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Journal

Alimentary Pharmacology & Therapeutics, 54(1)

ISSN

0269-2813

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Publication Date

2021-07-01

DOI

10.1111/apt.16392

Peer reviewed

HHS Public Access

Author manuscript

Aliment Pharmacol Ther. Author manuscript; available in PMC 2022 April 06.

Published in final edited form as:

Aliment Pharmacol Ther. 2021 July ; 54(1): 68–77. doi:10.1111/apt.16392.

The impact of genetic risk on liver fibrosis in non-alcoholic fatty liver disease as assessed by magnetic resonance elastography

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Summary

Background: Variants in multiple genetic loci modify the risk of non-alcoholic fatty liver disease (NAFLD) and cirrhosis but there are limited data on the quantitative impact of variant copies on liver fibrosis.

Aim: To investigate the effect of *PNPLA3, TM6SF2, MBOAT7, GCKR and HSD17B13* genotype on liver fibrosis assessed by magnetic resonance elastography (MRE), a reproducible, accurate, continuous biomarker of liver fibrosis.

Methods: This is a cross-sectional analysis derived from a well-characterised cohort at risk for NAFLD who underwent genotyping and MRE assessment. Liver stiffness (LS) was estimated using MRE and advanced fibrosis was defined as liver stiffness ≥3.63 kilopascals (kPa). Univariable and multivariable linear and logistic regression analysis, were used to assess the association between genotype and MRE.

Results: Two hundred sixty-four patients (63% women) with a mean age 53 (±17) years, and 31% Hispanic ethnicity with genotyping and MRE were included. The odds of advanced fibrosis were 3.1 (95% CI: 1.1–8.9, $P = 0.04$) for CG and 6.5 (95% CI: 2.2–18.9, $P < 0.01$) for GG compared to CC *PNPLA3* genotype. Each *PNPLA3* risk variant copy was associated with 0.40 kPa (95% CI: 0.19–0.61, $P < 0.01$) increase in LS on MRE in analysis adjusted for age, sex and BMI and there was significant genotype-age interaction ($P < 0.01$). Conversely, the protective TA

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Author contributions: VA: study concept and design, data analysis, drafting of the manuscript, critical revision and approval of the final manuscript. AL: drafting of the manuscript, critical revision and approval of the final manuscript. RB: data analysis, critical revision and approval of the final manuscript. DD: critical revision and approval of the final manuscript. LR: data collection, critical revision and approval of the final manuscript. RL: study concept and design, drafting of the manuscript, critical revision and approval of the final manuscript.

DATA AVAILABILITY STATEMENT

The summary statistics of data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

SUPPORTING INFORMATION

Additional supporting information will be found online in the Supporting Information section.

allele in *HSD17B13* was associated with a −0.41 kPa (95% CI: −0.76 to −0.05, $P = 0.03$) decrease in liver stiffness on MRE multivariable analysis.

Conclusion: Knowledge of *PNPLA3* and *HSD17B13* genotype may assist in the non-invasive risk stratification of NAFLD with closer monitoring recommended for those with high genetic risk.

1 | INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is emerging as a leading cause of chronic liver disease globally, yet heterogeneity in disease severity and natural history have limited our ability to identify and treat the highest risk population.[1,2] While NAFLD is closely tied to the presence of obesity and metabolic syndrome, approximately 20% of individuals with NAFLD have a body mass index (BMI) within the normal range.[3] Furthermore, while the presence of steatohepatitis on liver biopsy identifies a high-risk population, it is impractical to scale to the estimated affected population of 1 billion. Therefore, there is a need to identify NAFLD phenotypes at the greatest risk for morbidity and mortality.

The strong genetic underpinnings of NAFLD are clearly demonstrated by familial clustering of advanced liver disease and racial/ethnic predisposition to disease.[4,5] The most well-characterised common variant is the non-synonymous variant p.I148M in *PNPLA3*, which has increased prevalence in Hispanics[6] and is associated with an increased risk of hepatic steatosis,[6] non-alcoholic steatohepatitis (NASH),[7,8] cirrhosis[9] and hepatocellular carcinoma.[10] However, despite the strong association, the *PNPLA3* genotype has not been routinely incorporated into clinical practice. Additional loci including *TM6SF2*,[11,12] *MBOAT7*[13] and *GCKR*[14] have been associated with NAFLD risk and more recently a protective variant in *HSD17B13*[15] was identified.

The most commonly used currently available non-invasive assessments do not incorporate genetic data to assess NAFLD severity and *PNPLA3* genotype has performed poorly in discriminatory models identifying disease at a single time point. However, an unrealised value of genetic markers is to identify high-risk groups that may be at risk for an aggressive disease trajectory and future liver-related morbidity and mortality. The association between genotype and accurate, quantitative assessments of NAFLD activity is needed to better characterise the impact of genotype on NAFLD severity. While the liver biopsy is the reference standard, it evaluates only a small portion of the liver and utilises ordinal categories to stage fibrosis. In contrast, magnetic resonance elastography (MRE) is an accurate,[16] reproducible,[17] continuous measure of liver stiffness, which is associated with fibrosis and has been recently demonstrated to predict liver-related outcomes.[18,19] Therefore, we hypothesised that variants in *PNPLA3, TM6SF2, MBOAT7, GCKR and HSD17B13* would be associated with advanced fibrosis on MRE and that quantifying the magnitude of the association may assist in the non-invasive risk stratification of NAFLD patients. Using a well-characterised cohort of patients at risk for NAFLD, we evaluated the association between *PNPLA3, TM6SF2, MBOAT7, GCKR and HSD17B13* genotype and advanced fibrosis on MRE as well as the quantitative impact of those variants on liver fibrosis on MRE.

