Research Article
MASLD and Alcohol-Related Liver Diseases

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Differing genetic variants associated with liver fat and their contrasting relationships with cardiovascular diseases and cancer

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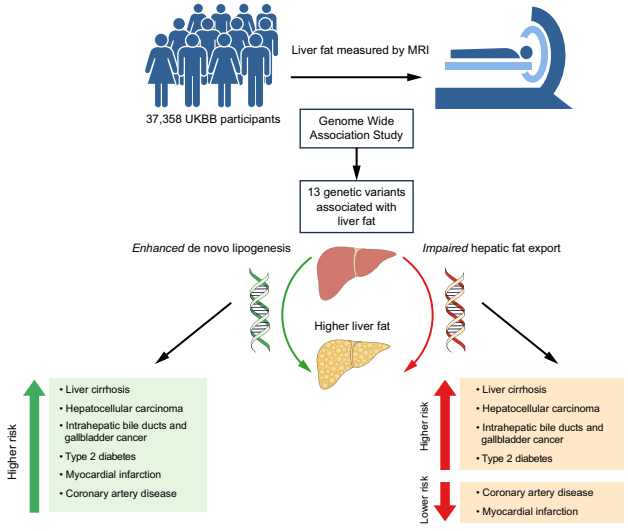
Graphical abstract



Highlights

- Genetic analysis of UK Biobank MRI data revealed 13 variants associated with liver fat.
- Key mechanisms for liver fat accumulation include lipid retention and enhanced *de novo* lipogenesis.
- Impaired triglyceride export lowers cardiovascular risk, while enhanced *de novo* lipogenesis increases it.
- Higher liver fat is causally linked to non-alcoholic cirrhosis, liver cancers, and type 2 diabetes, regardless of the underlying mechanism.
- Findings indicate the need for personalized treatment and risk assessment based on liver fat accumulation mechanisms.

Impact and implication

This research advances our understanding of the heterogeneity in mechanisms influencing liver fat accumulation, providing new insights into how liver fat accumulation may impact various health outcomes. The findings challenge the notion that liver fat is an independent risk factor for cardiovascular disease and highlight the mechanistic effect of some genetic variants on fat accumulation and the development of cardiovascular diseases. This study is of particular importance for healthcare professionals including physicians and researchers, as well as patients, as it allows for more targeted and personalised treatment by understanding the relationship between liver fat and various health outcomes. The findings emphasise the need for a personalised management approach and a reshaping of risk assessment criteria. It also provides room for prioritising a clinical intervention aimed at reducing liver fat content (likely via intentional weight loss) that could help protect against liver-related fibrosis and cancer.

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Differing genetic variants associated with liver fat and their contrasting relationships with cardiovascular diseases and cancer

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Background & Aims: The mechanisms underlying the association of steatotic liver disease with cardiovascular and cancer outcomes are poorly understood. We aimed to use MRI-derived measures of liver fat and genetics to investigate causal mechanisms that link higher liver fat to various health outcomes.

Methods: We conducted a genome-wide association study on 37,358 UK Biobank participants to identify genetic variants associated with liver fat measured from MRI scans. We used a Mendelian randomisation approach to investigate the causal effect of liver fat on health outcomes independent of BMI, alcohol consumption and lipids using data from published genome-wide association studies and FinnGen.

Results: We identified 13 genetic variants associated with liver fat that had differing effects on the risks of health outcomes. Genetic variants associated with *impaired* hepatic triglyceride export showed liver fat-increasing alleles to be correlated with a reduced risk of coronary artery disease and myocardial infarction but an elevated risk of type 2 diabetes, while variants associated with *enhanced de novo* lipogenesis showed liver fat-increasing alleles to be linked to a higher risk of myocardial infarction and coronary artery disease. Genetically higher liver fat content increased the risk of non-alcohol-related cirrhosis, hepatocellular carcinoma, and intrahepatic bile duct and gallbladder cancers, exhibiting a dose-dependent relationship, irrespective of the mechanism.

Conclusion: This study provides fresh insight into the heterogeneous effect of liver fat on health outcomes. It challenges the notion that liver fat *per se* is an independent risk factor for cardiovascular disease, underscoring the dependency of this association on the specific mechanisms that drive fat accumulation in the liver. However, excess liver fat, regardless of the underlying mechanism, appears to be causally linked to cirrhosis and cancers in a dose-dependent manner.

Introduction

Higher liver fat in the form of MASLD (metabolic dysfunction associated steatotic liver disease) has been linked to many disease outcomes by observational studies. These studies for example suggest that MASLD is an independent risk factor for acute myocardial infarction, stroke, coronary artery disease, and other atherosclerotic cardiovascular diseases independently of any shared risk factor (age, sex, adiposity measures and type 2 diabetes).[1,2] People with MASLD also have been found to have a twofold higher risk of developing type 2 diabetes[3] and a higher risk of thyroid cancer, lung cancer, hepatocellular carcinoma, colorectal cancer and breast cancer.[4] However, these studies do not explain the existence of people with MASLD (with a high degree of liver fat) who never develop cardiovascular disease or type 2 diabetes.

