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ORIGINAL ARTICLE

Alanine Transaminase Mediates the Causal Link between Chronic Heart Failure and Nonalcoholic Fatty Liver Disease

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ABSTRACT

Background: **Clinical evidence suggests an association between chronic heart failure (CHF) and liver disease; however, the causal relationship remains unclear. This study aimed to investigate the causal effects of CHF on common liver diseases, including non-alcoholic fatty liver disease (NAFLD), hepatitis, and cirrhosis, using a two-sample Mendelian randomization (MR) approach. Additionally, we evaluated whether four liver function indicators-alanine transaminase (ALT), aspartate transaminase (AST), bilirubin (BL), and albumin (AL)-mediate this relationship.**

Methods: **Genome-wide association study (GWAS) summary data were obtained from the UK Biobank database. CHF was treated as the exposure, while NAFLD, hepatitis, and cirrhosis were considered outcomes. ALT, AST, BL, and AL were analyzed as potential mediators. Five MR methods-Inverse Variance Weighted (IVW), MR-Egger, Weighted Median, MR-RAPS, and MR-PRESSO were applied to evaluate causal associations and mediation effects.**

Results: **MR analysis demonstrated a causal association between CHF and NAFLD by 48 SNPs (p = 0.0182), OR_{IVW} [95% CI] = 1.1352 [1.0218 - 1.2612]. No significant causal associations were observed between CHF and hepatitis or cirrhosis. Among the four liver function indicators, ALT was identified as a significant mediator in the CHF-NAFLD relationship, with a mediation effect of 0.0021 [0.0001 - 0.0040], and a mediation proportion of 1.6561 [0.0698, 3.1788] % (p = 0.0406). Sensitivity analyses, including funnel plots and leave-one-out analysis, confirmed the robustness of the findings.**

Conclusions: **These findings suggest that CHF may increase the risk of NAFLD, partially mediated through ALT levels, highlighting the potential role of liver function indicators in the pathophysiological link between CHF and NAFLD.**

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INTRODUCTION

Heart failure (HF) arises from a multitude of etiological factors leading to structural or functional abnormalities in the heart. This, in turn, results in disturbances in both systolic and diastolic functions, culminating in a syndrome characterized by circulatory disorders [1]. Primary clinical manifestations in HF patients encompass dyspnea, fatigue, peripheral edema, and fluid retention, marked by elevated morbidity and mortality rates, compromised functional capacity, and diminished quality of life [2]. Through clinical investigations, the team led by Zardi et al. [3] unveiled that chronic right heart failure can induce hepatic venous congestion, consequently elevating the risk of cirrhosis. In clinical scenarios, approximately 20% - 30% of acute HF patients exhibit impaired liver function, progressing to conditions such as fatty liver, congestive hepatopathy, and ultimately, liver failure [4]. This progression poses a severe threat to the lives of affected individuals. Itier et al. [5] systematically delved into the association between HF and non-alcoholic fatty liver disease (NAFLD, now termed metabolic dysfunction-associated steatotic liver disease [MASLD]), with a specific focus on the role of insulin resistance in the treatment of HF and NAFLD. Kavoliuniene et al. [6] conducted an analysis of liver damage associated with circulatory dysfunction, including hypoxic hepatitis and congestive hepatopathy, underscoring the critical importance of differential diagnosis of liver injury in the clinical practice of cardiologists. Furthermore, a meta-analysis corroborated a strong correlation between non-alcoholic fatty liver disease and HF, atrial fibrillation, ischemic stroke, and myocardial infarction [7]. In summation, HF is intricately linked to liver diseases.

Patients experiencing either acute or CHF often present with abnormal AST levels, a factor closely linked to a poorer prognosis. Notably, Allen et al. [8], in a clinical analysis, identified that approximately 3.1% of CHF patients exhibited elevated ALT levels, a factor associated with an unfavorable prognosis. Similarly, clinical studies conducted by van Deursen et al. [9] demonstrated that around 24 (12%) patients with acute HF had ALT levels surpassing the normal upper limit, correlating with heightened risks of patient mortality, readmission, and worsening of HF during hospitalization. The study conducted by Tomizawa et al. [10] suggested that ALT levels exceeding 19 IU/L can serve as valuable markers for screening NAFLD, with even higher ALT levels observed in cases of heightened uncertainty regarding the disease [11]. Collectively, these studies indicate elevated ALT levels in both CHF and NAFLD. Consequently, ALT emerges as a potential mediator between CHF and NAFLD.

Traditional observational cohort studies are unavoidably susceptible to various confounding factors and biases arising from reverse causation. While randomized controlled trials (RCTs) are deemed the gold standard for establishing causal relationships, their application is constrained by the substantial research costs and the imperative adherence to ethical standards [12]. The rapid advancement of Genome-Wide Association Studies (GWAS) and the extensive availability of public databases have led to the widespread adoption of MR analysis, particularly with Single Nucleotide Polymorphisms (SNPs) serving as instrumental variables (IVs) [13]. MR methods offer precise causal relationships without the need to consider research costs and ethical standards. Furthermore, the allele genes employed as IVs are randomly inherited to the next generation during meiosis, and the relationship between genes and outcomes remains unaffected by postnatal confounding factors such as environmental, social, and behavioral influences [14]. Consequently, MR has found broad application across various biomedical domains, yielding numerous valuable insights. In summary, this study intends to utilize MR methods to explore the causal relationship between CHF and liver diseases (NAFLD, hepatitis, cirrhosis), and to evaluate whether liver function indicators (ALT, AST, BL, and AL) serve as mediators between CHF and liver diseases.

