ISSN 1948-5182 (online)

World Journal of *Hepatology*

World J Hepatol 2024 August 27; 16(8): 1070-1198

Published by Baishideng Publishing Group Inc

World Journal of Hepatology

Contents

Monthly Volume 16 Number 8 August 27, 2024

EDITORIAL

1070 Intermittent fasting and the liver: Focus on the Ramadan model

Emara MH, Soliman H, Said EM, Elbatae H, Elazab M, Elhefnawy S, Zaher TI, Abdel-Razik A, Elnadry M

1084 *Rocahepevirus ratti*: An underrecognised cause of acute hepatitis

Gherlan GS

MINIREVIEWS

1091 Bridging the gap: Addressing disparities in hepatitis C screening, access to care, and treatment outcomes

Alenzi M, Almeqdadi M

ORIGINAL ARTICLE

Retrospective Cohort Study

1099 Alpha-1 antitrypsin deficiency and Pi*Z allele as important co-factors in the development of liver fibrosis

Ferreira AI, Guimarães C, Macedo Silva V, Xavier S, Magalhães J, Cotter J

Retrospective Study

1111 Successful treatment of acute liver failure due to Wilson's disease: Serendipity or fortuity?

Delle Cave V, Di Dato F, Calvo PL, Spagnuolo MI, Iorio R

Observational Study

1120 Correlation between non-alcoholic fatty liver disease and metabolic parameters in persons with newly diagnosed type 2 diabetes mellitus

Mukherjee S, Mukherjee S, Shing Kwok C, Phillips A

1131 Tissue inhibitor of metalloproteinase-3 expression affects clini-copathological features and prognosis of aflatoxin B1-related hepatocellular carcinoma

Liang QJ, Long QQ, Tian FQ, Su QY, Zhu XY, Long XD

Clinical and Translational Research

1145 Blood cell counts and nonalcoholic fatty liver disease: Evidence from Mendelian randomization analysis

Hu B, Wan AH, Xiang XQ, Wei YH, Chen Y, Tang Z, Xu CD, Zheng ZW, Yang SL, Zhao K

1156 Causal association between 731 immunocyte phenotypes and liver cirrhosis: A bidirectional two-sample mendelian randomization analysis

Li Y, Quan X, Tai Y, Wu YT, Wei B, Wu H

World Journal of Hepatology

Monthly Volume 16 Number 8 August 27, 2024

Contents

Basic Study

1167 Gadoxetic acid-enhanced magnetic resonance imaging in the assessment of hepatic sinusoidal obstruction syndrome in a mouse model

Chen YY, Yang L, Li J, Rao SX, Ding Y, Zeng MS

1177 Dynamics of glutamine synthetase expression in hepatic ischemia-reperfusion injury: Implications for therapeutic interventions

Huang ZH, Dong MQ, Liu FY, Zhou WJ

SYSTEMATIC REVIEWS

1185 Impact of non-alcoholic fatty liver disease on coronavirus disease 2019: A systematic review

Moeed A, Larik MO, Fahim MAA, Rahman HAU, Najmi L, Changez MIK, Javed MM, Hasibuzzaman MA

World Journal of Hepatology

Monthly Volume 16 Number 8 August 27, 2024

Contents

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INDEXING/ABSTRACTING

The *WJH* is now abstracted and indexed in PubMed, PubMed Central, Emerging Sources Citation Index (ESCI), Scopus, Reference Citation Analysis, China Science and Technology Journal Database, and Superstar Journals Database. The 2024 Edition of Journal Citation Reports® cites the 2023 journal impact factor (JIF) for *WJH* as 2.5; JIF Quartile: Q2. The *WJH*'s CiteScore for 2023 is 4.1 and Scopus CiteScore rank 2023: Hepatology is 41/82.

RESPONSIBLE EDITORS FOR THIS ISSUE

Production Editor: *Yi-Xuan Cai*.; **Production Department Director:** *Xiang Li*; **Cover Editor:** *Xiang Li*.

NAME OF JOURNAL
World Journal of Hepatology

ISSN
ISSN 1948-5182 (online)

LAUNCH DATE
October 31, 2009

FREQUENCY
Monthly

EDITORS-IN-CHIEF
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EDITORIAL BOARD MEMBERS
https://www.wjgnet.com/1948-5182/editorialboard.htm

PUBLICATION DATE
August 27, 2024

PUBLISHING PARTNER
Department of Infectious Diseases, the Second Affiliated Hospital of Xi'an Jiaotong University

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https://www.wjgnet.com/bpg/gerinfo/204

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https://www.wjgnet.com/bpg/GerInfo/239

ONLINE SUBMISSION
https://www.f6publishing.com

PUBLISHING PARTNER's OFFICIAL WEBSITE
http://2yuan.xjtu.edu.cn/Html/Departments/Main/Index_21148.html

World Journal of Hepatology

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World J Hepatol 2024 August 27; 16(8): 1145-1155

DOI: 10.4254/wjh.v16.i8.1145

ISSN 1948-5182 (online)

ORIGINAL ARTICLE

Clinical and Translational Research

Blood cell counts and nonalcoholic fatty liver disease: Evidence from Mendelian randomization analysis

Bin Hu, Ai-Hong Wan, Xi-Qiao Xiang, Yuan-Hao Wei, Yi Chen, Zhen Tang, Chang-De Xu, Zi-Wei Zheng, Shao-Ling Yang, Kun Zhao

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade B

Novelty: Grade B

Creativity or Innovation: Grade B

Scientific Significance: Grade B

P-Reviewer: Moriyama K

Received: May 19, 2024

Revised: July 3, 2024

Accepted: July 23, 2024

Published online: August 27, 2024

Processing time: 94 Days and 0.1 Hours

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Abstract

BACKGROUND

Previous research has highlighted correlations between blood cell counts and chronic liver disease. Nonetheless, the causal relationships remain unknown.

