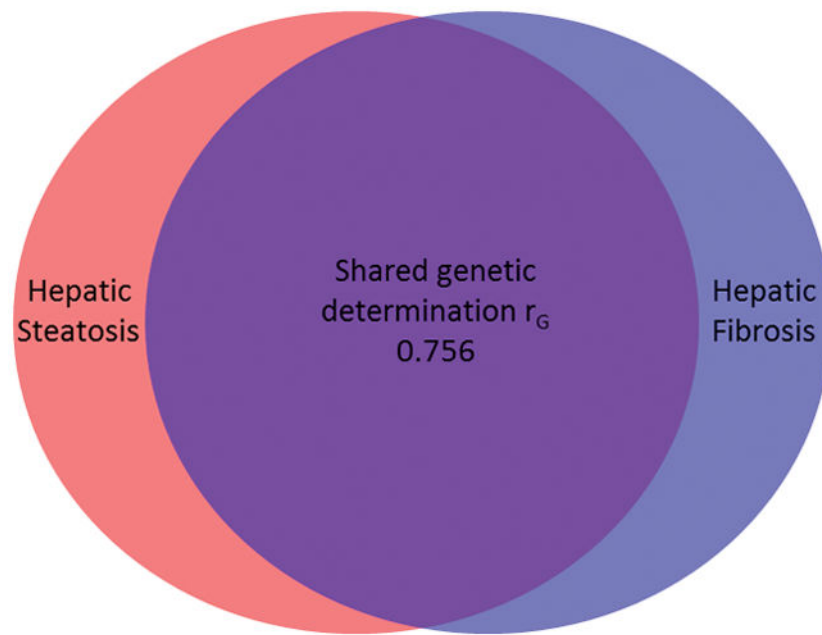
Figure 2.
Hepatic steatosis and fibrosis has a shared genetic determination rG of 0.756.
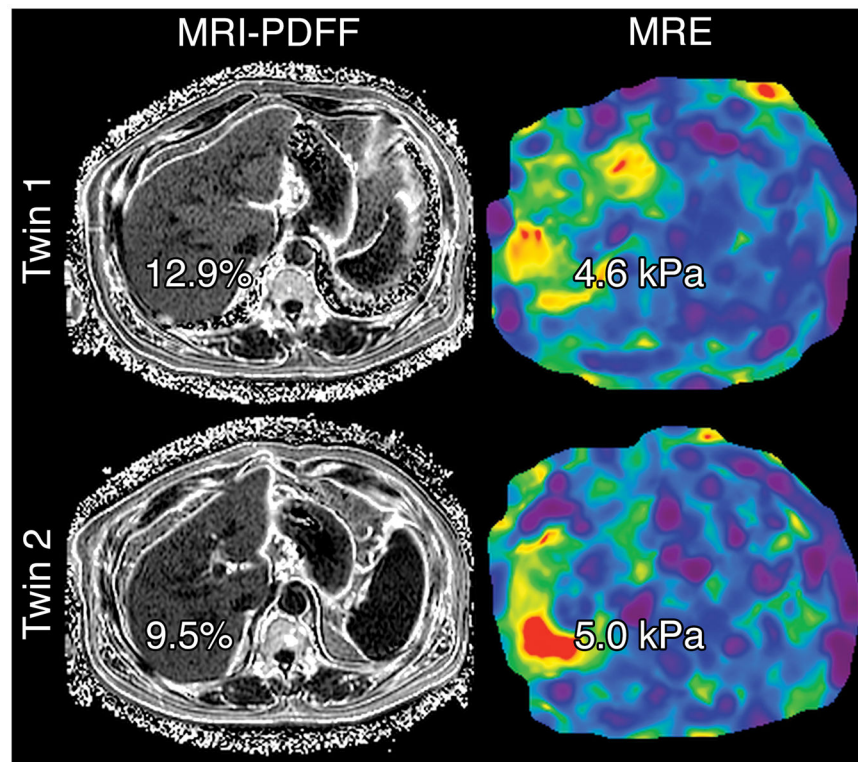
Figure 3.
Representative MRI-PDFF and MRE of a pair of 60 year old male twins concordant for both NAFLD (MRI-PDFF ≥5%) and hepatic fibrosis (MRE >3 kPa). Hepatic steatosis and fibrosis have significant shared gene effects with one another at 0.756 (95% CI: 0.716, 1), p=2.54e-5. Hepatic steatosis also has significant shared gene effects with BMI, systolic blood pressure, diastolic blood pressure, HDL cholesterol, triglycerides, glucose, HOMA-IR, insulin, and hemoglobin A1c, and hepatic fibrosis has significant shared gene effects with BMI, HDL cholesterol, triglycerides, glucose, HOMA-IR, insulin, and hemoglobin A1c.