Despite the mounting evidence linking MASLD to the increased risk of cardiovascular diseases, observational studies remain limited in their approach due to selection bias (owing to a lack of randomisation), presence of confounding factors (*e.g.* obesity), and reverse causation.[5] The evidence from Mendelian randomisation studies on the causal role of MASLD in cardiovascular disease is controversial. For example, genetically defined MASLD has been shown to be associated with a higher risk of arterial stiffness and heart failure but not with coronary artery disease, stroke, ischemic stroke and its subtypes.[6] Mendelian randomisation studies of the association between MASLD and cancers are scarce but suggest no

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association between genetically predicted MASLD and the risk of intrahepatic cholangiocarcinoma.[7]

While Mendelian randomisation studies have demonstrated their robustness in identifying the causal effect of MASLD on various disease outcomes, these investigations are not without their limitations. Notably, some studies aiming to assess the causal impact of MASLD have relied on circulating levels of liver enzymes as proxies for the condition. However, liver enzymes, such as alanine aminotransferase levels, are imperfect predictors of MASLD.[8] Additionally, definitions of MASLD can vary significantly depending on the diagnostic method employed, whether it be a liver biopsy, ultrasound, CT scan, or MRI scans. This variability in measurement methods introduces complexity and may influence the outcomes and comparability of different studies.[9] Furthermore, the binary definition of MASLD, as either present or absent, has constrained our ability to fully grasp the nuanced, continuous relationship between liver fat content and the risk of developing various diseases. By treating MASLD in a binary manner, the potential dose-response effect of increasing liver fat on disease risk is overlooked, limiting a comprehensive understanding of these complex associations.

In this study, we aimed to obtain a precise continuous measure of liver fat through gold standard MRI, transcending binary definitions. We conducted a genome-wide association study on 37,358 individuals from the UK Biobank to identify genetic variants associated with liver fat. We performed Mendelian randomisation analyses to investigate the causal relationships between elevated liver fat and a broad spectrum of health outcomes, including type 2 diabetes, different cardiovascular outcomes and liver cancer outcomes. Furthermore, we characterised each genetic variant's effects on lipids, BMI, and pancreas fat, to gain deeper mechanistic insights. Employing multivariable Mendelian randomisation techniques, we elucidated the unique and independent contributions of liver fat to each disease outcome.

Patients and methods

Study design

Fig. 1 summarizes our study design. To identify genetic determinants of liver fat, we performed a genome-wide association study (GWAS) of MRI-derived measures of liver fat. To understand the pleiotropic effect of each variant, we characterised the effect of variants on different liver-related outcomes. We performed a Mendelian randomisation study to understand whether there is a causal effect of genetically predicted liver fat on the risk of cardiovascular and liver cancer outcomes. We conducted a multivariable Mendelian randomisation study to infer the independent causal effect of liver fat on disease outcomes independent of six correlated risk factors (BMI,[10] HDL-C,[11] LDL-C,[11] VLDL-C,[12] triglycerides[11] and alcohol consumption;[13] Table S1).

Image-derived measures of liver fat

We used previously reported data from the UK Biobank MRI study.[14] In the current study, we included 37,358 individuals of White British ancestry who had MRI scans. The estimation of proton density fat fraction (PDFF) in liver slices was done using the PRESCO (Phase Regularized Estimation using Smoothing and Constrained Optimization) algorithm based on multi-echo data. For organ segmentation, manual annotations were performed on both GRE and IDEAL scans (Fig. S1). These annotations were thoroughly reviewed to ensure accuracy before

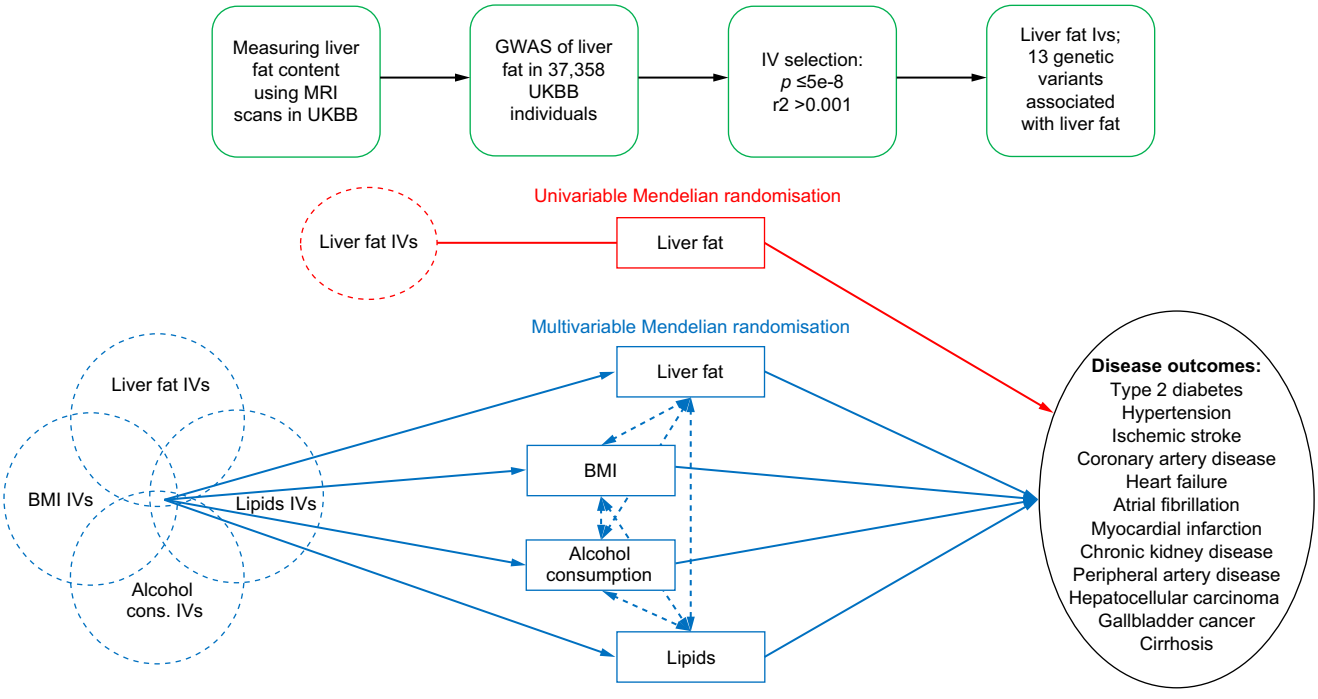

Fig. 1. Study design. Green flow diagram: we conducted a GWAS of MRI-measured liver fat and identified 13 genetic variants, which constituted our liver fat instrument. Red flow diagram: We performed univariable Mendelian randomisation to estimate the causal effect of genetically predicted liver fat on the risk of 12 disease outcomes, using data from FinnGen and published GWAS. Blue flow diagram: We performed multivariable Mendelian randomisation to estimate the direct causal effect of liver fat on the 12 disease outcomes accounting for correlated traits such as BMI, alcohol consumption and lipids. GWAS, genome-wide association study.

being utilised for modelling. We employed a customized U-net convolutional neural network for each imaging modality, which was then applied across all participant data. To combine data from IDEAL and multi-echo scans, each modality was first inverse rank normalized, and then these values were averaged between two scans, where both were available.

