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Causal associations of immunophenotypes with metabolic dysfunction-associated fatty liver disease and mediating pathways: a Mendelian randomization study

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Abstract

Background: Increasing evidence suggests that immunophenotypes play a crucial role in Metabolic dysfunction-associated fatty liver disease (MAFLD), but the specific immunophenotypes contributing to its pathogenesis remain unclear.

Objectives: This study aimed to elucidate the causal associations between immunophenotypes and MAFLD and identify the underlying mediation pathways involved.

Design: Mendelian randomization (MR) study.

Methods: This study is a quasi-causal inference analysis using univariable and multivariable MR (UVMR and MVMR). Five MAFLD genome-wide association studies (GWASs) and the largest immunophenotype GWAS were analyzed to assess their causal associations. Two-step MR identified potential mediators and quantified their mediation proportions. Comprehensive MR methods, multiple sensitivity analyses, meta-analyses, and false discovery rate (FDR) further enhanced the robustness of our findings.

Results: Pooled inverse-variance weighted (IVW) estimates in UVMR identified 47 immunophenotypes having a suggestive causal association with MAFLD. After adjusting for FDR, three lymphocyte phenotypes remained significant: CD20 on IgD$^-$CD24$^-$ B cells (OR: 1.035, p_{fdr}: 0.006), terminally differentiated CD8$^+$ T cells %T cells (OR: 1.052, p_{fdr}: 0.006), and CD4 on CD39$^+$ secreting CD4$^+$ regulatory T cells (OR: 1.036, p_{fdr}: 0.046). Meta-analysis of IVW MVMR estimates with confounders adjustment confirmed that CD20 on IgD$^-$CD24$^-$ B cells and terminally differentiated CD8$^+$ T cells %T cells had significant direct causal associations on MAFLD ($p_{fdr} < 0.05$). Additionally, two-step MR analysis identified the waist-to-hip ratio as a mediator, accounting for 42.64% of the causal association between CD20 on IgD$^-$CD24$^-$ B cells and MAFLD.

Conclusion: The causal associations of three lymphocyte phenotypes with increased MAFLD risk were identified in this study. CD20 on IgD$^-$CD24$^-$ B cells may both directly and indirectly elevate MAFLD risk, while terminally differentiated CD8$^+$ T cells have a direct causal relationship with MAFLD. These findings suggest new possibilities for targeted therapies and underscore the potential for personalized immunotherapy in managing MAFLD.

Keywords: causal inference, immune cell phenotypes, metabolic dysfunction-associated fatty liver disease, Mendelian randomization

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Introduction

Metabolic dysfunction-associated fatty liver disease (MAFLD) is the most common chronic liver disorder globally, affecting over one billion individuals with a prevalence of nearly 30%.[1–4] It has become the second leading indication for liver transplantation, significantly contributing to liver-related morbidity and mortality.[3,5] MAFLD includes a spectrum of liver conditions, from simple steatosis to metabolic dysfunction-associated steatohepatitis (MASH).[6] The progression from MAFLD to MASH substantially increases the risk of severe hepatic and extrahepatic complications.[1,2,5]

Despite the recent conditional approval of resmetirom by the Food and Drug Administration (FDA) for noncirrhotic MASH patients with moderate to advanced fibrosis, challenges in patient selection and treatment monitoring, as well as concerns about long-term safety, particularly regarding cardiovascular disease, limit its widespread clinical application.[2] Current therapeutic efforts primarily focus on weight loss and metabolic improvement, yet the rising prevalence and economic burden of MAFLD underscore the need for deeper insights into its pathogenesis and risk factors.[1–4,7,8]

Inflammation, particularly mediated by adaptive immunity, plays a critical role in the progression of MAFLD and its extrahepatic complications.[6,9] Evidence suggests that certain immune cell subsets, including B cells and T cells, are directly involved in MAFLD pathogenesis through mechanisms that promote liver inflammation, fibrosis, and insulin resistance.[6,10–12] For instance, CD8+ T cells have been shown to exacerbate liver inflammation, while B cells contribute to insulin resistance and metabolic dysregulation.[10,13,14] Immunotherapeutic interventions targeting specific inflammatory cells have shown promise in reducing steatohepatitis and fibrosis in MASH models.[6,10–12,15–17] However, conflicting data regarding the proinflammatory or protective roles of certain immune subsets, such as Kupffer cells, make it crucial to clarify these relationships.[9,10,15] The roles and pathways of specific inflammatory cell subset immunophenotypes, particularly lymphocyte-related traits, in MAFLD pathogenesis remain unclear.[9,10,18,19] This is intensified by methodological challenges, including small sample sizes, model heterogeneity, confounding factors, and reverse causality. Thus, rigorous study design and interdisciplinary research are crucial to comprehensively understand and delineate the causal mechanisms linking immunophenotypes with MAFLD.

