

ORIGINAL ARTICLE

Causal Associations of Perturbational Phenotyping of Human Blood Cells and CHB: a Mendelian Randomization Study

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ABSTRACT

Background: The dynamic interaction between the hepatitis B virus (HBV) and the host's inflammatory response plays a key role in determining the extent of hepatic injury and disease progression. This study aimed to elucidate the causal relationships among perturbational phenotypes of human blood cells, immune cell dynamics, and chronic hepatitis B infection (CHB) and to further validate these immune traits at the single-cell transcriptional level.

Methods: This study systematically summarized genome-wide association studies (GWAS) investigating 91 perturbational phenotypes of human blood cells in relation to CHB. Furthermore, a mediator Mendelian randomization analysis was performed to assess the potential mediating roles of immune cells in the causal framework linking perturbational phenotypes of human blood cells to CHB. To validate key MR-derived immune features, single-cell RNA-seq data from CHB patients and healthy controls were analyzed to delineate immune cell populations and characterize cell-state alterations.

Results: The analysis identified one perturbational phenotype of human blood cells that showed a causal association with CHB in individuals of European ancestry. This phenotype involved the eosinophil perturbation response, represented by the side scatter median of eosinophil 1 at baseline, measured using WDF dye. The findings suggest that this response mediates CHB risk through CD33 expressed on CD33^{br} HLA-DR⁺ CD14^{dim} cells. Two additional perturbational phenotypes of human blood cells were causally related to CHB: neutrophil perturbation response (neutrophil 2/neutrophil 4 ratio in response to water perturbation measured by WDF dye) and platelet perturbation response (side scatter standard deviation of platelets in response to captopril perturbation measured by WNR dye). The neutrophil perturbation response influenced CHB risk through Mo MDSC AC, HLA-DR on CD14⁺ CD16⁻ monocytes, and HLA-DR on CD14⁺ monocytes. The platelet perturbation response mediated CHB risk through Sw mem%B cells, secreting T_{reg} %CD4 cells, CD25⁺⁺ CD8^{br} %T cells, and CD28⁺ CD45RA⁻ CD8^{br} %CD8^{br} cells. Single-cell RNA-seq analysis further confirmed these MR findings by showing expanded inflammatory monocytes and MDSC-like cells, increased cytotoxic and regulatory T-cell activity, and reduced switched memory B-cell signatures in CHB.

Conclusions: This study provides genetic evidence supporting a potential causal relationship between perturbational phenotypes of human blood cells and CHB, indicating that immune cells may act as intermediaries in mediating this association. The integration of MR and single-cell profiling strengthens the biological plausibility of these pathways and highlights key immune states involved in HBV persistence and disease progression.

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INTRODUCTION

Chronic hepatitis B infection (CHB) is a major global public health concern. Currently, an estimated 400 million individuals are chronically infected with the hepatitis B virus (HBV), while approximately 2 billion people show serological evidence of previous exposure [1]. Chronic HBV infection predisposes affected individuals to severe hepatic complications, including liver failure, cirrhosis, and hepatocellular carcinoma (HCC) [2,3]. Epidemiological data indicate that the global incidence of HBV infection averages around 2.5%, with both incidence and mortality rates showing a decline attributable to widespread infant immunization, childhood vaccination, and antiviral therapy [4]. However, despite long-term NA therapy, a subset of patients continues to experience disease progression, likely reflecting the lack of curative antiviral strategies for HBV infection [3,5-7]. These observations imply the involvement of additional molecular or immunopathological mechanisms in disease advancement.

Perturbational phenotyping of human blood cells has revealed genetically determined latent characteristics associated with specific subsets of common diseases. Researchers have developed a framework employing human peripheral blood cells subjected to physical, chemical, and pharmacological perturbations, followed by flow cytometry-based functional assessments, to investigate underlying cellular processes. By using this approach, a genome-wide association study (GWAS) involving up to 2,600 individuals was conducted based on these inducible traits, leading to the identification of 119 genomic loci encompassing 96 genes linked to cellular responses and uncovering associations between induced blood phenotypes and subsets of common diseases [8]. The researchers identified a pro-inflammatory, anti-apoptotic neutrophil subset that was enriched in individuals with specific cardiometabolic disorders. A polygenic score model derived from blood cell phenotypic data enabled stratification of patients with differential risks for cardiometabolic, vascular, and inflammatory diseases, identifying subgroups potentially responsive to therapies targeting relevant biological pathways [7]. Peripheral blood may thus serve as a diagnostic window reflecting multiple organ systems and integrated physiological states [9-11]. Emerging evidence indicates that neutrophils play a pivotal role in the pathogenesis of viral infections [12]. Neutrophils are known to phagocytose viral particles and secrete cytokines that recruit other immune cells to sites of inflammation, contributing to hepatocellular injury and persistent HBV replication [13,14]. However, the specific perturbational

phenotypes or regulatory mechanisms by which neutrophil activity influences chronic HBV infection remain poorly defined.

Liver fibrosis represents a pathological consequence of hepatocyte injury mediated by cellular immune responses, and most patients with CHB show varying degrees of fibrosis [15]. In patients with cirrhosis, the body produces a PLT storage pool, leading to peripheral blood PLT depletion, and higher HBV DNA load is associated with a decrease in platelet count [16]. TH17, an effector T cell, promotes the generation of inflammatory factors after viral invasion to inhibit virus replication and limit disease progression [17]. However, excessive TH17 activity can result in overproduction of inflammatory mediators, aggravating organ damage, indicating its involvement in CHB onset and liver injury [18,19]. Peripheral blood cells, including neutrophils, PLT, and lymphocytes, are closely linked in CHB and contribute to the liver inflammatory response, potentially representing a novel pathogenic mechanism or therapeutic target.

