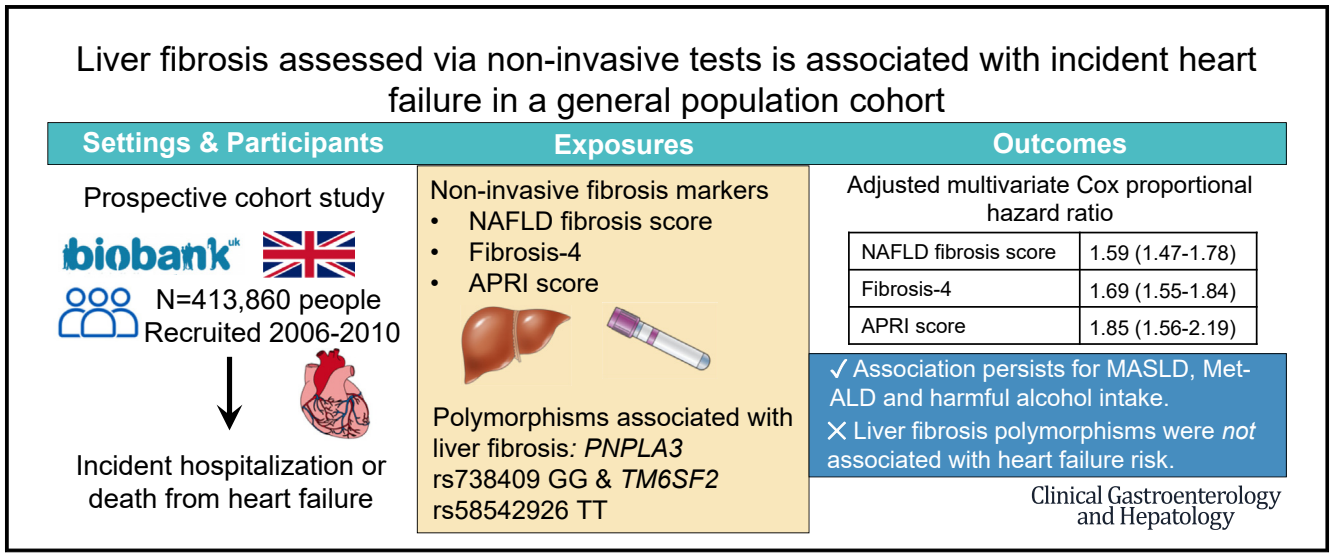
Liver Fibrosis Assessed Via Noninvasive Tests Is Associated With Incident Heart Failure in a General Population Cohort

Theresa J. Hydes,[1,2,3,*] Oliver J. Kennedy,[4,*] Kate Glyn-Owen,[4] Ryan Buchanan,[4,5] Julie Parkes,[4] Daniel J. Cuthbertson,[1,2,3] Paul Roderick,[4,§] and Christopher D. Byrne[5,6,§]

[1]*Department of Cardiovascular and Metabolic Medicine, Institute of Life Course and Medical Sciences, Faculty of Health & Life Sciences, University of Liverpool, Liverpool, United Kingdom;* [2]*University Hospital Aintree, Liverpool University Hospitals NHS Foundation Trust, Liverpool, United Kingdom;* [3]*Liverpool Centre for Cardiovascular Science, University of Liverpool, Liverpool, United Kingdom;* [4]*Primary Care, Population Sciences and Medical Education, Faculty of Medicine, University of Southampton, Southampton, United Kingdom;* [5]*Southampton National Institute for Health and Care Research, Biomedical Research Centre, University Hospital Southampton, Southamptom, United Kingdom; and* [6]*Nutrition and Metabolism, Human Development and Health, Faculty of Medicine, University of Southampton, Southampton, United Kingdom*



BACKGROUND & AIMS: **The aim of this study was to determine whether liver fibrosis is associated with heart failure in a general population cohort, and if genetic polymorphisms (*PNPLA3* rs738409; *TM6SF2* rs58542926), linked to increased risk of liver fibrosis and decreased risk of coronary artery disease, modify this association.**

METHODS: **Using UK Biobank data, we prospectively examined the relationship between noninvasive fibrosis markers (nonalcoholic fatty liver disease [NAFLD] fibrosis score [NFS], Fibrosis-4 [FIB-4] and aspartate transaminase [AST] to platelet ratio index [APRI]) and incident hospitalization/death from heart failure (n = 413,860). Cox-regression estimated hazard ratios (HRs) for incident heart failure. Effects of *PNPLA3* and *TM6SF2* on the association between liver fibrosis and heart failure were estimated by stratifying for genotype and testing for an interaction between genotype and liver fibrosis using a likelihood ratio test.**

*Authors share co-first authorship §Authors share co-senior authorship.

Abbreviations used in this paper: ACS, acute coronary syndrome; ALD, alcohol-related liver disease; APRI, AST platelet ratio index; AST, Aspartate aminotransferase; CI, confidence interval; CVD, cardiovascular disease; FIB-4, Fibrosis-4; HR, hazard ratio; ICD, International Classification of Diseases; IHD, ischemic heart disease; MASLD, metabolic dysfunction-associated steatotic liver disease; Met-ALD, metabolic alcoholic liver disease; MRI, magnetic resonance imaging; NAFLD, nonalcoholic fatty liver disease; NIT, noninvasive test; NFS, NAFLD fibrosis score; SNP, single nuclear polymorphism; T2D, type 2 diabetes; UKBB, UK Biobank.

Most current article

1542-3565
https://doi.org/10.1016/j.cgh.2024.03.045

RESULTS: **A total of 12,527 incident cases of heart failure occurred over a median of 10.7 years. Liver fibrosis was associated with an increased risk of hospitalization or death from heart failure (multivariable adjusted high-risk NFS score HR, 1.59; 95% confidence interval [CI],1.47-1.76; *P* < .0001; FIB-4 HR, 1.69; 95% CI, 1.55-1.84; *P* < .0001; APRI HR, 1.85; 95% CI, 1.56-2.19; *P* < .0001; combined fibrosis scores HR, 1.90; 95% CI, 1.44-2.49; *P* < .0001). These associations persisted for people with metabolic dysfunction-associated steatotic liver disease (MASLD), MASLD with alcohol consumption (Met-ALD), and harmful alcohol consumption. *PNPLA3* rs738409 GG and *TM6SF2* rs58542926 TT did not attenuate the positive association between fibrosis markers and heart failure. For *PNPLA3*, a statistically significant interaction was found between *PNPLA3* rs738409, FIB-4, APRI score, and heart failure.**

CONCLUSION: **In the general population, serum markers of liver fibrosis are associated with increased hospitalization/death from heart failure. Genetic polymorphisms associated with liver fibrosis were not positively associated with elevated heart failure risk.**

Keywords: Cirrhosis; Fibrosis; Heart Failure.