Mendelian randomization (MR) is a pivotal method in genomic research that discerns causal relationships between genetic variants and clinical outcomes.[20] MR uses genetic variants associated with exposures as instrumental variables to determine causality between exposures and outcomes.[21,22] This approach, often referred to as "nature's randomized trial," overcomes biases inherent in observational studies and provides the benefits of randomized controlled trials without their typical constraints.[23]

This study utilizes the largest available immunophenotype genome-wide association study (GWAS)[24] and five distinct GWASs on MAFLD[25–29] to conduct univariable MR (UVMR) analysis and meta-analysis. We aimed to elucidate the causal relationship between the immunophenotypes and MAFLD, providing new insights into MAFLD pathogenesis and identifying potential preventive and therapeutic strategies. Additionally, potential mediators were included in multivariate MR (MVMR) and mediation MR analyses to elucidate the direct causal effect of immunophenotypes on MAFLD and the possible underlying mechanisms. By identifying specific immune phenotypes causally linked to MAFLD, this research highlights the potential for targeted immunomodulatory therapies in managing MAFLD, emphasizing the clinical significance and value of these findings in guiding future treatment strategies.

Methods

Study design

This study is a quasi-causal inference analysis using UVMR and MVMR. MR relies on three key assumptions[21]: (1) genetic variants correlate with exposure; (2) genetic variants are unassociated with confounders; and (3) genetic variants influence outcomes exclusively through exposure. First, based on the above assumptions, bidirectional two-sample UVMR was used to estimate the causal relationship between the 731 immunophenotypes and MAFLD. Second, MVMR

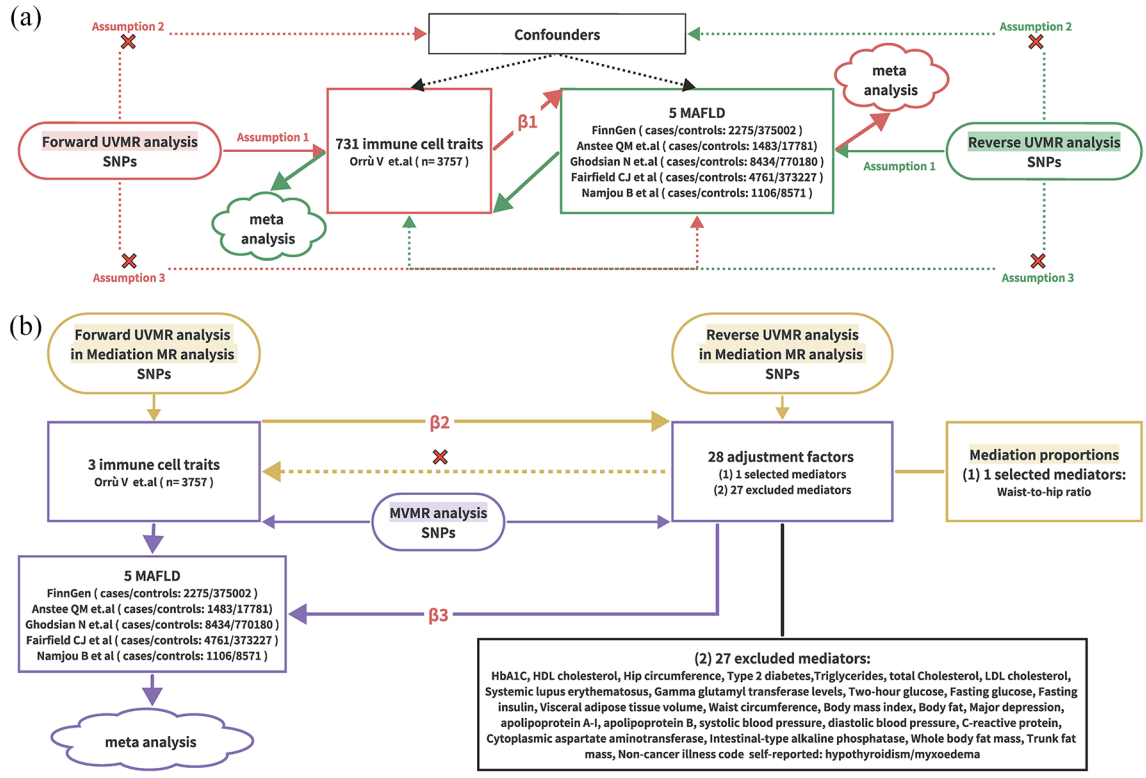

Figure 1. Overview of the study design. (a) Bidirectional univariable MR analysis and meta-analysis (red and green), utilizing the largest GWAS of 731 immunophenotypes and five MAFLD datasets, were conducted to establish the causal relationship between immunophenotypes and MAFLD. (b) Twenty-eight candidate mediators were included in the MVMR (purple) and mediation MR analyses (yellow) to reveal the direct effects and underlying mechanisms of immunophenotypes on MAFLD, which was supported by the meta-analysis.
MAFLD, metabolic dysfunction-associated fatty liver disease; MR, Mendelian randomization; MVMR, multivariable Mendelian randomization; UVMR, univariable Mendelian randomization.

was further applied to those immunophenotypes causally associated with MAFLD to assess their direct effects.[30] Finally, a two-step MR analysis was conducted to identify specific mediation.[30,31] A fixed-effect meta-analysis consolidated and confirmed the robustness of causal relationships identified by MR, utilizing data from five independent MAFLD GWASs (Figure 1). Figure S1 provides details of the process and the MR explanation.

