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Complex causal relationships between genetic predictions of 731 immune cell phenotypes and novel coronavirus: A two-sample Mendelian randomization analysis

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Abstract

Background: Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has had a significant impact on global health. While the virus primarily affects the respiratory system, the intricate interplay between immune cells and the virus remains poorly understood. This study investigates the causal relationship between 731 immune cell phenotypes and COVID-19 using Mendelian randomization (MR) analysis.

Methods: A bidirectional two-sample MR analysis was conducted using genetic variants strongly associated with immune cell phenotypes as instrumental variables. Data for 731 immune cell phenotypes were sourced from the Genome-Wide Association Study (GWAS) catalog, while data for COVID-19 susceptibility were obtained from the OPEN GWAS database. Five MR methods (inverse variance weighted [IVW], MR-Egger, weighted median, simple mode, and weighted mode) were used to estimate causal effects, with IVW as the primary analysis method.

Results: The study identified 57 immune cell phenotypes causally associated with COVID-19 risk across two independent GWAS datasets. Five immune cell phenotypes were consistently associated with COVID-19 risk across both datasets: CD3– lymphocyte %lymphocyte (protective), CD27 on CD20– (protective), CD20 on IgD+ CD38– unsw mem (increased risk), CD27 on IgD– CD38– (increased risk), and CD19 on B cell (increased risk). Sensitivity analyses confirmed the robustness of the findings.

Conclusion: This study provides compelling evidence for a causal relationship between specific immune cell phenotypes and COVID-19 risk. These findings highlight the potential for targeting these immune cell phenotypes as novel therapeutic targets for COVID-19 treatment and prevention.

Keywords: SARS-CoV-2; COVID-19; Mendelian randomization analysis

1. INTRODUCTION

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a newly emerged acute infectious disease primarily affecting the respiratory system.[1] It typically presents with initial symptoms such as sore throat, dry cough, and fever. Other common symptoms include fatigue, muscle pain, nausea, vomiting, diarrhea, headache, weakness, effusion, loss of smell, and loss of taste. The disease is usually self-limiting, with a favorable prognosis for most patients, although severe cases can lead to multiorgan failure.[2,3] As of April 2023, there have been over 670 million confirmed cases worldwide, resulting in nearly seven million deaths attributed to COVID-19. Despite extensive global vaccination efforts reducing incidence and severity, COVID-19 remains uncontrolled, with approximately 1000 deaths daily,[4] imposing significant burdens on society and families. The unclear pathogenesis of the novel coronavirus remains a key factor contributing to inadequate treatment and poor prognosis.

Immune cells, commonly referred to as white blood cells, include lymphocytes and various phagocytic cells, specifically referring to lymphocytes that recognize antigens and produce specific immune responses.[5] They play a critical role in the immune response against SARS-CoV-2 infection. Imbalances in lymphocyte subgroups may be a key factor in the progression of COVID-19. During the immune response process, pathological changes occur in the body when there are abnormalities in the quantity and function of various lymphocyte subgroups.[6] Therefore, this article primarily discusses the phenotypes of immune cells and the complex causality with novel coronaviruses.

Currently, it is recognized that certain viral proteins expressed by SARS-CoV-2 can evade and counteract recognition by the innate immune system, potentially contributing to the development of COVID-19.[7] The progression of COVID-19-induced

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pneumonia is primarily divided into two stages: direct virus–mediated lung tissue damage and recruitment of effector immune cells, leading to local and systemic inflammatory responses that persist even after viral clearance, thereby attacking tissues throughout the body and causing "long COVID".[8] In recent years, research on immune cells in COVID-19 has garnered increasing attention. Studies such as Xiong et al[9] have found significantly lower immune cell counts in COVID-19 patients who died than those who recovered, including total lymphocyte count, CD4+ T cells, CD8+ T cells, B cells, and natural killer (NK) cells. Inflammation in the lungs and other vital organs of patients with COVID-19 is characterized by neutrophil infiltration. In addition, compared to nonsevere cases, patients with severe COVID-19 exhibit lower percentages of lymphocytes, with both groups having absolute lymphocyte counts below normal values; severe cases particularly suffer from lymphopenia.[10,11] Yang et al[12] summarized recent international and domestic literature on COVID-19 and immune cells, concluding that the pathogenesis of COVID-19 mainly involves a dynamic interaction between the virus and the host immune system, often accompanied by immune cell imbalances.

All of these findings suggest a complex relationship between immune cells and COVID-19. However, current research on the pathogenesis of COVID-19 primarily relies on the aforementioned observational studies, which have limitations in inferring causality. Therefore, further evidence is needed to validate the causal relationship between immune cells and COVID-19. In this study, we conducted Mendelian randomization (MR) analysis to explore the intricate causal associations between 731 immune cell phenotypes and five immune cells in COVID-19.

