

1 **Liver fibrosis assessed via non-invasive tests is associated with incident heart failure in a general**  
2 **population cohort**

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## ABSTRACT

**Aims** 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 non-invasive fibrosis markers [NAFLD fibrosis score (NFS), Fibrosis-4 (FIB-4) and AST to platelet ratio index (APRI)] and incident hospitalization/death from heart failure (n=413,860). Cox-regression estimated hazard ratios (HR) 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.

**Results** 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 [1.47-1.76], p<0.0001; FIB-4 HR 1.69 [1.55-1.84], p<0.0001; APRI HR 1.85 [1.56-2.19], p<0.0001; combined fibrosis scores HR 1.90 [1.44-2.49], p<0.0001). 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.

**Key words** Heart failure; fibrosis; cirrhosis

## INTRODUCTION

In addition to adverse liver-related outcomes, liver disease is a significant risk factor for adverse cardiovascular outcomes.<sup>1</sup> 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.<sup>2,3</sup> 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.<sup>4</sup>

Liver fibrosis is a key histological stage associated with adverse liver-related outcomes for all etiologies of chronic liver disease.<sup>5,6</sup> Liver fibrosis is associated with extra-hepatic complications, in particular CVD,<sup>3,7</sup> and a higher all-cause mortality.<sup>8</sup> This association with CVD occurs irrespective of whether liver fibrosis is determined via non-invasive tests (NIT)<sup>7</sup> or histologically.<sup>3</sup> In a prospective, outpatient cohort of 898 people with non-alcoholic fatty liver disease (NAFLD), elevated fibrosis-4 (FIB-4) and NAFLD fibrosis scores (NFS) were independently associated with incident CVEs.<sup>7</sup> Furthermore, in a Swedish population-based cohort (n=10,422), rates of incident major adverse cardiac events (MACE) increased progressively with worsening histological NAFLD severity.<sup>3</sup> However, in 603 patients with biopsy-proven NAFLD and 6269 age/sex matched controls, although NAFLD patients were more likely to experience a CVD event (HR 1.54), histological fibrosis stage was not predictive factor.<sup>9</sup> Furthermore, in a large Danish population study performing Mendelian Randomisation 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);<sup>10</sup> and in a large (>300,000 individuals) exome wide association study was protective against IHD.<sup>11</sup>

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. While a link between NAFLD and heart failure has been confirmed in a recent meta-analysis of 11 cohort studies (pooled random-effects hazard ratio (HR) 1.50 [1.34-1.67]),<sup>12</sup> the association between liver fibrosis and heart failure in a general population with mixed risk factors for liver disease is unknown.<sup>14</sup> Multiple gene wide association studies (GWAS) and candidate gene studies have identified an association between single nuclear polymorphisms (SNP) in *PNPLA3* (rs738409 c.444 C>G, encoding Ile148Met (I148M)) and transmembrane 6 superfamily member 2 (*TM6SF2*) (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,<sup>13,14</sup> alcohol-related liver disease (ALD),<sup>15</sup> and chronic hepatitis C.<sup>16</sup> Considering the paradox whereby the SNPs rs738409 (*PNPLA3*) and rs58542926 (*TM6SF2*) are associated with liver fibrosis, and yet confer protection against CVD,<sup>11</sup> 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, i.e. 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.<sup>17</sup> Over 500,000 individuals, aged 40-69 years, identified from National Health Service registers were recruited between 2006-2010. 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.<sup>18</sup> 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.<sup>19</sup> Ethical approval was granted by the Northwest Multi-Centre Research Ethics Committee.

### **Inclusion and exclusion criteria**

All UK Biobank participants were initially included. Individuals with a baseline 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 Fibrosis-4 index (FIB-4)<sup>20</sup> >2.67, NAFLD fibrosis score (NFS)<sup>21</sup> >0.676, or AST to platelet ratio index (APRI)<sup>22</sup> ≥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.

### **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.<sup>23</sup> **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.<sup>24</sup>

### **Subgroup analyses**

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

### **Sensitivity analyses**

We performed two 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, arrhythmias.