Mendelian randomization (MR) uses GWAS to identify single-nucleotide polymorphisms (SNPs) that are strongly associated with specific outcomes. By leveraging randomly inherited genetic variation, MR provides a framework to infer causal relationships between an exposure and an outcome while reducing the influence of confounding factors [20]. To date, no MR studies have examined the relationship between perturbational phenotyping of human blood cells and CHB. In this study, it is hypothesized that perturbational phenotypes of human blood cells impact CHB through immune cell-mediated mechanisms. MR analysis is applied to assess the potential causal effects of 91 perturbational blood cell phenotypes on CHB and to investigate the regulatory role of 731 immune phenotypes in this context. To further validate the MR-derived immune features at the cellular and transcriptional level, single-cell RNA-seq data from CHB patients and healthy controls were analyzed to characterize immune landscapes and cell-state alterations. Together, these approaches aimed to deepen mechanistic understanding of CHB and identify novel immune-related risk factors.

MATERIALS AND METHODS

Data sources

A total of 91 perturbational phenotypes of human blood cells were obtained from the GWAS catalog database (accession numbers GCST90257017 - GCST90257105), as listed in Supplementary Table 1. Immune cell phenotypes were sourced from a publicly available GWAS database (registration numbers GCST90001391 - GCST90002121) and included relative count (RC), absolute count (AC), median fluorescence intensity (MFI), and morphological parameters (MP). These phenotypes reflect the levels of surface antigens on B cells, CDCs, mature T cells, monocytes, myeloid cells, TBNK

clusters (T cells, B cells, natural killer cells), and T_{reg} cells. The MP function further encompasses CDC and TBNK panels, with further details provided in Supplementary Table 2.

CHB-related GWAS data were retrieved from the GWAS website (<https://gwas.mrcieu.ac.uk/>), including 351,885 European samples (145 cases and 351,740 controls) and 171,822 Asian samples (2,234 cases and 169,588 controls). This study used publicly accessible, authoritative databases; therefore, ethics committee approval and informed consent from participants were not required.

Selection of instrumental variables (IVs)

In this study, IVs were derived from 91 perturbational phenotypes of human blood cell datasets and 731 immune cell datasets, followed by a systematic screening procedure. To strengthen the reliability of the IV estimates, variants with p-values below 1×10^{-5} were retained [21,22]. To reduce the influence of strong linkage disequilibrium (LD) on the analysis, a clustering approach with thresholds of $r^2 = 0.001$ and $kb = 10,000$ was applied to rigorously identify LD, and SNPs satisfying these criteria were selected from the outcome dataset. For each SNP, information including the effect allele, effect size (β), standard error (SE), and p-value was retrieved. The strength of the IVs was then assessed by calculating the proportion of variance explained (R^2) and the F statistic.

Single-cell transcriptomic analysis and immune-feature profiling in HBV and healthy controls

Public single-cell RNA-seq data from chronic hepatitis B (CHB) patients and healthy controls (HC) were obtained from Zenodo (record 8399409) and processed using Scanpy (v1.11). After filtering low-quality cells, 186,123 cells and 1,600 variable genes were retained. Data were normalized, log-transformed, and analyzed by PCA followed by neighborhood graph construction and UMAP embedding. Leiden clustering (resolution = 1.0) identified 38 immune cell clusters. Cell types were annotated using canonical markers (e.g. CD14/S100A8/A9 for monocytes, MS4A1/CD79A for B cells, FOXP3/IL2RA for Treg), confirmed by a DotPlot of lineage-defining genes. Based on the key immune-cell traits identified through Mendelian randomization, we further examined eight MR-derived immune features using violin plots, including CD25⁺ CD8⁺ bright T cells, CD28⁺ CD8⁺ bright memory/effector T cells, CD33⁺ bright HLA-DR⁺ CD14⁺ dim monocytes, HLA-DR⁺ CD14⁺ monocyte subsets, monocytic MDSC-like cells, secreting Treg, and switched memory B cells.

Data analysis

To assess the causal effects of multiple IVs, five widely used MR approaches were employed: inverse variance weighting (IVW), weighted mode, simple mode, weighted median, and MR-Egger regression. IVW served as the primary method for estimating causal effects,

whereas the other four approaches were applied as complementary analyses. Sensitivity analyses were performed to assess pleiotropy, heterogeneity, and the robustness of the MR estimates. Cochran's Q test was applied to quantify heterogeneity among IVs, with $p > 0.05$ indicating the absence of heterogeneity. The MR-Egger regression intercept was used to evaluate potential pleiotropy, where $p > 0.05$ indicated no significant pleiotropy. MR-PRESSO was employed to detect and correct for potential horizontal pleiotropy by identifying and removing outlier SNPs. A leave-one-out analysis was conducted to examine the influence of individual IVs on the overall estimates. Finally, statistical power was evaluated using the MR Analysis website (<https://mranalysis.cn/>) to determine whether the available sample size was adequate to support the study conclusions. To assess whether the available sample size was sufficient to detect meaningful causal effects, we performed a statistical power analysis following standard approaches for two-sample Mendelian randomization. Power was evaluated based on three key parameters: the proportion of variance in the exposure explained by the genetic instruments (R^2), the sample size of the outcome GWAS, and a conventional significance level ($\alpha = 0.05$). For each analysis, R^2 was calculated using the effect allele frequency and the per-allele effect size of each SNP. By using these values, we estimated the minimum detectable causal effect size under the given sample size and instrument strength. This analysis allowed us to determine whether the study had adequate power to detect effect sizes of practical relevance.