MR is a data analysis technique used to assess causal inference in epidemiological research. It uses genetic variants strongly correlated with exposure factors as instrumental variables (IVs) to evaluate causal relationships between exposure factors and outcomes.[13,14] Because IVs are genetically based, they are not influenced by confounding factors.[15] Current individual studies have revealed causal associations between immune cells and other chronic diseases.[16,17] However, the causal relationship between immune cells and COVID-19 remains unclear, warranting exploration of their correlation to provide new targets and directions for COVID-19 research.

2. METHODS

2.1. Study design

We evaluated the causal relationships between 731 immune cell phenotypes (seven groups, conventional dendritic cell (cDC) panel, regulatory cells (Treg) panel, B-cell panel, T cells, B cells and NK cells (TBNK) panel, maturation stages of T-cell panel, myeloid cell, monocyte panel) and the novel coronavirus using bidirectional two-sample MR analysis.[18] The study's workflow and experimental principles are illustrated in Fig. 1. (1) The association hypothesis: IVs are closely associated with exposure factors. (2) The independence hypothesis: IVs should not be influenced by known or unknown confounding factors. (3) The exclusion restriction hypothesis: IVs only affect outcome factors through exposure factors. The data collected for this study were sourced from the OpenGWAS public database, and before upload, the data were anonymized to ensure no personal or identifiable information was included. Because this study does not involve sensitive data or require institutional informed consent, ethical review is not applicable.

2.2. Data sources for exposure and outcomes

The data for COVID-19 were sourced from the OpenGWAS database and analyzed using two GWAS datasets: ebi-a-GCST011074 and ebi-a-GCST011081. ebi-a-GCST011074 includes genetic data from 1 348 701 individuals of European descent (n_{case} = 32 494, $n_{control}$ = 1 316 207), although ebi-a-GCST011081 includes data from 1 887 658 individuals of European descent (n_{case} = 9986, $n_{control}$ = 1 877 672). These datasets can be accessed at http://gwas.mrcieu.ac.uk. The GWAS data for 731 immune cell types were obtained from the GWAS catalog (including GCST0001391 to GCST0002121),[18] encompassing parameters related to immune cell morphology, relative cell counts, median fluorescence intensity reflecting surface antigen levels, and absolute cell counts.

2.3. Selection of single nucleotide polymorphism

On the basis of recent studies,[18,19] a significance threshold of 1×10^{-5} was set for each immune trait. Subsequently, single nucleotide polymorphisms (SNPs) in linkage disequilibrium (LD) were excluded, and the PLINK clumping algorithm (R^2 = 0.001, clumping distance = 10 000 kb) was used to cluster SNPs to ensure the independence of each SNP. For COVID-19 screening, a significance threshold of 5×10^{-6} was set to obtain a sufficient number of SNPs. In addition, an F statistic >10 was set to exclude weak IVs.

2.4. Statistical analysis

For MR analysis, we utilized the TwoSampleMR (version 0.5.6) package.[20] MR uses genetic variants as IVs to estimate the causal effects of risk factors on complex diseases (IV).[21] We used five methods: MR-Egger, weighted median, inverse variance

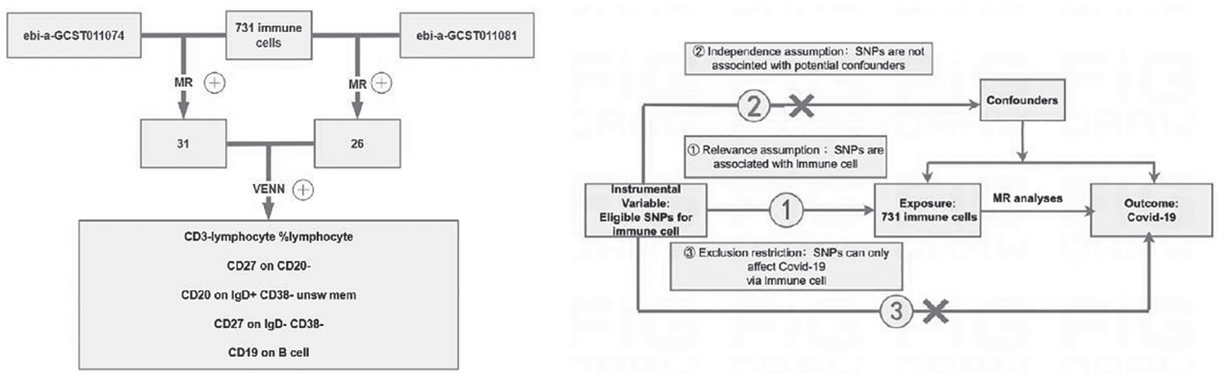

Fig. 1 Flowchart of the study process. The process and main assumptions of MR analysis are briefly introduced. MR = Mendelian randomization; SNP = single nucleotide polymorphism.

weighted (IVW), simple mode, and weighted mode with IVW as the primary analysis method.[22] MR-Egger was also used to detect pleiotropy, and a leave-one-out analysis was conducted to assess the influence of individual SNPs on the results. All analyses were conducted using R version 4.3.2 software (http://www.Rproject.org).