Table 1

Baseline characteristics stratified by NAFLD status in the Twin Cohort

	Overall	Twins with NAFLD (MRI-PDFF ≥ 5%)	Twins without NAFLD (MRI-PDFF < 5%)	p-value
N	130	26	104	
Demographics				
Age (years)	47.1 (21.9)	54.9 (17.3)	45.2 (22.5)	**.0417**
Sex (% male)	35 (26.9%)	11 (42.3%)	24 (23.1%)	**.0480**
Race				.0510
White	104 (80.0%)	18 (69.2%)	86 (82.7%)	
Hispanic	18 (13.9%)	5 (19.2%)	13 (12.5%)	
Asian	6 (4.6%)	1 (3.9%)	5 (4.8%)	
Hawaiian/Pac Islander	2 (1.5%)	2 (7.7%)	0 (0%)	
Physical				
Height (cm)	165.8 (8.2)	167.0 (10.7)	165.4 (7.5)	.4981
Weight (kg)	72.1 (17.9)	88.8 (20.5)	67.8 (14.3)	**<.0001**
Body mass index (kg/m2)	26.2 (5.8)	31.5 (4.8)	24.8 (5.1)	**<.0001**
Systolic blood pressure (mm Hg)	126.0 (19.6)	135.5 (16.7)	123.6 (19.6)	**.0052**
Diastolic blood pressure (mm Hg)	78.7 (12.4)	82.9 (13.0)	77.6 (12.1)	**.0494**
Waist circumference (cm)	88.8 (12.9)	100.0 (10.6)	85.9 (11.9)	**<.0001**
Hip circumference (cm)	99.6 (11.3)	107.5 (11.6)	97.7 (10.4)	**<.0001**
Laboratory data				
Glucose (mg/dL)	89.3 (18.5)	101.6 (34.3)	86.2 (9.5)	**.0013**
Insulin (U/L)	8.4(5.5)	12.6 (7.1)	7.4(4.5)	**.0005**
Hemoglobin A1c	5.8 (0.5)	6.1(0.7)	5.7(0.3)	**.0011**
HOMA-IR	1.9 (1.4)	3.1(1.9)	1.6(1.1)	**.0001**
AST (U/L)	23.6 (9.9)	27.0 (16.6)	22.7 (7.2)	.1676
ALT (U/L)	22.9 (18.1)	35.0(32.8)	19.8(10.0)	**.0008**
Alkaline Phosphatase (U/L)	69.1 (19.6)	69.7(15.1)	69.0(20.6)	.6589
Total bilirubin (mg/dL)	0.5(0.3)	0.5(0.4)	0.5(0.2)	.7893
Direct bilirubin (mg/dL)	0.1(0.1)	0.1(0.1)	0.1(0.1)	.4888
Albumin (g/dL)	4.5 (0.3)	4.5(0.2)	4.5(0.4)	.3867

