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Heritability of Hepatic Fibrosis and Steatosis Based on a Prospective Twin Study

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BACKGROUND & AIMS: Little is known about the heritability of hepatic fibrosis, and the heritability of hepatic steatosis has not been assessed systematically in adults. We investigated the heritability of hepatic fibrosis and steatosis in a community-dwelling twin cohort. **METHODS:** We performed a cross-sectional analysis of a cohort of well-characterized twins residing in Southern California including 60 pairs of twins (42 monozygotic and 18 dizygotic; average age, 45.7 ± 22.1 y; average body mass index, 26.4 ± 5.7 kg/m^2). We collected data on medical history, physical examinations, fasting laboratory test results, and liver health; all participants underwent an advanced magnetic resonance imaging (MRI) examination of the liver from January 2012 through January 2015. Hepatic steatosis was quantified noninvasively by MRI and determined based on the proton-density fat fraction (MRI-PDFF); liver fibrosis was measured based on stiffness measured by magnetic resonance elastography. **RESULTS:** Twenty-six of the 120 subjects (21.7%) had nonalcoholic fatty liver disease (defined as MRI-PDFF $\geq$ 5% after exclusion of other causes of hepatic steatosis). The presence of hepatic steatosis correlated between monozygotic twins ($r^2 = 0.70$; $P < .0001$) but not between dizygotic twins ($r^2 = 0.36$; $P = .2$). The level of liver fibrosis also correlated between monozygotic twins ($r^2 = 0.48$; $P < .002$) but not between dizygotic twins ($r^2 = 0.12$; $P = .7$). In multivariable models adjusted for age, sex, and ethnicity, the heritability of hepatic steatosis (based on MRI-PDFF) was 0.52 (95% confidence interval, 0.31–0.73; $P < 1.1 \times 10^{-11}$) and the heritability of hepatic fibrosis (based on liver stiffness) was 0.5 (95% confidence interval, 0.28–0.72; $P < 6.1 \times 10^{-11}$). **CONCLUSIONS:** A study of twins provides evidence that hepatic steatosis and hepatic fibrosis are heritable traits.

Keywords: Genetic Factors; Fatty Liver; NASH; NAFLD.

Q7 Q8 Q9 Nonalcoholic fatty liver disease (NAFLD) is characterized by hepatic steatosis in individuals who consume little or no alcohol and who have no other identifiable causes of steatosis.[1] It is the most common cause of chronic liver disease in the United States,[1–4] affecting 80–100 million Americans, of whom approximately 18 million are thought to have nonalcoholic steatohepatitis (NASH), a more advanced form that may lead to progressive fibrosis, cirrhosis, and hepatocellular carcinoma.[5–9]

Patients with hepatic fibrosis are at particularly high risk for developing cirrhosis and hepatocellular carcinoma, and require more intense monitoring and therapy.[6,10,11] Underpinning of genetic risk factors associated with hepatic steatosis and fibrosis in NAFLD is one of the top research priorities in the field.[12,13]

Hepatic steatosis is a key early event in the development of NAFLD, whereas hepatic fibrosis is a later event that has prognostic significance in predicting long-term outcomes related to liver disease.[5,10] Recent studies have suggested that there is a significant genetic association with the presence of hepatic steatosis.[14–18] The patatin-like phospholipase domain containing 3 (PNPLA-3) genotype has been linked to hepatic steatosis and also with features of NASH.[13,19,20] However, the PNPLA-3 genotype explains 10%–12% of the variance in the trait.[19] Therefore, 90% of the variance in the trait remains to be elucidated. Although significant progress has been made in assessing genetic risk factors associated with hepatic steatosis, there are limited human data in quantifying genetic risk factors associated with hepatic fibrosis in NAFLD. NAFLD is associated closely with metabolic traits.[21–24]

However, the heritability of NAFLD-associated hepatic steatosis in adults has not been examined systematically. Furthermore, there are no data regarding whether hepatic fibrosis is a heritable trait. A liver biopsy would not be ethical in patients without NAFLD, and an assessment of twins with and without NAFLD and fibrosis would be needed to assess the heritability of hepatic fibrosis.

Abbreviations used in this paper: **AE, additive genetic and environmental; ALT, alanine aminotransferase; CI, confidence interval; DZ, dizygotic; MRE, magnetic resonance elastography; MRI, magnetic resonance imaging; MZ, monozygotic; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; PDFF, proton density fat fraction; PNPLA-3, patatin-like phospholipase domain containing 3; UCSD, University of California at San Diego.**

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Therefore, accurate and precise noninvasive biomarkers were needed to document the heritability of hepatic fibrosis.