### **Gene effect analysis**

Overall, 488,377 individuals were genotyped in the UKBB study. We investigated the effect of the two 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 ACS**

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 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 non-invasive 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 (0.4%) of people 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 [5.81-6.73],  $p < 0.0001$ ), FIB-4 (HR 3.23 [3.00-3.49],  $p < 0.0001$ ) and APRI (HR 2.68 [2.29-3.13],  $p < 0.0001$ ) scores were associated with increased risk of incident heart failure (**Table 2**). Following multivariable adjustment, NFS (HR 1.59 [1.47-1.78],  $p < 0.0001$ ), FIB-4 (HR 1.69 [1.55-1.84],  $p < 0.0001$ ) and APRI scores (HR 1.85 [1.56-2.19],  $p < 0.0001$ ) 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 [1.60-2.02],  $p < 0.0001$ ), FIB-4 (HR 1.41 [1.23-1.61],  $p < 0.001$ ) and APRI score (HR 1.47 [1.08-2.01],  $p < 0.0001$ ) were associated with incident heart failure (**Supplementary Table 7**). For people with Met-ALD (n=76,385), both a high NFS (HR 1.87 [1.50-2.35],  $p < 0.0001$ ) and FIB-4 score (HR 1.82 [1.49-2.23],  $p < 0.001$ ) 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 [1.64-2.61],  $p < 0.0001$ ; FIB-4 HR 2.12 [1.76-2.56],  $p < 0.0001$ ; APRI HR 2.43 [1.86-3.17],  $p < 0.0001$ ) (**Supplementary Table 9**).



In people with MRI-PDFF 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 three non-invasive fibrosis scores remained positively associated with heart failure for people with and without obesity. For people with T2D, only the NFS score 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 three non-invasive 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 to 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 to 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.

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 ACS**

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 [1.03-1.37], p=0.0178; FIB-4 HR 1.12 [0.99-1.26], p=0.0609; APRI HR 1.03 [0.79-1.34], p=0.8396) (**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, six US centres), liver fibrosis was associated with a history of heart failure and with abnormal cardiac structure and function.<sup>26</sup> Simon *et al* demonstrated prospectively in >10,000 Swedish adults, an increased risk of MACE in people with NAFLD.<sup>3</sup> Amongst 1554 people with NAFLD fibrosis, 153 developed congestive heart failure (adjusted HR 2.04 [1.66-2.51]).<sup>3</sup> 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.<sup>27</sup> To our knowledge, no previous general 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 non-invasive 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.<sup>13-16</sup> Paradoxically, they confer protection against CVD.<sup>11</sup> 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.<sup>11</sup> This protection likely results from decreased export of very low density lipoprotein (VLDL) from the liver, lowering circulating VLDL and reducing the impact of the atherogenic lipoprotein phenotype that occurs with NAFLD on CVD effects.<sup>28</sup> 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 GWAS meta-analysis revealed no significant association between rs738409 (*PNPLA3*) and incident heart failure (beta 0.0116, p=0.2203), and a negative association between rs58542926 (*TM6SF2*) and incident heart failure (beta -0.0388, p=0.01081).<sup>29</sup> 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 (i.e. the risk between liver fibrosis and ACS is low compared to 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.<sup>30</sup> In people with NAFLD, gene signatures mediating collagen expression are also associated with significant liver fibrosis.<sup>31</sup> 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,<sup>32</sup> and here we demonstrate important prognostic value for incident heart failure. Thus, early identification of liver fibrosis may allow effective lifestyle and pharmacological intervention optimisation 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 two 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 hospitalisation 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 confidence

intervals. 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 modelling. Since repeat measurements are not available, this cohort was not appropriate to analyse 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 utilising other surrogate measures of liver fibrosis \(e.g. magnetic resonance elastography or multi-parametric MRI liver cT1 quantification\) 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.

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## Legends

**Table 1.** Etiology of liver disease for participants with advanced fibrosis according to non-invasive tests

**Table 2.** Association of non-invasive tests of liver fibrosis with hospitalization or death from heart failure in a general population cohort

**Table 3.** Association of non-invasive tests of liver fibrosis with hospitalization or death from heart failure stratified according to *PNPLA3* and *TM6SF2* polymorphisms in a general population cohort (univariate analysis)

**Figure 1.** Study flow chart

**Figure 2.** Hazard ratios and 95% confidence intervals for the association of non-invasive tests of liver fibrosis and hospitalization or death due to heart failure following full multivariable adjustment

**Figure 3.** Hazard ratios and 95% confidence intervals for association of combined non-invasive tests of liver fibrosis and hospitalization or death due to heart failure following full multivariable adjustment