	Overall	Twins with NAFLD (MRI-PDFF ≥ 5%)	Twins without NAFLD (MRI-PDFF < 5%)	p-value
GGT (U/L)	23.2 (19.9)	33.1(29.6)	20.7 (15.7)	**.0031**
Total cholesterol (mg/dL)	196.0 (40.8)	200.6 (36.1)	194.8 (42.1)	.3330
HDL–cholesterol (mg/dL)	66.4 (21.7)	51.0(15.9)	70.4(21.2)	**<.0001**
LDL–cholesterol (mg/dL)	111.0 (34.7)	119.8 (30.7)	108.8 (35.5)	**.0489**
Triglycerides (mg/dL)	92.8 (53.3)	150.7 (71.1)	78.0 (35.0)	**<.0001**
White blood cell count (x10³/uL)	5.7 (1.3)	6.4(1.5)	5.5(1.2)	**.0055**
Hemoglobin (g/dL)	13.7 (1.2)	14.0 (1.4)	13.6 (1.2)	.1038
Hematocrit (%)	40.6 (3.3)	41.5 (3.8)	40.4 (3.2)	.0832
Platelet count (x10³/uL)	251.7 (51.5)	253.0 (57.7)	251.4 (50.2)	.8753
INR	1.1 (0.3)	1.1(0.4)	1.1(0.3)	.6387
Ferritin (ng/mL)	100.9 (90.7)	134.4(144.4)	92.2(69.2)	.1769
Imaging data				
MRI-PDFF (%)	4.034 (4.11)	10.740 (5.08)	2.358 (0.87)	**<.0001**
MRE (Kpa)	2.327 (0.74)	3.004 (1.23)	2.152 (0.40)	**<.0001**
PNPLA3 phenotype (N=87)				0.8360
CC	39 (44.8%)	9 (45.0%)	30 (47.8%)	
CG	38 (43.7%)	8 (40.0%)	30 (44.8%)	
GG	10 (11.5%)	3 (15.0%)	7 (10.4%)	

Mean value are provided with standard deviation in parenthesis, unless otherwise noted as N(%). Differences between participants with and without NAFLD were evaluated with *t* tests or Wilcoxon Mann–Whitney for continuous variables and chi square or Fishers exact tests for categorical variables.

Abbreviations: NAFLD, non-alcoholic fatty liver disease; HOMA-IR, homeostatic model of insulin resistance; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma- glutamyl transpeptidase; LDL, low-density lipoprotein; HDL, high-density lipoprotein; INR, international normalized ratio; MRI, magnetic resonance imaging; PDFF, proton-density-fat-fraction. Significant p-values <0.05.

Table 2

AE model for shared genetic determination (pleiotropy) rG between hepatic steatosis, fibrosis, and metabolic risk factors

Trait	Hepatic Steatosis (MRI-PDFF)		Hepatic Fibrosis (MRE)	
	r_G estimate (CIs)	P-value	r_G estimate (CIs)	P-value
BMI	0.534 (0.305, 0.713)	**3.19e-5**	0.493 (0.493, 0.845)	**0.00649**
Blood pressure*				
Systolic	0.360 (0.052, 0.636)	**0.023**	0.308 (−0.137, 0.688)	0.160
Diastolic	0.444 (0.444, 0.742)	**0.0071**	0.257 (0.257, 0.723)	0.292
Total cholesterol	−0.021 (−0.397, −0.014)	0.903	−0.299 (−0.839, 0.218)	0.243
LDL-cholesterol	0.076 (−0.134, 0.090)	0.658	−0.127 (−0.609, −0.053)	0.600
HDL-cholesterol	−0.451 (−0.643, −0.216)	**3.57e-4**	−0.614 (−0.890, −0.614)	**5.74e-4**
Triglycerides	0.678 (0.585, 0.830)	**4.69e-8**	0.657 (0.657, 1)	**3.44e-4**
Ferritin	0.370 (−1, 1)	0.445	1 (−1, 1)	0.057
Glucose	0.716 (0.716, 1)	**1.64e-4**	0.746 (0.746, 1)	**0.0029**
HOMA-IR	0.490 (0.212, 0.739)	**8.71e-4**	0.610 (0.218, 1)	**0.0025**
Insulin	0.289 (0.017, 0.531)	**0.038**	0.429 (0.167, 0.735)	**0.023**
HbA1c	0.588 (0.588, 1)	**9.83e-4**	0.566 (0.566, 1)	**0.015**
Liver fibrosis (MRE)	0.756 (0.716, 1)	**2.54e-5**	N/A	N/A

Significant (p<0.05) coefficients are designated in **bold** type. **rG** indicates genetic co-variance that detects the shared genetic determination between two traits.