AIM

To evaluate the causal effect of blood cell traits on liver enzymes and nonalcoholic fatty liver disease (NAFLD) risk.

METHODS

Independent genetic variants strongly associated with blood cell traits were extracted from a genome-wide association study (GWAS) conducted by the Blood Cell Consortium. Summary-level data for liver enzymes were obtained from the

United Kingdom Biobank. NAFLD data were obtained from a GWAS meta-analysis (8434 cases and 770180 controls, discovery dataset) and the Fingen GWAS (2275 cases and 372727 controls, replication dataset). This analysis was conducted using the inverse-variance weighted method, followed by various sensitivity analyses.

RESULTS

One SD increase in the genetically predicted haemoglobin concentration (HGB) was associated with a β of 0.0078 (95%CI: 0.0059-0.0096), 0.0108 (95%CI: 0.0080-0.0136), 0.0361 (95%CI: 0.0156-0.0567), and 0.0083 (95%CI: 00046-0.0121) for alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase, and gamma-glutamyl transferase, respectively. Genetically predicted haematocrit was associated with ALP (β = 0.0078, 95%CI: 0.0052-0.0104) and ALT (β = 0.0057, 95%CI: 0.0039-0.0075). Genetically determined HGB and the reticulocyte fraction of red blood cells increased the risk of NAFLD [odds ratio (OR) = 1.199, 95%CI: 1.087-1.322] and (OR = 1.157, 95%CI: 1.071-1.250). The results of the sensitivity analyses remained significant.

CONCLUSION

Novel causal blood cell traits related to liver enzymes and NAFLD development were revealed through Mendelian randomization analysis, which may facilitate the diagnosis and prevention of NAFLD.

Key Words: Blood cell counts; Liver enzymes; Nonalcoholic fatty liver disease; Genome-wide association; Mendelian randomization study; Causal relationship

Core Tip: Mendelian randomization analysis revealed a novel evidence for a causal role of genetically predicted blood cell traits in liver injury and nonalcoholic fatty liver disease (NAFLD). The study found that genetically determined increases in hemoglobin concentration (HGB) and hematocrit levels were associated with elevated levels of liver enzymes. In addition, genetic determinants of HGB and reticulocyte ratio are associated with an increased risk of NAFLD. These findings may help in the diagnosis and prevention of NAFLD.

Citation: Hu B, Wan AH, Xiang XQ, Wei YH, Chen Y, Tang Z, Xu CD, Zheng ZW, Yang SL, Zhao K. Blood cell counts and nonalcoholic fatty liver disease: Evidence from Mendelian randomization analysis. *World J Hepatol* 2024; 16(8): 1145-1155
URL: https://www.wjgnet.com/1948-5182/full/v16/i8/1145.htm
DOI: https://dx.doi.org/10.4254/wjh.v16.i8.1145

INTRODUCTION

Chronic liver disease (CLD), including nonalcoholic fatty liver disease (NAFLD), is a significant cause of mortality and liver cancer, accounting for 3.5% of all deaths worldwide[1]. The incidence of NAFLD, a chronic metabolic stress-related liver disease, is increasing, contributing to the rapid increase in the global burden of liver disease[2]. The insidious onset and complex pathogenesis of NAFLD are not fully understood, but the disease is closely associated with insulin resistance (IR), genetic predisposition, and an increased risk of developing cirrhosis, end-stage liver disease, and hepatocellular carcinoma over time[3-5]. Serum liver enzymes, including alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl transferase (GGT), are considered important markers in the clinical assessment of liver injury, and they are associated with the risk of developing NAFLD[6,7]. Given the invasive nature of the gold standard for NAFLD biopsy and the limitations of diagnostic imaging criteria, there is a need to search for noninvasive circulating biomarkers. In addition, determining the causal relationships among diseases is the first step towards rational and individualized treatment.

Blood cell counts are quantitative clinical laboratory measures that characterize the production of haematopoietic progenitor cells, the synthesis of haemoglobin, and the clearance of mature or senescent blood cells from the circulation [8]. Considering the vital functions of blood cells in delivering tissue oxygen, managing inflammatory responses, addressing atherosclerosis, and preventing thrombosis, factors contributing to interpopulation variations in blood cell traits may significantly impact the development of CLD and contribute to health disparities among populations[9,10]. However, the limitations of these studies include potential confounding factors, reverse causality, and the inability to establish causality because of their cross-sectional or retrospective nature. Determining if blood cell counts are causally involved in the progression of NAFLD is of significant clinical importance

Mendelian randomization (MR), an emerging method, offers a novel approach to address these limitations. By utilizing single-nucleotide polymorphisms (SNPs) identified from genome-wide association studies (GWASs) as instrumental variables (IVs), MR analysis leveraging genetic variants randomly allocated at conception helps mitigate biases from confounders and reverse causation, resembling a "natural" randomized controlled trial[11]. Many powerful GWASs have identified thousands of SNPs associated with blood cell-related traits or circulating liver enzyme levels[9,12,13]. This creates an opportunity to test genetic, potentially causal relationships between blood cell traits and relevant CLD-

associated traits using the MR approach.

Therefore, in this context, we conducted a two-sample bidirectional MR design and systematically assessed the potential causal relationships between blood cell counts and CLD-associated traits.

MATERIALS AND METHODS

A summary of the research methodology is depicted in Figure 1. The study is reported according to the STROBE-MR checklist guidelines (https://www.strobe-mr.org/)[14]. The summary data from the GWAS employed in this research are accessible to the public for download, and every original investigation secured written consent from the subjects involved, with endorsement from ethical review boards. An overview of the GWAS data sources used for MR analysis is shown in Table 1.