In addition to adverse liver-related outcomes, liver disease is a significant risk factor for adverse cardiovascular outcomes.[1] In those with metabolic dysfunction-associated steatotic liver disease (MASLD), the most common etiology of chronic liver disease, cardiovascular disease (CVD) is the leading cause of mortality independent of shared cardiometabolic risk factors.[2,3] Higher alcohol consumption is also associated with an elevated risk of CVD, with a roughly linear association with increased risk of stroke, coronary disease (excluding myocardial infarction), heart failure, and hypertensive heart disease.[4]

Liver fibrosis is a key histological stage associated with adverse liver-related outcomes for all etiologies of chronic liver disease.[5,6] Liver fibrosis is associated with extrahepatic complications, in particular CVD,[3,7] and a higher all-cause mortality.[8] This association with CVD occurs irrespective of whether liver fibrosis is determined via noninvasive tests (NITs)[7] or histologically.[3] In a prospective, outpatient cohort of 898 people with nonalcoholic fatty liver disease (NAFLD), elevated Fibrosis-4 (FIB-4), and NAFLD fibrosis scores (NFS) were independently associated with incident cardiovascular events.[7] Furthermore, in a Swedish population-based cohort (n = 10,422), rates of incident major adverse cardiac events increased progressively with worsening histological NAFLD severity.[3] However, in 603 patients with biopsy-proven NAFLD and 6269 age/sex matched controls, although patients with NAFLD were more likely to experience a CVD event (hazard ratio [HR], 1.54), histological fibrosis stage was not predictive factor.[9] Furthermore, in a large Danish population study performing Mendelian randomization and meta-analysis, the gene variant encoding phospholipase domain-containing 3 (*PNPLA3*), I148M (rs738409), associated with increased liver fat content and fibrosis, was not casually associated with ischemic heart disease (IHD)[10]; and in a large (>300,000 individuals) exome-wide association study was protective against IHD.[11]

Few studies have examined the relationship between liver fibrosis and heart failure. This question is highly pertinent, from both a clinical and pathophysiological perspective, given the rising incidence of liver fibrosis and high morbidity, mortality, and economic burden associated with heart failure. Although a link between NAFLD and heart failure has been confirmed in a recent meta-analysis of 11 cohort studies (pooled random-effects HR, 1.50; 95% confidence interval [CI], 1.34-1.67),[12] the association between liver fibrosis and heart failure in a general population with mixed risk factors for liver disease is unknown.[13]

Multiple gene-wide association studies and candidate gene studies have identified an association between single nuclear polymorphisms (SNPs) in *PNPLA3* (rs738409 c.444 C>G, encoding Ile148Met [I148M]) and transmembrane 6 superfamily member 2 (*TM6SF*2) (rs58542926 c.449 C>T, p.Glu167Lys, E167K)) with more advanced liver disease, particularly fibrosis. People with the GG homozygous variant for *PNPLA3* and the TT homozygous variant for *TM6SF2* are at the highest risk of liver fibrosis. This association is valid for multiple etiologies of chronic liver disease including NAFLD,[13,14] alcohol-related liver disease (ALD),[15] and chronic hepatitis C.[16] Considering the paradox whereby the SNPs rs738409 (*PNPLA3)* and rs58542926 (*TM6SF2*) are associated with liver fibrosis, and yet confer protection against CVD,[11] the relationship between these genetic variants and incident heart failure is important. These data would help establish whether any causal relationship exists between liver and cardiac fibrosis and would influence clinical practice.

Using data from the UK Biobank (UKBB), in a general population cohort with mixed etiologies of liver disease, we aimed to examine the relationship between liver fibrosis, determined via NITs used in routine clinical practice, and incident heart failure. Secondly, we aimed to test whether there was any influence or interaction between genetic variants of *PNPLA3* and *TM6SF2* with liver fibrosis scores on incident heart failure (ie, can alleles associated with elevated risk of liver fibrosis, and lower risk of CVD, modify the risk of incident heart failure associated with liver fibrosis).

Methods

Study Population

The UKBB is a longitudinal, prospective study aimed at improving and preventing chronic disease through the identification of genetic and behavioral determinants of health in middle-aged and older adults.[17] Over 500,000 individuals, aged 40 to 69 years, identified from National Health Service registers were recruited between 2006 and 2010. At a baseline assessment visit, participants completed a questionnaire about their sociodemographic, lifestyle, and medical history. Self-reported doctor diagnosed medical conditions and medication history were verified and coded by nurses.[18] Anthropometric measurements were taken, and non-fasting blood and urine samples were collected. UKBB participants gave their consent to be continually followed-up through linkage to electronic health records, including records from the Office for National Statistics and the Registrar General's Office (death records and cancer registers) in addition to hospital records, held by the Department of Health's Hospital Episode Statistics and the Scottish Morbidity Records (http://content.digital.nhs.uk/services). At the time of analysis, mortality data were available up to January 2023. This investigation conforms with the principles outlined in the Declaration of Helsinki.[19] Ethical approval was granted by the North-west Multi-Centre Research Ethics Committee.

Inclusion and Exclusion Criteria

All UK Biobank participants were initially included. Individuals with a baseline International Classification of Diseases (ICD) or self-reported UKBB code for heart failure (Supplementary Methods), and those for whom there were insufficient data to calculate NITs for liver fibrosis, were excluded.

Ascertainment of Exposure

Advanced fibrosis was defined as a FIB-4[20] >2.67, NFS[21] >0.676, or aspartate transaminase (AST) to platelet ratio index (APRI)[22] ≥ 1.0. Absence of advanced fibrosis was defined as FIB-4 <1.3 (<2.0 if ≥ 65 years), NFS <-1.455 (<0.12 if ≥ 65 years) or APRI <1.0. Participants not falling into these groups were placed in an 'intermediate' fibrosis group.

Primary Outcome

The primary outcome was incident heart failure, defined as an ICD-9 (428.0-428.9) or ICD-10 (I50.0-I50.9) code for a new diagnosis of heart failure, or heart failure given as the primary/secondary cause of death on the death certificate. Fatal and non-fatal events were included. Where a participant had multiple admissions with heart failure, the first event was included.

What You Need to Know

Background

The relationship between liver fibrosis and heart failure is largely unknown. Polymorphisms associated with liver fibrosis (PNPLA3 rs738409; TM6SF2 rs58542926) are linked to decreased risk of coronary artery disease.

Findings

Liver fibrosis determined by noninvasive tests was associated with hospitalization or death from heart failure in a general population cohort. Polymorphisms associated with liver fibrosis did not attenuate this risk.

Implications for patient care

These data provide a rationale for greater focus on assessment and optimization of modifiable shared cardiometabolic risk factors to prevent the development of heart failure in people with hepatic fibrosis.