RESULTS

Selecting IVs

A total of 91 SNPs associated with human blood cell perturbation phenotypes were identified as IVs to investigate the relationship between these phenotypes and CHB. Among these, one perturbational phenotype was linked to CHB in the European population, while three were identified in the Asian population. All selected SNPs had F-statistics > 10 , indicating the absence of weak instrument bias. Further analysis incorporated combined SNP information, including the effect allele, alternative allele, β value, SE, and p-value. Detailed information on the selected IVs is presented in Supplementary Table 3.

MR analysis of perturbational phenotyping of human blood cells and CHB

The study workflow is illustrated in Figure 1, and Supplementary Table 4 provides detailed results of the comprehensive MR analyses of 91 perturbational phenotypes of human blood cells and CHB. IVW analysis indicated that, in the European population, an elevated Eosinophil perturbation response (side scatter median of eosinophil 1 at baseline measured by WDF dye) was associated with a protective effect against CHB (OR:

Table 1. The mediating effects of immune cells in causal association linking perturbational phenotyping of human blood cells to chronic hepatitis B infection.

Ancestral origin	Exposure	Mediator	The effect of exposure on outcome β	The effect of exposure on mediator β_1	The effect of mediator on outcome β_2	Mediation effect
European	Eosinophil perturbation response (side scatter median of eosinophil 1 at baseline measured by WDF dye)	CD33 on CD33br HLA DR ⁺ CD14dim	-0.093858758	-0.11917126	0.043491272	-0.00518291
East Asian	Neutrophil perturbation response (neutrophil 2/neutrophil 4 ratio in response to water perturbation measured by WDF dye)	Mo MDSC AC	0.11114602	0.109512126	0.219026958	0.023986108
		HLA DR on CD14 ⁺ CD16 ⁻ monocyte	0.11114602	-0.080486338	-0.224668343	0.018082732
		HLA DR on CD14 ⁺ monocyte	0.11114602	-0.07798135	-0.230220587	0.017952912
	Platelet perturbation response (side scatter standard deviation of platelet in response to captopril perturbation measured by WNR dye)	Sw mem %B cell	-0.022711936	0.02622163	0.200649498	0.005261357
		Secreting Treg %CD4	-0.022711936	0.028167489	0.147497936	0.004154646
		CD25 ⁺⁺ CD8br %T cell	-0.022711936	0.02764485	0.284179412	0.007856097
		CD28 ⁺ CD45RA ⁻ CD8br %CD8br	-0.022711936	0.028518768	0.107303181	0.003060154

0.91; 95% CI: 0.82 - 0.99; $p = 0.04$). In comparison, in the Asian population, an increased neutrophil perturbation response (neutrophil 2/neutrophil 4 ratio in response to water perturbation measured by WDF dye) was identified as a risk factor for CHB (OR: 1.117; 95% CI: 1.014 - 1.23; $p = 0.024$). An increased platelet perturbation response, measured as the forward scatter standard deviation of platelets in response to captopril perturbation using WNR dye (OR: 0.966; 95% CI: 0.936 - 0.997; $p = 0.032$) and the side scatter standard deviation of platelets under the same conditions (OR: 0.977; 95% CI: 0.957 - 0.997; $p = 0.031$), was associated with a protective effect against CHB. These associations were further supported by weighted mode, simple mode, weighted median, and MR-Egger regression analyses. Figure 2 illustrates the significant causal relationship between perturbational phenotypes of human blood cells and CHB. Reverse MR analysis indicated that CHB did not exert a causal effect on the perturbational phenotyping of human blood cells, as evidenced by $p > 0.05$ (Supplementary Table 5).

Immune cell-mediated effects between blood cell perturbational phenotypes and CHB

Using the IVW method, 37 causal associations between immune cell traits and hepatitis B infection were identified,

comprising 8 in the B cell group, 6 in the TBNK group, 6 in the T_{reg} group, 5 in the monocyte group, 3 in the cDC group, 5 in the myeloid cell group, and 4 in the T cell maturation stages group (Supplementary Tables 6 and 7). Furthermore, MR analysis was performed to explore the causal relationships between perturbational phenotypes of human blood cells and intermediate immune cell phenotypes. The study demonstrated that one immune cell marker was negatively associated with increased CHB risk in the European population: CD33 on CD33^{br} HLA DR⁺ CD14^{dim} (OR: 1.115; 95% CI: 1.018 - 1.22; $p = 0.018$). Five immune cell markers showed positive associations with elevated CHB risk: Mo MDSC AC (OR: 1.115; 95% CI: 1.018 - 1.22; $p = 0.018$), Sw mem %B cells (OR: 1.026; 95% CI: 1.006 - 1.046; $p = 0.008$), secreting T_{reg} %CD4 (OR: 1.028; 95% CI: 1.000 - 1.057; $p = 0.044$), CD25⁺⁺ CD8^{br} %T cells (OR: 1.028; 95% CI: 1.000 - 1.049; $p = 0.007$), and CD28⁺ CD45RA⁻ CD8^{br} %CD8^{br} (OR: 1.028; 95% CI: 1.000 - 1.051; $p = 0.011$). In comparison, HLA DR on CD14⁺ CD16⁻ monocytes (OR: 0.922; 95% CI: 0.86 - 0.98; $p = 0.0233$) and HLA DR on CD14⁺ monocytes (OR: 0.924; 95% CI: 0.862 - 0.991; $p = 0.028$) were associated with a reduced risk of CHB (Figure 3). Finally, mediating effects were estimated to identify specific factors mediating the relationship between perturbation-