Data sources for the exposure, mediators, and outcomes

Exposure. GWAS data on 731 immunophenotypes, from a cohort of 3757 Sardinians,[24] revealed approximately 22 million genetic variants related to these immune cell traits adjusted for age and sex. The immunophenotypes were categorized into seven panels—TBNK cells, regulatory T cells (Tregs), T-cell maturation stages, DCs, B cells, monocytes, and myeloid cells. These genes were further divided into four types of immunological signatures: absolute cell counts (ACs; $n = 118$), relative cell counts (RCs; $n = 192$), median fluorescence intensities of surface antigens ($n = 389$), and morphological parameters (MPs; $n = 32$). Additional details are available in the original article.[24]

Outcomes. Five GWASs on MAFLD were included[25–29]: (1) FinnGen, with MAFLD based on clinical diagnosis (ICD-10: K76.0), including 2275 cases and 375,002 controls; (2) several leading European tertiary liver centers, with MAFLD diagnosed via biopsy, biochemical tests, or ultrasonography, including 1483 cases and 17,781 controls; (3) a genome-wide meta-analysis of four

cohorts, documented in electronic health records as MAFLD among participants of European ancestry, including 8434 cases and 770,180 controls; (4) the UK Biobank, utilizing recorded MAFLD diagnoses based on diagnostic codes from recent consensus guidelines, including 4761 cases and 373,227 controls; and (5) the Electronic Medical Records and Genomics Network, including 1106 cases and 8571 controls. For more information, please see the original article.

Mediators. Considering the factors influencing MAFLD reported in previous studies,[1] we identified 28 candidate mediators and sourced their corresponding GWAS data from the Integrative Epidemiology Unit (IEU) GWAS database project (https://gwas.mrcieu.ac.uk/) for MVMR and mediator analysis. The candidate mediators are categorized into eight adiposity traits (body mass index, body fat, whole body fat mass, trunk fat mass, visceral adipose tissue volume, hip circumference, waist circumference, and waist-to-hip ratio), six lipids (triglycerides, total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein cholesterol, Apo-Lipoprotein A-I, and Apo-Lipoprotein B), four glucose metabolism-related traits (fasting glucose, 2-h glucose, fasting insulin, and glycated hemoglobin levels), two blood pressures (systolic and diastolic blood pressure), four blood proteins (aspartate aminotransferase, intestinal-type alkaline phosphatase, gamma glut-amyl transferase levels, and C-reactive protein), and four diseases (systemic lupus erythematosus, major depression, type 2 diabetes, and hypothyroidism/myxedema). Table S1 provides detailed sources, reasons, and references for inclusion of these factors.

Bidirectional UVMR analysis

The following criteria were applied to select genetic variants as instrumental variables (IVs) for MR analysis: (1) ensuring a robust association between IVs and the exposure traits, minimizing false positives (immune cell traits: $p < 1 \times 10^{-5}$; MAFLD: $p < 5 \times 10^{-6}$); (2) no linkage disequilibrium (immune cell traits: $R^2 < 0.1$ within 500 kb; MAFLD: $R^2 < 0.01$ within 5000 kb); and (3) no correlation with outcome. Specifically, unqualified IVs were eliminated using the MR Pleiotropy RESidual Sum and Outlier (MR-PRESSO) test and Steiger filtering.[32,33] The MR-PRESSO test identifies and corrects for outliers indicative of pleiotropy ($p \leqslant 0.05$).[32] Steiger filtering ensures that IVs predominantly influence the exposure rather than the outcome; if an instrumental variable satisfies the criteria, the direction of the instrument is "TRUE"; otherwise, it is "FALSE."[33] Therefore, only the IVs with the direction of "TRUE" were retained to avoid the effects of reverse causality bias. Additionally, IVs related to potential confounders associated with outcomes, such as body mass index and obesity, were identified and removed using PhenoScannerV2 (http://www.phenoscanner.medschl.cam.ac.uk/).[34,35] Excluding IVs with F-statistics $\leqslant 10$, calculated via $(\text{beta}/\text{SE})^2$, ensures that only robustly associated genetic variants are utilized, minimizing the potential for weak instrument bias.[36] Finally, single-nucleotide polymorphisms (SNPs) not present in the outcome dataset were excluded and proxy SNPs were not used to ensure that the genetic tool could link exposures to outcomes.