Statistical Analysis

Univariate and multivariable Cox proportional hazards models were used to calculate HRs for the relationship between NITs for liver fibrosis and heart failure. Non-cases were censored at the date of loss to follow-up, date of death, or end of follow-up. Potential confounding factors were identified from the literature and entered into multivariate regression models, using data from the baseline assessment. Model 1 was adjusted for age, sex, ethnicity, and Townsend deprivation index.[23] Model 2 was additionally adjusted for alcohol (weekly grams; continuous) and smoking status (never, previous, current). Model 3 was additionally adjusted for type 2 diabetes (T2D), waist circumference, hypertension, and dyslipidemia. Model 4 was additionally adjusted for a prior history of acute coronary syndrome (ACS), valvular disease, cardiomyopathy, and arrhythmias. Full definitions of all covariates are provided in the Supplementary Methods. The linearity of the effect of each continuous variable in the adjusted models was determined using univariate Cox hazard regression with penalized splines. Where a Wald-type test using the nonlinear coefficient estimates indicated significant non-linearity, splines were used in further analyses. The validity of the proportional hazards assumption for each variable was determined by examining correlations between scaled Schoenfeld residuals and time. The statistical package used was R. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines were followed in reporting this study.[24]

Subgroup Analyses

We repeated the analyses in participants with a baseline diagnosis of MASLD and metabolic ALD (Met-ALD),[25] participants consuming harmful levels of alcohol, and people living with/without obesity or T2D. See Supplementary Methods for definitions. In addition, we analyzed the relationship between NITs for liver fibrosis and heart failure in people with/without liver steatosis, determined via magnetic resonance imaging (MRI)-proton-density fat fraction (liver fat threshold $\geq$5.56%).

Sensitivity Analyses

We performed 2 sensitivity analyses; the first excluding individuals who were either former or harmful drinkers (women drinking >280 grams per week, or men drinking >400 grams per week), and the second excluding people with baseline CVD, including heart failure, ACS, valve disease, cardiomyopathy, or arrhythmias.

Gene Effect Analysis

Overall, 488,377 individuals were genotyped in the UKBB study. We investigated the effect of the 2 SNPs of interest, known to be associated with liver fibrosis: rs738409 (*PNPLA3)* (chromosome 22, location 44324727) and rs58542926 (*TM6SF2*) (chromosome 19, location 19268740). We explored the association between liver fibrosis and heart failure stratified by these genotypes. The interaction between NITs and genotype, in relation to heart failure risk, was evaluated using a likelihood ratio test to determine whether the inclusion of an interaction term in the Cox model significantly improved the model fit.

Association Between Serum Fibrosis Markers and Acute Coronary Syndrome

As IHD is a risk factor for heart failure, we determined whether similar associations between NITs for fibrosis and ACS were evident (definition in Supplementary Methods). All UKBB participants were included, excluding individuals with a baseline diagnosis of ACS or insufficient data to calculate fibrosis scores.

Results

Study Population and Baseline Demographics

Following the exclusions described, the final study population consisted of 413,860 participants (Figure 1). Baseline demographics are shown in Supplementary Table 1. The baseline characteristics of individuals excluded did not differ significantly to the overall UKBB population (Supplementary Table 2). In total 1.3% (n = 5400), 2.2% (n = 9226), and 0.5% (n = 2169) of people in the UKBB had evidence of liver fibrosis according to NFS, FIB-4, and APRI scores, respectively. We attempted to determine the etiology of liver disease for participants with raised NITs for liver fibrosis (Table 1; Supplementary Table 3). Frequencies of MASLD were

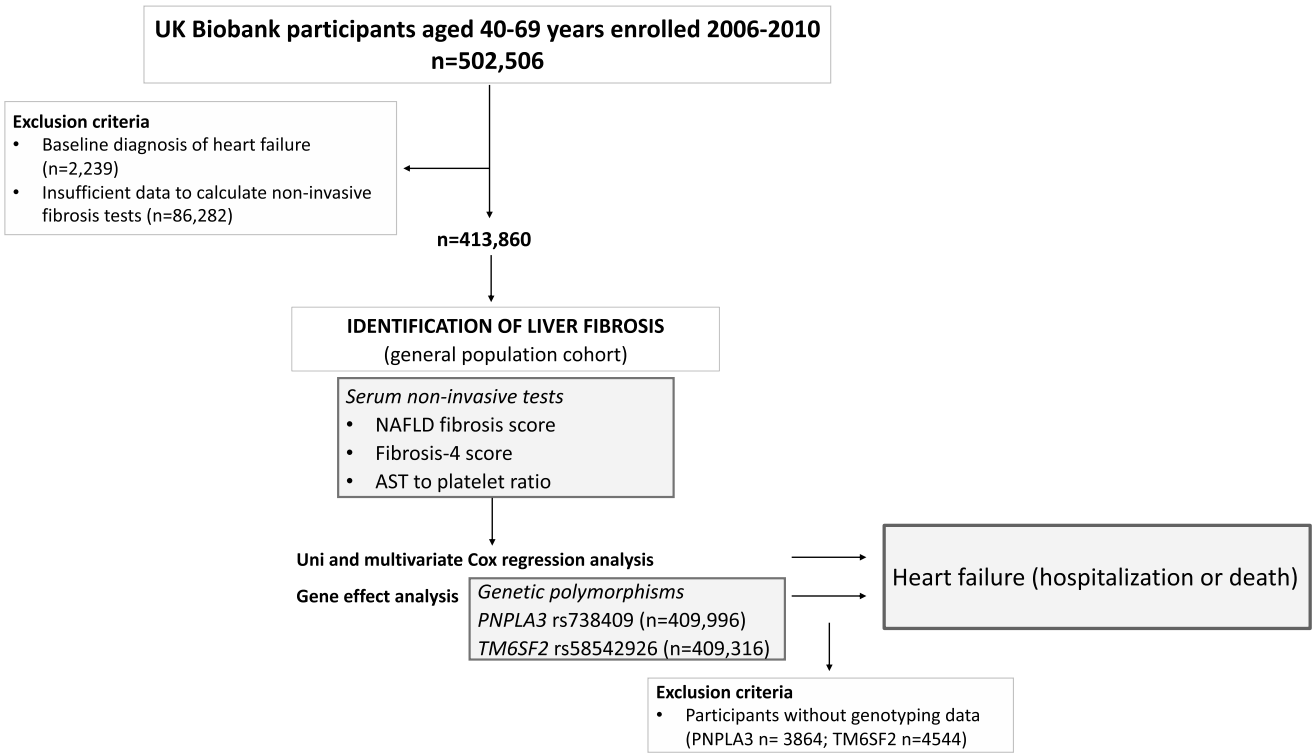


Figure 1. Study flow chart. AST, Aspartate aminotransferase; NAFLD, nonalcoholic fatty liver disease.