MATERIALS AND METHODS

Study design

In this study, CHF was chosen as the exposure variable, and SNPs associated with CHF were employed as instrumental variables. Three liver diseases (NAFLD, hepatitis, cirrhosis) were designated as outcome variables, while four liver function indicators (ALT, AST, BL, and AL) were identified as mediators. The analysis employed a two-sample MR approach to investigate the causal relationship between CHF and liver diseases, exploring the potential role of liver function indicators as mediators. Subsequently, Cochran's Q and MR Egger algorithms were applied to assess heterogeneity and horizontal pleiotropy. Lastly, funnel plots and leave-one-out sensitivity analysis were conducted to evaluate the stability of the results.

Data source

All the aggregated data used in this study are publicly accessible and retrieved from the MRC IEU OpenGWAS database (https://opengwas.io/). The study selected the most extensive and recent GWAS statistical data, serving as exposure or outcome factors, with detailed specifications provided in Table 1. For the cirrhosis outcome, the GWAS dataset (GWAS ID: finn-b-CIRRHOSIS_BROAD) was derived from the FinnGen consortium, and the corresponding phenotype definition

is publicly available through the FinnGen Risteys portal (https://r8.risteys.finngen.fi/phenocode/CIRRHOSIS_BROAD). Given that all original GWAS studies included in this research have obtained ethical and institutional review board approval from local authorities, as documented in each respective original publication, no additional ethical approval is deemed necessary for this study.

Instrumental variable selection

Rigorous criteria were applied in the selection of qualified IVs. Initially, SNPs closely associated with the European CHF population were identified as candidate IVs ($p < 1 \times 10^{-05}$) [23]. The PLINK clustering method, with a clustering threshold ($r^2 < 0.001$, kb = 10,000), was then employed to mitigate potential biases arising from linkage disequilibrium. Subsequently, SNPs not provided in the outcome GWAS results were excluded, and no proxy SNPs were utilized in the analysis. Following this, F-statistics were calculated to assess the strength between IVs and CHF, retaining SNPs with an F-statistic > 10 ($F = [(N - K - 1)/K]/[R^2/(1 - R^2)]$, where K is the number of genetic variants, and N is the sample size) [24]. Finally, eligible and non-pleiotropic SNPs were included in the instrumental variable set for MR analysis.

Statistical analysis

In the realm of statistical analysis, this study employed the "TwoSampleMR" package (Version: 0.6.22) within R software (Version: 4.5.1) for conducting two-sample MR analyses. The assessment of MR pleiotropy residual sum and outlier analysis was executed using the "MRPRESSO" package (Version: 1.0). Additionally, PhenoScanner was utilized to explore and evaluate all known phenotypes associated with the IVs employed in the analysis. The results were primarily scrutinized using five MR algorithms, namely MR-Egger, IVW, Weighted Median, MR-RAPS, and MR-PRESSO. For enhanced result interpretation, β and SE values were transformed into Odds Ratios (OR), and 95% Confidence Intervals (CI) were calculated.

To assess the reciprocal causal relationship between CHF and NAFLD, a two-sample MR approach was employed, characterized as the overall effect. The validity of MR analysis relies on three crucial assumptions: 1) the selected IVs must exhibit a close association with the exposure, representing the relevance assumption; 2) the chosen IVs should demonstrate independence from potential confounding factors, ensuring the independence assumption; 3) the selected IVs should solely influence the outcome through exposure and not through alternative pathways, emphasizing the exclusivity assumption, as illustrated in Figure 1A.

A two-step MR approach was employed to delve into the potential mediation of liver function indicators in the causal relationship between CHF and NAFLD, as illustrated in Figure 1B. The overall effect is dissected into two components: the direct effect without mediation and the indirect effect mediated through intermediaries. The total effect of CHF on NAFLD is the sum of its direct effect and the indirect effect facilitated by intermediaries. This study calculated the percentage of mediation effect by dividing the indirect effect by the total effect. Additionally, the Delta method was applied to estimate standard errors (SE) and 95%CI for mediation effects [25]. Furthermore, a parametric bootstrap approach (10,000 simulations) was used to assess robustness. Indirect effects were estimated as the product of simulated coefficients, with 95% confidence intervals derived from the empirical distribution. And R package "gsmr2" package (Version: 1.1.1) was applied to perform the HEIDI (Heterogeneity in Dependent Instruments) test.

In this study, the causal direction of each extracted SNP in relation to exposure and outcome was determined using MR Steiger filtering tests, excluding SNPs with conflicting directions. Heterogeneity was assessed through Cochran's Q statistics, while MR-Egger intercept and MR-PRESSO were employed for gene-level pleiotropy detection. In cases of outlier detection, anomalous SNPs were removed, and the MR causality was re-evaluated. If heterogeneity persisted post-removal, the stability of results was assessed using a random-effects model. Furthermore, leave-one-out analysis was conducted to validate the influence of each SNP on the overall causal estimation. Finally, to evaluate the potential influence of pleiotropy and confounding factors, we conducted a supplementary sensitivity analysis using PhenoScanner (http://www.phenoscanner.medschl.cam.ac.uk/). SNPs associated with key confounders - including body mass index (BMI), diabetes mellitus, hypertension, cancer, severe COVID-19, and alcohol intake - were identified and excluded (Supplementary Table 1), after which the MR analysis was repeated.