2 | MATERIALS AND METHODS

2.1 | Study design

This is a cross-sectional analysis derived from a well-characterised prospective cohort of patients who had genotyping data and MRE assessment. This study included 264 uniquely phenotyped patients who underwent a standardised research visit: history, physical exam, *PNPLA3* genotyping and MRE assessment between 2011 and 2017 at the University of California, San Diego NAFLD Research Center.[20–24] All patients provided written informed consent prior to enrolling in the study and the study was approved by the UCSD Institutional Review Board.

2.2 | Inclusion and exclusion criteria

Patients ≥18 years of age with written informed consent were included. Participants meeting any of the following criteria were excluded from the study: significant alcohol consumption (defined as ≥14 drinks/week for men or ≥7 drinks/week for women) within the previous 2-year period; evidence of active substance use; clinical or laboratory evidence of secondary causes or chronic conditions associated with hepatic steatosis including nutritional disorders, human immunodeficiency virus infection, and use of steatogenic drugs such as amiodarone, glucocorticoids, methotrexate, l-asparaginase and valproic acid; underlying liver disease other than NAFLD including viral hepatitis (assessed with serum hepatitis B surface antigen and hepatitis C RNA assays), haemochromatosis, Wilson's disease, alpha-1 antitrypsin deficiency, glycogen storage disease, autoimmune hepatitis and cholestatic or vascular liver disease; major systemic illnesses; decompensated liver disease (defined as Child-Pugh score >7 points); contraindications to magnetic resonance imaging (MRI) including metallic implants, claustrophobia and body circumference exceeding the imaging chamber capacity; pregnancy or attempting to be pregnant; any other conditions believed by the principal investigator to affect patient's competence or compliance to complete the study.

2.3 | Clinical assessment and laboratory tests

All patients underwent a standardised clinical evaluation including a detailed history and a physical examination, which included vital signs, height, weight and anthropometric measurements, was performed by a trained clinical investigator. BMI 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 (AUDIT) and the Skinner questionnaire. Other causes of liver disease were systematically ruled out based on history and laboratory tests. Patients underwent the following biochemical tests: glucose, albumin, haemoglobin A1c, alanine aminotransferase, aspartate aminotransferase, total bilirubin, alkaline phosphatase, fasting lipid panel, platelets, insulin, international normalised ratio. FIB-4,[25] NAFLD Fibrosis Score[26] were calculated as described previously. Participants were instructed to fast for a minimum of 8 hours prior to collection of laboratory tests.

2.4 | *PNPLA3, TM6SF2, MBOAT7, GCKR and HSD17B13* genotyping

Whole-blood specimens collected during the research visit were used, and DNA was extracted using Qiagen's DNeasy® Blood & Tissue Kit. *PNPLA3, TM6SF2, MBOAT7, GCKR and HSD17B13* genotyping was performed in triplicate using single-nucleotide polymorphism assays from Applied Biosystems and analysed using Quant Studio.

2.5 | Magnetic resonance imaging

Abdominal MRI was obtained on a single 3 Tesla magnetic resonance scanner (GE Signa EXCITE HDxt, GE Healthcare) at the UCSD MR3T Research Laboratory using previously described methods.[27–31] MRI proton density fat fraction (MRI-PDFF) was used to measure hepatic steatosis and where NAFLD is defined as having an MRI-PDFF ≥5%. Liver stiffness was estimated using two-dimensional MRE, which is the most accurate biomarker for the quantitative assessment of liver stiffness as a surrogate for hepatic fibrosis.[32–35] A passive driver was fitted around the body over the liver and connected to an acoustic active driver that delivered continuous vibrations at 60 hertz to produce shear waves in the liver, which were processed to generate elastograms depicting liver stiffness. Four slices were assessed, and co-localised regions of interest were manually specified as previously described.[36]

2.6 | Definition of advanced fibrosis

Participants were considered to have advanced fibrosis if a liver stiffness of ≥3.63 kilopascals (kPa) was found on MRE. Previous studies have shown that ≥3.63 kPa on MRE provides an accuracy of 0.92 for the detection of advanced fibrosis, and it is the most accurate non-invasive test for the diagnosis of advanced fibrosis.[32–34]

2.7 | Outcome measures

The **primary outcome** was the assessment of the association of *PNPLA3* genotype with advanced fibrosis on MRE.

Secondary outcomes were assessing the association of *TM6SF2, MBOAT7, GCKR and HSD17B13* with advanced fibrosis on MRE, *PNPLA3, TM6SF2, MBOAT7, GCKR and HSD17B13* genotype on MRE as a continuous measure and the association between *PNPLA3* genotype and MRI-PDFF.

2.8 | Statistical analysis

We hypothesised that there would be an association between *PNPLA3* risk variants and advanced fibrosis on MRE. Power analysis performed assuming that 60% of the cohort had one or two risk variants and that the presence of risk variants was associated with four times higher odds of advanced fibrosis (5% in CC and 20% in CG/GG) showed that a sample size of 190 would provide 80% power with a two-tailed alpha of 0.05 and therefore, we had adequate power to test and confirm our hypothesis. For patient characteristics, an ANOVA was performed on continuous variables presented as mean (SD), Kruskal-Wallis performed on those presented as median (IQR). Chi-square or Fisher's exact test as appropriate on all categorical variables. Unadjusted logistic regression analyses were conducted to assess the odds ratio (OR) of various factors on the presence of advanced fibrosis (MRE ≥3.63).