Table 1. Etiology of Liver Disease for Participants With Advanced Fibrosis According to NITs

	NFS >0.676	FIB-4 >2.67	APRI ≥1.0
MASLD	3478 (64.4)	3720 (40.3)	840 (38.7)
Met-ALD	683 (12.6)	1377 (14.9)	359 (16.6)
ALD	608 (11.3)	1427 (15.5)	592 (27.3)
Viral hepatitis	37 (0.7)	81 (0.9)	60 (2.8)
Autoimmune and biliary liver disease	29 (0.5)	63 (0.7)	29 (1.3)
Hemochromatosis	19 (0.4)	31 (0.3)	10 (0.5)
Other (vascular, drug-induced, inherited liver disease)	2 (0.04)	5 (0.1)	2 (0.1)
No clearly identifiable cause of liver disease	544 (10.1)	2522 (27.3)	277 (12.8)

Note: Data are presented as number (%).
Note: MASLD: Hepatic steatosis index >30, or an ICD code for NAFLD plus a metabolic risk factor, excluding people drinking >140 grams per week (women) or >210 grams per week (men), or UKBB or ICD code for alcohol excess, or a non-NAFLD causes of liver disease.
Note: Met-ALD: Hepatic steatosis index >30, or an ICD code for NAFLD plus a metabolic risk factor, plus alcohol intake women 140–350 g/week, men 210–420 g/week.
Note: ALD: People drinking at harmful levels (>350 grams per week women >420 grams per week men), or ICD code for ALD, or alcohol dependency.
Note: Viral hepatitis: UKBB or ICD code for viral hepatitis.
Note: Autoimmune and biliary liver disease: UKBB or ICD code for autoimmune hepatitis, primary biliary cholangitis, or primary sclerosing cholangitis.
Note: Hemochromatosis: UKBB or ICD code for haemochromatosis.
Note: Other: UKBB or ICD code for vascular, drug-induced or inherited liver disease.
Note: No identifiable cause: Not meeting criteria for any of the above.
ALD, Alcohol-related liver disease; APRI, AST platelet ratio index; AST, aspartate transaminase; FIB-4, Fibrosis-4; ICD, International Classification of Diseases; MASLD, Metabolic dysfunction-associated steatotic liver disease; Met-ALD, metabolic alcoholic liver disease; NAFLD, nonalcoholic fatty liver disease; NFS, NAFLD fibrosis score; NITs, noninvasive tests; UKBB, UK Biobank.

64.4%, 40.3%, and 38.7%; Met-ALD 12.6%, 14.9%, and 16.6%; and ALD 11.3%, 15.5%, and 27.3% for people with a raised NFS score, FIB-4 score, and APRI score, respectively.

Association Between Noninvasive Fibrosis Markers and Incident Heart Failure

The median follow-up period for combined fatal and non-fatal incident heart failure was 10.7 years. In total, 12,527 cases of hospitalization or death from heart failure were recorded (Table 2). Overall, 15.4% of cases of incident heart failure were associated with (a code for) ACS within the previous year, and only 53 people (0.4%) who developed heart failure had (a code for) alcoholic cardiomyopathy. Raised NITs for liver fibrosis at baseline were associated with higher event rates of incident heart failure (Table 2).

Univariate analysis of baseline factors associated with heart failure are shown in Supplementary Table 4. High NFS (HR, 6.25; 95% CI, 5.81-6.73; $P < .0001$), FIB-4 (HR, 3.23; 95% CI, 3.00-3.49; $P < .0001$), and APRI (HR, 2.68; 95% CI, 2.29-3.13; $P < .0001$) scores were associated with increased risk of incident heart failure (Table 2).

Following multivariable adjustment, NFS (HR, 1.59; 95% CI, 1.47-1.78; $P < .0001$), FIB-4 (HR, 1.69; 95% CI, 1.55-1.84; $P < .0001$), and APRI scores (HR, 1.85; 95% CI, 1.56-2.19; $P < .0001$) remained significantly associated with the development of heart failure (Table 2; Figure 2). Where liver fibrosis was defined using combinations of NITs, people who scored 'high' on more than one fibrosis marker were at higher risk of developing heart failure (Supplementary Table 5; Figure 3).

Subgroup Analyses

Results are presented for multivariate models (see Supplementary Table 6 for baseline demographics). For participants with a baseline diagnosis of MASLD (n = 220,771), a high NFS (HR, 1.80; 95% CI, 1.60-2.02; $P < .0001$), FIB-4 (HR, 1.41; 95% CI, 1.23-1.61; $P < .001$), and APRI score (HR, 1.47; 95% CI, 1.08-2.01; $P < .0001$) were associated with incident heart failure (Supplementary Table 7). For people with Met-ALD (n = 76,385), both a high NFS (HR, 1.87; 95% CI, 1.50-2.35; $P < .0001$) and FIB-4 score (HR, 1.82; 95% CI, 1.49-2.23; $P < .001$) were associated with the development of heart failure (Supplementary Table 8). For people with harmful alcohol consumption (n = 39,100), all three fibrosis scores were associated with heart failure (NFS HR, 2.07; 95% CI, 1.64-2.61; $P < .0001$; FIB-4 HR, 2.12; 95% CI, 1.76-2.56; $P < .0001$; APRI HR, 2.43; 95% CI, 1.86-3.17; $P < .0001$) (Supplementary Table 9).

In people with MRI-proton-density fat fraction-determined liver steatosis (n = 8175), there was a trend (non-significant) between fibrosis scores and incident heart failure, although the low overall event numbers implied a lack of statistical power (Supplementary Table 10).

All 3 noninvasive fibrosis scores remained positively associated with heart failure for people with and without obesity. For people with T2D, only the NFS score

Table 2. Association of NITs of Liver Fibrosis With Hospitalization or Death From Heart Failure in a General Population Cohort