Before MR analysis, exposure and outcome data were harmonized to align the effect alleles to the forward strand, and the palindromic genetic variants were discarded for further MR analysis. The primary method used was inverse-variance weighted (IVW) MR,[37] with weighted-median,[38] MR-Egger,[39] and MR-PRESSO[32] as complementary approaches. IVW MR can combine SNP-specific estimates calculated using Wald ratios, assuming that there is no directional pleiotropic effect of each SNP.[37] A weighted-median estimator provides reliable estimates if more than 50% of the instrumental variables are valid.[38] MR-Egger yields directional horizontal pleiotropy corrected causal estimates, albeit with significantly less efficiency (i.e., wider confidence intervals).[39] The MR-PRESSO test is used to assess significant outliers and to correct for horizontal pleiotropy effects by removing outliers.[32]

MVMR analysis

MVMR extends MR by using genetic variants associated with both primary and mediator exposures, allowing the isolation of the direct effect of primary exposure on the outcome.[30] In this study, 28 candidate mediators were adjusted in the MVMR analysis (Table S1), with the IVW method chosen as the primary analytical approach.

Mediation MR analysis

The causal mediators should fulfill the following conditions[30]: (1) a causal association between the immunophenotype and the mediator; (2) the effect of the immunophenotype on the mediator must be unidirectional, confirming the validity of the mediation model; and (3) if the immunophenotype is a risk factor for MAFLD, the associations between the immunophenotype and the mediator and between the mediator and MAFLD should be consistent. Otherwise, the direction should be the opposite; and (4) the causal association between the mediator and MAFLD persists after adjusting for the immunophenotype.

Two-step MR was used to assess the mediating effect of the causal associations between immunophenotypes and MAFLD.[30] First, UVMR was employed to evaluate the direct effects between immunophenotypes and mediators. Additionally, reverse causality between mediators and immunophenotypes was tested to ensure that there was no bidirectional causality, which might influence the validity of the mediator model. Second, MVMR evaluates the causal effect of the mediator on MAFLD across five datasets, adjusting for immunophenotypes (beta 3). Similarly, the multivariable IVW (MV-IVW) method was applied for the main analysis. Finally, the mediation proportion of each mediator in the total effect of immune cell traits on MAFLD was calculated by [(beta 2 $\times$ beta 3_{pooled})/beta 1], and beta 1 was the total effect, a combined estimate of the immunophenotypes to MAFLD obtained from the previously described two-sample UVMR. The delta method was applied to derive the 95% CIs of the mediation proportions.[40]

Sensitivity analysis

A series of sensitivity analyses were performed to assess the robustness of the results. First, Cochran's Q test was used to detect heterogeneity. If heterogeneity was present ($p_{heterogeneity} \leqslant 0.05$), a random-effects IVW model was adopted; otherwise, a fixed-effects IVW model was used.[41] Next, the intercept of an MR-Egger regression was used to assess horizontal pleiotropy, which indicates the presence of pleiotropy when the intercept deviates from zero.[39] Finally, a leave-one-out analysis was performed by sequentially excluding each SNP and applying the IVW method to the remaining SNPs to determine the impact of specific variants on the results.[42] The false discovery rate (FDR) correction was used to ensure the reliability of the statistical significance.[43] All analyses were carried out using the R packages "TwoSampleMR," "MRPRESSO," "MendelianRandomization," "MVMR," and "meta" in R software (version: 4.3.0, hosted by the Vienna University of Economics and Business and supervised by the R Foundation for Statistical Computing, https://www.r-project.org/).

Results

Baseline characteristics

This study included 731 immunophenotypes categorized into seven panels—TBNK, Tregs, maturation stages of T cells, DCs, B cells, monocytes, and myeloid cells. Additionally, five distinct GWASs on MAFLD were analyzed. We examined between 4 and 969 SNPs, as detailed in Tables S2 and S3. The meta-analysis of IVW estimates from the UVMR analysis revealed 60 immunophenotypes with significant causal effects on MAFLD risk ($p_{pooled} < 0.05$; Tables S4–S6). Forty-seven immunophenotypes remained as robust causal factors without pleiotropy concerns ($p_{pleiotropy} > 0.05$; Table S4), after excluding 13 with pleiotropy ($p_{pleiotropy} < 0.05$; Table S5). Among these, 7 displayed heterogeneity ($p_{heterogeneity} < 0.05$) and were analyzed using a random-effects model, while 40 were assessed using a fixed-effects model due to lack of heterogeneity ($p_{heterogeneity} > 0.05$; Table S4). Excluding immunophenotypes with pleiotropy ensures the specificity of causal associations while accounting for heterogeneity ensures the robustness of our findings. Of the 47 immunophenotypes, 18 were protective against MAFLD, and 29 were hazardous.