GWAS summary statistics of 17 blood cell count traits

Summary statistics for the 17 blood cell traits were derived from comprehensive GWASs conducted by the Blood Cell Consortium and the United Kingdom Biobank (UKB), which represents the largest GWAS on blood cell traits to date, encompassing 563085 participants of European ancestry[9,10]. In the original GWAS, meticulous efforts were made to correct for potential confounders, including age, sex, the first 10 principal components of genetic ancestry, and cohort-specific covariates, to ensure the robustness of the genetic associations identified. The specific blood cell traits analysed included 8 red blood cell traits [red blood cell count (RBC); haemoglobin concentration (HGB); haematocrit (HCT); mean corpuscular haemoglobin (MCH); mean corpuscular volume; mean corpuscular haemoglobin concentration; red cell distribution width (RDW)], 2 immature red cell traits [reticulocyte count (RET); reticulocyte fraction of red cells (RET%)], 6 white blood cell traits [white blood cell count (WBC); neutrophil count (NEU); lymphocyte count (LYM); monocyte count (MONO); basophil count (BASO); eosinophil count (EO)], and 2 platelet traits [platelet count (PLT); mean platelet volume (MPV)]. Complete summary statistics from GWASs of blood cell traits can be downloaded from the GWAS catalogue (https://www.ebi.ac.uk/gwas/).

GWAS summary statistics of liver enzymes and NAFLD

Genetic associations with ALP, ALT, and GGT were extracted from GWASs conducted by Pazoki *et al*[12]. We utilized data from the UKB and included 437267 individuals aged between 40 and 69 years. Study participants were identified through the United National Health Service Registers across 22 centres in the UKB between the years 2006 and 2010. The study included individuals of European ancestry, following quality measures and exclusions such as sex, high missingness, and/or heterozygosity. The summary datasets of AST were derived from a GWAS meta-analysis of 411048 individuals in the UKB (adjusted for various factors, including the age at recruitment, sex, body mass index, and the first 12 principal components of genetic ancestry)[13].

The summary level GWAS statistics for NAFLD in the primary analysis were obtained from the Ghodsian *et al*'s study [15], which included 8434 NAFLD cases and 770180 controls of European ancestry (discovery dataset). The study integrated data from four additional GWASs: The Electronic Medical Records and Genomics, the FinnGen consortium, the UKB, and the Estonian Biobank. NAFLD diagnosis was determined on the basis of electronic health records or hospital records for all participants. Specifically, NAFLD was defined by the use of EHR codes and the International Classification of Diseases (ICD) (ICD9: 571.5, ICD9: 571.8, ICD9: 571.9, ICD10: K75.81, ICD10: K76.0 and ICD10: K76.9). To validate our results *via* replication analysis and meta-analysis, we used NAFLD (2275 NAFLD cases and 375002 controls) and data from the FinnGen consortium (replication dataset), which is publicly available at the following website (https://R10.finngen.fi/).

Instrumental variable selection

We extracted all SNPs that strongly and independently predicted 17 blood cell traits at the threshold of genome-wide significance ($P < 5 \times 10^{-8}$). After the significant SNPs corresponding to each blood cell trait were extracted, a linkage disequilibrium (LD) evaluation was conducted utilizing the European genetic profiles from a cohort of 1000 subjects as a benchmark and excluding potential LD (with $R^2 < 0.001$ within a 10000 kb region), palindromic structural SNPs (minor allele frequency > 0.42), and incompatible SNPs[16]. We calculated the F statistic ($F = \beta^2/SE^2$) for each SNP (F statistic range 29.75 to 1600.00), $F > 10$, suggesting the absence of weak instrumental bias[17]. In addition, we aimed to estimate the causal effects of genetically predicted liver enzymes and NAFLD on blood cell traits. We further performed reverse MR analyses. Data harmonization and MR methods were also performed as described in the forwards MR analysis. Comprehensive details regarding these exposures, outcomes, datasets, and IVs are presented in Supplementary Table 1.

MR and sensitivity analyses

Before conducting the MR analysis, SNPs directly related to the liver enzymes and NAFLD data were removed to avoid potential horizontal pleiotropy ($P < 5 \times 10^{-8}$). The primary analysis used inverse-variance weighted (IVW) with a random effects model[18]. In addition, a range of sensitivity analyses, such as MR-Egger[19], weighted median[20], weighted mode[21], simple mode, and penalized weighted median methods, were performed to evaluate the potential bias in the MR setting. In addition, RadialMR and MR pleiotropy residual sum and outlier (MRPRESSO, NbDistribution = 3000) were utilized to identify outliers with multiple effects across all levels for variables exhibiting significant causal links, followed by a re-evaluation of the causality estimates subsequent to the exclusion of these outliers[22,23]. Furthermore, we also performed sensitivity analyses using MR-Egger regression (assessing the presence of directional pleiotropy) to

Table 1 Characteristics of summary-level data used in the Mendelian randomization analysis

Phenotypes	Trait name	Unit	Sample size	No. of cases	Ethnicity	Adjustment	Ref.
Red blood cells	RBC	per pL	563946	545203	European	Age, sex, the first 10 principal components, and cohort-specific covariates were corrected in the original GWAS	[9]
	HGB	g/dL	563946	563946	European		
	HCT	%	563946	562259	European		
	MCH	pg	563946	491553	European		
	MCV	fL	563946	544127	European		
	MCHC	g/dL	563946	491553	European		
	RDW	fL	563946	531774	European		
Immature red cells	RET	pL	408112	408112	European		[10]
	RET%	%	408112	408112	European		
White blood cells	WBC	per nL	563946	562243	European		[9]
	NEU	per nL	563946	519288	European		
	LYM	per nL	563946	524923	European		
	MONO	per nL	563946	521594	European		
	BASO	per nL	563946	474001	European		
	EO	per nL	563946	474237	European		
Platelets	PLT	per nL	563946	542827	European		
	MPV	fL	563946	460935	European		
Covariates	WHR	SD	694649	694649	European	BMI	[27]
	T2D	Log (OR)	933970	80154	European	Age, sex, study-specific covariates, and principal components	[28]
Liver enzymes	ALP	SD	437438	437438	European	Age and sex and principal components of genetically inferred ancestry	[12]
	ALT	SD	437267	437267	European		
	GGT	SD	437194	437194	European		
	AST	SD	411048	411048	European	Age sex, BMI, and the first 12 principal components of genetic ancestry	[13]
Chronic liver diseases	NAFLD (Ghodsian *et al*[15], 2020)	Log (OR)	778614	8434	European	BMI	[15]
	NAFLD (Fingen 10)	Log (OR)	375002	2275	European	Age, sex, the first 10 genetic principal components, and genotyping batch	-