3. RESULTS

3.1. Screening of IVs

In this study, IVs screening was conducted on GWAS data for 731 immune cell phenotypes. All IVs had *F* statistics >10, ensuring no weak IV bias. The numbers and types of SNPs selected for all positive results are detailed in Supplementary Table 1, http://links.lww.com/JCMA/A303.

3.2. To explore the causal relationship between immune cell phenotype and COVID-19

The IVW method revealed significant evidence of causal relationships between certain immune cell phenotypes and the risk of COVID-19 (details are found in Supplementary Table 1, http://links.lww.com/JCMA/A303 and Supplementary Table 2, http://links.lww.com/JCMA/A304). We identified 57 immune cell phenotypes across both samples, showing potential pathogenic links to COVID-19 (Figs. 2 and 3). In the ebi-a-GCST011074 sample, the IVW results for immune cell phenotypes and their genetic predisposition to COVID-19 are depicted in Figure 2, indicating that 31 immune cell phenotypes are associated with genetic susceptibility to COVID-19, with 14 immune cell phenotypes positively correlated with genetic susceptibility to COVID-19 (odds ratio [OR] < 1, $p < 0.05$). In addition, the remaining 17 phenotypes are associated with a reduced incidence of COVID-19 (OR < 1, $p < 0.05$). Among these 31 statistically significant immune cell phenotypes, eight belong to the B-cell panel, seven to the TBNK panel, six to the Treg panel, four to the myeloid cell panel, three to the maturation stages of the T-cell panel, two to the monocyte panel, and one to the cDC panel (for details, see Supplementary Table 3, http://links.lww.com/JCMA/A305). In the ebi-a-GCST011081 sample, the IVW results for immune cell phenotypes and their genetic predisposition to COVID-19 are shown in Fig. 3, indicating that 26 immune cell phenotypes are associated with genetic susceptibility to COVID-19, with 16 immune cell phenotypes positively correlated with genetic susceptibility to COVID-19 (OR > 1, $p < 0.05$). In addition, the remaining 10 phenotypes are associated with a reduced incidence of COVID-19 (OR < 1, $p < 0.05$). Among these 26 statistically significant immune cell phenotypes, eight belong to the B-cell panel, eight to the TBNK panel, three to the Treg panel, three to the maturation stages of the T-cell panel, two to the myeloid cell panel, and two to the cDC panel (for details, see Supplementary Table 3, http://links.lww.com/JCMA/A305).