Moreover, the association between genotype and additional MRE cut points, 3.0 and 5.0 kPa was evaluated by logistic regression. In addition, logistic regression analyses, multivariable-adjusted for age, sex, BMI, diabetes mellitus (DM) and FIB-4, were conducted to assess the OR of five genetic loci on the presence of advanced fibrosis (MRE ≥3.63). Furthermore, linear regression was performed with MRE as a continuous measure as the outcome and each of the five genetic loci as the predictor. Interaction terms between age, BMI and *PNPLA3* genotype were evaluated by adding cross-product terms to regression models. All statistical analyses were performed using SAS 9.4 (SAS Institute), and a *P* value less than 0.05 was considered statistically significant.

3 | RESULTS

3.1 | Characteristics of the study population

Two hundred sixty-four patients with genotype data and MRE were included. Participants had a mean age of 53 (±17) years and were predominantly female (63%); 55% white and 31% Hispanic ethnicity. The risk allele frequency for *PNPLA3, TM6SF2, MBOAT7 and GCKR* was 0.42 G allele, 0.06 T allele, 0.40 T allele and 0.43 T allele respectively. The protective TA allele frequency in *HSD17B13* was 0.18. The mean BMI was 29 (±7) kg/m^2. One hundred and twenty-two (46%) had NAFLD (MRI-PDFF ≥5%) and 34 (13%) had advanced fibrosis (MRE ≥3.63 kPa). (Table 1) A greater number of *PNPLA3* risk alleles were associated with Hispanic ethnicity (G allele frequency 0.66 vs 0.30 in non-Hispanics $P < 0.01$), higher BMI ($P = 0.01$), increased family history of liver disease ($P < 0.01$) and higher alanine aminotransferase (ALT) ($P = 0.02$), aspartate aminotransferase (AST) ($P = 0.01$), alkaline phosphatase ($P = 0.04$), homeostatic model assessment method for insulin resistance (HOMA-IR) ($P < 0.01$), triglycerides ($P = 0.0353$) and high-d ensity lipoprotein cholesterol (HDL) ($P = 0.0263$) (Table 1). The mean (±SD) MRI-PDFF values for CC, CG and GG genotypes were 5.8% (±5.8), 7.6% (±7.9) and 11.1% (±8.8), respectively, $P < 0.01$, and the Mean (±SD) MRE values for CC, CG and GG genotypes were 2.4 kPa (±0.6), 2.9 kPa (±1.3) and 3.3 kPa (±2.2), respectively, $P < 0.01$ (Figure 1).

3.2 | Factors associated with advanced fibrosis on MRE

Higher AST (OR = 1.05 [95% CI: 1.02–1.07, $P < 0.01$]), ALT (OR = 1.01 [95% CI: 1.00–1.03, $P = 0.02$]), alkaline phosphatase (OR = 1.02 [95% CI: 1.01–1.04, $P < 0.01$]), total bilirubin (OR = 6.60 [95% CI: 2.9–15.0, $P < 0.01$]) and HOMA-IR (OR = 1.07 [95% CI: 1.03–1.12, <0.01]) were associated with advanced fibrosis. Lower albumin (OR = 0.09 [95% CI: 0.03–0.29, $P < 0.01$]), HDL (OR = 0.96 [95% CI: 0.94–0.99, $P < 0.01$]), LDL-cholesterol (OR = 0.98 [95% CI: 0.963–0.990, $P < 0.01$]) and platelet count (OR = 0.98 [95% CI: 0.97–0.99, $P < 0.01$]) were associated with advanced fibrosis (Table 2).

In addition, a one-unit increase in FIB-4 (OR = 1.62 [95% CI: 1.3–2.0, $P < 0.01$]) and NAFLD Fibrosis Score (OR = 2.29 [95% CI: 1.7–3.0, $P < 0.01$]) was associated with higher odds of advanced fibrosis. The number of *PNPLA3* risk variants was strongly associated with advanced fibrosis on MRE, (OR = 3.1 [95% CI: 1.1–8.9, $P = 0.04$]) for CG and (OR = 6.5 [95% CI: 2.2–18.9, $P < 0.01$]) for GG compared to CC. The association between *PNPLA3* risk variants and advanced fibrosis on MRE persisted in models for age and sex

(OR = 3.1 [95% CI: 1.1–9.4, $P = 0.04$]) for CG and (OR = 7.4 [95% CI: 2.4–22.7, $P < 0.01$]) for GG compared to CC. Furthermore, *PNPLA3* genotype was associated with advanced fibrosis despite adjustment for age, sex, BMI and FIB-4 score (OR = 2.94 [95% CI: 1.06–8.14, $P = 0.04$]) (Table 3). In addition, adjusted analysis PNPLA3 risk alleles remained associated with advanced fibrosis in a model adjusted for age, sex and DM (OR = 3.21 [95% CI: 3.21–9.61, $P = 0.04$]) for CG and (OR = 7.56 [95% CI: 2.45–23.3, $P < 0.01$]) for GG compared to CC. Finally, on analysis stratified by Hispanic ethnicity the association between PNPLA3 genotype and liver stiffness on MRE in the Hispanic population (N = 81) did not meet the threshold for statistical significance with wide confidence intervals, however, CG/GG genotype was associated with increased liver stiffness compared to CC in an analysis restricted to the non-Hispanic population (Table S1).