GWAS for liver PDFF were performed using REGENIE version v3.1.1.[15] We included only participants who self-reported their ancestry as 'White British' and who clustered with this group in a principal components analysis. We further excluded participants exhibiting sex chromosome aneuploidy, with a discrepancy between genetic and self-reported sex, heterozygosity and missingness outliers, and genotype call rate outliers.[16] Age, age^2, sex, genotyping array, imaging centre, and the first 10 principal components of the genotype relatedness matrix were included. Liver PDFF was inverse normal transformed before performing the association study. Imputed single nucleotide polymorphisms were filtered to a minor allele frequency >0.01 and INFO score >0.9, leaving 9,788,243 single nucleotide polymorphisms included in the final association study.

Outcome data

We selected 12 health outcomes including type 2 diabetes, hypertension, ischemic stroke, coronary artery disease, heart failure, atrial fibrillation, myocardial infarction, peripheral artery disease, chronic kidney disease, cirrhosis, hepatocellular carcinoma, and intrahepatic bile ducts and gallbladder cancer. The definition of cases and controls and sample size are summarized in Table S2. We obtained genome-wide summary level data for the 12 outcomes from FinnGen consortium Data Freeze 7 and 10. For all outcomes except cirrhosis, hepatocellular carcinoma, intrahepatic bile duct and gallbladder cancer and peripheral artery disease, we obtained genome-wide summary level data from other independent published GWAS.[17–24] We meta-analysed the Mendelian randomisation results for eight outcomes available from both FinnGen and published GWAS.

Mendelian randomisation

Mendelian randomisation is a statistical method that uses genetic variants as an instrument to infer the causal effect of an exposure (*e.g.*, liver fat) on an outcome of interest (*e.g.*, coronary heart disease). We defined the instruments using independent genetic variants (p ≤5x10^{-8}, linkage disequilibrium pruning of r2 >0.001 in a window of 10 Mb with the inclusion of unrelated white Europeans from the 1000 Genomes reference panel).

We applied different methods of Mendelian randomisation. For the main analysis, we used the inverse variance weighted (IVW) method. However, it is important to acknowledge that the IVW estimates could be susceptible to two principal sources of bias: instrumental variable bias and horizontal pleiotropy. The instrumental variable bias typically occurs due to a weaker association between the instrument and exposure proportional to the strength of the instrument and skewed towards the confounded direction of the association. Horizontal pleiotropy occurs when the instrument exhibits an association with the outcome via a pathway different from the exposure, violating the third assumption of Mendelian randomisation.[25]

To mitigate these potential biases, we performed different sensitivity analyses. MR-Egger was applied to check for horizontal pleiotropy through an examination of the Egger intercept. Additionally, MR-PRESSO, weighted median, simple mode, and weighted mode tests were conducted as robustness checks.[26]

Since liver fat, BMI, alcohol consumption and lipids are correlated risk factors, we performed a multivariable Mendelian randomisation analysis to understand the direct causal effect of liver fat on disease outcomes. Multivariable Mendelian randomisation is an advanced form of the univariable method that enables the assessment of multiple exposures on an outcome of interest. The method provides a direct causal estimate for each exposure while accounting for other exposures in the model.[27,28]

All Mendelian randomisation analyses were performed using "TwoSampleMR" package version 0.5.6.[29] We used the "metafor" package for meta-analysis of results from FinnGen and published GWAS. We used Benjamini-Hochberg–adjusted p values <0.05 to classify significant IVW causal associations.

Data and resource availability

All FinnGen outcome data used in this study are available from FinnGen Data Freezes 7 and 10 available at (https://www.finngen.fi/en/access_results). Outcome data for type 2 diabetes are available from (https://kp4cd.org/node/872), hypertension, ischemic stroke, coronary heart disease, heart failure, atrial fibrillation and myocardial infarction are available from (https://gwas.mrcieu.ac.uk/), and chronic kidney disease data is available from (https://ckdgen.imbi.uni-freiburg.de).

Results

We measured liver PDFF in 37,358 individuals of White British ancestry from the UK Biobank. The median age of participants was 64 years (IQR 59-70), the median liver PDFF was 4.8% (IQR 2-5), and 27% of participants had a liver PDFF greater than 5% (Table 1). We identified 13 independent variants strongly associated with liver fat. Together, these variants explained 35.7% of the variation in liver fat, with a high degree of statistical reliability (I^2 0.98; mean F-statistics 156 [min: 31, max: 856]; Table 2). Among these variants, rs6446296 (*CDHR4*) emerged as a novel finding, while others have been previously reported in GWAS studies of MASLD[30–34] (including rs738408 (*PNPLA3*), rs58542926 (*TMS6SF2*), rs429358 (*APOE*), rs1260326 (*GCKR*), rs2642438 (*MARC-1*), rs1229984 (*ADH1B*), rs28601761(*TRIB1*), and rs7029757 (*TOR1B*)) or were in linkage disequilibrium with previously reported variants[31] (including rs7096937 (*GPAM*), rs11867241 (*DRG2*) and rs188247550 (*SUGP1*)).