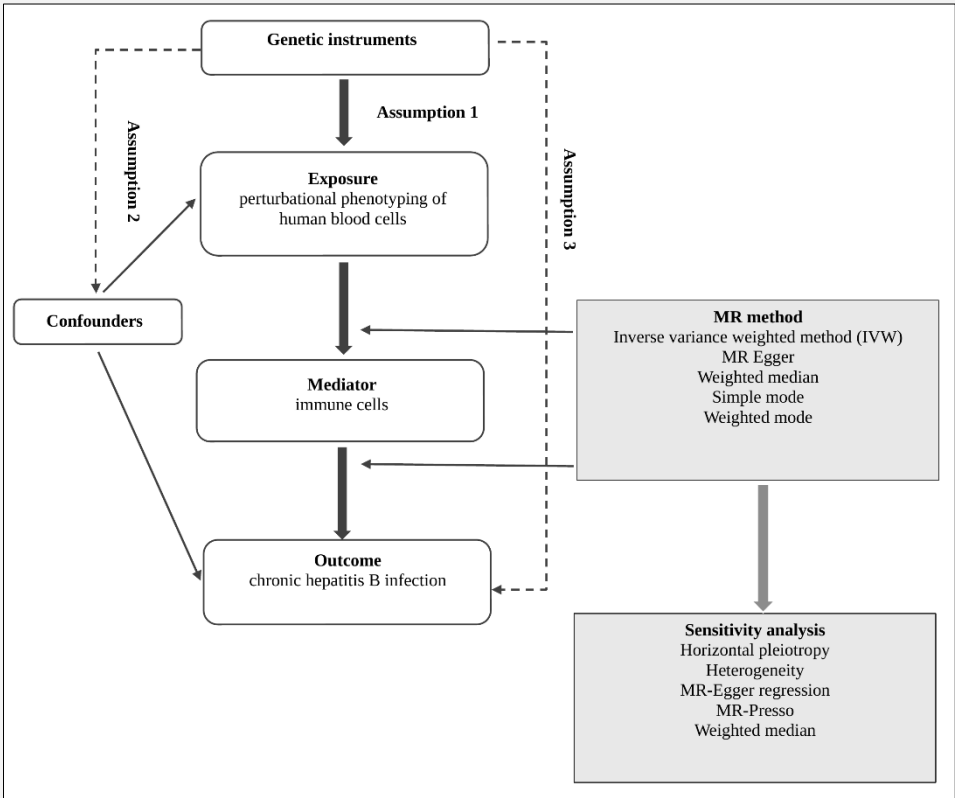


Figure 1. Flowchart of the MR-analysis process.

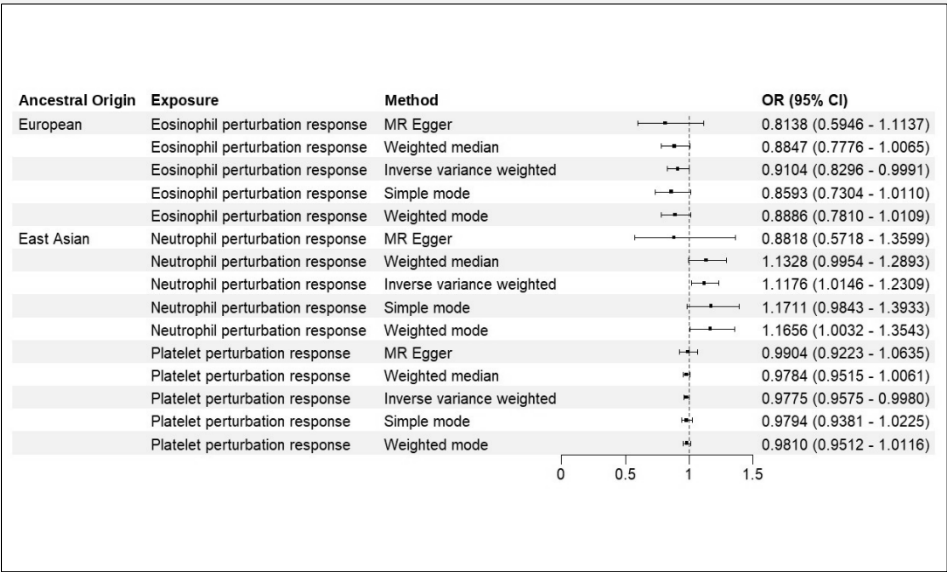


Figure 2. The forest plot shows the mendelian randomization results of the causal relationship between the perturbational phenotyping of human blood cells on chronic hepatitis B infection. OR: odds ratio, CI: confidence interval.