Risk variants in TM6SF2, MBOAT7 and GCKR did not have a statistically significant association with advanced fibrosis (Table S2), but the point estimates were greater than 1 compared when compared to no risk variants. The protective HSD17B13:TA variant was protective against advanced fibrosis (OR = 0.337 [95% CI: 0.124–0.915], $P = 0.0329$) compared to the referent genotype TT. Fifty-nine and nineteen patients had a liver stiffness ≥3.0 and 5.0 kPa respectively. In sensitivity analyses for lower (3.0 kPa) and higher cut points (5.0 kPa), indicating moderate fibrosis and cirrhosis with a high risk of decompensation, respectively, *PNPLA3* GG genotype remained associated with moderate fibrosis at the 3.0 kPa cut-point compared to CC, however, other risk variants did not meet the threshold of statistical significance (Table S3). When evaluating the higher cut-point of 5.0 kPa, no *PNPLA3* CC (referent) patients had liver stiffness ≥5.0 kPa limiting the analysis for that genotype. The *HSD17B13* TA variant remained significantly protective, albeit with only one patient with the TA allele with liver stiffness ≥5.0 kPa (Table S4).

3.3 | Quantitative impact of *PNPLA3* risk variants on hepatic steatosis and fibrosis on MRI-PDFF and MRE

Each *PNPLA3* risk variant copy was associated a 2.56% (95% CI: 1.39–3.72, $P < 0.01$) increase in hepatic steatosis on MRI-PDFF on linear regression and remained similar in multivariable analysis adjusted for age, sex and BMI, 2.11% (95% CI: 1.02–3.21, $P < 0.01$). Each *PNPLA3* risk variant copy was associated a 0.45 kPa (95% CI: 0.23–0.66, $P < 0.01$) increase in liver stiffness on MRE on linear regression and remained similar in multivariable analysis adjusted for age, sex and BMI, 0.40 kPa (95% CI: 0.19–0.61, $P < 0.01$). Graphical representation of the interaction between *PNPLA3* genotype and BMI suggested demonstrated that higher BMI category combined with increased risk variants increased liver stiffness (Figure 2), however, the *P*-value for an interaction term between BMI and *PNPLA3* genotype was not statistically significant, ($P = 0.40$). An interaction for age and PNPLA3 genotype was significant, ($P = 0.002$) and demonstrates similar liver stiffness in patients <50 regardless of *PNPLA3* genotype. However, in populations age 50–64 years and ≥65 years *PNPLA3* genotype is strongly associated with increased liver stiffness (Figure 3) demonstrating the value of incorporating genotype into the frequency of monitoring particularly as patients age.

3.4 | Quantitative impact of *TM6SF2, MBOAT7, GCKR and HSD17B13* variants on fibrosis on MRE

Risk variants in *TM6SF2, MBOAT7 and GCKR* did not have a statistically significant association with liver stiffness on MRE on linear regression, however, the point estimates were greater than 0; 0.12 (95% CI: −0.45 to 0.69, $P = 0.6705$), 0.14 (95% CI: −0.1 to 0.39, $P = 0.2557$) and 0.14 (95% CI: −0.11 to 0.39, $P = 0.282$). On multivariable analysis adjusted for age, sex and BMI, each *TM6SF2, MBOAT7 and GCKR* risk variant copy did not have a statistically significant association with liver stiffness on MRE; 0.11 (95% CI: −0.44 to 0.66, $P = 0.6814$), 0.14 (95% CI: −0.1 to 0.38, $P = 0.2495$) and 0.21 (95% CI: −0.03 to 0.45, $P = 0.092$) respectively. The protective TA allele in *HSD17B13* was less common in Hispanic compared to non-Hispanic populations (minor allele frequency 0.09 vs 0.23, $P < 0.01$) and was associated with a −0.40 (95% CI: −0.77 to −0.02, $\mathbf{P = 0.0387}$) decrease in liver stiffness on MRE on unadjusted linear regression and on multivariable analysis adjusted for age, sex and BMI was associated with a −0.41 (95% CI: −0.76 to −0.05, $\mathbf{P = 0.0263}$) decrease in liver stiffness on MRE.

4 | DISCUSSION

Using a diverse, well-characterised cohort of patients with *PNPLA3* genotype and MRE assessment, we demonstrate that *PNPLA3* risk alleles are strongly associated with advanced fibrosis and each *PNPLA3* risk allele is associated with a 0.40 kPa increase in liver stiffness on MRE adjusting for age, sex and BMI. This strong association quantifies the impact of *PNPLA3* genotype on liver fibrosis in patients at risk for NAFLD. Conversely, the protective TA variant in *HSD17B13* is associated with a −0.41 kPa decrease in liver stiffness on MRE on adjusted analysis. In the context of previously described cut-points the difference between CC and GG genotype could translate to the difference between minimal fibrosis (stage 0–1) and advanced fibrosis (stage 3–4). Importantly, a strong interaction between age and PNPLA3 genotype reveals the potential clinical utility of genotype on identifying patients at risk for a more aggressive disease trajectory. Currently, liver biopsy or non-invasive tests, including liver stiffness measurements, are used to determine the frequency of monitoring and choice of intervention in patients with NAFLD. However, we demonstrate that the presence of *PNPLA3* risk variants is associated with a divergent and more aggressive disease trajectory in patients 50 years or older, which may warrant closer monitoring or early intervention.

4.1 | In context with published literature

The severity of fibrosis is the strongest predictor of outcomes in patients with NAFLD.[37] Generally, clinical prediction rules are used for the high negative predictive value to identify patients at low risk for advanced fibrosis who require only limited monitoring,[38] however, a subset of patients can progress rapidly from minimal to advanced fibrosis.[39] Importantly paired biopsy studies have demonstrated that 40%−50% of patients with NAFLD without NASH can progress to NASH on a subsequent biopsy, which is associated with progression to significant fibrosis.[40] The underlying factors associated with a transition to more severe disease and the optimal strategy to monitor these patients is undefined.