GWAS: Genome-wide association study; OR: Odds ratio; BMI: Body mass index; RBC: Red blood cell count; HGB: Haemoglobin concentration; HCT: Haematocrit; MCH: Mean corpuscular haemoglobin; MCV: Mean corpuscular volume; MCHC: Mean corpuscular haemoglobin concentration; RDW: Red cell distribution width; RET: Reticulocyte count; RET%: Reticulocyte fraction of red cells; WBC: White blood cell count; NEU: Neutrophil count; LYM: Lymphocyte count; MONO: Monocyte count; BASO: Basophil count; EO: Eosinophil count; PLT: Platelet count; MPV: Mean platelet volume; WHR: Waist-to-hip ratio; T2D: Type 2 diabetes; ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; GGT: Gamma-glutamyl transferase; AST: Aspartate aminotransferase; NAFLD: Nonalcoholic fatty liver disease.

determine the robustness of the IVW results, in which a P value for the intercept < 0.05 was considered statistically significant[24]. Cochran's Q statistic checks the heterogeneity of individual causal effects, and leave-one-out analysis examines the effects of outlying and pleiotropic SNPs on causal estimates[25,26]. To control the influence of pleiotropy within this research, we proceeded to perform multivariable MR (MVMR) analyses, which accounted for the waist-to-hip ratio (WHR)[27] and type 2 diabetes (T2D)[28]. MVMR facilitates the concurrent assessment of causal relationships among various predictors and outcomes[29]. MVMR was executed employing the IVW approach as the principal technique and the Egger regression method for supplementary evaluations.

All the statistical analyses were performed using the TwoSampleMR (version 0.5.4), RadialMR (version 1.0), and MRPRESSO (version 1.0) packages in R software (version 4.2.2). The MR estimates are reported as effect sizes (β) or odds ratios (ORs) along with 95%CIs. The statistical significance threshold was defined as a P value < 5.88×10^{-4} (0.05/17 exposure × 5 outcome) after Bonferroni correction to address multiple testing issues[30]. If a P value was between 5.58 ×