The liver fat-increasing alleles had a consistent dose-dependent effect on a higher risk of cirrhosis, hepatocellular carcinoma, and intrahepatic bile duct and gallbladder cancer (Fig. 2). However, they exhibited a heterogenous effect on other outcomes clustering into three main groups (Fig. 3). The first group included *TOR1B, MBOAT7, MARC1,* and *GPAM* where liver fat-increasing alleles were associated with lower triglycerides and higher LDL-C and HDL-C. The second group included variants with liver fat-increasing alleles associated with lower triglycerides and lower LDL-C. These variants included those in *PNPLA3, TMS6SF2, APOE,* and *SUGP1*. The liver fat-increasing alleles at these variants were associated with a higher risk of type 2 diabetes but a lower risk of coronary artery disease and myocardial infarction. The third group

Table 1. Characteristics of UK Biobank participants in the MRI-liver imaging cohort.

Sex	Number	Age	BMI	Diabetes%	Townsend deprivation index	Liver fat%	Liver fat >5%	Excess alcohol consumption%
Both	37,357	64 [59-70]	26.5 [23.5-28.8]	4.54	-2.02 [-3.93–0.71]	4.84 [2.12-5.29]	27	20
Female	19,154	64 [58-70]	26.0 [22.7-28.5]	2.95	-1.98 [-3.89–0.65]	4.18 [1.89-4.21]	20	6
Male	18,203	65 [59-71]	27.0 [24.3-29.1]	6.21	-2.06 [-3.96–0.77]	5.54 [2.48-6.45]	34	16

Diabetes%: percentage of individuals with diabetes; Liver fat >5%: percentage of individuals with liver fat greater than 5%; Excess alcohol consumption%: percentage of individuals with alcohol consumption above the recommended units per week.

included variants in *TRIB1, GCKR, ADH1B,* and *CDHR4* with liver fat-increasing alleles associated with higher triglycerides and higher LDL-C, and lower risk of type 2 diabetes and higher risk of coronary artery disease and myocardial infarction.

In our univariable Mendelian randomisation using IVW, a one standard deviation (SD) increase in genetically determined liver fat level (equivalent to a 5% increase in liver fat fraction) was associated with a higher risk of cirrhosis (odds ratio [OR] 6.33; 95% CI 4.6-8.60; Benjamini-Hochberg–adjusted $p = 6 \times 10^{-30}$), hepatocellular carcinoma (OR 13.1; 95% CI 9.30-18.7; Benjamini-Hochberg–adjusted $p = 2 \times 10^{-45}$) and intrahepatic bile duct and gallbladder cancer (OR 3.7; 95% CI 2.90-4.70; Benjamini-Hochberg–adjusted $p = 1 \times 10^{-25}$; Fig. 4), with consistent direction of effect across different sensitivity tests (Table S3). No evidence of pleiotropy was observed for these associations (Egger intercept *p* value >0.05; Cochran's Q <0.05; Table S4). These associations remained unchanged even after correction for the causal role of correlated risk factors (*i.e.*, BMI, alcohol consumption, triglycerides, HDL-C and LDL-C; Fig. 4 and Fig. S2, Table S5). The sensitivity analysis excluding *ADH1B* and *MBOAT7* variants from the liver fat instrument, due to their association with alcohol metabolism or alcohol-related liver disease, did not change our results (Fig. S3; Table S6).

There was a suggestive causal effect on risk of type 2 diabetes (OR 1.2; 95% CI 1.10-1.32), which became stronger after correcting for correlated risk factors in our multivariable Mendelian randomisation (OR 1.3; 95% CI 1.23–1.40; Benjamini-Hochberg–adjusted $p = 6 \times 10^{-20}$) (Fig. 5, Table S5). No evidence of a causal association was detected between genetically determined liver fat and hypertension, ischemic stroke, coronary artery disease, heart failure, atrial fibrillation, myocardial infarction, chronic kidney disease or peripheral artery disease (Fig. 5). However, MR-Egger intercept *p* value and Cochran's Q indicated evidence of pleiotropy for coronary artery disease, atrial fibrillation, myocardial infarction, intrahepatic bile duct and gallbladder cancer and peripheral artery disease, type 2 diabetes, hypertension, heart failure, and chronic kidney disease. Consequently, we performed MR-PRESSO for these outcomes to correct for pleiotropy. The global test was significant (*p* value <0.001) indicating the presence of outliers ranging from 1 to 5 variants. However, there was no significant difference in estimated causal effect except for heart failure and atrial fibrillation, where the direction of effect was reversed, *i.e.* one SD increase in genetically determined liver fat was associated with higher risk of developing heart failure and atrial fibrillation (Table S7).

Given the heterogeneity in the effect of liver fat variants on other outcomes, we conducted a secondary Mendelian randomisation analysis using the three main groups of variants as exposures. All three groups were associated with higher risk of cirrhosis, hepatocellular carcinoma, and intrahepatic bile duct and gallbladder cancer with consistent effect (Fig. S4). However, the effect on other outcomes differed across the three groups of exposures (Fig. S4). Group one (including variants in *TOR1B, MBOAT7, MARC1,* and *GPAM*) showed no association with cardiovascular outcomes. Group two (*PNPLA3* locus*, TMS6SF2, APOE,* and *SUGP1*) was associated with a higher risk of type 2 diabetes and lower risk of coronary artery disease and myocardial infarction, while group three (*TRIB1, GCKR, ADH1B,* and *CDHR4*) was associated with a higher risk of coronary artery disease, heart failure, myocardial infarction, and a lower risk of chronic kidney disease.