The p.I148M-variant in *PNPLA3* is the most well-characterised common variant in NAFLD and is associated with the full spectrum of disease in NAFLD including hepatic steatosis,[6] NASH,[8,41] advanced fibrosis[7] and hepatocellular carcinoma.[10] Furthermore, a population-based study of the National Health and Nutrition Examination Survey demonstrated that homozygosity with the risk allele is associated with a >8 fold increased hazard ratio for liver-related death.[42] Stender and colleagues evaluated the quantitative impact of *PNPLA3* risk variants on hepatic steatosis using magnetic resonance spectroscopy and described an important interaction between adiposity and genotype that impacts hepatic steatosis and the risk of cirrhosis.[43] While previous studies demonstrated an association with fibrosis, an accurate continuous measure of liver fibrosis is required to quantify the impact of risk variants on liver fibrosis. Krawczyk et al, demonstrated an association between *PNPLA3* genotype and liver stiffness on vibration-controlled transient elastography (VCTE), however, VCTE has inferior diagnostic accuracy and repeatability[44] when compared to MRE, limiting its precision to quantify the impact of risk variants on liver fibrosis. Furthermore, their study cohort primarily consisted of patients with viral hepatitis with only 7.1% with NAFLD. This study utilised MRE, the most accurate, non-invasive, continuous marker of liver fibrosis in NAFLD. Furthermore, we demonstrated that the association between genotype and advanced fibrosis persisted despite adjustment for FIB-4 and quantified the association between genotype and hepatic steatosis using MRI-PDFF. Importantly, we demonstrated a strong interaction between age, *PNPLA3* risk variants and fibrosis on MRE that began to diverge at age 50. Moreover, all patients with a very high liver stiffness, ≥5.0 kPa, carried at least one copy of the *PNPLA3* risk variant, further underscoring its importance as a major risk factor for progressive liver fibrosis. Taken together, closer monitoring may be warranted for patients with *PNPLA3* risk allele, even if they are low-risk based on non-invasive biomarker assessment, particularly when age 50 years or older.

Furthermore, we also evaluated additional genetic loci associated with NAFLD severity including *TM6SF2, MBOAT7, GCKR and HSD17B13*. Each risk allele had a point estimate consistent with a minor, non-significant impact on liver stiffness on MRE. However, the protective TA variant in *HSD17B13* had a strong protective association on liver stiffness on MRE of similar magnitude to *PNPLA3*. To date, studies have focused on assessing the protective effect of *HSD17B13* on aminotransferases[15,45] or semi-quantitative measures on liver biopsy.[46] This study demonstrates the magnitude of the variant on well-phenotyped patients using the most accurate, continuous biomarker of liver fibrosis, which can be used in future polygenic assessments of fibrosis risk.

4.2 | Strengths and limitations

Although this study evaluates a diverse, prospectively recruited well-phenotyped cohort at risk for NAFLD, certain limitations merit acknowledgment. First, this is a cross-sectional study and to better evaluate the impact of genotype on transitioning to higher risk NAFLD, longitudinal studies will be required. However, the association between risk alleles and liver fibrosis persisted despite multivariable adjustment and we demonstrated a strong interaction between *PNPLA3* genotype and age. Second, this is a single-centre study, and our sample size limited the ability to evaluate for interactions with obesity. However, despite being a single-centre study, our population was diverse with approximately one-third of patients of

Hispanic ethnicity. Finally, we evaluated five loci with prior studies demonstrating a strong association with NAFLD. Additional, loci including *NCAN*,[47] *PPP1R3B*[48] and *LYPLAL1*[49] were not assessed. Despite this, few studies have evaluated the five included loci in patients with a detailed NAFLD assessment. Further, multi-centre studies utilising MRE may be required to better evaluate if a significant interaction between age, obesity, genetic risk and liver fibrosis in NAFLD and quantify a smaller impact of risk alleles in *TM6SF2, MBOAT7* and *GCKR*.

4.3 | Implications for future research

This study quantifies the impact of *PNPLA3* genotype on liver fibrosis and demonstrates a strong association that persists despite adjustment for FIB-4 score. The clinical utility of genotyping for common variants, including *PNPLA3*, will be to identify patients at risk for a more aggressive disease trajectory. Rather than basing ongoing monitoring and interventions on a clinical prediction rule or liver stiffness measurement, consideration of genetic risk may focus interventions on patients with mild disease who are at risk for progression, particularly as they age beyond 50 years. *HSD17B13* genotype may ameliorate the genetic risk of liver fibrosis with a similar magnitude as *PNPLA3* risk variants increase risk and should be incorporated into a genetic risk assessment. In addition, emerging evidence suggests that *PNPLA3* genotype may be associated with a differential response to certain treatments and *PNPLA3* expression may represent a target for future pharmacotherapy of affected individuals.[50]

Currently, clinical trials of therapeutic agents in NAFLD must account for the heterogeneous trajectory of NASH including an up to 30% placebo response rate, which mandates larger sample sizes with substantially higher associated costs. Through the assessment of genetic risk, clinical trials can enrich populations at greatest risk for disease progression. By utilising an accurate, continuous biomarker of fibrosis, this study quantifies the effect of risk and protective variants and provides regression coefficients that may be incorporated into the calculation of polygenic risk. For example an individual who is *PNPLA3* G/G, *HSD17B13* T/TA may be intermediate risk and comparable to *PNPLA3* C/G, *HSD17B13* T/T. As regulatory bodies in Europe and the United States have emphasised improvement in fibrosis as a key endpoint, assessment of polygenic risk for fibrosis may substantially improve the trial design.