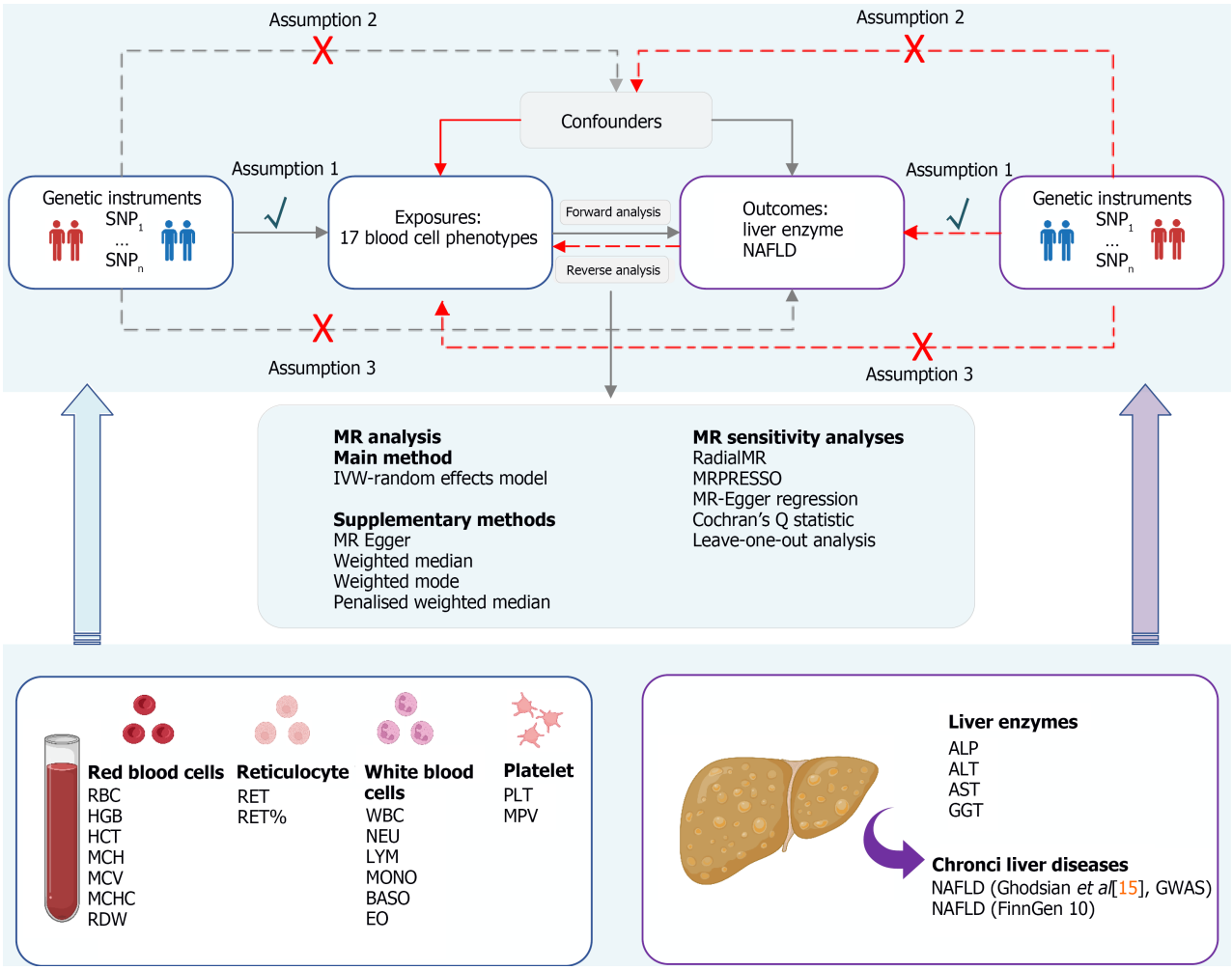

Figure 1 Mendelian randomization assumptions and study design. Mendelian randomization (MR) analysis relies on three key assumptions. Assumption 1: Genetic variants are closely associated with exposure (blood cell traits); Assumption 2: Genetic variants are not associated with any potential confounders; Assumption 3: Genetic variants are not associated with outcome (liver enzymes and nonalcoholic fatty liver disease) except *via* exposure. MR: Mendelian randomization; MRPRESSO: Mendelian randomization pleiotropy residual sum and outlier; RBC: Red blood cell count; HGB: Haemoglobin concentration; HCT: Haematocrit; MCH: Mean corpuscular haemoglobin; MCV: Mean corpuscular volume; MCHC: Mean corpuscular haemoglobin concentration; RDW: Red cell distribution width; WBC: White blood cell count; NEU: Neutrophil count; LYM: Lymphocyte count; MONO: Monocyte count; BASO: Basophil count; EO: Eosinophil count; PLT: Platelet count; MPV: Mean platelet volume; RET: Reticulocyte count; RET%: Reticulocyte fraction of red cells; ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; GGT: Gamma-glutamyl transferase; AST: Aspartate aminotransferase; NAFLD: Nonalcoholic fatty liver disease. Created with BioRender.com.

10^{-4} and 0.05, it was considered to indicate a potential causal relationship with nominal significance. For the MVMR analysis, a P value < 0.05 was considered statistically significant.

RESULTS

Causal estimates between blood cell traits and liver enzymes

Specifically, IVW MR analysis revealed a statistically significant association between genetically predicted higher RBC levels and ALP (β = 0.0043, 95%CI: 0.002-0.0065, PIVW = 1.84E-04 per 1-SD increase). Higher HGB levels were significantly associated with ALP (β = 0.0079, 95%CI: 0.0051-0.0107, PIVW = 3.07E-08), ALT (β = 0.0128, 95%CI: 0.0085-0.0170, PIVW = 4.69E-09), AST (β = 0.0713, 95%CI: 0.0395-0.1031, PIVW = 1.12E-05) and GGT (β = 0.0108, 95%CI: 00049-0.0168, PIVW = 3.73E-04; Supplementary Table 2). In addition, genetically determined higher HCT levels (per 1-SD increase) were significantly associated with the levels of ALP (β = 0.0060, 95%CI: 0.0033-0.0087, PIVW = 1.14E-05) and ALT (β = 0.0075, 95%CI: 0.0034-0.0116, PIVW = 3.05E-04). The RadialMR and MRPRESSO tests were used to identify any level of multieffect outliers and reassessed the causal effect estimates after removing outliers, and the results remained significant (Supplementary Table 3).

Another aspect, genetically determined higher circulating levels of WBC, NEU, and BASO, was associated with increased levels of ALP (β = 0.0031, 95%CI: 0.0005-0.0056, PIVW = 1.89E-02 per 1-SD increase in WBC; β = 0.0035, 95%CI: 0.0007-0.0062, PIVW = 1.25E-02 per 1-SD increase in NEU; β = 0.0041, 95%CI: 0.0004-0.0077, PIVW = 2.87E-02 per 1-SD increase in BASO; Supplementary Table 2). Genetically predicted RBC, MCH, RET, RET%, and LYW were associated with

the levels of ALT (β = 0.0040, 95%CI: 0.0005-0.0075, PIVW = 2.42E-02 per 1-SD increase in RBC; β = 0.0042, 95%CI: 0.0012-0.0071, PIVW = 5.26E-03 per 1-SD increase in MCH; β = 0.0052, 95%CI: 0.0017-0.0087, PIVW = 4.00E-03 per 1-SD increase in RET; β = 0.0050, 95%CI: 0.0019-0.0080, PIVW = 1.58E-03 per 1-SD increase in RET%; β = 0.005, 95%CI: 0.0013-0.0086, PIVW = 7.88E-03 per 1-SD increase in LYM).

We detected a positive correlation between HCT levels and AST (β = 0.0452, 95%CI: 0.0162-0.0742, PIVW = 2.23E-03 per 1-SD increase in HCT). Similarly, we observed that the levels of RBC, HCT, and PLT were suggestively associated with the levels of GGT, with β values of 0.0078 (95%CI: 0.003-0.0126 PIVW = 1.35E-03), 0.0064 (95%CI: 0.001-0.0118 PIVW = 2.09E-02), and β of 0.0068 (95%CI: 0.0027-0.0109 PIVW = 1.06E-03), respectively. Interestingly, genetically predicted higher RDWs were inversely associated with the levels of ALT (β = -0.0034, 95%CI: -0.0067 to -0.0002, PIVW = 3.95E-02) and AST (β = -0.0248, 95%CI: -0.046 to -0.0035, PIVW = 2.21E-02). No significant causal associations were found between other genetically driven blood cell traits and liver enzymes.