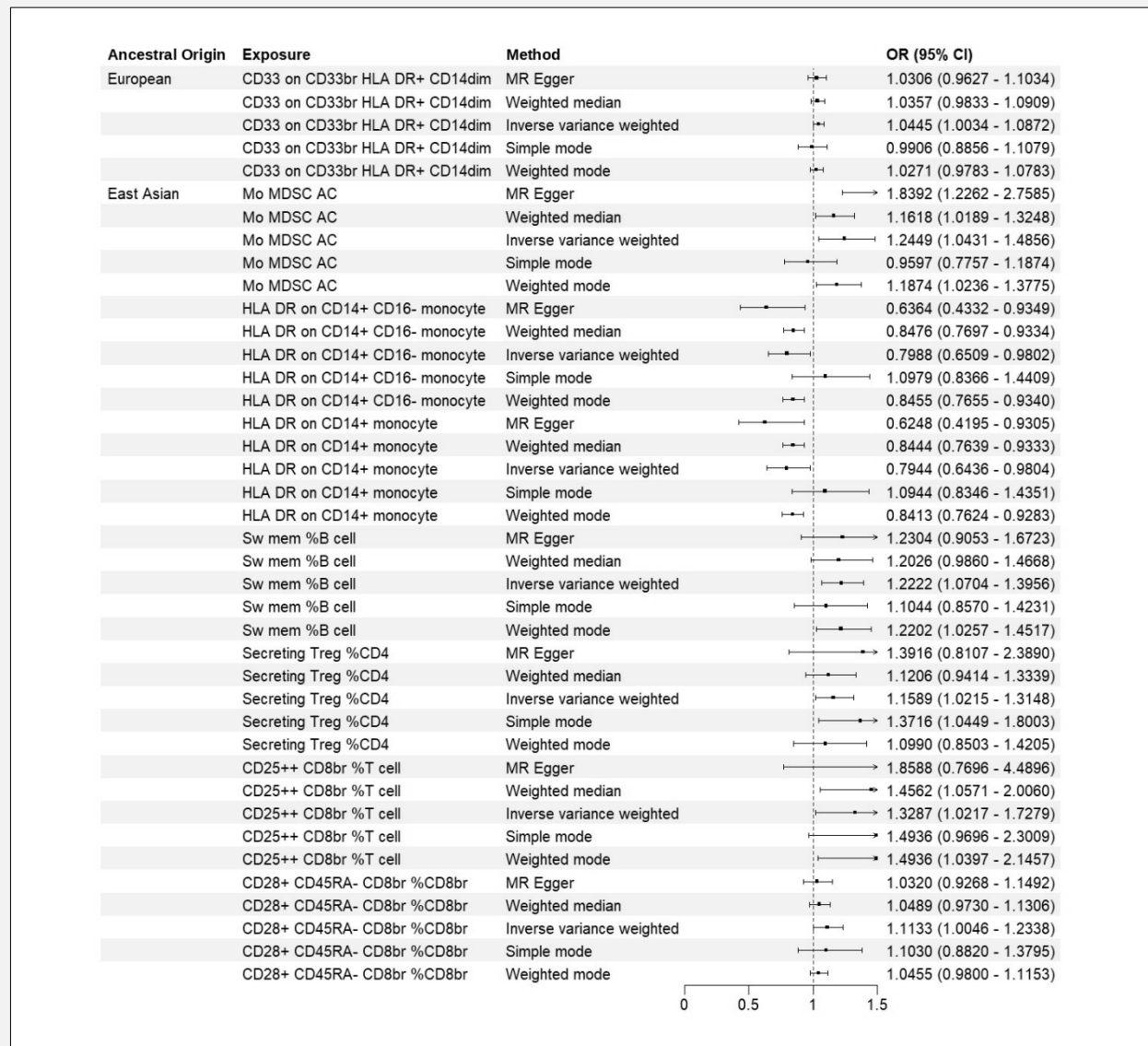


Figure 3. The forest plot shows the Mendelian randomization results of the causal relationship between the immune cells on chronic hepatitis B infection.

OR: odds ratio, CI: confidence interval.

al phenotypes of human blood cells and CHB. For the Eosinophil perturbation response, CD33 on CD33^{br} HLA DR⁺ CD14^{dim} served as the mediator. For the Neutrophil perturbation response, mediators included Mo MDSC AC (OR: 0.023; 95% CI: -0.016 - 0.063), HLA DR on CD14⁺ CD16⁻ monocytes (OR: 0.018; 95% CI: -0.028 - 0.064), and HLA DR on CD14⁺ monocytes (OR: 0.017; 95% CI: -0.030 - 0.066). For the Platelet perturbation response, mediating factors comprised Sw-m %B cells (OR: 0.005; 95% CI: -0.021 - 0.031),

secreting Treg %CD4 (OR: 0.004; 95% CI: -0.014 - 0.022), CD25⁺⁺ CD8^{br} %T cells (OR: 0.007; 95% CI: -0.066 - 0.082), and CD28⁺ CD45RA⁻ CD8^{br} %CD8^{br} (OR: 0.003; 95% CI: -0.007 - 0.014) (Table 1, Supplementary Table 8).

Sensitivity analysis

Sensitivity analyses were performed to evaluate the consistency and reliability of the IVs (Figure 4). Heterogeneity in the MR analysis was assessed using Coch-