RESULTS

Instrumental variables extraction

First, based on the default threshold for genome-wide statistical significance ($p < 5 \times 10^{-08}$) and using the clustering threshold in the PLINK clustering method ($r^2 < 0.001$, kb = 10,000), a preliminary screening was conducted. On this basis, the R^2 and F statistics of each SNP were calculated, and SNPs with an F statistic less than 10 were excluded. Eventually, 7 SNPs related to chronic heart failure were selected (Table 2). However, it should be noted that only 1 SNP in the ALT GWAS dataset could not be subjected to downstream analysis. Therefore, the screening threshold was adjusted to $p < 1 \times 10^{-05}$, resulting in 68 SNPs related to chronic heart failure. To ensure that at least 3 SNPs were detected in all outcome GWAS datasets, low-quality SNPs with incompatible alleles or palindromic with intermediate allele frequencies were excluded from the outcome datasets (Table 2, Figure 2).

Table 1. Detailed information of included data sources in this study.

Phenotypes	GWAS ID	Cases/controls	Number Of SNPs	Population
CHF [15]	ebi-a-GCST90018806	14,262/471,898	24,178,220	European
NAFLD [16]	ebi-a-GCST90054782	4,761/373,227	9,097,254	European
Cirrhosis [17]	finn-b-CIRRHOSIS_BROAD	1,931/216,861	16,380,466	European
Hepatitis [18]	ebi-a-GCST90038627	2,450/482,148	958,7836	European
ALT [19]	ebi-a-GCST004940	9,731	1,698,168	European
AST [20]	ebi-a-GCST90013996	388,490	10,783,671	European
BL [21]	ebi-a-GCST90025973	436,055	4,231,416	European
AL [22]	ebi-a-GCST90092807	115,064	11,590,399	European

Table 2. Statistics of instrumental variables extraction.

Exposure	Threshold	SNPs	R^2 quartile	F quartile	Exposure	Detected SNPs
CHF	$p < 5 \times 10^{-08}$	7	0.0081 [0.0080 - 0.0094]	53.9100 [52.1100 - 54.1000]	NAFLD	7
					Cirrhosis	7
					Hepatitis	6
					ALT	1
					AST	7
					BL	7
					AL	7
CHF	$p < 1 \times 10^{-05}$	68	0.0066 [0.0064 - 0.0071]	32.1700 [31.6200 - 32.4000]	NAFLD	68
					Cirrhosis	68
					Hepatitis	61
					ALT	14
					AST	68
					BL	12
					AL	68

Relationship between CHF and liver diseases

In this study, MR is utilized to explore the causal connection between CHF and the development of liver diseases, including NAFLD, hepatitis, and cirrhosis (Table 3, Figure 3, 4, Supplementary Figure 1). The IVW analysis revealed a significant causal relationship between CHF and NAFLD (p = 0.0182), OR [95% CI] = 1.1352 [1.0218 - 1.2612], while no discernible causal links are observed with hepatitis or cirrhosis. Although the MR-PRESSO analysis showed a non-significant result (p = 0.736), the IVW analysis supported a significant causal association between CHF and NAFLD. This discrepancy may be related to differences in statistical power and methodological assumptions among MR approaches. Rigorous examination of the MR results for CHF and NAFLD, incorporating Cochran's Q and Horizontal Pleiotropy tests, provides evidence of the absence of heterogeneity and gene-level pleiotropy. In addition, Cochran's Q test indicated that there was a certain degree of heterogeneity in the MR Egger and IVW algorithms for hepatitis ($p < 0.05$). Therefore, the IVW algorithm was corrected, and the results were recalculated using IVW (multiplicative random effects). In summary, CHF demonstrated a noteworthy correlation with NAFLD.

Relationship between CHF and liver function indicators

This study employed MR to investigate the causal relationship between CHF and liver function indicators, including ALT, AST, BL, and AL (Table 4, Figure 3, 4, Supplementary Figure 1). Cochran's Q test indicated that there was a certain degree of heterogeneity in the MR Egger and IVW algorithms for ALT, AST and BL

Table 3. MR Results for the impact of CHF on liver diseases (NAFLD, hepatitis, and cirrhosis).

MR info							Cochran's Q			Horizontal pleiotropy		
Outcomes	Snps (n)	method	β	SE	OR [95% CI]	p	Q	df	p	intercept	SE	p
NAFLD	48	MR Egger	0.1187	0.1249	1.1260 [0.8815 - 1.4385]	0.3470	50.1371	46	0.4099	0.0007	0.0098	0.9428
		IVW	0.1268	0.0537	1.1352 [1.0218 - 1.2612]	0.0182	50.1428	47	0.4490	-	-	-
		Weighted median	0.1467	0.0754	1.1580 [0.9989 - 1.3424]	0.0517	-	-	-	-	-	-
		MR-RAPS	0.0042	0.0121	1.0042 [0.9807 - 1.0282]	0.7310	-	-	-	-	-	-
		MR-PRESSO	0.0041	0.0120	1.0041 [0.9808 - 1.0279]	0.736	-	-	-	-	-	-
Cirrhosis	59	MR Egger	0.0421	0.1914	1.0430 [0.7167 - 1.5179]	0.8266	61.0842	57	0.3315	0.0046	0.0140	0.7418
		IVW	0.0994	0.0813	1.1045 [0.9419 - 1.2952]	0.2211	61.2017	58	0.3618	-	-	-
		Weighted median	0.0511	0.1136	1.0524 [0.8424 - 1.3148]	0.6527	-	-	-	-	-	-
		MR-RAPS	0.1122	0.0887	1.1187 [0.9403 - 1.331]	0.2058	-	-	-	-	-	-
		MR-PRESSO	0.0232	0.0226	1.0235 [0.9791 - 1.0699]	0.3094	-	-	-	-	-	-
Hepatitis	62	MR Egger	0.0007	0.0010	1.0007 [0.9988 - 1.0026]	0.4655	88.0296	60	0.0107	-4.37e-05	7.36e-05	0.5555
		IVW (multiplicative random effects)	0.0994	0.0813	1.1045 [0.9419 - 1.2952]	0.2211	88.5453	61	0.0121	-	-	-
		Weighted median	0.0007	0.0005	1.0007 [0.9997 - 1.0017]	0.1573	-	-	-	-	-	-
		MR-RAPS	0.0009	0.0011	1.0009 [0.9987 - 1.0032]	0.4348	-	-	-	-	-	-
		MR-PRESSO	1.30e-05	8.89e-05	1.0000 [0.9998 - 1.0002]	0.8839	-	-	-	-	-	-