id.exposure	exposure	Method	nSNP	OR(95%CI)	P.value
ebi-a-GCST90001396	IgD+ CD38- AC	Inverse variance weighted	18	1.05(1.00 to 1.09)	0.043867919
ebi-a-GCST90001453	CD11c+ CD62L- monocyte %monocyte	Inverse variance weighted	18	0.96(0.92 to 1.00)	0.038900689
ebi-a-GCST90001523	CD33- HLA DR+ AC	Inverse variance weighted	28	0.98(0.96 to 1.00)	0.014932285
ebi-a-GCST90001526	CD33dim HLA DR+ CD11b+ %CD33dim HLA DR+	Inverse variance weighted	23	1.02(1.00 to 1.04)	0.035853424
ebi-a-GCST90001561	CD45RA+ CD8br %CD8br	Inverse variance weighted	21	0.98(0.96 to 1.00)	0.028650991
ebi-a-GCST90001604	T cell %lymphocyte	Inverse variance weighted	17	1.05(1.00 to 1.09)	0.037398104
ebi-a-GCST90001635	CD8dim NKT %lymphocyte	Inverse variance weighted	25	1.04(1.01 to 1.07)	0.016226424
ebi-a-GCST90001640	CD3- lymphocyte %lymphocyte	Inverse variance weighted	6	0.91(0.84 to 0.98)	0.013498390
ebi-a-GCST90001661	CD28- CD8dim %T cell	Inverse variance weighted	22	0.97(0.94 to 1.00)	0.026364907
ebi-a-GCST90001673	CD28- CD127- CD25++ CD8br %T cell	Inverse variance weighted	19	1.05(1.01 to 1.09)	0.005020745
ebi-a-GCST90001695	CD45RA- CD28- CD8br AC	Inverse variance weighted	243	1.00(1.00 to 1.00)	0.005028451
ebi-a-GCST90001699	CD45RA+ CD28- CD8br %CD8br	Inverse variance weighted	29	1.00(1.00 to 1.00)	0.039992067
ebi-a-GCST90001750	CD20 on IgD+ CD38- unsw mem	Inverse variance weighted	18	1.04(1.00 to 1.08)	0.041162889
ebi-a-GCST90001765	CD24 on IgD+ CD24+	Inverse variance weighted	24	0.98(0.96 to 1.00)	0.029472822
ebi-a-GCST90001782	CD25 on IgD+ CD38- unsw mem	Inverse variance weighted	23	0.99(0.97 to 1.00)	0.044889711
ebi-a-GCST90001795	CD25 on transitional	Inverse variance weighted	21	0.96(0.93 to 0.99)	0.023351983
ebi-a-GCST90001796	CD27 on CD20-	Inverse variance weighted	15	0.96(0.93 to 0.99)	0.011127419
ebi-a-GCST90001802	CD27 on IgD- CD38-	Inverse variance weighted	29	1.03(1.01 to 1.06)	0.018999969
ebi-a-GCST90001810	CD38 on CD3- CD19-	Inverse variance weighted	16	0.97(0.94 to 1.00)	0.027119820
ebi-a-GCST90001844	CD3 on TD CD4+	Inverse variance weighted	23	1.04(1.01 to 1.07)	0.010226088
ebi-a-GCST90001892	CD28 on CD39+ CD4+	Inverse variance weighted	19	0.98(0.96 to 1.00)	0.030464656
ebi-a-GCST90001893	CD28 on CD28+ CD45RA- CD8br	Inverse variance weighted	18	1.04(1.00 to 1.07)	0.043076614
ebi-a-GCST90001907	CCR7 on naive CD4+	Inverse variance weighted	22	1.03(1.01 to 1.05)	0.012132956
ebi-a-GCST90001912	CD45 on HLA DR+ NK	Inverse variance weighted	13	0.96(0.93 to 1.00)	0.031872943
ebi-a-GCST90001918	CD45 on HLA DR+ T cell	Inverse variance weighted	15	0.95(0.92 to 0.98)	0.000349964
ebi-a-GCST90001919	CD45 on NKT	Inverse variance weighted	21	0.97(0.95 to 1.00)	0.022409762
ebi-a-GCST90001996	CX3CR1 on CD14+ CD16+ monocyte	Inverse variance weighted	31	0.98(0.96 to 1.00)	0.022097626
ebi-a-GCST90002012	CX3CR1 on CD14- CD16+ monocyte	Inverse variance weighted	20	0.97(0.95 to 1.00)	0.033285537
ebi-a-GCST90002019	CD14 on Mo MDSC	Inverse variance weighted	23	0.98(0.96 to 1.00)	0.025037341
ebi-a-GCST90002028	CD19 on B cell	Inverse variance weighted	25	1.05(1.02 to 1.09)	0.003426207
ebi-a-GCST90002093	CD11b on CD66b++ myeloid cell	Inverse variance weighted	16	1.03(1.01 to 1.06)	0.013447199

P<0.05 was considered statistically significant

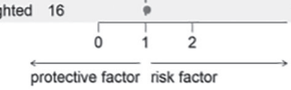

Fig. 2 Forest plot illustrating causal relationships between 731 immune cell phenotypes and the GWAS of ebi-a-GCST011074. Forest plot of MR results showing causal relationships between 731 immune cell phenotypes and ebi-a-GCST011074 GWAS. CI = confidence interval; GWAS = Genome-Wide Association Study; MR = Mendelian randomization; nSNP = number of single nucleotide polymorphism; OR = odds ratio; SNP = single nucleotide polymorphism.