	Risk fibrosis	Participants	Events (fatal)	Median follow-up, *months*	Event rate	Univariate HR (95% CI)	Model 1 HR (95% CI)	Model 2 HR (95% CI)	Model 3 HR (95% CI)	Model 4 HR (95% CI)
NFS	Low	327,270	8248 (55)	123.8	2.19	1.00 Ref.	1.00 Ref.	1.00 Ref.	1.00 Ref.	1.00 Ref.
	Intermediate	81,190	3498 (27)	128.4	0.90	1.76 (1.70–1.83)[b]	1.77 (1.7–1.85)[b]	1.77 (1.69–1.85)[b]	1.33 (1.26–1.4)[b]	1.30 (1.24–1.37)[b]
	High	5400	781 (14)	122.4	0.21	6.25 (5.81–6.73)[b]	3.61 (3.35–3.89)[b]	3.69 (3.39–4.01)[b]	1.72 (1.56–1.9)[b]	1.59 (1.47–1.78)[b]
FIB-4	Low	271,951	7514 (59)	126.8	1.95	1.00 Ref.	1.00 Ref.	1.00 Ref.	1.00 Ref.	1.00 Ref.
	Intermediate	132,683	4292 (29)	129.4	1.09	1.24 (1.19–1.29)[b]	1.07 (1.03–1.12)[a]	1.13 (1.08–1.18)[b]	1.33 (1.26–1.4)[b]	1.17 (1.12–1.23)[b]
	High	9226	721 (8)	120.9	0.20	3.23 (3.00–3.49)[b]	1.66 (1.54–1.8)[b]	1.82 (1.67–1.98)[b]	1.72 (1.56–1.9)[b]	1.69 (1.55–1.84)[b]
APRI	Low	411,691	12366 (94)	126.1	3.22	1.00 Ref.	1.00 Ref.	1.00 Ref.	1.00 Ref.	1.00 Ref.
	High	2169	161 (2)	129.1	0.04	2.68 (2.29–3.13)[b]	2.28 (1.95–2.66)[b]	2.38 (2.00–2.82)[b]	1.88 (1.58–2.23)[b]	1.85 (1.56–2.19)[b]

Note: NFS: low risk, <-1.455 (<0.12 if ≥ 65 years); intermediate risk, -1.455 to 0.676 (0.12-0.676 if ≥ 65 years); high risk >0.676.
Note: FIB-4 score: low risk, <1.3 (<2.0 if ≥ 65 years); intermediate risk, 1.3–2.67 (2.0-2.67 if ≥ 65 years); high risk, >2.67.
Note: APRI: low risk <1.0; high risk ≥ 1.0.
Note: Multivariable model 1: Adjusted for age, sex, deprivation, ethnicity.
Note: Multivariable model 2: Adjusted for age, sex, deprivation, ethnicity, alcohol (continuous), smoking.
Note: Multivariable model 3: Adjusted for age, sex, deprivation, ethnicity, alcohol (continuous), smoking, diabetes, waist circumference, hypertension, dyslipidemia.
Note: Multivariable model 4: Adjusted for age, sex, deprivation, ethnicity, alcohol (continuous), smoking, diabetes, waist circumference, hypertension, dyslipidemia baseline acute coronary syndrome, valve disease, cardiomyopathy.
APRI, AST platelet ratio index; AST, aspartate transaminase; CI, confidence interval; FIB-4, Fibrosis-4 score; HR, hazard ratio; NAFLD, nonalcoholic fatty liver disease; NITs, non-invasive tests; NFS, NAFLD fibrosis score; Ref, reference.
[a]$P < .001$.
[b]$P < .0001$.

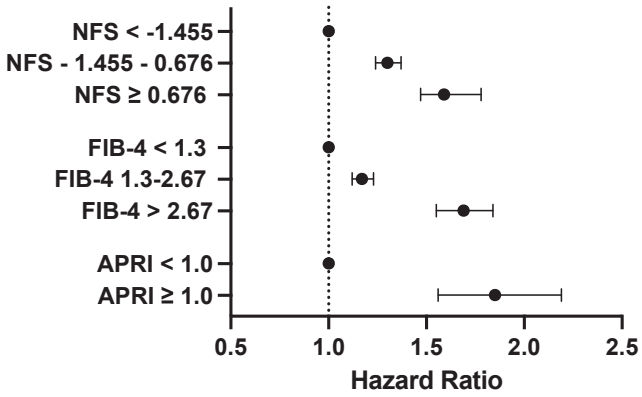


Figure 2. HRs and 95% CIs for the association of NITs of liver fibrosis and hospitalization or death due to heart failure following full multivariable adjustment.

remained associated with heart failure, although there was a trend towards increased risk with FIB-4 and APRI.

Sensitivity Analyses

We performed sensitivity analyses excluding individuals who were either former or harmful drinkers (Supplementary Table 11), and people with baseline CVD (Supplementary Table 12). For both analyses, all 3 noninvasive markers of fibrosis continued to be significantly associated with an elevated risk of hospitalization or death from heart failure.

Genetic Effect Analysis

Within our study cohort for *PNPLA3,* 4.8% expressed the variant GG, 33.7% the variant GC, and 61.5% CC. For *TM6SF2,* 85.6% carried the polymorphisms CC, 13.8% TC, and 0.6% TT. In univariate analysis, we observed no attenuation in risk of incident heart failure for people with evidence of liver fibrosis determined via a NIT for people with *PNPLA3* GG (high-risk allele for liver fibrosis) homozygosity compared with those with the heterozygous state, or *PNPLA3* CC homozygosity (Table 3). Similarly, there was no significant attenuation in risk for *TM6SF2* TT (high-risk allele for liver fibrosis) homozygosity compared with heterozygotes, or *TM6SF2* CC for people with liver fibrosis determined via any of the NITs at baseline (Table 3). For *PNPLA3* GG, we observed a non-significant trend towards an increase in risk of incident heart failure.

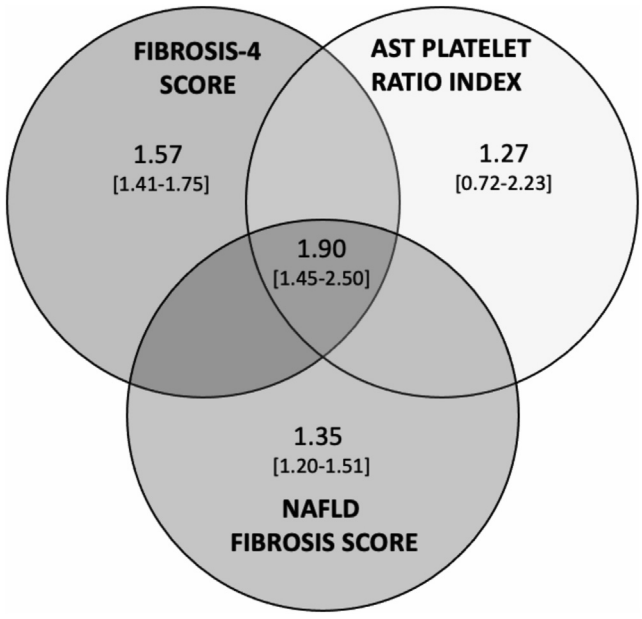


Figure 3. HRs and 95% CIs for association of combined NITs of liver fibrosis and hospitalization or death due to heart failure following full multivariable adjustment.

We incorporated interaction terms into our models to examine how *PNPLA3* and *TM6SF2* influence the relationships between fibrosis scores and heart failure risk. The interactions involving *PNPLA3* rs738409 with both FIB-4 and APRI were statistically significant. No statistically significant interaction was found with *TM6SF2*, although the overall number of people with *TM6SF2* TT homozygosity and a heart failure event were small.