NAFLD: Nonalcoholic fatty liver disease, OR and 95% CI were calculated from unrounded beta estimates.

($p < 0.05$), and the results were recalculated using IVW (multiplicative random effects). The multiplicative random effects (IVW) analysis revealed a significant causal association between CHF and ALT ($p = 0.0279$), OR [95% CI] = 1.0420 [1.0045 - 1.0810]. And the result of Weighted Median and MR-PRESSO methods also support a substantial causal relationship between CHF and ALT ($p = 0.0012$, 0.0483), OR [95% CI] = 1.0656 [1.0253 - 1.1075] and 1.0420 [1.0045 - 1.0810]. However, no causal links are observed between CHF and AST, BL, and AL ($p > 0.05$). Horizontal pleiotropy tests on the MR results for CHF and ALT indicate the absence of gene-level pleiotropy ($p > 0.05$). In summary, a robust correlation between CHF and ALT is established. In addition, the colocalization analysis was supplemented by the HEIDI (Heterogeneity in Dependent Instruments) test. The results showed that there was no significant colocalization between the SNPs of CHF and those of NAFLD and ALT ($p > 0.05$).

Relationship between ALT and NAFLD

MR was employed to investigate the causal relationship between ALT and NAFLD, as outlined in Supplementary Table 2. The IVW analysis revealed a causal association between ALT and NAFLD (OR [95% CI] = 1.0510 [1.0320 - 1.0710], $p < 0.0001$). Additionally, the weighted median (OR [95% CI] = 1.0530 [1.0310 - 1.0750], $p < 0.0001$) and MR-PRESSO (OR [95% CI] = 1.0490 [1.0330 - 1.0660], $p = 0.0090$) results substantiate the conclusions drawn by IVW. Cochran's Q and horizontal pleiotropy tests on the MR results for ALT and NAFLD indicate the absence of heterogeneity and gene-level pleiotropy ($p > 0.05$).

Table 4. MR Results of CHF on liver function indicators (ALT, AST, BL, and AL).

MR info							Cochran's Q			Horizontal pleiotropy		
Outcomes	Snps (n)	method	β	SE	OR [95% CI]	p	Q	df	p	intercept	SE	p
ALT	13	MR Egger	-0.0038	0.0388	0.9962 [0.9232 - 1.0749]	0.9230	20.0738	12	0.0657	0.0081	0.0050	0.1281
		IVW (multiplicative random effects)	0.0412	0.0187	1.0420 [1.0045 - 1.0810]	0.0279	24.5429	13	0.0264	-	-	-
		Weighted median	0.0635	0.0197	1.0656 [1.0253 - 1.1075]	0.0012	-	-	-	-	-	-
		MR-RAPS	0.0337	0.0182	1.0342 [0.9979 - 1.0719]	0.0649	-	-	-	-	-	-
		MR-PRESSO	0.0412	0.0187	1.0420 [1.0045 - 1.0810]	0.0483	-	-	-	-	-	-
AST	54	MR Egger	-0.0038	0.0388	0.9962 [0.9232 - 1.0749]	0.9230	558.7900	52	< 0.0001	-0.0004	0.028	0.8956
		IVW (multiplicative random effects)	-0.0116	0.0161	0.9884 [0.9577 - 1.0201]	0.4697	558.9769	53	< 0.0001	-	-	-
		Weighted median	-0.0111	0.0081	0.9890 [0.9733 - 1.0049]	0.1723	-	-	-	-	-	-
		MR-RAPS	-0.0044	0.0102	0.9956 [0.9759 - 1.0158]	0.6681	-	-	-	-	-	-
		MR-PRESSO	0.0006	0.0034	1.0006 [0.9940 - 1.0072]	0.8690	-	-	-	-	-	-
BL	12	MR Egger	-0.012	0.032	0.9880 [0.9270 - 1.0520]	0.712	27.4286	10	0.0022	0.0037	0.0026	0.1812
		IVW (multiplicative random effects)	-0.0122	0.0323	0.9878 [0.9273 - 1.0523]	0.7123	33.0945	11	0.0005	-	-	-
		Weighted median	0.0288	0.0157	1.0292 [0.9980 - 1.0614]	0.0667	-	-	-	-	-	-
		MR-RAPS	0.0186	0.0136	1.0187 [0.9920 - 1.0462]	0.1717	-	-	-	-	-	-
		MR-PRESSO	0.0252	0.0140	1.0255 [0.9978 - 1.0539]	0.0711	-	-	-	-	-	-
AL	65	MR Egger	0.0076	0.0233	1.0076 [0.9627 - 1.0546]	0.7462	81.2656	60	0.0605	-0.0007	0.0018	0.6875
		IVW	-0.0009	0.0101	0.9991 [0.9794 - 1.0192]	0.9308	81.4762	61	0.0693	-	-	-
		Weighted median	-0.0053	0.0137	0.9947 [0.9684 - 1.0217]	0.6961	-	-	-	-	-	-
		MR-RAPS	-0.0002	0.0108	0.9998 [0.9788 - 1.0212]	0.9838	-	-	-	-	-	-
		MR-PRESSO	0.0002	0.0024	1.0002 [0.9954 - 1.0050]	0.9410	-	-	-	-	-	-