Three lymphocyte immunophenotypes increased MAFLD risk

After FDR adjustment ($p_{fdr} < 0.05$), three lymphocyte immunophenotypes were strongly associated with increased MAFLD risk (Figure 2). Specifically, the pooled odds ratios (ORs) of CD20 on $IgD^{-}CD24^{-}$ B cells, terminally differentiated $CD8^{+}$ T cells %T cells, and CD4 on $CD39^{+}$ secreting CD4 Tregs on MAFLD risk were estimated to be 1.035 (95% confidence interval (CI): 1.019, 1.052; p value < 0.001; p_{fdr}: 0.006, Figure 2), 1.052 (95% CI: 1.029, 1.076;

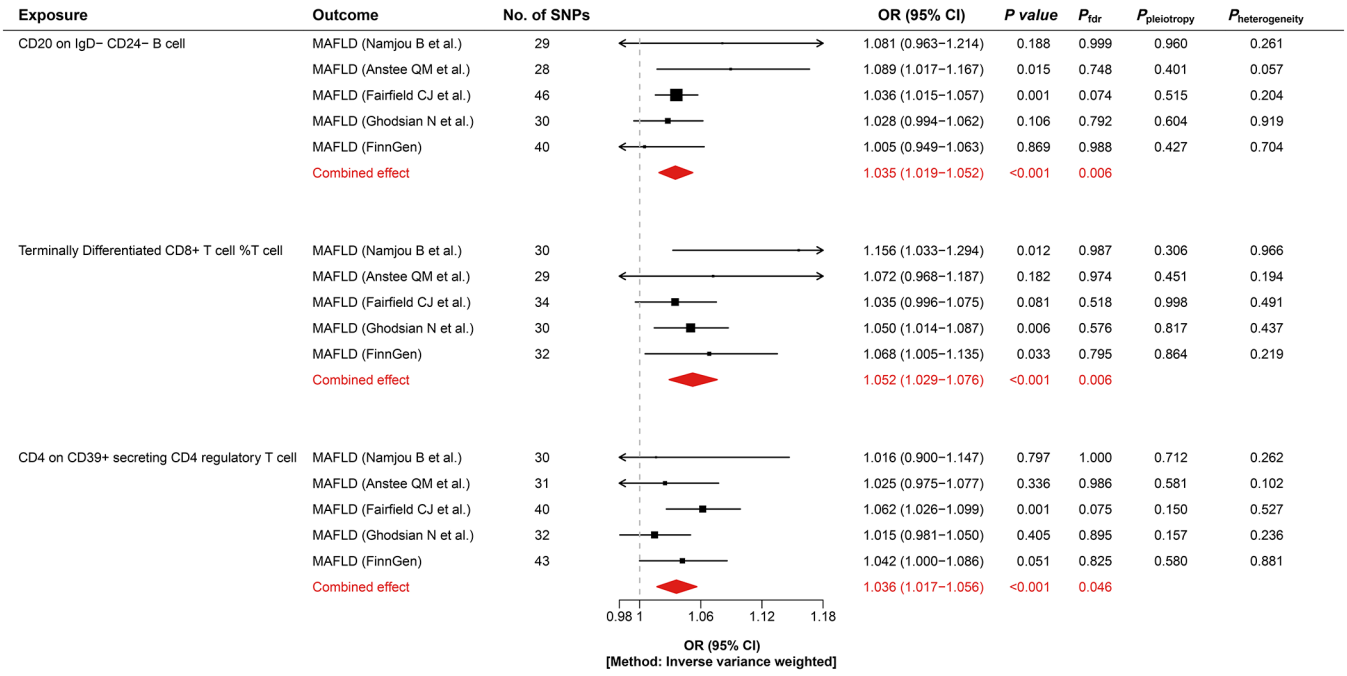

Figure 2. UVMR revealed causal associations between the three immunophenotypes and MAFLD after FDR adjustment.
CI, confidence interval; FDR, false discovery rate; MAFLD, metabolic dysfunction-associated fatty liver disease; OR, odds ratio; SNPs, single-nucleotide polymorphisms; UVMR, univariable Mendelian randomization.

p value < 0.001; p_{fdr}: 0.006), and 1.036 (95% CI: 1.017, 1.056; p value < 0.001; p_{fdr}: 0.046), respectively, according to the meta-analyses of IVW estimates. Similar results were obtained from the meta-analysis of the weighted-median, MR-Egger, and MR-PRESSO methods for determining the causal effect of CD20 on IgD$^-$CD24$^-$ B cells and terminally differentiated CD8$^+$ T cells %T cells on MAFLD (Table S4). F-statistics above 10 across databases indicated robustness against weak instrument bias, and MR-Egger intercepts confirmed no pleiotropy, which reinforced the stability of our findings (Table S4). Scatter plots and leave-one-out analysis further validated these causal associations (Figures S2 and S3).