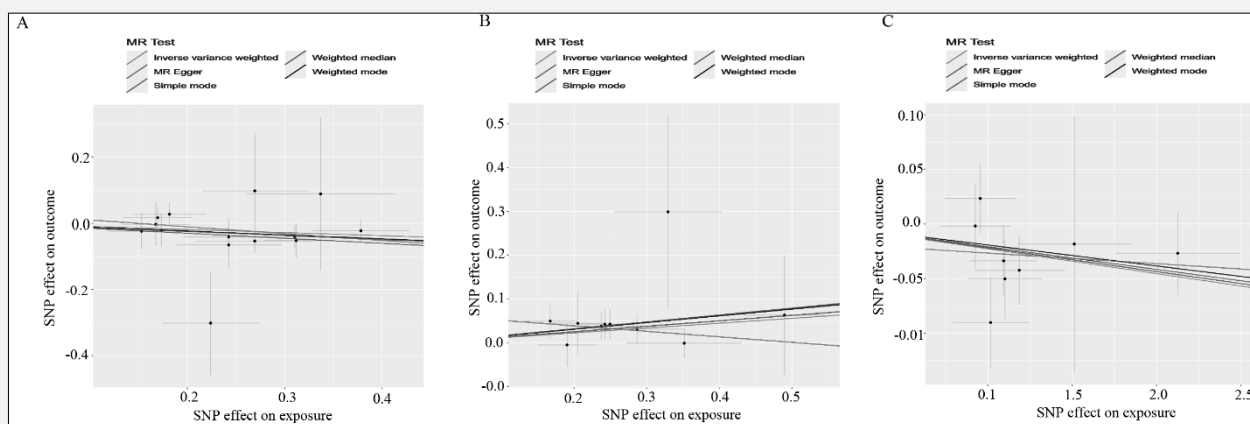


Figure 4. Scatter plots of the causal effect of perturbational phenotyping of human blood cells on chronic hepatitis B infection.

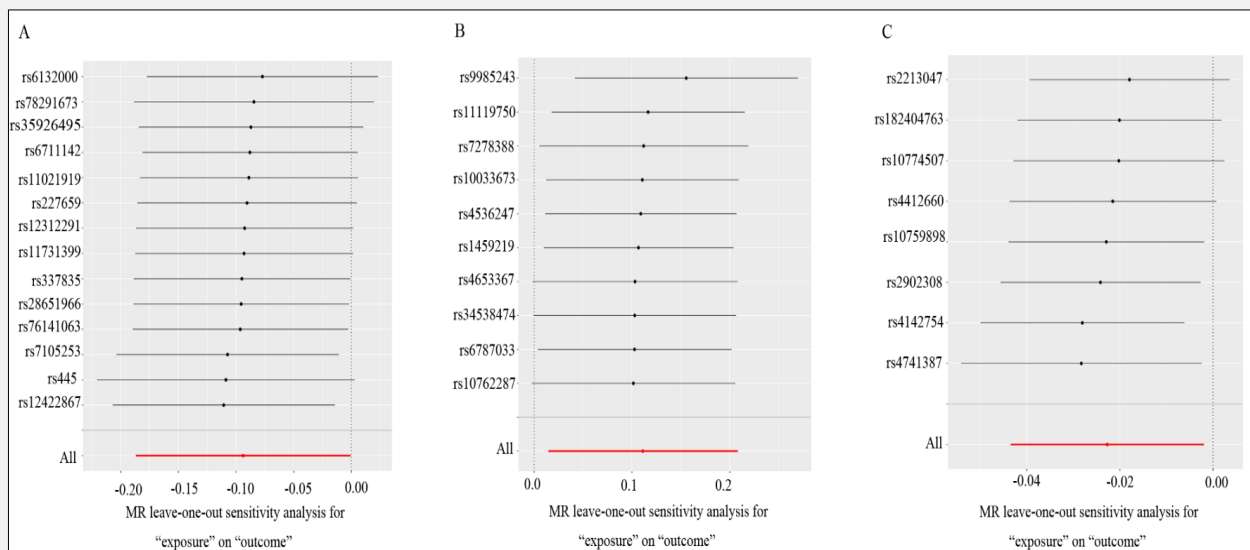


Figure 5. The Leave-one-out method results of perturbational phenotyping of human blood cells on chronic hepatitis B infection.

ran's Q test and MR-Egger regression. Results indicated no evidence of heterogeneity, as the Q statistic was non-significant ($p > 0.05$). MR-PRESSO was applied to detect and correct for outliers, addressing potential within-study heterogeneity, and no significant heterogeneity was observed ($p > 0.05$). Furthermore, the MR-Egger intercepts were not significantly different from zero

($p > 0.05$), further supporting the absence of within-study heterogeneity (Supplementary Tables 9 - 14). A leave-one-out analysis was conducted to determine whether individual SNPs disproportionately influenced the causal estimates (Figure 5). In this study, three human blood cell phenotypes were used as exposures, with CHB as the outcome. Using the R^2 values from the

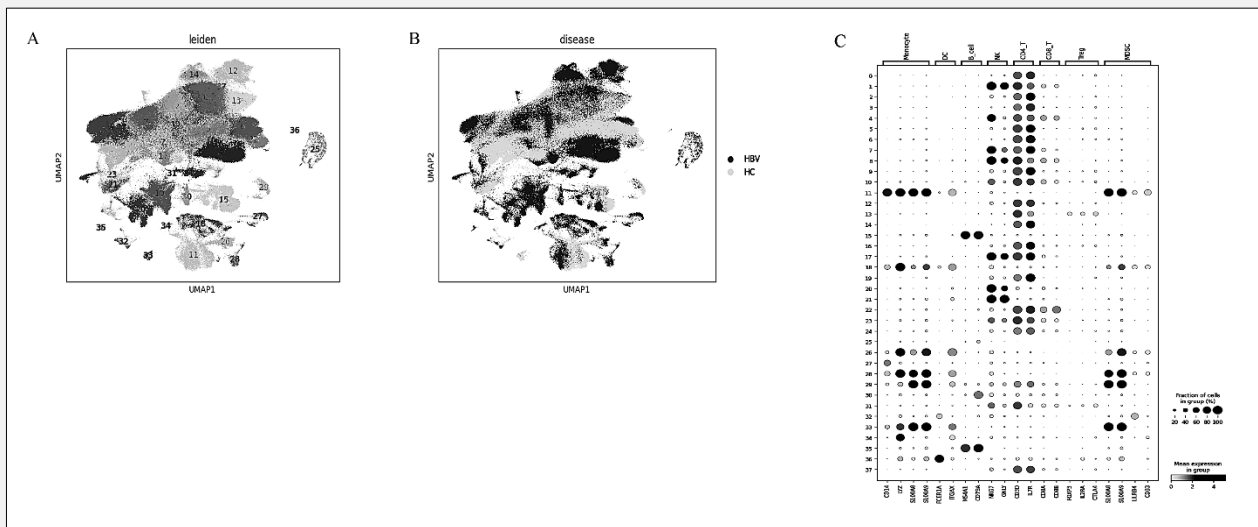


Figure 6. UMAP visualization and immune cell annotation of HBV and healthy PBMCs.