Table 2. Characteristics of the genetic variants associated with liver fat.

rsID	Chromosome	Position	Effect allele	Other allele	EAF	BETA	SE	*p* value	Gene	F-statistics
rs2642438	1	220970028	G	A	0.705	0.057	0.008	9.80E-13	*MARC1*	50.9
rs1260326	2	27730940	T	C	0.391	0.056	0.007	4.70E-14	*GCKR*	56.9
rs6446296	3	49838052	A	G	0.791	0.050	0.009	2.30E-08	*CDHR4*	31.2
rs1229984	4	100239319	C	T	0.976	0.157	0.024	3.80E-11	*ADH1B*	43.7
rs28601761	8	126500031	C	G	0.579	0.062	0.007	5.90E-17	*TRIB1*	70.0
rs7029757	9	132566666	G	A	0.904	0.072	0.012	6.40E-09	*TOR1B*	33.7
rs7096937	10	113950418	T	C	0.270	0.062	0.008	4.60E-14	*GPAM*	56.9
rs11867241	17	17988586	C	T	0.301	0.044	0.008	2.80E-08	*DRG2*	30.9
rs58542926	19	19379549	T	C	0.075	0.313	0.014	6.80E-115	*TM6SF2*	519.0
rs188247550	19	19396616	T	C	0.015	0.305	0.031	3.20E-22	*SUGP1*	94.0
rs429358	19	45411941	T	C	0.848	0.123	0.010	2.00E-34	*APOE*	149.7
rs626283	19	54677189	T	C	0.438	0.044	0.007	2.10E-09	*MBOAT7*	35.9
rs738408	22	44324730	T	C	0.214	0.257	0.009	4.60E-188	*PNPLA3*	855.5

Effect allele: liver fat-increasing allele; Other allele: liver fat-decreasing allele; BETA: effect size; Gene: nearest protein-coding gene; F-statistics: average strength of the association of each variant with the instrument (*i.e.* liver fat). Positions are based on Build 37. EAF, effect allele frequency; SE, standard error.

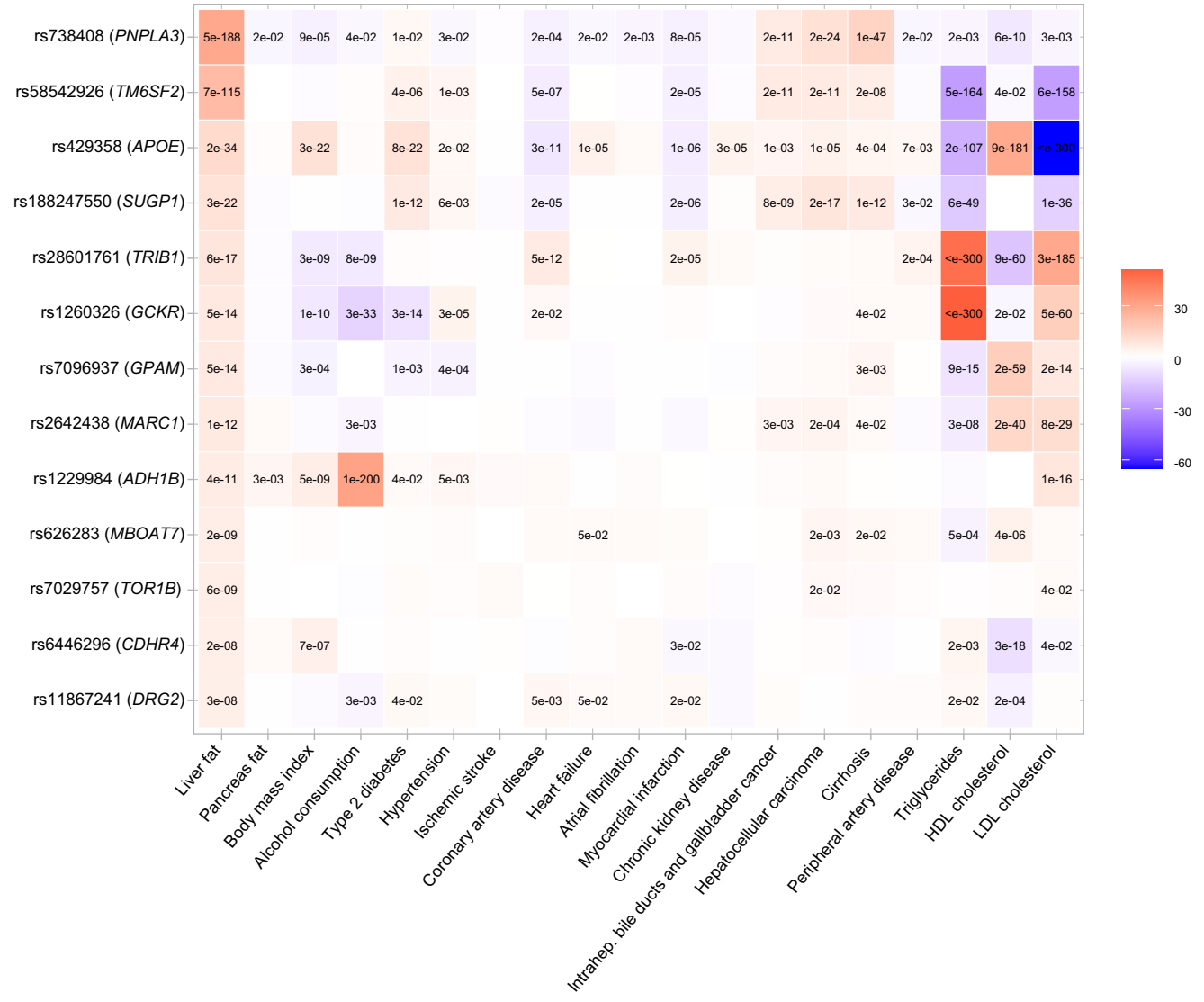

Fig. 2. The effect of liver fat variants on health outcomes. Effect of 13 liver fat-increasing alleles on 18 health outcomes. Colors and intensity represent the direction and magnitude of Z-scores from linear regression in the genome-wide association model. Benjamini-Hochberg adjusted *p* values <0.05 are provided for each association.