id.exposure	exposure	Method	nSNP	OR(95%CI)	P.value
ebi-a-GCST90001470	CD62L- plasmacytoid DC AC	Inverse variance weighted	22	1.06(1.01 to 1.12)	0.0278798490
ebi-a-GCST90001534	CD45RA- CD4+ AC	Inverse variance weighted	12	0.92(0.86 to 0.99)	0.0292265777
ebi-a-GCST90001602	Lymphocyte %leukocyte	Inverse variance weighted	20	1.05(1.01 to 1.10)	0.0247317103
ebi-a-GCST90001605	T cell %leukocyte	Inverse variance weighted	17	1.06(1.01 to 1.11)	0.0256112944
ebi-a-GCST90001626	HLA DR+ CD4+ %lymphocyte	Inverse variance weighted	17	0.93(0.87 to 1.00)	0.0492804221
ebi-a-GCST90001639	CD3- lymphocyte AC	Inverse variance weighted	17	0.92(0.86 to 0.99)	0.0202572908
ebi-a-GCST90001640	CD3- lymphocyte %lymphocyte	Inverse variance weighted	6	0.80(0.70 to 0.91)	0.0008766991
ebi-a-GCST90001666	CD28+ CD45RA+ CD8dim AC	Inverse variance weighted	36	1.01(1.00 to 1.03)	0.0465055103
ebi-a-GCST90001734	CD19 on IgD- CD38br	Inverse variance weighted	15	1.09(1.02 to 1.15)	0.0068307514
ebi-a-GCST90001737	CD19 on naive-mature B cell	Inverse variance weighted	25	0.95(0.91 to 1.00)	0.0292903412
ebi-a-GCST90001750	CD20 on IgD+ CD38- unsw mem	Inverse variance weighted	18	1.09(1.03 to 1.15)	0.0039915915
ebi-a-GCST90001793	CD25 on sw mem	Inverse variance weighted	19	1.06(1.00 to 1.12)	0.0476864292
ebi-a-GCST90001796	CD27 on CD20-	Inverse variance weighted	15	0.94(0.89 to 1.00)	0.0330281116
ebi-a-GCST90001802	CD27 on IgD- CD38-	Inverse variance weighted	27	1.05(1.00 to 1.10)	0.0343256340
ebi-a-GCST90001804	CD27 on IgD- CD38dim	Inverse variance weighted	28	1.05(1.00 to 1.09)	0.0295017450
ebi-a-GCST90001819	CD38 on transitional	Inverse variance weighted	22	0.95(0.90 to 1.00)	0.0431736618
ebi-a-GCST90001832	CD62L on CD62L+ plasmacytoid DC	Inverse variance weighted	14	1.05(1.00 to 1.10)	0.0426589436
ebi-a-GCST90001840	CD3 on TD CD8br	Inverse variance weighted	23	1.07(1.01 to 1.13)	0.0230669254
ebi-a-GCST90001847	CD3 on HLA DR+ T cell	Inverse variance weighted	25	1.04(1.00 to 1.07)	0.0488513046
ebi-a-GCST90001950	CD33 on Gr MDSC	Inverse variance weighted	17	1.04(1.01 to 1.08)	0.0175541705
ebi-a-GCST90001952	CD33 on Mo MDSC	Inverse variance weighted	18	1.03(1.00 to 1.06)	0.0347160076
ebi-a-GCST90002028	CD19 on B cell	Inverse variance weighted	25	1.07(1.01 to 1.13)	0.0239879848
ebi-a-GCST90002034	CD39 on monocyte	Inverse variance weighted	20	1.03(1.00 to 1.05)	0.0368225932
ebi-a-GCST90002056	CD8 on EM CD8br	Inverse variance weighted	22	0.93(0.87 to 0.99)	0.0208604477
ebi-a-GCST90002068	CD4 on secreting Treg	Inverse variance weighted	24	0.95(0.91 to 1.00)	0.0356209242
ebi-a-GCST90002076	SSC-A on NK	Inverse variance weighted	19	0.92(0.86 to 0.99)	0.0210165538

P<0.05 was considered statistically significant

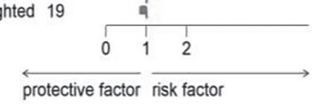

Fig. 3 Forest plot illustrating causal relationships between 731 immune cell phenotypes and the GWAS of ebi-a-GCST011081. Forest plot of MR results showing causal relationships between 731 immune cell phenotypes and ebi-a-GCST011081 GWAS. CI = confidence interval; GWAS = Genome-Wide Association Study; MR = Mendelian randomization; nSNP = number of single nucleotide polymorphism; OR = odds ratio; SNP = single nucleotide polymorphism.