ALT: Alanine Transaminase, AST: Aspartate Transaminase, BL: Bilirubin, AL: Albumin, OR and 95%CI were calculated from unrounded Beta estimates.

Table 5. Results of ALT-mediated relationship between CHF and NAFLD.

Methods	Total effect (β [95% CI])	Indirect effect (β [95% CI])	Mediator effect proportion [95% CI] (%)	p
Delta	0.1268 [0.0752, 0.1784]	0.0021 [0.0001, 0.0040]	1.6561 [0.0698, 3.1788] %	0.0406
Bootstrap	-	0.0020 [0.0002, 0.0042]	-	-

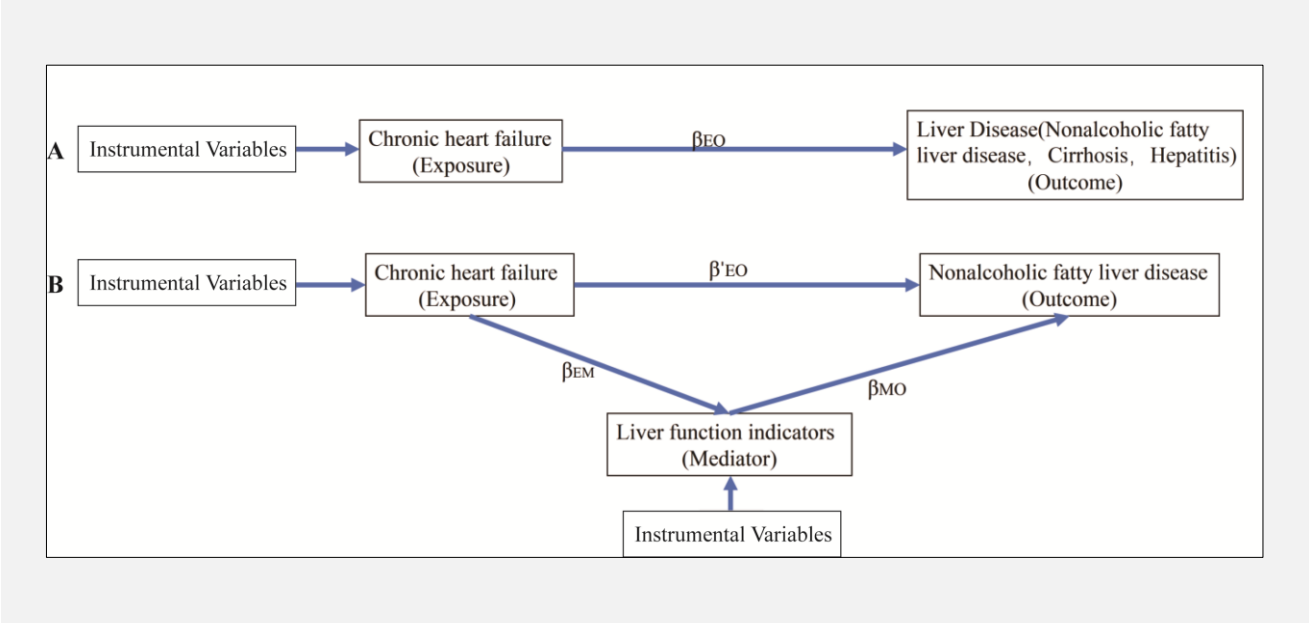


Figure 1. Exploration of relationships in this study.

A: The overall effect between CHF and liver diseases (NAFLD, cirrhosis, and hepatitis). β_{EO} represents the comprehensive impact of predicting CHF as exposure and liver diseases as outcomes. B: The total effect is disentangled into components: indirect effects using a two-step method (β_{EM} as the total effect of CHF on liver function indicators, and β_{MO} denoting the effect of liver function indicators on NAFLD) and the product method ($\beta_{EM} \times \beta_{MO}$), along with the direct effect ($\beta'_{EO} = \beta_{EO} - \beta_{EM} \times \beta_{MO}$). The proportion mediated is calculated by dividing the indirect effect by the total effect.

Proportion of ALT-Mediated Relationship Between CHF and NAFLD

Given the established correlations among CHF, NAFLD, and ALT, an intermediate MR analysis was conducted (Supplementary Table 2). The proportion of the ALT-mediated effect was calculated by dividing the indirect effect by the total effect of CHF on NAFLD. The mediation analysis revealed that ALT, as an intermediary, and the mediating effect value estimated using the delta method was 0.0021 [0.0001, 0.0040]. Notably, ALT contributed to only 1.6561 [0.0698, 3.1788] % of the total effect (p = 0.0406) in influencing the risk of NAFLD associated with CHF. Using the parametric bootstrap approach, the corresponding estimate was 0.0020 [0.0002, 0.0042] (Table 5). The bootstrap-derived confidence intervals were broadly consistent with those obtained using the delta method, supporting the robustness of the mediation effect.