The MVMR analysis was conducted to evaluate the direct causal effect of the three immunophenotypes—CD20 on IgD$^-$CD24$^-$ B cells, terminally differentiated CD8+ T cells %T cells, and CD4 on CD39+ secreting CD4 Tregs—on MAFLD, adjusting for twenty-eight candidate mediators (Figure 3, Table S9). According to the pooled MVMR results of the IVW estimates, 12 direct causal associations involving 3 immunophenotypes and 10 candidate mediators ($p_{pooled} < 0.05$) were found without pleiotropy ($p_{pleiotropy} > 0.05$; Figure 3, Table S9). Subsequent adjustments for FDR refined these to 3 significant associations involving two immunophenotypes and three candidate mediators ($p_{fdr} < 0.05$). Most SNPs showed no heterogeneity, reinforcing the robustness of our MVMR findings. Supplementary methods, especially MR-PRESSO MR estimates, provided consistent support. Specifically, the two immunophenotypes demonstrated causative effects on MAFLD both in UVMR and in MVMR after FDR adjustment ($p_{fdr} < 0.05$). Specifically, CD20 on IgD$^-$CD24$^-$ B cells (MV-IVW OR: 1.071; 95% CI: 1.05, 1.091; p value < 0.001; $p_{fdr} < 0.001$) increased MAFLD risk after adjusting for major depression. Similarly, increased terminally differentiated CD8+ T cells %T cells were associated with increased MAFLD risk after adjusting for several factors, including cytoplasmic aspartate aminotransferase (MV-IVW OR: 1.063; 95% CI: 1.031, 1.097; p value < 0.001; $p_{fdr} < 0.001$) and intestinal-type alkaline phosphatase (MV-IVW OR: 1.065; 95% CI: 1.03, 1.102; p value < 0.001; $p_{fdr} < 0.001$). Furthermore, CD4 on CD39+ secreting CD4+ Tregs appeared to increase MAFLD risk, after adjustment for intestinal-type alkaline phosphatase (MV-IVW OR: 1.036; 95%

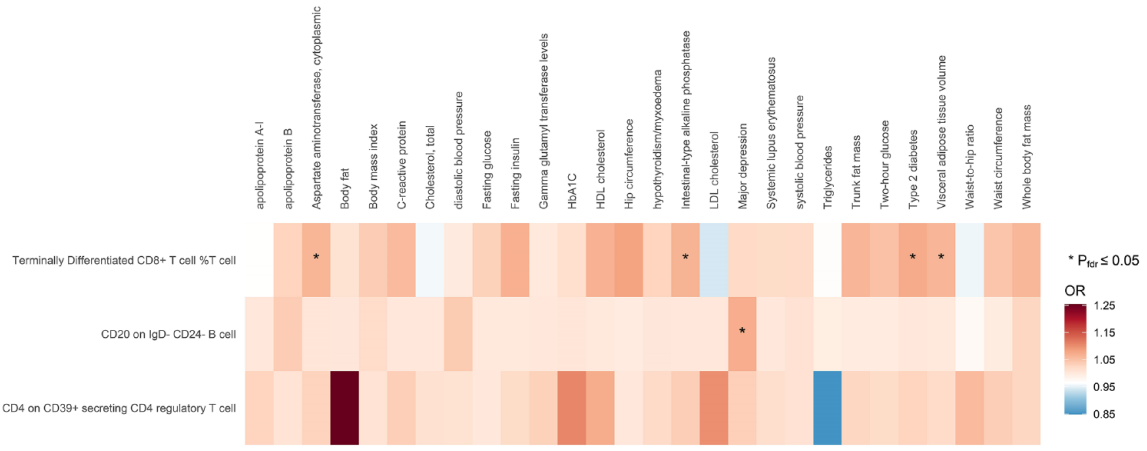

Figure 3. MVMR revealed causal associations between the three immunophenotypes and MAFLD after adjustment for 28 candidate mediators.
MAFLD, metabolic dysfunction-associated fatty liver disease; MVMR, multivariable Mendelian randomization; OR, odds ratio. Immunophenotypes that remained statistically significant after adjusting for FDR with a significance threshold of 0.05 in the forward UVMR analysis and meta-analysis of 731 immunophenotypes and five MAFLD datasets are highlighted in bold red.

CI: 1.01, 1.063; *p* value: 0.007; *p*fdr: 0.220), triglycerides (MV-IVW OR: 0.849; 95% CI: 0.742, 0.97; *p* value: 0.016; *p*fdr: 0.315), C-reactive protein (MV-IVW OR: 1.035; 95% CI: 1.006, 1.065; *p* value: 0.018; *p*fdr: 0.329), cytoplasmic aspartate aminotransferase (MV-IVW OR: 1.03; 95% CI: 1.005, 1.055; *p* value: 0.018; *p*fdr: 0.329), and LDL cholesterol (MV-IVW OR: 1.1; 1.012, 1.197; *p* value: 0.026; p_{fdr}: 0.370). However, none of these associations reached an FDR-adjusted significance level of 0.05. Further details are listed in Table S9.

Waist-to-hip ratio mediates the effect of immunophenotypes on MAFLD

Using two-step MR and sensitivity analyses, only one of the twenty-eight mediators was ultimately selected for its role in the association between immunophenotype and MAFLD, with no detected pleiotropy (Table S10). As shown in Table 1, the waist-to-hip ratio mediated 42.64% (95% CI: 2.52%, 82.75%) of the causal effect of CD20 expression on IgD$^-$CD24$^-$ B cells on MAFLD.