Abbreviations: MRI-PDFF, magnetic resonance imaging-proton density fat fraction; MRE, magnetic resonance elastography; BMI, body mass index; CI: confidence interval; LDL, low-density lipoprotein; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; HbA1c, hemoglobin A1c.

Table 3

AE model for shared environmental determination (pleiotropy) rE between hepatic steatosis, fibrosis, and metabolic risk factors

Trait	Hepatic Steatosis (MRI-PDFF)		Hepatic Fibrosis (MRE)	
	r_E estimate (CIs)	P-value	r_E estimate (CIs)	P-value
BMI	0.087 (−0.188, 0.352)	0.0538	−0.067 (−0.345, 0.226)	0.654
Blood pressure*				
Systolic	0.177 (−0.098, 0.426)	0.204	0.116 (−0.161, 0.378)	0.411
Diastolic	0.181 (−0.104, 0.438)	0.211	0.129 (−0.160, 0.399)	0.381
Total cholesterol	0.178 (−0.128, 0.452)	0.252	−0.023 (−0.322, 0.277)	0.883
LDL-cholesterol	0.213 (−0.075, 0.467)	0.146	−0.065 (−0.343, 0.223)	0.660
HDL-cholesterol	−0.229 (−0.488, −0.069)	0.129	0.030 (−0.279, 0.327)	0.851
Triglycerides	0.236 (−0.045, 0.481)	0.097	0.123 (−0.190, 0.415)	0.445
Ferritin	0.307 (0.019, 0.544)	**0.037**	−0.189 (−0.415, 0.083)	0.167
Glucose	−0.070 (−0.332, 0.202)	0.617	−0.006 (−0.271, 0.262)	0.968
HOMA-IR	0.015 (−0.271, 0.298)	0.917	−0.166 (−0.430, 0.125)	0.261
Insulin	0.088 (−0.211, 0.370)	0.565	−0.210 (−0.477, 0.096)	0.176
HbA1c	0.200 (−0.098, 0.460)	0.185	0.089 (−0.195, 0.360)	0.543
Liver fibrosis (MRE)	−0.052 (−0.359, 0.278)	0.758	N/A	N/A

Significant (p<0.05) coefficients are designated in **bold** type. **r_E** indicates environmental co-variance that suggests the shared environmental determination between two traits.

Abbreviations: MRI-PDFF, magnetic resonance imaging-proton density fat fraction; MRE, magnetic resonance elastography; BMI, body mass index; CI: confidence interval; LDL, low-density lipoprotein; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; HbA1c, hemoglobin A1c.