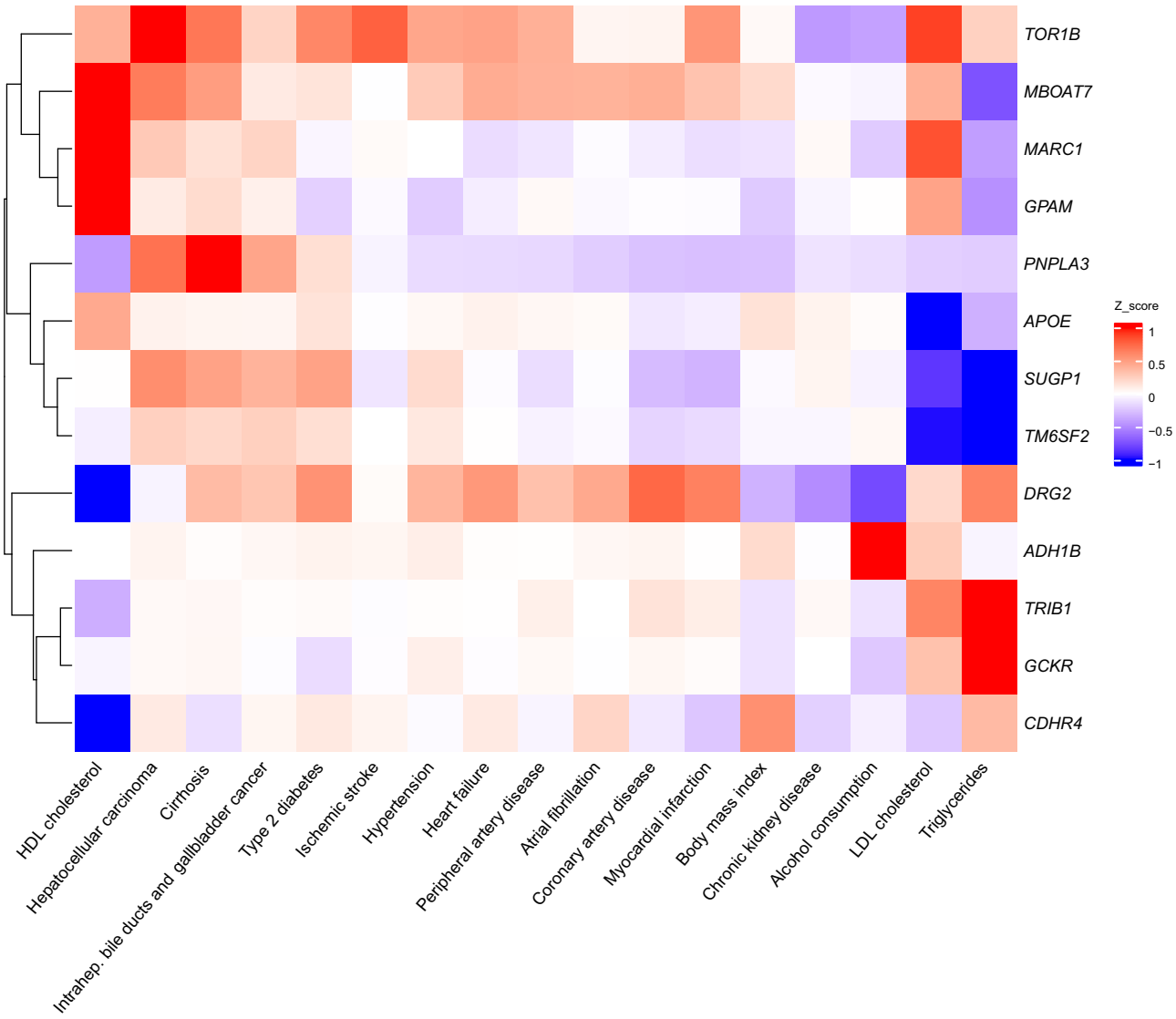

Fig. 3. The heterogeneous effect of liver fat-increasing alleles on different health outcomes. Twelve of the 13 liver fat-increasing alleles are clustered into three groups (y-axis) based on their effect on 17 health outcomes (x-axis). Z score are scaled to the range of [-1:1] with red indicating positive association and blue indicating negative association.

Discussion

We measured liver fat fraction from MRI scans of 37,358 participants from the UK Biobank study and used its genetic determinants to understand its causal role in cardiovascular and liver cancer outcomes. Our findings suggest that liver fat is a heterogeneous phenotype, with distinct mechanisms capable of increasing liver fat, some of which exhibit opposing effects on the risk of diseases outside of the liver. Our study provides evidence that genetically determined higher liver fat accumulation is causally associated with an increased risk of cirrhosis, hepatocellular carcinoma, and intrahepatic bile duct and gallbladder cancer. We did not, however, find evidence of a causal association between genetically determined liver fat and cardiovascular diseases like hypertension, ischemic stroke, and coronary artery disease.

The characterisation of liver fat variants reveals the involvement of diverse mechanisms contributing to higher fat accumulation in the liver, consistent with recent findings.[30,31] One group associated with *impaired* hepatic triglyceride export. This group includes four variants that increase liver fat by increasing triglyceride accumulation within hepatocytes through various pathways. *PNPLA3* liver fat-increasing allele is associated with impairing lipid droplet remodelling and turnover leading to retention of triglycerides in hepatocytes.[35,36] *TM6SF2* is involved in lipoprotein lipidation and the liver fat-increasing allele at this locus is associated with a decreased secretion of VLDL from the liver, lower serum cholesterol and triglyceride levels accompanied by an accumulation of hepatic triglycerides.[36,37] *APOE* encodes for a primary component of VLDL and chylomicrons. The liver fat-increasing allele at *APOE* could diminish the liver's ability to produce VLDL leading to hepatic triglyceride accumulation.[38] While the *SUGP1* variant exhibits a similar pattern, there is comparatively less evidence about its role in lipid metabolism.