Sensitivity analysis

In order to investigate the impact of each SNP on the overall causal estimates, this study conducted sensitivity analyses using funnel plots and leave-one-out analysis. The funnel plots, depicting the causal relationships among these SNPs, revealed no evidence of asymmetry, as illustrated in Figure 4. Through the leave-one-out analysis, systematically removing each SNP and re-evaluating the remaining SNPs, the results remained consistent. This suggests that all SNPs were computed to establish significant causal relationships, as demonstrated in Figure 4. Excluding SNPs associated with key confounders (BMI, T2D, hypertension, cancer, severe COVID-19, and alcohol intake) yielded MR estimates largely consistent with the main analysis (Supplementary Table 1), supporting the robustness of our findings.

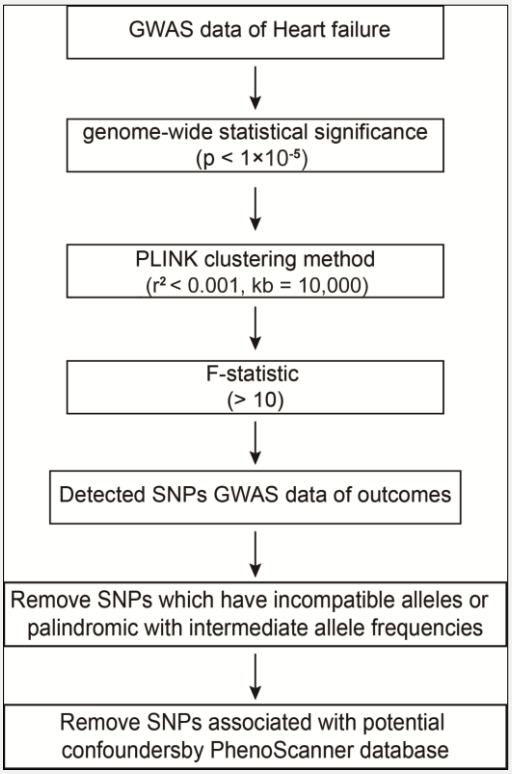


Figure 2. Flow diagram showing the IV selection and filtering process.

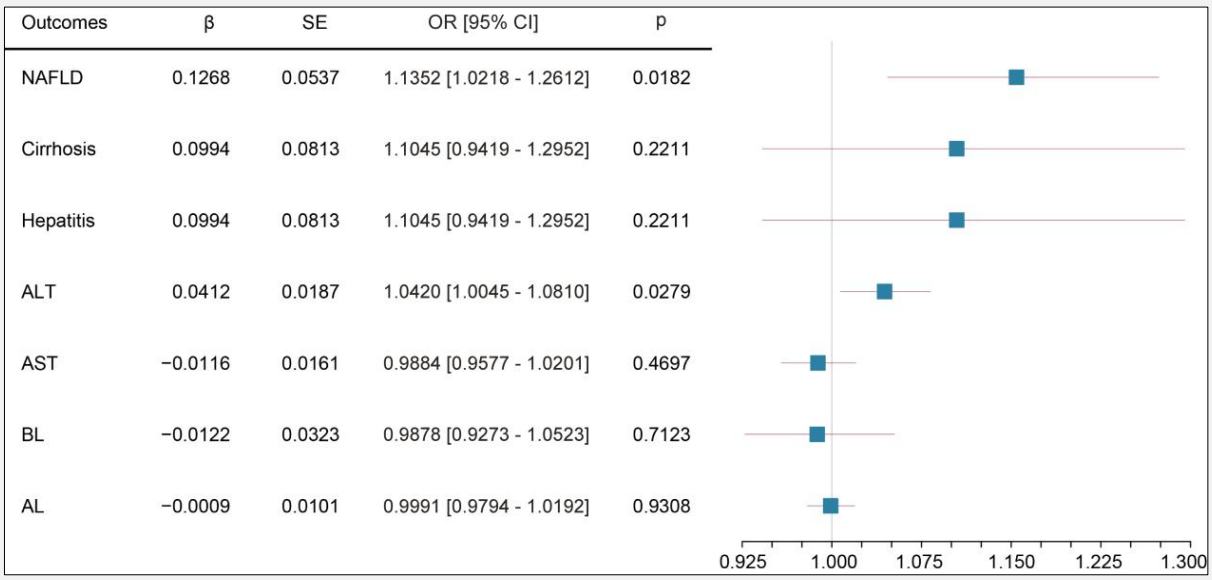


Figure 3. Forest plot for the main results of the Mendelian randomization analysis based on IVW method.