3.3. Phenotypic differential analysis of immune cells

We conducted a differential analysis of immune cell phenotypes associated with COVID-19 in the two samples, and the intersecting results are shown in Fig. 4. The Venn diagram indicates that there are five immune cell phenotypes common to both datasets. The MR results for these five immune cell phenotypes associated with COVID-19 are depicted in Fig.5: CD3– lymphocyte %lymphocyte (OR = 0.80, 95% confidence interval [CI] = 0.70-0.91, p = 0.0008) and CD27 on CD20– (OR = 0.94, 95% CI = 0.89-1.00, p = 0.033) have been confirmed in both samples to be protective against the occurrence of COVID-19. Conversely, CD20 on IgD+ CD38– unsw mem (OR = 1.09, 95% CI = 1.03-1.15, p = 0.004), CD27 on IgD– CD38– (OR = 1.05, 95% CI = 1.00-1.10, p = 0.034), and CD19 on B cell (OR = 1.07, 95% CI = 1.01-1.13, p = 0.02) are causally associated with an increased risk of COVID-19. Among these five immune cell phenotypes, three of them are from the B-cell panel (CD20 on IgD+ CD38– unsw mem, CD27 on CD20–, CD27 on IgD– CD38–), and the other two are from the TBNK panel (CD3– lymphocyte %lymphocyte, CD19 on B cell).

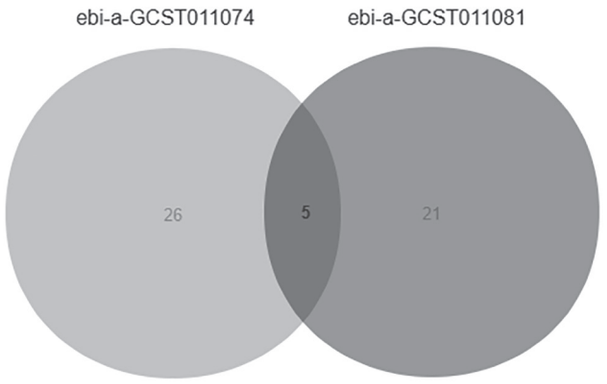

Fig. 4 Venn diagram of two significant MR results. A Venn diagram was generated to show the intersection of results from MR analysis using two different GWAS datasets (ebi-a-GCST011074 and ebi-a-GCST011081). GWAS = Genome-Wide Association Study; MR = Mendelian randomization.

3.4. Sensitivity analysis

We conducted sensitivity analyses on the MR of 731 immune cell phenotypes associated with COVID-19, assessing their stability through scatter plots and leave-one-out analyses. Furthermore, we displayed the sensitivity analyses of the final five immune cell traits in Fig. 6 and Supplementary Fig. 1, http://links.lww.com/JCMA/A310, confirming the robustness of the MR analysis results. According to the MR-Egger regression intercept method, there is no evidence suggesting horizontal pleiotropy for these five immune cell traits, indicating a causal relationship with COVID-19 (Supplementary Table 4, http://links.lww.com/

id.exposure	exposure	id.outcome	nSNP	OR(95%CI)	P.value
ebi-a-GCST90001640	CD3- lymphocyte %lymphocyte	ebi-a-GCST011081	6	0.80(0.70 to 0.91)	0.0008766991
		ebi-a-GCST011074	6	0.91(0.84 to 0.98)	0.0134983900
ebi-a-GCST90001750	CD20 on IgD+ CD38- unsw mem	ebi-a-GCST011081	18	1.09(1.03 to 1.15)	0.0039915915
		ebi-a-GCST011074	18	1.04(1.00 to 1.08)	0.0411628888
ebi-a-GCST90001796	CD27 on CD20-	ebi-a-GCST011081	15	0.94(0.89 to 1.00)	0.0330281116
		ebi-a-GCST011074	15	0.96(0.93 to 0.99)	0.0111274192
ebi-a-GCST90001802	CD27 on IgD- CD38-	ebi-a-GCST011081	27	1.05(1.00 to 1.10)	0.0343256340
		ebi-a-GCST011074	29	1.03(1.01 to 1.06)	0.0189999692
ebi-a-GCST90002028	CD19 on B cell	ebi-a-GCST011081	25	1.07(1.01 to 1.13)	0.0239879848
		ebi-a-GCST011074	25	1.05(1.02 to 1.09)	0.0034262070

P<0.05 was considered statistically significant

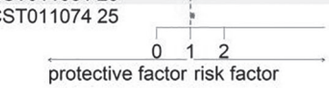

Fig. 5 MR results for five different immune cell phenotypes (CD3– lymphocyte %lymphocyte, CD20 on IgD+ CD38– unsw mem, CD27 on CD20–, CD27 on IgD– CD38–,CD19 on B cell). CI = confidence interval; MR = Mendelian randomization; nSNP = number of single nucleotide polymorphism; OR = odds ratio; SNP = single nucleotide polymorphism.