Until recently, accurate and precise noninvasive quantification of hepatic fibrosis was not feasible, and, therefore, the heritability of hepatic fibrosis could not be examined. With the recent advances in magnetic resonance elastography (MRE), it has now become feasible to assess hepatic fibrosis noninvasively with increased accuracy and precision.[25–29]

Hence, using a twin study design, we conducted a cross-sectional analysis of a prospective cohort study in community-dwelling monozygotic and dizygotic adult twins to examine the heritability of hepatic steatosis (as assessed by magnetic resonance imaging [MRI]) and hepatic fibrosis (as assessed by MRE).

Materials and Methods

Setting and Participants

This was a cross-sectional analysis of a prospective cohort study of twin-pairs residing in Southern California that was designed with the primary goal to study NAFLD. The cohort was derived from newspaper advertisement and also access to a twin-birth registry. Study participants were twin volunteers from urban Southern California (principally the San Diego area). All participants underwent a standardized clinical research visit including detailed medical history, past medical history, alcohol quantification using the Skinner and Audit questionnaire, physical examination, and testing to rule out other causes of chronic liver diseases (see the Inclusion and Exclusion Criteria section for further details), fasting laboratory tests (see the biochemical and metabolic traits subsection for further details), and then underwent an advanced MR examination of the liver between January 2012 and January 2015. Hepatic steatosis was quantified noninvasively by MRI-determined proton-density fat-fraction (MRI-PDFF) and liver fibrosis was quantified by MRE-determined stiffness (MRE-stiffness) as previously published.[26,30–33] Research visits and MRI procedures for each twin-pair were performed on the same day. Written informed consent was obtained from each participant, and the research protocol was approved by the University of California at San Diego (UCSD) Institutional Review Board.

Inclusion and Exclusion Criteria

Inclusion criteria were as follows: participants must be twins aged 18 years or older and willing and able to complete all research procedures and observations. Participants were fully informed and personally signed and dated the written Informed Consent and Health Insurance Portability and Accountability Act provisions.

Exclusion criteria were as follows: Pregnancy or nursing at the time of study procedures; contraindications for MRI including severe claustrophobia, metal implants, or body circumference greater than the imaging chamber; use of steatogenic medications including amiodarone, methotrexate, glucocorticoids, L-asparaginase, and valproic acid for at least 3 months in the past 6 months; chronic diseases other than NAFLD that may be associated with hepatic steatosis including cystic fibrosis, human immunodeficiency virus, hepatitis C or B, Wilson's disease, glycogen storage disease, lipodystrophy, celiac disease, or type 1 diabetes mellitus; significant alcohol consumption (defined as $>$ 10 g/day in females and $>$ 20 g/day in males, on average) for more than 3 consecutive months in the past 12 months or an inability to reliably quantify alcohol consumption; prior bariatric surgery (eg, gastroplasty, Roux-en-Y gastric bypass); low α1-antitrypsin level and ZZ phenotype; dysbetalipoproteinemia; phenotypic hemochromatosis including the presence of iron overload on MRI; polycystic liver disease; clinical or laboratory evidence of systemic infectious disease; or clinical evidence of other causes of liver disease.

Definition of NAFLD

NAFLD was defined as the presence of hepatic steatosis on MRI-PDFF of 5% or greater without any secondary causes of hepatic steatosis such as significant alcohol use or use of steatogenic medications or other causes of liver disease (see the exclusion criteria listed previously for further details), consistent with NAFLD practice guidelines.[1]

Assessment of Twin-Ship Status

Detailed information regarding participants twin-ship status (monozygotic [MZ] or dizygotic [DZ]) was obtained. The majority (34 twin-pairs) were diagnosed by a physician as either MZ or DZ by genetic testing. Participants were asked the following questions to further confirm twin-ship status by using the previously published and well-accepted questionnaire (Appendix 3) developed by Boyd et al.[34]

Clinical Research Visit and Laboratory Tests

All participants underwent a uniform and standardized clinical research visit at the UCSD NAFLD Translational Research Unit. Participants underwent a detailed medical history, including history of liver disease and other comorbid conditions, medication use, and alcohol consumption. The Alcohol Use Disorders Identification Test questionnaire and Skinner Lifetime Drinking history were administered to record and quantify alcohol use. A physical examination including vital signs, height, weight, and anthropometric measurements was performed by a trained investigator. Body mass index was calculated by dividing body weight (in kilograms) by the square of the height (in meters). After completion of the earlier-described elements of the history and physical examination, participants had fasting laboratory work performed including a 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), hemoglobin A1c, hepatic panel (total bilirubin, direct bilirubin, aspartate aminotransferase, alanine aminotransferase [ALT], alkaline phosphatase, γ-glutamyltransferase, albumin, prothrombin time, and international normalized ratio), lipid profile, and glucose-insulin levels.

Genotyping

Whole-blood specimens collected during the research visit were used and DNA was extracted. PNPLA-3 genotyping was conducted and its association in explaining the variance in hepatic steatosis and hepatic fibrosis was examined. The genotyping was performed by Human Longevity, Inc (San Diego, CA).