Causal estimates between blood cell traits and the risk of NAFLD

After removing outliers, the MR results revealed that genetically predicted HGB and RET% increased the risk of NAFLD (OR = 1.199, 95%CI: 1.087-1.322, PIVW = 2.70E-04) and (OR = 1.157, 95%CI: 1.071-1.250, PIVW = 2.24E-04; Supplementary Table 4). Nevertheless, we observed a potential positive correlation between genetically elevated levels of RET and the risk of NAFLD (OR = 1.137, 95%CI: 1.051-1.229, PIVW = 1.29E-03). In addition, MR analysis suggested that a genetic predisposition to higher HGB, RET, and RET% was causally associated with an increased risk of NAFLD in both discovery, replication, and meta-analyses (Figure 2).

Sensitivity analysis and MVMR results

No evidence of heterogeneity (Cochranes's *Q*, *P* value > 0.05) or horizontal pleiotropy (MR-Egger intercept, *P* value > 0.05) was observed in the MR estimates (Supplementary Table 5). The global tests from RadialMR and MRPRESSO were utilized to identify outliers with multiple effects across all levels for associations with significant causality and to reevaluate the causality estimates after these outliers were removed. The results remained stable (Supplementary Tables 3 and 4). Additionally, a leave-one-out sensitivity analysis was conducted, successively excluding each SNP associated with risk factors and recalculating the MR outcomes for the remaining SNPs. Notably, this analysis confirmed the stability of the outcomes (Supplementary Figures 1-10). The visual representations, including scatter plots, funnel plots, forest plots, and leave-one-out plots, are shown in Supplementary Figures 1-10.

Within the MVMR framework, we adjusted for two key confounding factors (WHR and T2D). Our findings revealed that the causal relationships remained robust and consistent, notwithstanding the consideration of a range of influencing factors (Figure 3A and Supplementary Table 6). However, the associations between genetically determined HGB and GGT levels were nonsignificant (β = 0.0128, 95%CI: 0.0024-0.0233, PIVW = 1.62E-02 per 1-SD increase in HGB) (MVMR-IVW method) and (β = 0.0134, 95%CI: 0.0028-0.0238, PIVW = 1.26E-02 per 1-SD increase in HGB) (MVMR-Egger method). Importantly, most of the *P* values of the MVMR-Egger intercept were > 0.05, suggesting a low likelihood of pleiotropy.

The positive effects of RET (OR = 1.266, 95%CI: 1.070-1.498, PIVW = 1.29E-03, per 1-SD increase) and RET% (OR = 1.264, 95%CI: 1.165-1.371, PIVW = 1.59E-08) remained following adjustments for WHR and T2D in the multivariable MR analysis (Figure 3B and Supplementary Table 6). Furthermore, there was an inconsistency between the MVMR-Egger results and the MVMR-IVW analysis results. For example, genetically predicted HGB and the risk of NAFLD became nonsignificant (OR = 1.165, 95%CI: 0.987-1.373, PIVW = 7.03E-04, per 1-SD increase in HGB). In addition, most of the *P* values of the MVMR-Egger intercept were > 0.05, indicating a low likelihood of pleiotropy.

Causal estimates between liver enzymes, NAFLD, and blood cell counts

We performed reverse MR analysis on the significant results of the positive MR (after Bonferroni correction). Characteristics of the genetic variants were associated with liver enzyme levels and NAFLD (Supplementary Table 7). The IVW MR analysis revealed that ALP had significant causal effects on increases in RBC (β = 0.5365, 95%CI: 0.2636-0.8094, PIVW = 1.17E-04) and HCT (β = 0.4562, 95%CI: 0.2046-0.7077, PIVW = 3.78E-04; Supplementary Table 8). In addition, higher ALT levels were significantly associated with HGB levels (β = 0.3576, 95%CI: -0.1262 to 0.8412, PIVW = 5.83E-05). All additional methods yielded similar results, except for MR-Egger and weighted methods (Supplementary Table 8). In addition, genetic susceptibility to NAFLD risk was not associated with HGB (β = 0.2892, 95%CI: -0.0139 to 0.5923, PIVW = 3.52E-03), RET (β = 0.0014, 95%CI: -0.0731 to 0.0759, PIVW = 9.70E-01), or RET% (β = -0.0097, 95%CI: -0.0876 to 0.0683, PIVW = 8.08E-01).

DISCUSSION

In this MR study, we provided new novel evidence for the causal roles of 17 genetically predicted blood cell traits in liver injury and the development of NAFLD. We found that genetically predicted HGB and HCT were causally associated with a wide range of levels of liver injury. Elevated HGB, RET, and RET% were strong indicators of an increased risk of NAFLD. Similarly, the multivariate MR and sensitivity analyses remained significant. These findings highlight the roles of blood cell traits in the pathogenesis of NAFLD and provide new evidence for understanding the impact of abnormal liver function on blood biology, which may facilitate its diagnosis and prevention.

Blood is described as a viscous non-Newtonian fluid, with more than 95% of blood being red blood cells, thus determining its critical role in whole blood viscosity (WBV). WBV is a crucial physiological and pathological marker that often increases with the onset of chronic conditions such as inflammation, aging, and NAFLD[31-33]. Understanding the