Association Between Serum Fibrosis Markers and Acute Coronary Syndrome

Within a cohort of n = 321,095 participants, 11,229 experienced an ACS event (3.5%). Following full multivariate adjustment, only the NFS remained significantly associated with ACS events (NFS HR, 1.19; 95% CI, 1.03-1.37; $P = .0178$; FIB-4 HR, 1.12; 95% CI, 0.99-1.26; $P = .0609$; APRI HR, 1.03; 95% CI, 0.79-1.34; $P = .8396$) (Supplementary Table 13).

Discussion

We show that liver fibrosis markers are associated with an increased risk of hospitalization or death from heart failure in a prospective general population cohort and could be used for risk stratification. This association persists for people with MASLD and harmful alcohol consumption. Polymorphisms identifying people at risk of liver fibrosis (*PNPLA3* rs738409 GG and *TM6SF2* rs58542926 TT) were not associated with incident heart failure; therefore, causality remains uncertain.

In the Multi-Ethnic Study of Atherosclerosis (cross-sectional, 6 United States centers), liver fibrosis was associated with a history of heart failure and with abnormal cardiac structure and function.[26] Simon et al demonstrated prospectively in >10,000 Swedish adults, an increased risk of major adverse cardiac events in people with NAFLD.[3] Among 1554 people with NAFLD fibrosis, 153 developed congestive heart failure (adjusted HR, 2.04; 95% CI, 1.66-2.51).[3] Within the Korean National Health Insurance datasets, in people with NAFLD, liver fibrosis (defined using BARD scores), was associated with increased incident and hospitalized heart failure.[27] To our knowledge, no previous general

Table 3. Association of NITs of Liver Fibrosis With Hospitalization or Death From Heart Failure Stratified According to *PNPLA3* and *TM6SF2* Polymorphisms in a General Population Cohort (Univariate Analysis)

			NFS			FIB-4 score			APRI	
			Low	Intermediate	High	Low	Intermediate	High	Low	High
PNPLA3 (rs738409)	CC	No. cases HF	5150	2159	466	4790	2583	402	7697	78
		HR (95% CI)	1.00 Ref.	1.72 (1.64- 1.81)[d]	6.20 (5.64- 6.82)[d]	1.00 Ref.	1.20 (1.14- 1.25)[d]	2.99 (2.70- 3.31)[d]	1.00 Ref.	2.68 (2.15- 3.35)[d]
	CG GC	No. cases HF	2690	1143	269	2386	1455	261	4046	56
		HR (95% CI)	0.96 (0.92- 1.01)	1.74 (1.63- 1.85)[d]	5.90 (5.21- 6.67)[d]	0.94 (0.89- 0.98)[b]	1.21 (1.14- 1.29)[d]	3.28 (2.89- 3.71)[d]	0.97 (0.94-1.01)	2.20 (1.69- 2.87)[d]
	GG	No. cases HF	342	169	41	283	216	53	526	26
		HR (95% CI)	0.89 (0.80- 0.99)[a]	1.89 (1.63- 2.21)[d]	7.67 (5.64-10.43)[d]	0.84 (0.75- 0.95)[a]	1.26 (1.10- 1.44)[b]	4.02 (3.06- 5.26)[d]	0.94 (0.86- 1.02)	4.70 (3.20- 6.90)[d]
TM6SF2 (rs58542926)	CC	No. cases HF	7000	2981	674	6388	3672	595	10,530	125
		HR (95% CI)	1.00 Ref.	1.75 (1.68- 1.83)[d]	6.28 (5.80- 6.80)[d]	1.00 Ref.	1.24 (1.19- 1.29)[d]	3.17 (2.91- 3.45)[d]	1.00 Ref.	2.54 (2.13- 3.03)[d]
	CT TC	No. cases HF	1132	476	95	1025	561	117	1671	32
		HR (95% CI)	1.01 (0.95- 1.07)	1.93 (1.76- 2.12)[d]	6.16 (5.03- 7.54)[d]	1.00 (0.94- 1.07)	1.26 (1.16- 1.38)[d]	3.73 (3.10- 4.48)[d]	1.01 (0.96- 1.06)	3.52 (2.49- 4.98)[d]
	TT	No. cases HF	33	16	6	35	18	2	53	2
		HR (95% CI)	0.76 (0.54- 1.08)	1.51 (0.93- 2.47)	6.59 (2.96-14.67)[d]	0.89 (0.64- 1.24)	1.00 (0.63- 1.59)	1.17 (0.29- 4.67)	0.82 (0.63- 1.08)	2.33 (0.58- 9.33)

Note: Grey shaded boxes represent risk alleles associated with an increased risk of liver fibrosis.
Note: NFS: low risk, <-1.455 (<0.12 if $\geq$65 years); intermediate risk, -1.455–0.676 (0.12–0.676 if $\geq$65 years); high risk, >0.676.
Note: FIB-4 score: low risk, <1.3 (<2.0 if $\geq$65 years); intermediate risk, 1.3–2.67 (2.0–2.67 if $\geq$ 65 years); high risk, >2.67.
Note: APRI: low risk, <1.0; high risk, ≥ 1.0.
APRI, AST platelet ratio index; AST, aspartate transaminase; CI, confidence interval; FIB-4, Fibrosis-4 score; HF, heart failure; HR, hazard ratio; NAFLD, nonalcoholic fatty liver disease; NITs, non-invasive tests; NFS, NAFLD fibrosis score; Ref, reference.
[c]$P < .001$.
[a]$P < .05$.
[b]$P < .01$.
[d]$P < .0001$.

population-based studies have examined the relationship between liver fibrosis and heart failure, nor have studies tested whether genetic polymorphisms, identifying people at risk of liver fibrosis, modify risk of incident heart failure. We also provide novel evidence that liver fibrosis in people with ALD is a risk factor for heart failure. Shared mechanistic pathways may therefore exist across different liver disease etiologies.