A: UMAP plot showing the 38 Leiden clusters, each representing a transcriptionally distinct immune cell population. **B:** UMAP plot illustrating disease-based distribution of single cells, showing distinct spatial patterns between patients with HBV infection and healthy controls. **C:** Dotplot displaying the expression of lineage-defining marker genes across major immune cell groups, enabling identification of cell-type identities.

harmonized datasets, the genetic instruments explained 24.31% of the exposure variance in the European ancestry analysis (Total $R^2 = 0.2431$), but only 0.316% and 0.27% in the Asian ancestry analyses (Total $R^2 = 0.003164$ and 0.00269). Despite the modest variance explained in the Asian datasets, all Mendelian randomization analyses remained adequately powered owing to the large outcome sample sizes ($n = 351,885$ for European ancestry; $n = 171,822$ for Asian ancestry). Under a conventional significance level ($\alpha = 0.05$), the study had sufficient power to detect causal effects of practical relevance, although very small effects may be underpowered. Overall, the sample sizes in both the European and Asian ancestry analyses were sufficiently large to ensure adequate power and reinforce the robustness of the MR results. These findings support the validity of the study design and provide a strong foundation for further investigation of the causal relationships and underlying mechanisms (Table 1).

MR-identified immune traits validated by single-cell transcriptomic profiling

UMAP revealed a complex but coherent immune landscape shared by HBV and healthy controls (HC), with CHB samples showing preferential enrichment in monocyte-dominant and cytotoxic T-cell regions. Leiden clustering resolved 38 transcriptionally distinct immune states, and lineage-marker enrichment in the Dot-

Plot clearly delineated monocytes, dendritic cells, NK cells, $CD4^+$ and $CD8^+$ T-cell subsets, B cells, Treg cells, and MDSC-like clusters (Figure 6). The detailed cell-type annotation of all 38 Leiden clusters is provided in Supplementary Table 15. Analysis of the eight MR-derived immune features demonstrated broad Immunological remodeling in CHB. These immunophenotypes from the blood-cell GWAS (e.g. “Mo MDSC AC”, “HLA-DR on $CD14^+$ monocytes”, “Switched memory %B cells”, “Secreting Treg % $CD4^+$ ”, “ $CD25^{++}$ $CD8^-$ bright %T cells”, and “ $CD28^+$ $CD45RA^-$ $CD8^{bright}$ % $CD8^{bright}$ ”) were mapped to corresponding cell populations in the scRNA-seq data by using canonical marker genes ($CD14$, $CD33$, $HLA-DRA$, $FOXP3$, $IL2RA$, $CD8A/B$, $MS4A1$, etc.) and cluster-level annotations (monocytes, Mo-MDSC-like cells, B cells, Treg, and $CD8$ T cells). $CD25^+$ $CD8^{bright}$ T cells ($IL2RA/CD8A$) and cytotoxic $CD8$ phenotypes (high $GZMB$) were expanded in CHB, whereas reduced $CD28$ expression indicated activation accompanied by partial exhaustion. Several monocyte subsets exhibited increased $HLA-DRA$ and $CD33$, together with elevated $S100A8/A9$, consistent with inflammatory activation and expansion of MDSC-like myeloid populations. Treg-associated genes ($FOXP3$, $IL2RA$, $CTLA4$) were also up-regulated in CHB, suggesting enhanced regulatory activity. In contrast, switched memory B-cell markers ($CD27$, $IGHG1$) were more abundant in healthy con-

trols, indicating impaired B-cell maturation in CHB (Supplementary Figure 1).

DISCUSSION

HBV does not directly damage hepatocytes; instead, viral antigenic epitopes are expressed on infected liver cells, which are recognized and processed by antigen-presenting cells (APCs). These epitopes are presented via MHC class I molecules, leading to the activation of CD8⁺ T cells and a cellular immune response targeting infected cells, contributing to disease pathology. CD8⁺ T cell activation relies on recognition by the innate immune system. The nature and magnitude of the host immune response to HBV determine the clinical manifestations and outcomes of HBV infection and associated liver diseases across individuals [23]. HBV can disrupt the antiviral immune response through multiple mechanisms, facilitating persistent infection. Although current therapies for CHB can slow disease progression, complete viral clearance remains challenging. Understanding the immune pathogenesis of CHB is therefore critical for developing more effective treatment strategies.