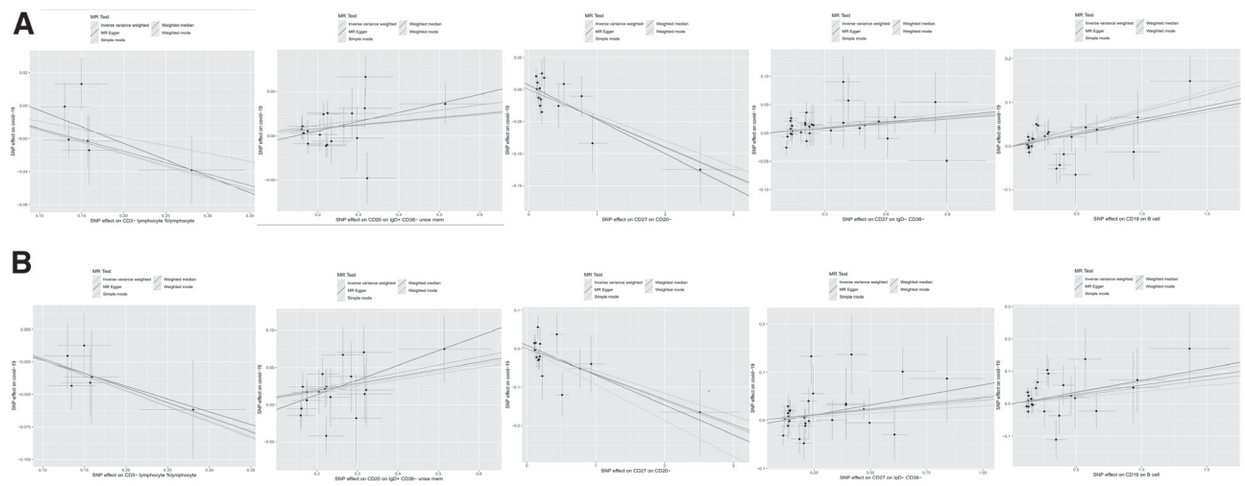

Fig. 6 Scatter plot analyses of genetic correlations in five immune cell phenotypes using MR methods. We conducted scatter plot analyses using five MR methods to assess the genetic correlations of five immune cell phenotypes. Specifically, (A) and (B) represent the scatter plot analyses for the outcomes ebi-a-GCST011074 and ebi-a-GCST011081. MR = Mendelian randomization; SNP = single nucleotide polymorphism.

JCMA/A306). In addition, we observed that the removal of any single IV for these five immune cell analyses did not significantly impact the results. Furthermore, MR-PRESSO tests showed no outliers in the results.

4. DISCUSSION

The pathogenesis of COVID-19 is complex, involving interactions between the virus and various molecules and cells. Current research primarily focuses on clinical studies for therapeutic purposes, with limited studies on etiology. To date, the causal relationship between immune cells and COVID-19 remains uncertain. This study utilized large-scale statistical data from GWAS on circulating immune cell phenotypes and COVID-19 to explore the causal relationships between 731 immune cell types (including cDC panel, Treg panel, B-cell panel, TBNK panel, maturation stages of T-cell panel and myeloid cell, monocyte panel) and COVID-19. We identified a total of 52 immune cell phenotypes that are causally associated with COVID-19. Interestingly, the differential analysis revealed that five immune cell phenotypes show significant causal relationships with COVID-19 risk in two independent datasets. Among these, three phenotypes are positively correlated with COVID-19 risk, whereas two are negatively correlated.

Our study found that the expression of CD3– lymphocyte %lymphocyte is associated with a reduced risk of developing COVID-19, potentially offering insights for future treatments of COVID-19. The term "CD3– lymphocyte %lymphocyte" describes the percentage of lymphocytes that do not express CD3 among lymphocytes. According to the classification of immune cell surface markers, CD3 is identified as a surface marker for B lymphocytes and NK cells.[23] B lymphocytes play a crucial role in defending against human viral infections by producing specific antibodies that trigger virus-specific immune responses. These B cells transform into memory B cells after disease resolution, thereby providing long-term immunity and playing a critical role in host protection.[24] Ellsworth et al[25] explored through animal experiments that upregulating the expression of B cells effectively alleviates symptoms induced by SARS-CoV-2 in mice, consistent with our findings. Therefore, certain subgroups of B cells may play a protective rather than a promoting role in COVID-19. On the other hand, NK cells, as one of the main players in adaptive immunity, also play a crucial role in controlling viral infections.[26] Studies have observed significant impairments in NK cell counts and cytolytic activity in severe COVID-19 patients. Animal experiments have shown that cells treated with NK-derived extracellular vesicles (NK-EV) exhibit upregulation of antiviral microRNA (miRNA) cargoes (miR-27a, miR-27b, miR-369-3p, and miR-491-5p) effectively reducing the expression of proinflammatory cytokines (tumor necrosis factor alpha [TNF-α] and interleukin 8 [IL-8]) in SARS-CoV-2–infected mice. These findings are consistent with our study.[27] However, there

is currently no reported research on the association of CD27 on CD20– with patients with COVID-19. Our study is the first to discover a negative causal relationship between CD27 on CD20– and COVID-19, providing new avenues for future research.