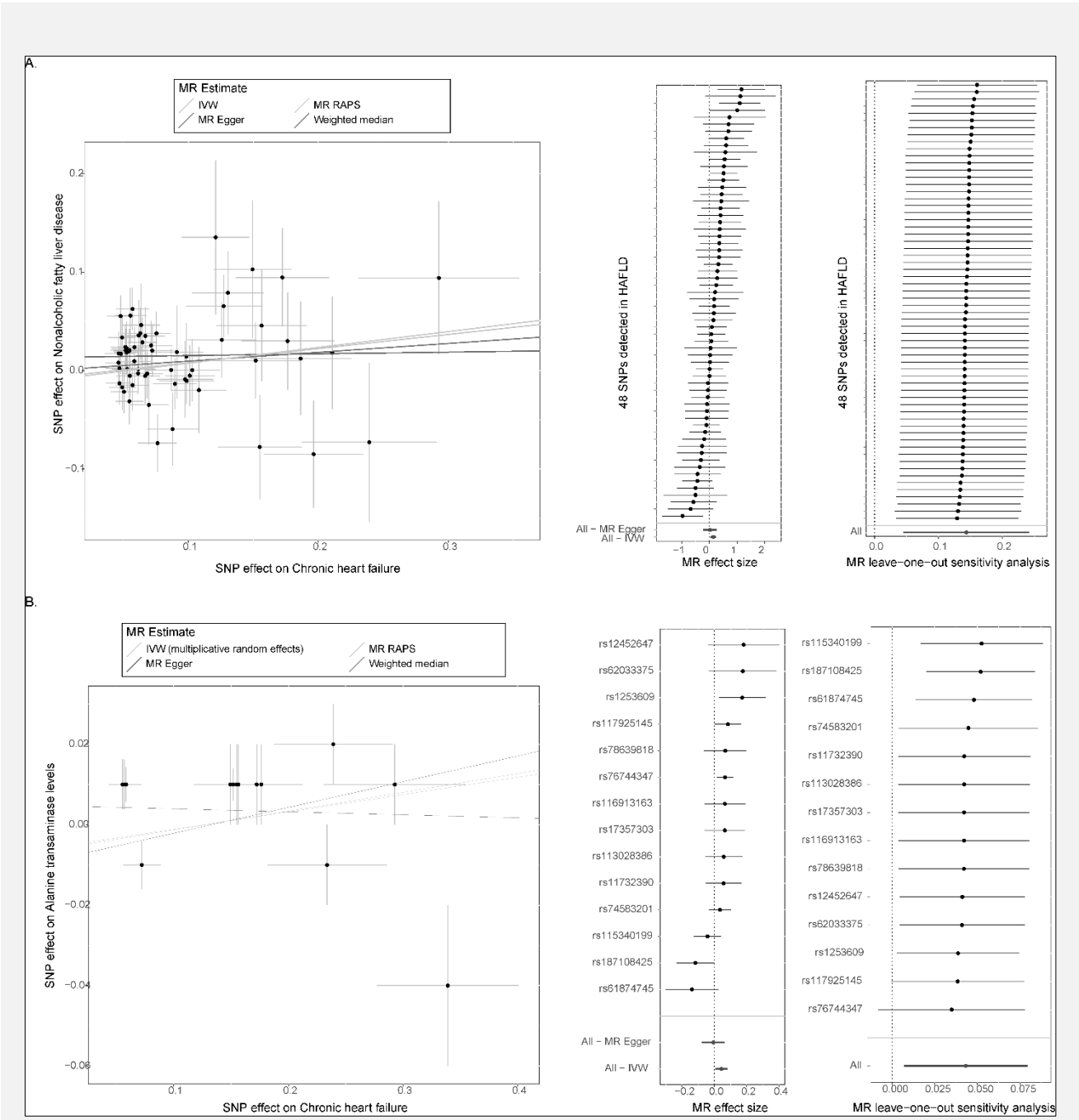


Figure 4. Scatter plot, SNP effect size and leave one out forest plot for the main results of the Mendelian randomization analysis.

A: Result of NAFLD. B: Result of ALT.

DISCUSSION

HF is a chronic disease resulting from impaired cardiac function, affecting approximately 1% - 2% of the adult population. HF not only diminishes the quality of life for patients but, in severe cases, poses a life-threatening condition. Within the spectrum of HF patient treatment, a subset also experiences damage to other circulatory-sensitive tissues, such as the liver. This can lead to the development of common liver diseases, including

NAFLD, hepatitis, or cirrhosis [26,27]. The global prevalence of NAFLD has been on the rise, driven by escalating levels of obesity, type 2 diabetes, and metabolic syndrome [28]. Beyond being a standalone ailment, NAFLD emerges as a complication in various diseases, particularly prevalent in cardiovascular conditions [29]. This study provides further genetic evidence regarding the potential causal relationship between CHF and liver diseases using a two-sample MR approach. Recently, Zhang et al. [30] conducted a bidirectional MR study and reported a potential causal association between heart failure and NAFLD. Consistent with their findings, our results support a causal effect of CHF on NAFLD, while further extending previous evidence by exploring the potential mediating role of liver function indicators, particularly ALT, in this causal pathway. The MR results underscore that CHF significantly heightens the risk of NAFLD. ALT emerges as an identified intermediate factor, contributing to a mediation effect of 0.0021 [0.0001, 0.0040], constituting 1.6561 [0.0698, 3.1788] % of the total effect ($p = 0.0406$).