The second group of variants increase the accumulation of fat in the liver through increased fat synthesis or inhibition of

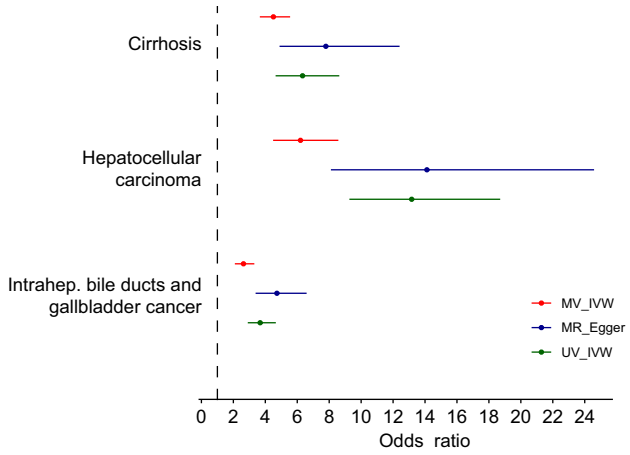

Fig. 4. The total and independent causal effect of liver fat on cirrhosis and cancer. The x-axis shows the odds ratio and 95% CIs for the association between genetically predicted higher liver fat and the risk of cirrhosis (1,266 cases *vs.* 407,801 controls), hepatocellular carcinoma (500 cases *vs.* 314,193 controls), and intrahepatic bile duct and gallbladder cancer (1,207 cases *vs.* 314,193 controls) from FinnGen. Results are shown from IVW method from univariable Mendelian randomisation (red), MR-Egger method from univariable Mendelian randomisation (blue), and IVW method from multivariable Mendelian randomisation (green). IVW, inverse variance weighted.

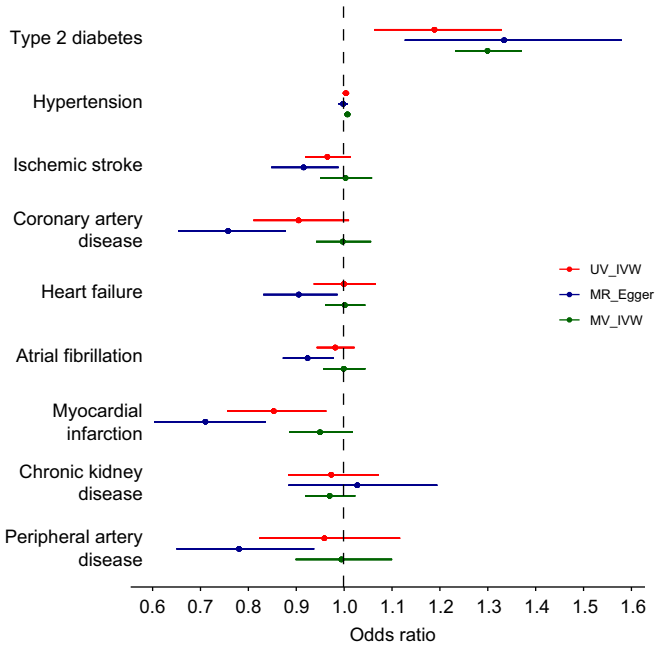

Fig. 5. The total and independent causal effect of liver fat on type 2 diabetes and cardiovascular disease. The x-axis shows the odds ratio and 95% CIs for the association between genetically predicted higher liver fat and the risk of nine outcomes, meta-analysed from FinnGen and Published GWAS: type 2 diabetes (139,209 cases *vs.* 1,159,118 controls), hypertension (177,354 cases *vs.* 697,769 controls), ischemic stroke (51,074 cases *vs.* 689,168 controls), coronary artery disease (73,822 cases *vs.* 407,751 controls), heart failure (76,981 cases *vs.* 1,312,519 controls), atrial fibrillation (111,363 cases *vs.* 288,638 controls), myocardial infarction (73,822 cases *vs.* 345,759 controls), chronic kidney disease (51,434 cases *vs.* 836,009 controls), and peripheral artery disease (11,924 cases *vs.* 407,751 controls). Results are shown from IVW method from univariable Mendelian randomisation (red), MR-Egger method from univariable Mendelian randomisation (blue), and IVW method from multivariable Mendelian randomisation (green). IVW, inverse variance weighted.

lipid breakdown. This group includes variants in *GCKR* and *TRIB1*. The *GCKR* liver fat-increasing allele increases glucokinase enzyme activity in the liver which promotes *de novo* lipogenesis leading to the accumulation of hepatic triglycerides.[36,39] *TRIB1* is involved in regulating hepatic glycogenesis and lipogenesis. The *TRIB1* liver fat-increasing allele is associated with higher levels of plasma triglycerides and LDL-C and, consequently, more hepatic triglyceride accumulation.[40] The rest of the variants have diverse effects on different outcomes.

Our results suggest that the association of higher liver fat with the risk of cardiovascular diseases, specifically myocardial infarction and coronary artery disease, depends on the specific mechanism by which fat accumulates in the liver. For example, if the underlying mechanism is through *impaired* hepatic triglyceride export (as we see for variants in *PNPLA3, TM6SF2, APOE* and *SUGP1*), higher liver fat is accompanied by lower circulatory triglycerides and lower LDL-C and is linked to lower risk of myocardial infarction and coronary artery disease. On the other hand, if the underlying mechanism is through increased *de novo* lipogenesis (for example through *TRIB1 and GCKR*), higher liver fat is accompanied by higher triglycerides and higher LDL-C, leading to more systemically delivered atherogenic lipids and a higher risk of myocardial infarction and coronary artery disease. It is important to note that increased *de novo* lipogenesis is likely one of several mechanisms by which these genetic variants influence liver fat accumulation. Both mechanisms were associated with higher risk of type 2 diabetes as previously reported.[41]