No statistically significant causal effect of MAFLD on immunophenotypes

The meta-analysis of IVW estimates in reverse UVMR identified sixty-five causal links from MAFLD to immunophenotypes ($p_{pooled} < 0.05$), all without pleiotropy (*Ppleiotropy* > 0.05) or heterogeneity ($p_{heterogeneity} > 0.05$; Tables S7 and S8). Among these, four immunophenotypes exhibited bidirectional causal relationships with MAFLD, including CD4 on CD39+ secreting CD4 Tregs, CD24 on IgD$^-$CD38$^-$ B cells, CD25++ CD45RA+ CD4 but not Tregs %T cells, and IgD$^-$CD38$^-$ B cells %B cells. However, after adjusting for FDR with a significance threshold of 0.05, none of these causal links remained statistically significant (Table S7).

Discussion

This study used bidirectional MR analysis and meta-analysis to explore the causal role of immune markers in MAFLD. UVMR identified 47 immune phenotypes linked to MAFLD, with three—CD20 on IgD$^-$CD24$^-$ B cells, CD4 on CD39$^+$ secreting CD4 Tregs, and terminally differentiated CD8$^+$ T cells % T cells—remaining significant after FDR adjustment. MVMR and mediation MR further analysis confirmed these associations, highlighting the relevance of these immune phenotypes in MAFLD pathogenesis.

Interestingly, our findings suggest that lymphocyte-related immune responses, particularly those involving T and B cells, play a key role in MAFLD progression. Lymphocyte activation, such as that indicated by CD20, has been implicated in more severe stages of MAFLD, including MASH.[6,10,12,44]

Table 1. The mediation proportion of CD20 on IgD⁻CD24⁻ B cell on MAFLD via waist-to-hip ratio.

Exposure	Mediator	Outcome	Total effect	Direct effect A	Direct effect B	Mediation proportion (%)
			β (95% CI)	β (95% CI)	β (95% CI)	(95% CI)
CD20 on IgD⁻CD24⁻ B cell	Waist-to-hip ratio	MAFLD	0.034 (0.019–0.051)	0.026 (0.004–0.047)	0.574 (0.339–0.808)	42.64% (2.52%–82.75%)

"Total effect" indicates the effect of CD20 on IgD⁻CD24⁻ B cell on MAFLD, "direct effect A" indicates the effect of CD20 on IgD⁻CD24⁻ B cell on waist-to-hip ratio, "direct effect B" indicates the effect of waist-to-hip ratio on MAFLD. Total effect, direct effect A, and direct effect B were derived by IVW; the 95% CI of mediation proportion was derived by using the delta method.
IVW, inverse-variance weighted; MAFLD, metabolic dysfunction-associated fatty liver disease.

Our findings align with existing literature suggesting that lymphocyte infiltration and activation are associated with worsened inflammation and fibrosis in MAFLD.[10]

B cells, particularly the proinflammatory B2 subset, play a crucial role in the pathogenesis of MAFLD.[10] Current literature supports the therapeutic value of B cell depletion in reducing inflammation and fibrosis in MAFLD.[14,45,46] Our data indicate that CD20 on IgD⁻CD24⁻ B cells showed both direct and indirect associations with MAFLD. This highlights the potential therapeutic value of targeting CD20+ B cells to reduce inflammation and fibrosis.[45,46] CD20 on IgD⁻CD24⁻ B cells may also serve as a biomarker for stratifying MAFLD risk. In clinical practice, incorporating these markers could improve risk assessment and treatment strategies.

Our study identified the waist-to-hip ratio as a significant mediator in the causal pathway between CD20 on IgD⁻CD24⁻ B cells and MAFLD, accounting for 42.64% of this association. Waist-to-hip ratio is a key indicator of central adiposity and is strongly associated with insulin resistance and inflammation, both of which are critical in MAFLD pathogenesis.[47–50] Central obesity, as indicated by a high waist-to-hip ratio, reflects an accumulation of visceral fat, which is known to contribute to the development of metabolic syndrome and related liver conditions.[51,52] Visceral fat is metabolically active and secretes proinflammatory cytokines, such as TNF-α and IL-6, which can exacerbate liver inflammation and insulin resistance.[53,54] Several studies have demonstrated that an increased waist-to-hip ratio is linked to a higher risk of MAFLD and its progression.[52,55,56] The mediation role of waist-to-hip ratio in our study highlights the interplay between immune markers and metabolic factors in MAFLD. The presence of CD20 on IgD⁻CD24⁻ B cells may influence metabolic pathways that affect fat distribution, leading to an increased waist-to-hip ratio and consequently, a higher risk of MAFLD. This mechanism suggests that targeting waist-to-hip ratio-related pathways could be a potential strategy for managing MAFLD. The identification of waist-to-hip ratio as a mediator in the relationship between immune phenotypes and MAFLD underscores the complex interactions between metabolic and immune factors in liver disease. T lymphocytes, including CD4⁺ and CD8⁺ subsets, have been linked to the progression of MAFLD.[16,17,44,57] Studies have reported that increased infiltration of CD8⁺ T cells exacerbates liver injury in MAFLD, which aligns with our findings that terminally differentiated CD8⁺ T cells are associated with an elevated risk of MAFLD.[17,44,57] These cells, defined by CCR7⁻CD45RA⁺ markers, are known as terminally differentiated effector memory T cells (TEMRA), which have been shown to predict severe liver fibrosis and steatosis in MAFLD.[24,13] The activation of CD8⁺ T cells may impair insulin sensitivity and enhance gluconeogenesis, contributing to the progression of MAFLD.[17,57]