The intricate relationship between NAFLD and HF has been extensively confirmed in numerous studies. For example, Peng et al. [31], through a two-sample MR analysis, identified a substantial correlation between NAFLD and atherosclerosis (β [95% CI] = 0.04 [0.02 - 0.06], $p < 0.01$) as well as HF (OR [95% CI] = 1.08 [1.02 - 1.14], $p < 0.01$). Li et al. [32], in a meta-analysis of observational studies, reported an increased risk of HF in NAFLD patients (HR [95% CI] = 1.47 [1.25 - 1.75], $I2 = 99\%$). In line with these findings, our study indicates that CHF heightens the risk of NAFLD. Zhang et al. [33], in a clinical investigation, suggested that NAFLD might be a contributing factor to the progression of CHF, aligning with our conclusion. Presently, HF is recognized as a risk factor for the occurrence of NAFLD, although the precise pathological and physiological mechanisms between them remain incompletely understood. Most studies propose that NAFLD may impact the risk of cardiovascular diseases and cardiac complications (functional, structural, and arrhythmic) through various pathways. These pathways include abnormalities in lipid metabolism leading to atherosclerosis, influence on liver metabolism, chronic inflammation, imbalance in procoagulant and anticoagulant factors, insulin resistance, and increased oxidative stress [34,35]. But the funnel plot (Figure 4A) for CHF→ NAFLD shows some asymmetry, suggesting that individual SNPs do not fully capture the association between CHF and NAFLD and that an integrated approach should be adopted.

ALT is widely regarded by clinicians as a pivotal marker for assessing NAFLD activity and the severity of liver damage [36]. A study by Verma et al. [37] involving 222 NAFLD patients revealed a significantly lower proportion of lung fibrosis in the group with normal ALT levels compared to the elevated ALT group. Another study by Zheng et al. [38] found a positive correlation between the risk of NAFLD and ALT expression levels, with higher overall mortality and cardiovascular mortality rates observed in patients with normal or lower ALT expression. Consistent with these findings, patients with acute or CHF often report elevated ALT levels, indicative of a poorer prognosis [8,39]. This aligns with the conclusion of our study, where the more severe liver and kidney impairment in CHF patients is reflected in elevated ALT expression levels [8]. Altogether, ALT emerges as a key indicator connecting CHF and NAFLD, holding the potential to monitor the risk of NAFLD occurrence.

Despite numerous clinical studies indicating a correlation between CHF, NAFLD, and ALT, it remains uncertain whether a causal relationship exists among them. Firstly, RCTs, observational cohorts, and cross-sectional studies might be influenced by external confounding factors and reverse causation, thereby reducing the reliability of the results. Secondly, variations in cases, gender, environment, and the severity of CHF and NAFLD across different studies lead to diverse conclusions, impacting the accuracy of the results. Lastly, while some meta-analyses suggest a higher risk of HF in NAFLD patients [32], the inclusion of observational studies prevents randomization, and causality remains unconfirmed. This study employed MR to elucidate the causal relationship between CHF and NAFLD, offering a novel research approach to substantiate the "heart-liver" axis theory. The strengths of MR studies lie in reducing the impact of confounding factors and reverse causation, significantly enhancing the accuracy of conclusions. Furthermore, utilizing GWAS data from European populations minimizes biases from external factors such as environment, population, and diet. Furthermore, the validity of the mediation analysis relies on the assumption that no interaction exists between CHF and ALT. If this assumption is violated, the decomposition of the total effect into direct and indirect components may be biased. This assumption cannot be formally tested in a two-sample Mendelian randomization framework due to the use of summary-level data. However, ALT is widely recognized as a biomarker of liver function and metabolic status, and there is limited evidence to suggest that CHF substantially modifies the downstream effects of ALT on NAFLD. Nevertheless, this assumption should be interpreted with caution, and future studies using individual-level data are warranted to further investigate potential interactions.

This study has the following strengths: 1) This study extends previous MR investigations by applying a two-sample MR framework to systematically evaluate the causal relationships between CHF and various liver diseases, while further exploring whether liver function indicators may serve as potential mediators. 2) MR methodology mitigates confounding bias, yielding robust estimates of causal effects. 3) The exposure GWAS data employed is the most recent and extensive dataset for CHF, and rigorous adherence to MR's three fundamental assumptions was observed during the selection of instrumental variable SNPs. 4) The study employs five

different MR analysis methods, assessing all MR results for stability through Cochran's Q statistics, MR Egger regression tests, funnel plots, and leave-one-out analysis, enhancing result validation. However, similar to other MR studies, this research has the following limitations: 1) All included GWAS data originates from European populations, limiting the generalizability of the results. In addition, NAFLD cases in the UK Biobank were identified mainly through ICD coding, which may have limited sensitivity and resulted in potential phenotype misclassification. 2) The SNP threshold for CHF GWAS is broad ($p = 1 \times 10^{-05}$), potentially resulting in a lower association strength between selected instrumental variables and exposure. 3) Despite employing various methods to exclude outlier variants, the study cannot completely eliminate the impact of horizontal pleiotropy on the results. 4) Stratification based on CHF severity, gender, and age factors was not conducted. 5) The study primarily focuses on individuals of European descent, potentially diminishing the universality of the findings.

CONCLUSION

In summary, this study represents the first application of MR analysis to investigate the causal link between CHF and liver diseases, including NAFLD, hepatitis, and cirrhosis. Additionally, the study explores whether liver function indicators (ALT, AST, BL, and AL) act as intermediate factors in this relationship. The findings reveal a causal association between CHF and NAFLD, while no such causal links were observed with hepatitis or cirrhosis. Furthermore, ALT was identified as an intermediary factor between CHF and NAFLD. Rigorous analyses, including Cochran's Q statistics, MR Egger regression, funnel plots, and leave-one-out method, affirm the stability and reliability of the MR results.

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The authors declare no competing interests.

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