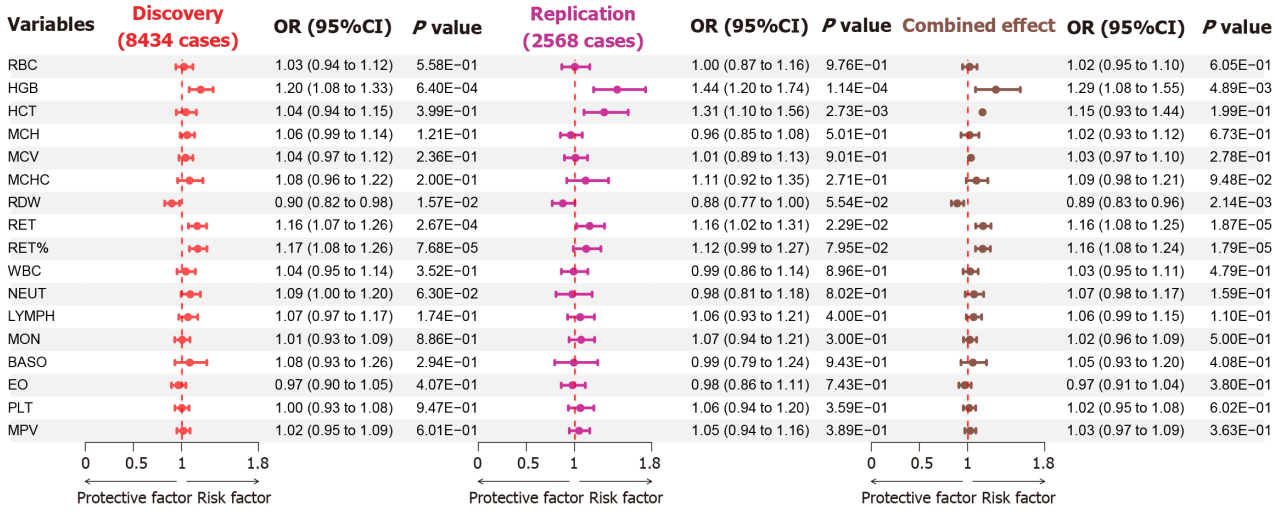

Figure 2 Associations of genetically predicted blood cell counts with the risk of nonalcoholic fatty liver disease in the discovery, replication, and combined datasets. OR: Odds ratio; RBC: Red blood cell count; HGB: Haemoglobin concentration; HCT: Haematocrit; MCH: Mean corpuscular haemoglobin; MCV: Mean corpuscular volume; MCHC: Mean corpuscular haemoglobin concentration; RDW: Red cell distribution width; WBC: White blood cell count; NEU: Neutrophil count; LYM: Lymphocyte count; MONO: Monocyte count; BASO: Basophil count; EO: Eosinophil count; PLT: Platelet count; MPV: Mean platelet volume; RET: Reticulocyte count; RET%: Reticulocyte fraction of red cells; ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; GGT: Gamma-glutamyl transferase; AST: Aspartate aminotransferase; NAFLD: Nonalcoholic fatty liver disease.

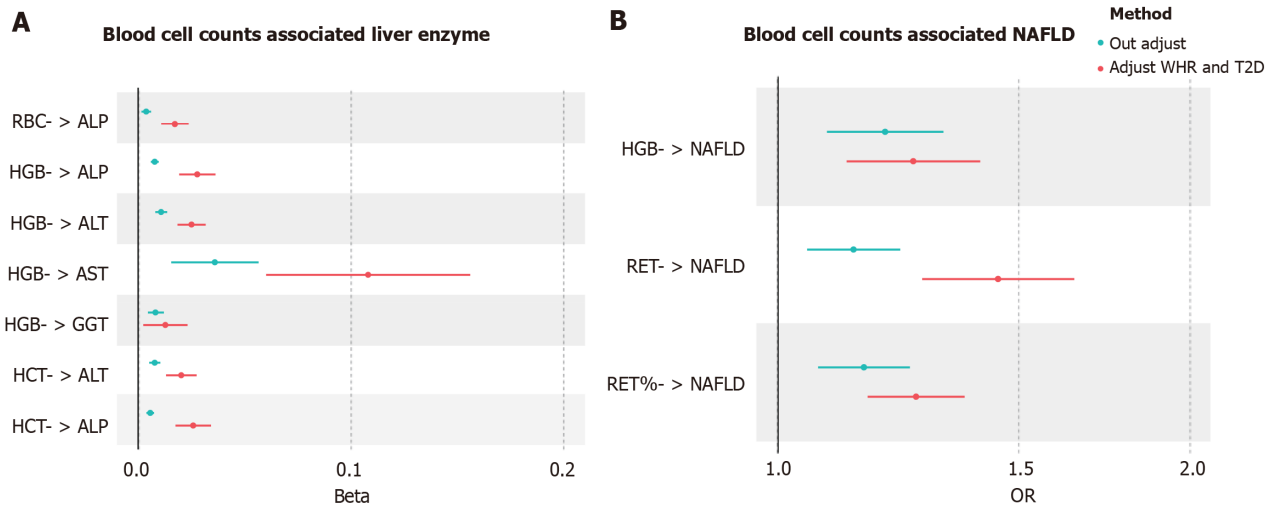

Figure 3 Inverse-variance weighted–Mendelian randomization results before and after correcting for waist-to-hip ratio and type 2 diabetes using the multivariable Mendelian randomization framework. A: Blood cell counts associated liver enzyme; B: Blood cell counts associated liver enzyme. OR: Odds ratio; RBC: Red blood cell count; HGB: Haemoglobin concentration; HCT: Haematocrit; ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; GGT: Gamma-glutamyl transferase; AST: Aspartate aminotransferase; NAFLD: Nonalcoholic fatty liver diseases; T2D: Type 2 diabetes.

pivotal role of WBV as a physiological and pathological marker provides valuable insights into the interconnected mechanisms at play. Prior research has underscored the robust correlation between elevated RBC levels and various health conditions, such as IR, metabolic syndrome (Mets), and NAFLD, and emphasized the significance of hematological parameters in assessing metabolic health[34-36]. Moreover, in the context of NAFLD, elevated RBC counts have been identified as an independent predictor of disease progression, suggesting their potential utility as a diagnostic biomarker [37]. The widely accepted "multiple parallel hits" hypothesis offers a comprehensive understanding of NAFLD pathogenesis, highlighting the interplay of IR, oxidative stress, and inflammatory responses within this intricate framework[38,39]. As ongoing research continues to unveil the nuanced connections between blood traits and systemic health, these findings contribute to a broader understanding of the intricate web of factors influencing metabolic and liver-related disorders.