We provide new insights exploring the association of noninvasive fibrosis markers with heart failure, stratified according to genetic polymorphisms associated with liver fibrosis. Both rs738409 GG for *PNPLA3* and rs58542926 for *TM6SF2* confer increased risk of advanced liver disease across etiologies.[13–16] Paradoxically, they confer protection against CVD.[11] An exome-wide association study showed that *TM6SF2* TT and *PNPLA3 GG* genotypes are involved in hepatic production of triglyceride-rich lipoproteins, and are associated with increased risk of T2D and lower risk of coronary artery disease.[11] This protection likely results from decreased export of very low-density lipoprotein from the liver, lowering circulating very low-density lipoprotein and reducing the impact of the atherogenic lipoprotein phenotype that occurs with NAFLD on CVD effects.[28] It might therefore be predicted that genetic variants associated with liver fibrosis are protective against heart failure due to reduced incidence of IHD. Data from a gene-wide association study meta-analysis revealed no significant association between rs738409 (*PNPLA3*) and incident heart failure (beta, 0.0116; $P = .2203$), and a negative association between rs58542926 (*TM6SF2*) and incident heart failure (beta, −0.0388; $P = .01081$).[29] In this study, we identify *PNPLA3* rs738409 GG and *TM6SF2* rs58542926 TT are not positively associated with an elevated risk of incident heart failure; however, this risk of heart failure is not attenuated for people with high liver fibrosis scores who carry these polymorphisms. Furthermore, we demonstrate in this general population cohort that only the NFS score remains associated with ACS incidence after full multivariate adjustment with only a modest elevation in risk (ie, the risk between liver fibrosis and ACS is low compared with liver fibrosis and heart failure). Additional pathological pathways, other than IHD, therefore likely explain this positive association including endothelial dysfunction, increased epicardial adipose tissue, coronary microcirculatory dysfunction, cardiac hypertrophy, ventricular myopathy, myocardial fibrosis, and altered cardiac bioenergetics resulting from a pro-fibrinogenic state in patients with chronic liver disease.[30] In people with NAFLD, gene signatures mediating collagen expression are also associated with significant liver fibrosis.[31] We speculate that in MASLD, there is a predisposition to fibrinogenesis in other extrahepatic tissues, including the heart.

NITs are currently used for risk stratification of liver-related events,[32] and here, we demonstrate important prognostic value for incident heart failure. Thus, early identification of liver fibrosis may allow effective lifestyle and pharmacological intervention optimization to prevent clinically significant liver and cardiac disease. Such findings support targeted screening for fibrosis in people with risk factors for liver disease and a focus on cardiometabolic multimorbidity management in people with liver fibrosis.

The UKBB is a substantial (0.5 million participants) prospective multi-modality dataset with nearly 17 years of clinical event follow-up time. Baseline data were available to calculate multiple well-validated prediction scores for risk stratification for advanced liver fibrosis, enabling examination of the incremental risk between levels of exposure (liver fibrosis) and incident heart failure. We were additionally able to adjust for multiple potential confounders and identify incident cases of both fatal and non-fatal heart failure using linked datasets. Finally, we were able to undertake a genetic association study to investigate the potential modifying influence of SNPs in 2 genes associated with increased risk of liver fibrosis.

Accurate identification of liver fibrosis is challenging, with high rates of false-positive scores with NITs. We relied upon ICD coding for heart failure, and these results capture only people with incident heart failure associated with hospitalization or death. Despite adjusting for multiple covariates, the primary outcome may still be influenced by residual confounding, although multiple sensitivity analyses were conducted. We could not explore different heart failure phenotypes, and a small proportion of individuals may have had cirrhotic cardiomyopathy-related heart failure. Patients with heart failure are at increased risk of congestive hepatopathy and ischemic hepatitis, which can influence NITs. However, fibrosis markers were calculated at baseline with individuals with pre-existing heart failure excluded. In addition, a sensitivity analysis, excluding those with underlying CVD on study entry, did not alter our findings. In genetic analyses, the case numbers of heart failure were small for those in the group with the greatest risk (high risk NITs and *PNPLA3* rs738409 GG) leading to wide CIs. This issue was more pronounced for *TM6SF2* rs58542926 TT. Analysis of the interaction of *TM6SF2* rs58542926 was also likely underpowered, given the low number of people with *TM6SF2* TT homozygosity. Finally, the White British predominance within UKBB means our findings may not be transferable to other ethnicities.

We were unable to perform time-dependent regression modeling. Because repeat measurements are not available, this cohort was not appropriate to analyze whether fibrosis markers can improve existing risk-prediction tools for heart failure. We were unable to prove causality or derive mechanistic insight into the association between liver fibrosis and heart failure in this observational study; exploration of this relationship may reveal novel therapeutic targets. Future studies utilizing other surrogate measures of liver fibrosis (eg, magnetic resonance elastography or multi-parametric MRI liver cT1 quantification) may help support our findings.

To conclude, serum markers assessing probability of advanced liver fibrosis, are associated with an increased risk of hospitalization or death from heart failure in a general population. These data provide a compelling rationale for multimorbidity assessment and aggressive cardiovascular risk-factor management to prevent heart failure in people with hepatic fibrosis determined via NITs.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Clinical Gastroenterology and Hepatology* at www.cghjournal.org, and at https://doi.org/10.1016/j.cgh.2024.03.045.