Future studies will need to incorporate polygenic risk and the interaction with lifestyle factors to better classify the risk of liver-related and all-cause mortality in patients at risk for NAFLD. In conclusion, our study demonstrates that *PNPLA3* genotype is associated with liver fibrosis assessed on MRE and that the impact of being homozygous for the risk allele compared to wild-type could translate into the difference between minimal/no fibrosis and advanced fibrosis. Importantly, the impact of *PNPLA3* risk alleles becomes pronounced in patients 50 years or older. These data require further validation in longitudinal multicentre studies to identify if *PNPLA3* genotype can predict patients at risk for a more aggressive disease trajectory and if protective variants in *HSD17B13* minimise that risk.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENT

Declaration of personal interests: Potential conflict of interest for Rohit Loomba: Dr Loomba serves as a consultant or advisory board member for Bird Rock Bio, Celgene, Enanta, GRI Bio, Madrigal, Metacrine, NGM, Sanofi, Arrowhead Research, Galmed, NGM, GNI, NovoNordisk, Merck, Siemens, Pfizer, Gilead. Glympsebio, In addition, his institution has received grant support from Allergan, BMS, BI, Daiichi-Sankyo Inc, Eli-Lilly, Galectin, Galmed, GE, Genfit, Intercept, Janssen Inc, Madrigal, Merck, NGM, Pfizer, Prometheus, Siemens, and Sirius. He is also co-founder of Liponexus Inc

Funding information

RL receives additional funding support from NIEHS (5P42ES010337), NCATS (5UL1TR001442), and NIDDK (R01DK106419 and P30DK120515) and DOD PRCRP (CA170674P2). VA is supported by NIDDK (K23DK119460). DD was supported by an ACTRI/NIH grant KL2TR001444, the San Diego Digestive Diseases Research Center (NIH DK120515) and the Southern California Research Center for ALPD and Cirrhosis funded by the National Institute on Alcohol Abuse and Alcoholism of the National Institutes of Health under Award Number 5P50AA011999.

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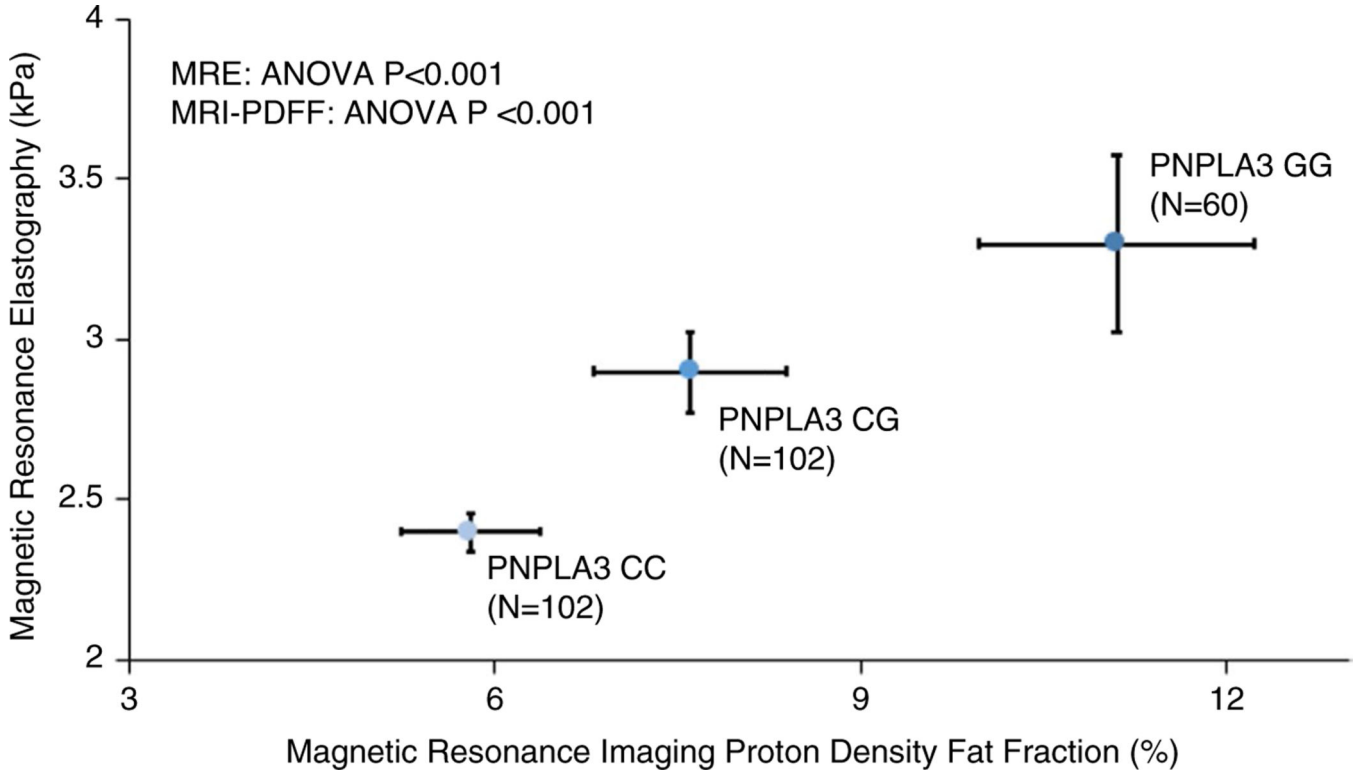

FIGURE 1.