Notably one previous Mendelian randomisation study which used MASLD as exposure also found no causal association between MASLD and cardiovascular diseases including coronary artery disease and stroke.[42] This finding and our novel extension here appear somewhat contrary to evidence from several observational studies showing an association between MASLD and a higher risk of cardiovascular diseases.[43–45] These contradictory findings may be attributed to the fact that observational studies are confounded by obesity (and its downstream metabolic consequences) which is a shared risk factor for both MASLD and cardiovascular diseases, with 50-90% of people with MASLD living with obesity.[46] The current guidelines by the UK NICE (National Institute for Health and Care Excellence) state that '*cardiovascular disease is the most common cause of death in people with MASLD*'.[47] Our results emphasise that higher fat accumulation in the liver may not be an independent risk factor for cardiovascular diseases and is not a single disease; the association depends on the underlying lipid regulation mechanism. It is important to emphasise that the potential benefits of a novel drug for MASLD could be counteracted by an elevated risk of cardiovascular disease if the drug simultaneously increases plasma lipid levels.[48]

This study provides strong evidence of a causal association between genetically determined higher liver fat levels and an increased risk of cirrhosis, hepatocellular carcinoma, and intrahepatic bile duct and gallbladder cancer, consistent with previous reports.[31,49] These findings resonate with those of Bianco *et al.* (2021)[50] who reported that a genetic risk score for hepatic accumulation can predict the risk of hepatocellular carcinoma, both in at-risk individuals with MASLD and in the general population. Our results suggest the effect of liver fat variants on the risk of liver cirrhosis and cancer is proportional to their effect on liver fat; the bigger the effect on liver fat, the bigger the effect on

the risk of cirrhosis and cancer, and vice versa. These findings suggest that higher liver fat increases the risk of cirrhosis, hepatocellular carcinoma, and intrahepatic bile duct and gallbladder cancer in a dose-dependent pattern and irrespective of the mechanism by which candidate genes exert their effect on liver fat (*i.e.* higher fat import and export to and from the liver). In light of the poor prognosis of these diseases, these findings provide a strong case for a clinical intervention aimed at reducing liver fat content (likely predominantly by intentional weight loss) regardless of a binary definition for MASLD. Clinicians must think about reshaping the risk assessment approach and prioritise interventions (perhaps including novel incretin-based weight loss drugs) that may mitigate the risk of liver fibrosis and cancers.

Our study has some limitations. First, we used genetic variants associated with a lifetime predisposition to accumulated liver fat. Consequently, the study does not take into consideration the impact of short-term liver fat change (including those influenced by dietary changes, medication or lifestyle) on the risk of cardiovascular and metabolic diseases. Second, although our study provides novel findings underpinning the two main mechanisms involved in hepatic fat accumulation, we acknowledge that we have used circulatory lipid levels, BMI, alcohol consumption and disease outcomes for clustering liver fat variants which may not provide a comprehensive understanding of the heterogeneity and complexity of liver fat accumulation. Future research utilising unsupervised clustering of liver fat variants and multiomics data could provide better understanding. Third, although the study identified various genetic variants that are strongly associated with liver fat accumulation and possibly the risk of cardiovascular, metabolic and oncologic outcomes, the mechanisms by which those variants exert their effect are not fully understood, consequently limiting the ability of this study to fully explain the effect of those variants on health-related outcomes. Fourth, we limited our discovery of liver fat associated genetic variants to the White British population, which could negatively impact the generalisability of this study. Identifying genetic variants associated with liver fat in other ethnic groups might reveal some novel insight and a different effect on liver fat accumulation and the risk of those outcomes. Fifth, our study did not exclude the possibility that excess alcohol intake, chronic viral hepatitis C, and Mendelian disorders (*e.g.*, hypobetalipoproteinaemia), known to affect hepatic fat accumulation, could be contributing factors in this subset of UK Biobank participants. Conducting sensitivity analyses to examine the association of genetic variants with hepatic fat in specific subgroups (*e.g.*, with and without excess alcohol intake) would help verify the consistency of these effects.[51]

Our results warrant further research to elucidate the short-term effect of change in liver fat and the mechanisms behind the genetic determinant of liver fat, as well as to explore such effects in different populations.

This study provides a multifaceted understanding of the association between liver fat accumulation and various health outcomes. Our findings confirm and challenge some pre-existing knowledge. While we provided evidence for the adverse consequence of higher fat accumulation in the liver, our findings challenge previous assumptions from observational studies that liver fat *per se* increases the risk of cardiovascular diseases. The association between liver fat and cardiovascular disease is determined by the underlying mechanisms that increase fat accumulation in the liver and is probably influenced in large part through lipid regulation. These distinct findings could have implications for clinical practice and emphasise the need for more personalised treatment options that prioritise other complications of liver fat accumulation such as liver fibrosis and cancers.

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Abbreviations

GWAS, genome-wide association study; IVW, inverse variance weighted; MASLD, metabolic dysfunction associated steatotic liver disease; OR, odds ratio; PDFF, proton density fat fraction; SD, standard deviation.

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Conflict of interest

NS has received grant and personal fees from AstraZeneca, Boehringer Ingelheim, and Novartis; a grant from Roche Diagnostics; and personal fees from Abbott Laboratories, Afimmune, Amgen, Eli Lilly, Hanmi Pharmaceuticals, Merck Sharp & Dohme, Novo Nordisk, Pfizer, and Sanofi outside the submitted work. MC is employee of Calico Life Sciences LLC.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

A.A. analysed the data. H.Y. designed the study. A.A., N.S. and H.Y. wrote the manuscript. M.C. and J.D.B. provided the MRI-derived measures of liver fat and performed the genome-wide association study of liver fat. H.Y. is the guarantor of this work and, as such, has full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Data availability statement

Full data including individual liver PDFF measures will be returned to UK Biobank and made publicly available via application (amsportal.ukbiobank.ac.uk).

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Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2024.06.030.

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