References

1. Roca-Fernandez A, Banerjee R, Thomaides-Brears H, et al. Liver disease is a significant risk factor for cardiovascular outcomes - a UK Biobank study. J Hepatol 2023;79:1085–1095.
2. Targher G, Tilg H, Byrne CD. NAFLD: a multisystem disease requiring a multidisciplinary and holistic approach. Lancet Global Health 2021;6:578–588.
3. Simon TG, Roelstraete B, Hagström H, et al. Non-alcoholic fatty liver disease and incident major adverse cardiovascular events: results from a nationwide histology cohort. Gut 2021; 71:1867–1875.
4. Wood AM, Kaptoge S, Butterworth AS, et al. Emerging Risk Factors Collaboration/EPIC-CVD/UK Biobank Alcohol Study Group. Risk thresholds for alcohol consumption: combined analysis of individual-participant data for 599,912 current drinkers in 83 prospective studies. Lancet 2018;391:1513–1523.
5. Ng CH, Lim WH, Hui Lim GE, et al. Mortality outcomes by fibrosis stage in nonalcoholic fatty liver disease: a systematic review and meta-analysis. Clin Gastroenterol Hepatol 2022; 21:931–939.e5.
6. Lackner C, Spindelboeck W, Haybaeck J, et al. Histological parameters and alcohol abstinence determine long-term prognosis in patients with alcoholic liver disease. J Hepatol 2017; 66:610–618.
7. Baratta F, Pastori D, Angelico F, et al. Nonalcoholic fatty liver disease and fibrosis associated with increased risk of cardiovascular events in a prospective study. Clin Gastroenterol Hepatol 2020;18:2324–2331.e4.
8. Kim D, Kim WR, Kim HJ, et al. Association between noninvasive fibrosis markers and mortality among adults with nonalcoholic fatty liver disease in the United States. Hepatology 2013;57:1357–1365.
9. Hagström H, Nasr P, Ekstedt M, et al. Cardiovascular risk factors in non-alcoholic fatty liver disease. Liver Int 2019;39:197–204.
10. Lauridsen BK, Stender S, Kristensen TS, et al. Liver fat content, non-alcoholic fatty liver disease, and ischaemic heart disease: Mendelian randomization and meta-analysis of 279,013 individuals. Eur Heart J 2018;39:385–393.
11. Liu DJ, Peloso GM, Yu H, et al; Charge Diabetes Working Group; EPIC-InterAct Consortium; EPIC-CVD Consortium; GOLD Consortium; VA Million Veteran Program. Exome-wide association study of plasma lipids in >300,000 individuals. Nature Genetics 2017;49:1758–1766.
12. Mantovani A, Petracca G, Csermely A, et al. Non-alcoholic fatty liver disease and risk of new-onset heart failure: an updated meta-analysis of about 11 million individuals. Gut 2022; 72:372–380.
13. Liu YL, Reeves HL, Burt AD, et al. TM6SF2 rs58542926 influences hepatic fibrosis progression in patients with non-alcoholic fatty liver disease. Nat Commun 2014;5:4309.
14. Valenti L, Al-Serri A, Daly AK, et al. Homozygosity for the patatin-like phospholipase-3/adiponutrin I148M polymorphism influences liver fibrosis in patients with nonalcoholic fatty liver disease. Hepatology 2010;51:1209–1217.
15. Buch S, Stickel F, Trépo E, et al. A genome-wide association study confirms PNPLA3 and identifies TM6SF2 and MBOAT7 as risk loci for alcohol-related cirrhosis. Nat Genet 2015;47:1443–1448.
16. Trépo E, Pradat P, Potthoff A, et al. Impact of patatin-like phospholipase-3 (rs738409 C>G) polymorphism on fibrosis progression and steatosis in chronic hepatitis C. Hepatology 2011;54:60–69.
17. About UK Biobank. Available at: http://Www.Ukbiobank.Ac.Uk/about-Biobank-Uk/. Accessed June 3, 2024.
18. UKBB Self-Reported Non-Cancer Illness Codes. Available at: https://Biobank.Ctsu.Ox.Ac.Uk/Crystal/Field.Cgi?Id=20002. Accessed June 3, 2024.
19. Rickham PP. Human experimentation. Code of ethics of the World Medical Association. Declaration of Helsinki. Br Med J 1964;2:177.
20. Sterling RK, Lissen E, Clumeck N, et al. , APRICOT Clinical Investigators. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. Hepatology 2006;43:1317–1325.
21. Angulo P, Bugianesi E, Bjornsson ES, et al. Simple noninvasive systems predict long-term outcomes of patients with nonalcoholic fatty liver disease. Gastroenterology 2013;145:782–789.e4.
22. Wai C, Greenson JK, Fontana RJ, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. Hepatology 2003;38:518–526.
23. Mackenbach JP. Health and deprivation. In: Townsend P, Phillimore P, Beattie A, eds. Inequality and the North. Croom Helm Ltd, 1987.
24. Von Elm E, Altman D, Egger M, et al; STROBE Initiative. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies. BMJ 2007;335:806–808.
25. Rinella ME, Lazarus JV, Ratziu V, et al; NAFLD Nomenclature consensus group. A multisociety Delphi consensus statement on new fatty liver disease nomenclature. Hepatology 2023; 78:1966–1986.
26. Ostovaneh M, Ambale-Venkatesh B, Fuji T, et al. Association of liver fibrosis with cardiovascular diseases in the general population: the Multi-Ethnic Study of Atherosclerosis (MESA). Circ Cardiovasc Imaging 2018;11:e007241.
27. Park J, Kim G, Kim H, et al. The association of hepatic steatosis and fibrosis with heart failure and mortality. Cardiovasc Diabetol 2021;20:1–14.
28. Borén J, Adiels M, Björnson E, et al. Effects of TM6SF2 E167K on hepatic lipid and very low-density lipoprotein metabolism in humans. JCI Insight 2020;5:e144079.
29. Shah S, Henry A, Roselli C, et al. Genome-wide association and Mendelian randomisation analysis provide insights into the pathogenesis of heart failure. Nat Commun 2020;11:163.
30. Packer M. Atrial fibrillation and heart failure with preserved ejection fraction in patients with nonalcoholic fatty liver disease. Am J Med 2020;133:170–177.

31. Bilson J, Oquendo CJ, Read J, et al. Markers of adipose tissue fibrogenesis associate with clinically significant liver fibrosis and are unchanged by synbiotic treatment in patients with NAFLD. Metabolism 2024;151:155759.
32. European Association for the Study of the Liver (EASL), European Association for the Study of Diabetes (EASD), European Association for the Study of Obesity (EASO). EASL–EASD–EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. J Hepatol 2016;64:1388–1402.

Correspondence
Address correspondence to: Theresa J. Hydes, MBBS, PhD, Department of Cardiovascular and Metabolic Medicine, Institute of Life Course and Medical Sciences, Faculty of Health and Life Sciences, University of Liverpool, Clinical Sciences Building (3rd floor) University Hospital Aintree, L9 7AL, Liverpool, United Kingdom e-mail: theresa.hydes@liverpool.ac.uk; tel: 0151 529 0228.

CRediT Authorship Contributions

Theresa J. Hydes, MBBS, PhD (Conceptualization: Supporting; Data curation: Equal; Formal analysis: Supporting; Investigation: Supporting; Methodology: Supporting; Project administration: Lead; Writing – original draft: Lead; Writing – review & editing: Supporting)

Oliver J. Kennedy (Conceptualization: Supporting; Data curation: Equal; Formal analysis: Lead; Investigation: Lead; Methodology: Lead; Validation: Lead; Writing – review & editing: Supporting)

Kate Glyn-Owen (Conceptualization: Supporting; Formal analysis: Supporting; Investigation: Supporting; Methodology: Supporting; Writing – review & editing: Supporting)

Ryan Buchanan (Conceptualization: Supporting; Formal analysis: Supporting; Methodology: Supporting; Resources: Lead; Writing – review & editing: Supporting)

Julie Parkes (Conceptualization: Supporting; Methodology: Supporting; Resources: Equal; Supervision: Supporting; Writing – review & editing: Supporting)

Daniel J. Cuthbertson (Conceptualization: Supporting; Formal analysis: Supporting; Investigation: Supporting; Methodology: Supporting; Supervision: Supporting; Writing – review & editing: Supporting)

Paul J. Roderick (Conceptualization: Equal; Formal analysis: Equal; Investigation: Equal; Methodology: Equal; Supervision: Equal; Writing – review & editing: Equal)

Chris D. Byrne (Conceptualization: Equal; Formal analysis: Equal; Investigation: Equal; Methodology: Equal; Supervision: Equal; Writing – review & editing: Equal)

Conflicts of interest
authors disclose no conflicts.

Funding
Chris D. Byrne is supported in part by the Southampton National Institute for Health and Care Research Biomedical Research Centre (NIHR203319), United Kingdom.

Data Availability
The original data are available through access to the UK Biobank.