PNPLA3 risk alleles increase both mean liver stiffness of magnetic resonance elastography and liver fat on magnetic resonance imaging proton density fat fraction shown with standard error bars

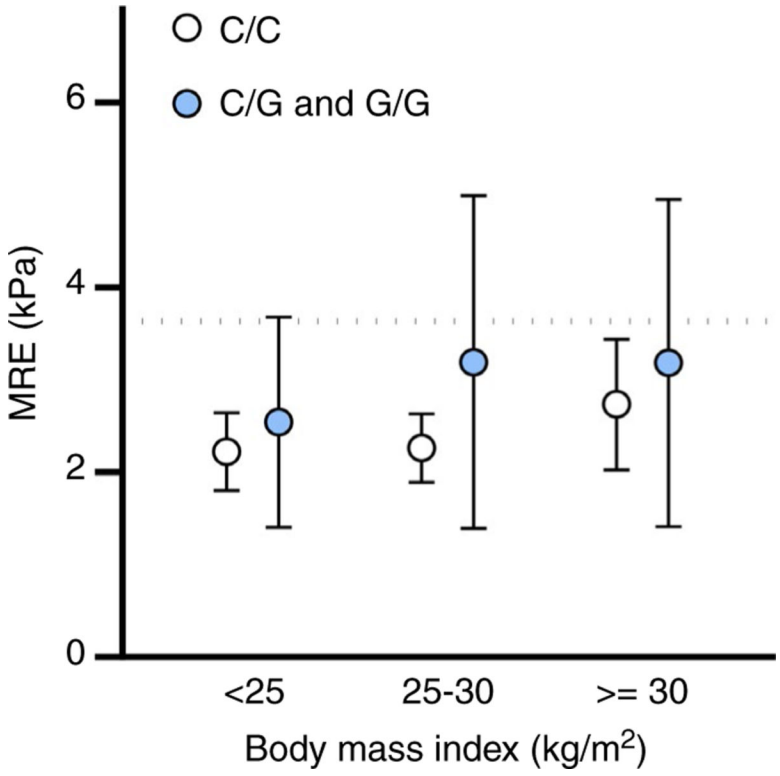

FIGURE 2.
Liver stiffness on magnetic resonance elastography by *PNPLA3* genotype and body mass index

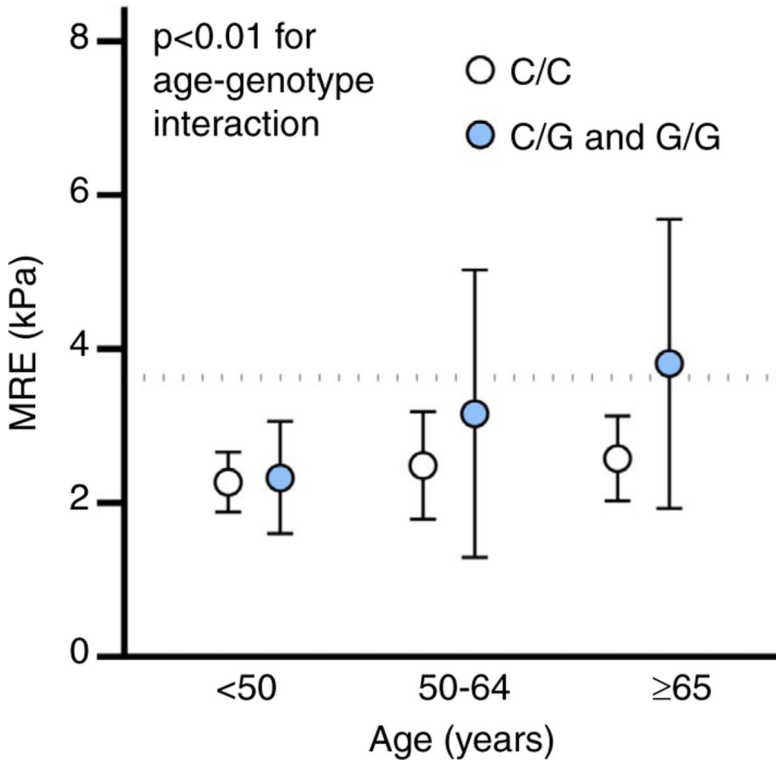

FIGURE 3.
Liver stiffness on magnetic resonance elastography by *PNPLA3* genotype and age