Our study also revealed a positive correlation between CD19 on B cells and the progression of COVID-19. The CD19 antigen is a 95 kDa glycosylated type I transmembrane protein on the surface of B lymphocytes, belonging to the immunoglobulin superfamily. It is expressed from early B-cell progenitors in the bone marrow throughout B-cell maturation, persisting until differentiation into plasma cells, and serves as a surface marker for the B-cell lineage.[28,29]

A study found that, on average, about 40% of B cells (CD19+) in the peripheral blood of patients infected with SARS-CoV-2 were infected by the virus, whereas <5% of T cells (CD4+ and CD8+) and approximately 15% of monocytes (CD14+) were infected. Therefore, B cells (CD19+) show a stronger correlation with the progression of COVID-19.[30] In addition, Sosa-Hernández et al[31] found that expression of CD19+ B cells increases in severe cases of COVID-19 compared to mild cases, which is consistent with our study. However, there is currently limited research in this area, and future studies should use more refined methods to explore the diversity of B cells and their role in COVID-19.

Previous studies have found that CD38 is predominantly present in B lymphocytes. During the development of B cells, based on the expression of IgD and CD38, most mature B cells can be classified into five types: "naïve" immature cells (IgD+ CD38–), activated immature cells (IgD + CD38 lower), pregerminal center cells (IgD + CD38++), germinal center cells (centroblasts or centrocytes: IgD– CD38++), and memory cells (IgD– CD38+/–).[32] Therefore, "CD20 on IgD+ CD38– unsw mem" refers to CD20+ unswitched memory naive B cells, where CD20 is typically highly expressed in all B-lineage cells except for pro B cells and plasma cells.[33]

Jing et al,[34] through characterization of B cell immune phenotypes in recovered patients with COVID-19, found a decrease in the percentage of unswitched memory B cells upon B-cell receptor (BCR) stimulation compared to healthy controls. Castleman et al[35] also observed that the frequency of unswitched memory B cells decreases with severe SARS-CoV-2 infection. In contrast, our study found a positive correlation between "CD20 on IgD+ CD38– unsw mem" and the risk of COVID-19, which contradicts the aforementioned observational studies. This discrepancy may be attributed to the phenotype of B cells ("naïve" immature cells, IgD+ CD38–), although currently there are no studies directly addressing this relationship. Therefore, extensive research is needed to fully confirm the association between "naïve" immature B cells and COVID-19. The term "CD27 on IgD– CD38–" denotes switched memory B cells, where based on CD27 and IgD expression, memory cells are further classified into unswitched memory B cells (IgD+ CD27+) and switched memory B cells (IgD– CD27+).[36] Çölkesen et al,[37] in a single-center retrospective study, found a significant decrease in switched memory B-cell levels in deceased patients compared to recovered patients (both $p < 0.05$), with a significant negative correlation observed with the severity of COVID-19. On the other hand, Bean et al[38] discovered an increase in activated switched memory B-cell expression in moderate patients compared to severe cases and healthy controls. Our study found a positive correlation between "CD27 on IgD– CD38–" and the risk of COVID-19, consistent with Bean et al's[38] findings but differing from Çölkesen et al's[37] results. These conflicting conclusions may be attributed to varying disease severity among patients, indicating the need for more detailed research to determine the relationship between "CD27 on IgD– CD38–" and COVID-19.

In this study, we conducted MR analysis based on a previously published set of large-scale GWAS cohorts. Our conclusions rely on genetic IVs and causal inference using MR analysis, demonstrating robustness against horizontal pleiotropy and other factors. To mitigate the inherent stochasticity of single GWAS studies, we analyzed two GWAS datasets of the same phenotype and intersected the results to enhance the persuasiveness of the final phenotype outcomes. Nonetheless, our study has several limitations. First, the reliance on a European database may constrain the generalizability of our findings to other ethnic groups. Second, the lack of access to individual-level data due to the absence of public databases impedes stratified analyses. Third, future MR studies should incorporate data from diverse populations to validate causal relationships.

In conclusion, in this research, we used systematic MR analysis to establish causal links between specific immune phenotypes and COVID-19, shedding new light on the intricate connections between various immune cell types and COVID-19. This offers a fresh theoretical foundation for investigating early interventions and therapies. Nevertheless, additional experimental and clinical studies are necessary to validate and extend our discoveries.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data related to this article can be found at http://links.lww.com/JCMA/A303.

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