Odds of NAFLD and Fibrosis

	Odds of NAFLD		Odds of Fibrosis	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Demographics				
Age	1.02 (1.00–1.05)	0.0832	1.05 (0.99–1.11)	0.1071
Age 45	2.92 (0.85–10.05)	0.0894	5.78 (0.69–48.21)	0.1053
Male	2.75 (0.92–8.24)	0.0713	**4.65 (1.09–19.87)**	**0.0379**
Hispanic	1.67 (0.47–5.91)	0.4289	3.26 (0.54–19.70)	0.1971
Anthropometric				
Height (cm)	1.02 (0.95–1.09)	0.6515	0.99 (0.90–1.08)	0.7534
Weight (kg)	**1.07 (1.04–1.11)**	**<0.0001**	**1.05 (1.02–1.08)**	**0.0008**
Body mass index (kg/m2)	**1.26 (1.12–1.43)**	**0.0002**	**1.21 (1.10–1.32)**	**<0.0001**
BMI > 30	**6.42 (2.40–17.16)**	**0.0002**	**7.72 (1.65–27.39)**	**0.0079**
SBP (mm Hg)	**1.03 (1.01–1.04)**	**0.0108**	**1.04 (1.01–1.08)**	**0.0172**
DBP (mm Hg)	1.02 (0.99–1.05)	0.1533	1.04 (1.00–1.08)	0.0548
Waist (cm)	**1.10 (1.05–1.15)**	**0.0002**	**1.08 (1.03–1.13)**	**0.0018**
Hip (cm)	**1.09 (1.04–1.14)**	**0.0002**	**1.04 (1.00–1.08)**	**0.0274**
Laboratory data				
Glucose (mg/dL)	**1.03 (1.01–1.05)**	**0.0132**	1.03 (0.99–1.07)	0.1075
Insulin (U/L)	1.12 (1.00–1.26)	0.0563	1.10 (0.97–1.24)	0.1253
Hemoglobin A1c	**4.31 (1.75–10.65)**	**0.0015**	**3.48 (1.04–11.61)**	**0.0426**
HOMA-IR	**1.63 (1.08–2.44)**	**0.0189**	**1.70 (1.04–2.79)**	**0.0359**
AST (U/L)	1.01 (0.98–1.04)	0.6153	**1.04 (1.00–1.09)**	**0.0427**
ALT (U/L)	**1.03 (1.01–1.06)**	**0.0104**	**1.03 (1.01–1.05)**	**0.0046**
Alkaline Phosphatase (U/L)	1.00 (0.98–1.01)	0.5761	1.01 (0.99–1.02)	0.4051
Albumin (g/dL)	1.02 (0.37–2.85)	0.9699	0.62 (0.09–4.25)	0.6250
GGT (U/L)	1.01 (0.99–1.03)	0.3642	**1.03 (1.01–1.06)**	**0.0175**
Total cholesterol (mg/dL)	1.00 (0.99–1.01)	0.5128	1.00 (0.99–1.02)	0.6757
HDL-cholesterol (mg/dL)	**0.95 (0.92–0.97)**	**0.0002**	**0.90 (0.85–0.95)**	**0.0002**
LDL-cholesterol (mg/dL)	1.01 (1.00–1.02)	0.1528	1.01 (0.99–1.03)	0.3180

	Odds of NAFLD		Odds of Fibrosis	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Triglycerides (mg/dL)	**1.03 (1.01–1.04)**	**<0.0001**	**1.02 (1.01–1.03)**	**<0.0001**
WBC (x10³/uL)	**1.74 (1.26–2.40)**	**0.0008**	1.27 (0.80–2.04)	0.3122
Hemoglobin (g/dL)	1.45 (0.96–2.19)	0.0788	1.01 (0.64–1.59)	0.9581
Hematocrit (%)	1.13 (0.98–1.31)	0.1025	1.01 (0.86–1.18)	0.9357
Platelets (x10³/uL)	1.00 (0.99–1.01)	0.4749	1.00 (0.98–1.01)	0.5051
INR	0.94 (0.35–2.55)	0.9002	**10.34 (2.03–52.69)**	**0.0049**
Ferritin (ng/mL)	**1.00 (1.00–1.00)**	**0.0479**	1.00 (1.00–1.01)	0.1294

Odds ratios derived from generalized estimating equations (PROC GENMOD) in SAS software version 9.4, to account for intra-pair correlations within twinships. A two-tailed p-value < 0.05 is considered statistically significant (in bold type). Abbreviations for table: NAFLD, non-alcoholic fatty liver disease; HOMA-IR, homeostatic model of insulin resistance; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma- glutamyl transpeptidase; LDL, low- density lipoprotein; HDL, high-density lipoprotein; INR, international normalized ratio. Significant p-values <0.10.