TABLE 1

Clinical, demographic and imaging characteristics by PNPLA3 genotype

PNPLA3 genotypes	**Total N**	**C/C N = 102**	**C/G N = 102**	**G/G N = 60**	***P***
Demographic					
Age in years, mean (SD)	263	52.8 (17.0)	53.4 (17.2)	53.2 (16.5)	0.9727
Male, n (%)	264	41 (40.2%)	34 (33.3%)	22 (36.7%)	0.5964
BMI (kg/m^2), mean (SD)	256	28.9 (6.6)	28.7 (7.9)	32.0 (5.4)	0.0103
White, n (%)	260	73 (71.6%)	56 (56.0%)	14 (24.1%)	<0.0001
Diabetes, n (%)	264	48 (47.1%)	43 (42.2%)	25 (41.7%)	0.7188
Hypertension, n (%)	264	46 (45.1%)	35 (34.3%)	27 (45.0%)	0.2241
Hyperlipidaemia, n (%)	176	11 (19.3%)	15 (20.8%)	11 (23.4%)	0.8763
Family history					
Liver disease, n (%)	176	5 (8.8%)	19 (26.4%)	26 (55.3%)	<0.0001
Biochemical profile					
HbA1c (%), median (IQR)	259	6(1.3)	5.9 (0.9)	6 (1.4)	0.5073
AST (U/L), median (IQR)	262	20.5 (8)	24 (13)	23 (20)	0.0147
ALT (U/L), median (IQR)	261	19 (14)	24.5 (19)	26 (22)	0.0201
Alkaline phosphatase (U/L), median (IQR)	262	71 (26)	73 (37)	79 (42)	0.0433
Total bilirubin (mg/dL), median (IQR)	262	0.5 (0.3)	0.4 (0.3)	0.5 (0.3)	0.0791
Albumin (g/dL), median (IQR)	262	4.5 (0.3)	4.5 (0.4)	4.4 (0.4)	0.1288
HOMA-IR median (IQR)	248	2.7 (3)	3.2 (4.3)	5.5 (10.4)	<0.0001
Triglycerides (mg/dL), median (IQR)	260	96 (75)	102 (103)	125 (72.5)	0.0353
HDL (mg/dL), median (IQR)	260	60 (27)	55 (28)	50 (18.5)	0.0263
LDL (mg/dL), median (IQR)	259	99 (42)	96 (43)	106 (53)	0.1444
Platelet count (10^9/L), median (IQR)	262	239.5 (84)	236.5 (92.5)	238.5 (117.5)	0.8124
INR, median (IQR)	262	1 (0.1)	1 (0.1)	1 (0.1)	0.2528
Clinical prediction rule					
FIB-4, median (IQR)	259	1 (0.7)	1.1 (1)	1.1 (1.2)	0.5398
NAFLD Fibrosis Score, median (IQR)	252	−1.3 (1.8)	−1.8 (2.6)	−1.2 (3.6)	0.7150
Imaging					
MRI-PDFF (%), mean (SD)	264	5.8 (5.8)	7.6 (7.9)	11.1 (8.8)	<0.0001

PNPLA3 genotypes	Total N	C/C N = 102	C/G N = 102	G/G N = 60	*P*
MRE (kPa), mean (SD)	264	2.4 (0.6)	2.9 (1.3)	3.3 (2.2)	0.0003

Note: ANOVA performed on continuous variables presented as mean (SD), Kruskal-Wallis performed on all other continuous variables. Chi-square or Fisher's exact test as appropriate on all categorical variables.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; FIB-4, fibrosis index based on the 4 factor; HbA1c, haemoglobin A1c; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment method for insulin resistance (calculated as (fasting insulin (μU/mL) * fasting glucose (mmol/L))/22.5); INR, International normalised ratio; IQR, interquartile range; LDL, low-density lipoprotein; MRE, magnetic resonance elastography; MRI-PDFF, magnetic resonance imaging proton density fat fraction; SD, standard deviation.

TABLE 2

Factors associated with advanced fibrosis on MRE

	Advanced fibrosis on MRE (≥3.63) OR (95% CI)	*P*-value
PNPLA3 genotype		
CC (ref)	1	
CG	3.086 (1.068–8.918)	0.0374
GG	6.467 (2.214–18.892)	0.0006
Demographic & biochemical		
BMI (kg/m^2)	1.030 (0.981–1.081)	0.2398
HbA1c (%)	1.134 (0.888–1.446)	0.3135
AST (U/L)	1.046 (1.024–1.068)	<0.0001
ALT (U/L)	1.014 (1.002–1.026)	0.0247
Alkaline phosphatase (U/L)	1.024 (1.012–1.036)	<0.0001
Total bilirubin (mg/dL)	6.604 (2.917–14.951)	<0.0001
Albumin (g/dL)	0.094 (0.031–0.286)	<0.0001
HOMA-IR	1.071 (1.026–1.118)	0.0016
Triglycerides (mg/dL)	1.003 (0.999–1.007)	0.1405
HDL (mg/dL)	0.960 (0.935–0.986)	0.0024
LDL (mg/dL)	0.977 (0.963–0.990)	0.0007
Platelet count (10^9/L)	0.977 (0.970–0.985)	<0.0001
Clinical prediction rules		
FIB-4	1.619 (1.291–2.031)	<0.0001
NAFLD Fibrosis Score	2.287 (1.725–3.032)	<0.0001
Imaging		
MRI-PDFF	0.992 (0.944–1.042)	0.7357

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; FIB-4, fibrosis index based on the 4 factor; HbA1c, haemoglobin A1c; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment method for insulin resistance (calculated as (fasting insulin (μU/mL) * fasting glucose (mmol/L))/22.5); INR, international normalised ratio; LDL, low-density lipoprotein; MRI-PDFF, magnetic resonance imaging proton density fat fraction.

TABLE 3

Multivariable models assessing the association of PNPLA3 genotype with advanced fibrosis

	Advanced fibrosis on MRE (≥3.63) OR (95% CI)	*P*-value
Model 1		
CC (ref)	1	
CG	3.120 (1.051–9.383)	0.0405
GG	7.380 (2.405–22.650)	0.0005
Model 2: Model 1 + BMI		
CC (ref)	1	
CG	3.154 (1.059–9.395)	0.0391
GG	6.829 (2.176–21.428)	0.0010
Model 3: Model 2 + FIB 4		
CC (ref)	1	
CG	2.417 (0.783–7.456)	0.1247
GG	5.257 (1.616–17.104)	0.0058
Model 4: Model 1 + DM		
CC (ref)	1	
CG	3.205 (1.068–9.614)	0.0377
GG	7.564 (2.452–23.334)	0.0004

Note: Model 1: Adjusted for age and sex.

Abbreviations: BMI, body mass index; DM, diabetes mellitus; FIB 4, fibrosis index based on the 4 factor; MRE, magnetic resonance elastography.