Obesity also increases visceral adipose tissue CD8⁺ T cells, raising IFN-γ and Granzyme B levels, which further worsen MAFLD conditions.[58] Our data suggest that the direct effect of terminally differentiated CD8⁺ T cells on MAFLD risk persists even after adjusting for obesity and insulin resistance. This indicates that these cells may play a role beyond obesity-related inflammation. Given this, terminally differentiated CD8+ T

cells could serve as a potential biomarker for assessing MAFLD risk, particularly in identifying patients at risk for severe fibrosis.[13]

Tregs play a crucial role in modulating immune responses in MAFLD, with our study finding a significant association between CD4 expression on $CD39^+$ Tregs and increased susceptibility to the disease.[59] Previous studies have shown that a reduction in total $CD4^+$ T cells accelerates MAFLD progression, as these cells contribute to immune homeostasis through their immunosuppressive functions.[16–19] However, while Tregs initially protect against liver inflammation, their immunosuppressive activity can paradoxically exacerbate MAFLD and potentially lead to malignant changes.[18,19] Tregs also secrete TGF-β, which promotes fibrosis, further contributing to liver steatosis and progression.[60–62] Our findings suggest that targeting CD4 on $CD39^+$ Tregs may offer prognostic value in MAFLD by reducing liver inflammation and fibrosis, potentially improving clinical outcomes. However, more research is needed to clarify the exact role these Tregs play in disease progression and to assess their therapeutic potential.

The causal inferences between immunophenotypes and MAFLD were approached with caution. This study used bidirectional UVMR analysis, supported by extensive GWAS datasets and multiple MR methods, to investigate these relationships. The use of clinically diagnosed MAFLD outcomes, instead of self-reported data, enhances the validity of our findings. Furthermore, MVMR analysis accounted for 28 MAFLD-related factors, allowing us to pinpoint the direct effects of specific immunophenotypes while reducing potential confounding biases. We also minimized the risk of weak instrument bias by managing sample overlap between exposure and outcome sources, which strengthens the reliability of our causal inferences.

There are some limitations in this study. First, the examined peripheral lymphocyte patterns did not directly reflect the hepatic immunokinetics. The frequencies of several lymphocyte subsets differ between the peripheral blood and liver.[63,64] Second, the exclusive focus on participants of European ancestry limits the generalizability of our results to other ethnicities and geographic regions, where MAFLD prevalence and genetic backgrounds may differ significantly.[1,3,4,8] Third, the lack of individual-level data prevented stratified analyses by important variables such as age, sex, or histological stage, which are crucial given the demographic disparities in MAFLD incidence[1,2,4] and the varied inflammatory profiles observed in conditions such as MASH.[9,11] Fourth, inherent pleiotropy is a consideration in MR studies. However, our extensive sensitivity analyses across various assumptions consistently supported our findings, with robust methods like MR-PRESSO correction and data integration from multiple databases further reinforcing the stability of our conclusions. Finally, although our primary results yielded consistent trends across all five data (Figure 2), particularly in line with the trend from Anstee et al. based on biopsy-confirmed cases, we should acknowledge that the diagnosis of MAFLD in the outcome portion of the data derived from the analysis may be biased by misclassification of cases and controls using hospital records (i.e., ICD code). Therefore, future replication of the study on a larger scale in patients with MAFLD diagnosed by the gold standard (i.e., liver biopsy) is essential.

Conclusion

This study provides significant insights into the immunopathogenesis of MAFLD, identifying CD20 on IgD^-CD24^- B cells, CD4 on $CD39^+$ secreting Tregs, and terminally differentiated $CD8^+$ T cells as key immunophenotypes with causal relationships to MAFLD. Overall, these insights highlight the potential for personalized immunotherapy in MAFLD management, emphasizing the need for further research to translate these findings into clinical practice. The identification of specific immune phenotypes as causal factors in MAFLD may open a new avenue for targeted therapies to improve clinical outcomes.

Declarations

Ethics approval and consent to participate

This study is based on publicly available, summary-level data, and does not involve any direct research with human or animal participants.

Therefore, informed consent is not applicable. All original studies from which this data was derived had received prior ethical approval.

Consent for publication
Not applicable.

Author contributions
Kexin Xie: Conceptualization; Formal analysis; Investigation; Methodology; Supervision; Validation; Visualization; Writing – original draft; Writing – review & editing.

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Competing interests
The authors declare that there is no conflict of interest.

Availability of data and materials
Data were extracted from the following sources: the IEU OpenGWAS project (https://gwas.mrcieu.ac.uk/); the GWAS Catalog (https://www.ebi.ac.uk/gwas/home); and the FinnGen (https://www.finngen.fi/fi).

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Supplemental material
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