HGB is the most common component of erythrocytes and is the carrier of carbon dioxide and oxygen through the vascular network to body cells. The intricate relationship between elevated HGB levels and increased risk of NAFLD is underscored by various physiological mechanisms. Elevated HGB not only is positively correlated with NAFLD risk but also serves as a diagnostic indicator for disease progression[39-41]. The repercussions of increased HGB include increased blood viscosity, compromised hepatic blood flow perfusion, and impaired microcirculation, culminating in hepatocyte

ischemia and hypoxia. Furthermore, the cascade of events includes hypoxia-induced oxidative stress, disruptions in hepatic glycogen deposition, and diminished insulin sensitivity. This complex interplay also involves the upregulation of hepatic lipid synthesis genes and the downregulation of lipid metabolism genes[33,42]. These molecular and physiological alterations collectively contribute to increased susceptibility to NAFLD. Additionally, the robustness of these findings is supported by MR analysis, which establishes a causal relationship between genetically predisposed HGB levels and the levels of four liver enzymes, as well as the incidence of NAFLD. Importantly, even after adjusting for confounding factors such as the WHR and T2D status in multivariable MR analyses, the persistent causal association underscores the critical role of HGB in the development and progression of NAFLD and its impact on liver health.

RET serves as an indicator reflecting the haematopoietic function of bone marrow, drawing considerable attention from scholars worldwide for its applications in diagnosing and treating anaemic conditions, as well as in radiotherapy for patients with tumours. Notably, previous findings revealed marked increases in RET% and RET levels within the chronic hepatitis cohort, suggesting a potential link between RET and liver health[43]. Additionally, other studies have confirmed that RET can indicate liver fibrosis severity to a certain extent, thus serving as a diagnostic marker for liver fibrosis and its routine monitoring[44]. Moreover, anaemia resulting from malabsorption of iron, folic acid, and vitamin B12 in patients with CLDs may present as elevated RET levels[45]. Expanding on these observations, our MR study adds a genetic perspective, indicating that genetically predicted higher RET% levels increase the risk of NAFLD. This aligns with findings from independent datasets, collectively reinforcing the validity of the conclusion and providing robust genetic support for previously observed associations. The convergence of evidence from various sources underscores the multifaceted role of RET in liver health and positions it as a valuable marker for both haematopoietic function and liver-related conditions.

A key advantage of this research is its use of the MR approach, which effectively mitigates the impact of confounding factors and the issue of reverse causality typically encountered in observational research. This method provides a thorough examination of the causal links between blood cell trait circulation and the levels of liver enzymes, as well as the risk of NAFLD. In addition, we used data from an independent GWAS in NAFLD to validate our findings in the discovery data and combined causal effect estimates from both datasets through meta-analysis to increase statistical power and improve estimation accuracy. The associations remained consistent in the sensitivity analyses, and no indication of unbalanced pleiotropy was detected. Furthermore, we performed MVMR analyses for traits with strong phenotypes, and the adjusted associations remained substantially stable.

Interpreting the results of our MR study requires careful consideration of several limitations. First, MR provides only a preliminary assessment of the causality between exposure and disease, without delving into the specific biological pathways that underpin these relationships. Our study acknowledges the potential for residual confounding factors that may not have been identified or documented in the current literature. Additionally, MR studies do not offer individual-level data, which limits the ability to examine potential nonlinear relationships. Consequently, robust individual-level data are essential to better understand the impact of elevated blood cell counts on the development of NAFLD.

Finally, the findings from this MR study are grounded in GWAS summary statistics from the European population. However, the generalizability of these results to other ethnic groups remains an open question that necessitates further investigation. To address this, cross-ethnic group studies should be undertaken. These studies compare genetic relationships with NAFLD across different populations, aiming to identify any disparities in the gene-NAFLD association. Expanding the research to integrate multiomics data encompassing transcriptomics and proteomics with clinical data from a variety of ethnic backgrounds can deepen our comprehension of the complex gene-NAFLD interplay. Incorporating MR analysis within this framework is advantageous, as it employs genetic variants as IVs. This approach offers robust evidence for the causal relationship between blood traits and NAFLD, enhancing our grasp of the underlying mechanisms involved.

CONCLUSION

Taking into account both our MR and MVMR results, the associations between high HGB, HCT, and RET% with liver injury and the risk of NAFLD may act independently. These findings have clinical implications, as they suggest the future potential for evaluating blood cell traits as targets for liver injury and NAFLD prevention or treatment.

ACKNOWLEDGEMENTS

The authors acknowledge the researchers of the original studies for sharing the GWAS data used in this project.

FOOTNOTES

Author contributions: Yang SL and Zhao K conceptualized and designed the research; Hu B and Wan AH wrote the original draft of the manuscript, contributed equally to this work and share first authorship; Xiang XQ and Wei YH performed the statistical analysis; Chen Y, Tang Z, Xu CD, and Zheng ZW contributed to the acquisition, analysis, or interpretation of the data. Both Yang SL and Zhao K have played important and indispensable roles in the study design, data interpretation, and manuscript preparation as the co-corresponding authors. Yang SL was instrumental in conceptualizing the study's framework, devising innovative methodologies, and overseeing the experimental design., and also obtained the funds for this research project. Zhao K excelled in leading the data analysis process, applying

sophisticated statistical techniques to interpret the results. This collaboration between Yang SL and Zhao K is crucial for the publication of this manuscript and other manuscripts still in preparation.

Supported by the Shanghai Natural Science Foundation of China, No. 23ZR1447800; and the Fengxian District Science and Technology Commission Project, China, No. 20211838.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Country of origin: China

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S-Editor: Li L
L-Editor: A
P-Editor: Che XX

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