This study presents an approach that integrates cell phenotypes with GWAS to uncover novel genetic loci exerting significant effects on cellular biology. Using a multi-gene model derived from selected cell phenotypes, the study identified clinical phenotypes linked to significant alterations in disease risk mediated by these intermediate cellular traits. The analysis emphasizes cellular responses in peripheral blood, facilitating the identification of previously unrecognized disease-related intracellular phenotypes. This approach enables the identification of common alleles with large β -values, which may serve as potential drug targets. It allows for the detection of alleles involved in key cellular processes, such as neutrophil activation and apoptosis, across complex diseases beyond hematological disorders. Dysregulation of hematopoietic processes can drive disease progression through mechanisms such as inflammation contributing to atherosclerosis or insulin resistance, or excessive thrombosis in pathological contexts. Induced cellular responses in peripheral blood offer an additional means to characterize existing phenotypes and can uncover genes and pathways with translational potential. The present MR study provides genetic evidence supporting a causal relationship between perturbational phenotypes of human blood cells and CHB. The eosinophil perturbation response, measured as the side scatter median of eosinophil 1 at baseline using WDF dye, mediates the risk of CHB through CD33 on CD33^{br} HLA DR⁺ CD14^{dim} cells. The neutrophil perturbation response, defined as the neutrophil 2/neutrophil 4 ratio following water perturbation measured by WDF dye, was associated with a reduced CHB risk, partially mediated via HLA DR on CD14⁺ CD16⁻ monocytes (0.922, [0.86 - 0.98], $p = 0.0233$) and HLA DR on CD14⁺ monocytes (0.924, [0.862 - 0.991], $p = 0.028$). Compar-

atively, Mo MDSC AC was linked to an elevated CHB risk (1.115, [1.018 - 1.22], $p = 0.018$). The platelet perturbation response, measured as the side scatter standard deviation of platelets following captopril perturbation using WNR dye, was associated with increased CHB risk through four immune mediators: Sw mem% B cell (1.026, [1.006 - 1.046], $p = 0.008$), Secreting T_{reg} %CD4 (1.028, [1.00 - 1.057], $p = 0.044$), CD25⁺⁺ CD8^{br} %T cell (1.028, [1.00 - 1.049], $p = 0.007$), and CD28⁺ CD45RA⁻ CD8^{br} %CD8^{br} (1.028, [1.00 - 1.051], $p = 0.011$). The differences observed between the two ancestries are not unexpected. Genetic architecture, allele frequencies, and linkage disequilibrium patterns vary substantially between East Asian and European populations, and environmental exposures may further modify genetic effects. Therefore, inconsistent MR estimates likely reflect true population-specific heterogeneity rather than analytical errors. Previous studies have demonstrated that CHB is associated with elevated levels of peripheral blood inflammatory mediators. Compared with healthy controls, CHB patients show a significant inflammatory imbalance, characterized by an increased MDP score and a distinctive network of correlations among plasma biomarker concentrations [24]. At the cellular level, CHB infection is characterized by inflammation-driven hepatocyte apoptosis, arising from sustained immune activation of infected liver cells and compensatory tissue regeneration [25-27]. This immune response is primarily characterized by T cell proliferation and exhaustion [28-32]. These findings highlight the impact of peripheral blood perturbations on systemic inflammation in CHB and identify the immune cell mediators involved. The results provide deeper insight into the host systemic response to HBV during active viral replication.

Integration of UMAP clustering, lineage marker annotation, and targeted violin-plot profiling demonstrates coordinated immune dysregulation in chronic HBV infection. CHB is characterized by activated, yet partially exhausted CD8 T cells, hyperactivated antigen-presenting monocytes, expansion of inflammatory MDSC-like populations, and increased Treg suppressive activity. Simultaneously, reduced switched memory B-cell signatures suggest impaired humoral differentiation. These cellular alterations align with known mechanisms of HBV persistence-sustained antigen stimulation, myeloid-driven inflammation, regulatory suppression, and defective B-cell immunity - and provide functional immune-cell evidence that complements and reinforces the Mendelian randomization findings.

This study has some limitations. First, a relatively lenient threshold was applied in selecting IVs for each immune cell trait to increase statistical power and reduce weak instrument bias; however, this may increase the risk of false positives or residual confounding. Second, the potential specific or synergistic effects of perturbations in peripheral blood cell counts on various immune cell traits or other influential factors could not be fully elucidated. Third, the conclusions of this study

represent genetic causal estimates derived from Mendelian randomization, reflecting the effects predicted by genetic instruments rather than direct observational or experimental evidence. Due to constraints in research resources, challenges in sample collection, and the prolonged duration required for cohort studies, independent cohort validation has not yet been performed.

In future work, we aim to integrate clinical collaborative resources, partner with relevant medical institutions to collect blood samples and clinical data from CHB patients and healthy controls, and progressively advance cohort studies. This approach will enable further verification of causal relationships between blood cell perturbational phenotypes and disease, as well as their underlying mechanisms, providing stronger clinical evidence to support present findings.

In conclusion, the study reveals a complex interplay between perturbational phenotyping of human blood cells and CHB infection, encompassing both protective and pathogenic effects. In individuals of European ancestry, a single perturbational phenotype negatively modulates CHB risk via a specific immune cell. In the Asian population, one perturbational phenotype and two immune cell traits are inversely associated with CHB, whereas two perturbational phenotypes and five immune cell traits are positively associated with the disease. Single-cell RNA-seq analysis further reinforced these findings by demonstrating expansion of inflammatory monocytes and MDSC-like cells, heightened cytotoxic and regulatory T-cell activity, and reduced switched memory B-cell signatures in CHB-patterns consistent with the immune mediators identified through MR. These findings indicate that perturbational phenotyping of human blood cells offers novel insights into CHB pathogenesis and may guide the development of future therapeutic strategies.

Data Availability Statement:

The source code is available at: <https://github.com/CS7012/Manuscript-250620-Chen>.

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Declaration of Generative AI in Scientific Writing:

The authors confirm that generative AI tools were not used at any stage of the manuscript preparation.

Declaration of Interest:

The authors declare no conflicts of interest.

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