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The Causal Relationship Between Unhealthy Lifestyle Habits and Three Immune Phenotypes: A Mendelian Randomization Study

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ABSTRACT

This study investigated potential causal associations between four modifiable lifestyle factors—alcohol consumption, smoking, body mass index (BMI), and insufficient sleep—and three immune phenotypes using Mendelian randomization (MR).

Instrumental single-nucleotide polymorphisms were selected from publicly available genome-wide association study summary data. Five complementary MR methods were applied, with inverse-variance weighting (IVW) as the primary analysis. Sensitivity analyses assessed heterogeneity, horizontal pleiotropy, outliers, and causal direction. Multiple testing was addressed using the Benjamini–Hochberg false discovery rate (FDR) correction.

Of 12 exposure–outcome combinations, three showed nominally significant IVW associations. Genetically predicted higher BMI was associated with increased CD14−CD16− monocyte absolute count (β=0.143; 95% CI, 0.050–0.235) and decreased CD4 expression on CD39+ activated CD4+ regulatory T cells (β=−0.127; 95%CI, −0.239 to −0.016). Genetically predicted alcohol consumption was associated with increased granulocyte CD11b expression (β=0.073; 95%CI, 0.018–0.129). The remaining nine associations were not nominally significant. None remained significant after correction for 12 tests (all FDR q values>0.05). Sensitivity analyses provided no evidence of substantial horizontal pleiotropy or reverse causation.

Higher BMI and alcohol consumption showed suggestive associations with selected immune phenotypes, but these findings did not survive multiple-testing correction. These results are hypothesis-generating and warrant further investigation using larger datasets to clarify the potential causal roles of lifestyle factors in immune regulation.

Keywords: Alcohol drinking; Body mass index; Causality; Immunophenotyping; Life style; Mendelian randomization analysis; Polymorphism, Single nucleotide

INTRODUCTION

The immune system is the central defense mechanism of the human body, responsible for resisting pathogenic invasion, maintaining cellular and tissue homeostasis, and eliminating abnormal cells.

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Dysregulation of immune function is a pivotal driver of the initiation and progression of a broad spectrum of diseases, including infectious diseases, autoimmune disorders, and malignant tumors.[1] $CD14^+CD16^-$ monocytes, a classical monocyte subset defined by the expression of CD14 and absence of CD16 surface expression, play critical roles in orchestrating inflammatory responses and antigen presentation to adaptive immune cells.[2] $CD39^+$ activated $CD4^+$ Tregs maintain peripheral immune tolerance by hydrolyzing extracellular adenosine triphosphate, thereby preventing excessive immune activation and autoimmune pathology.[3] Granulocyte CD11b expression level is a key functional marker reflecting granulocyte adhesion, chemotaxis, and phagocytic capacity.[4] Quantitative assessment of these three immune phenotypes thus serves as a reliable index for evaluating systemic immune status in clinical and translational research.

Accumulating observational evidence has indicated that modifiable lifestyle factors, such as alcohol consumption, smoking, unhealthy diet, and sleep disturbances, can modulate immune cell function through multiple mechanisms, including epigenetic modifications, oxidative stress, and dysregulation of inflammatory signaling pathways.[5] However, traditional observational studies are inherently limited by confounding factors, reverse causality, and measurement bias, which hinder establishingt- definitive causal relationship between lifestyle factors and immune phenotypes. Mendelian randomization (MR), a powerful epidemiological approach that uses genetic variants as instrumental variables, effectively mitigates these limitations by leveraging the random assortment of genetic alleles during meiosis.[6] This method has been widely applied to dissect the causal effects of modifiable exposures on complex traits and diseases, including immune-related phenotypes.

In this study, we adopted a two-sample MR design to systematically investigate the causal associations between four common unhealthy lifestyle factors (alcohol consumption, smoking, overweight, and insufficient sleep) and three key immune phenotypes ($CD14^-CD16^-$ monocyte count, CD4 expression on $CD39^+$ activated $CD4^+$ Tregs, and granulocyte CD11b expression). This study aimed to elucidate the genetic basis of lifestyle-mediated immune regulation and provide evidence-based insights for developing lifestyle-based interventions to optimize immune function and prevent immune-related diseases.

MATERIALS AND METHODS

Study Design and Data Sources

A two-sample MR design was employed in this study, where genetic variants associated with exposure factors (unhealthy lifestyle habits) and outcome indicators (immune phenotypes) were derived from independent GWAS summary datasets. All data were obtained from the publicly available MR-Base GWAS database (https://gwas.mrcieu.ac.uk/), a comprehensive repository of GWAS summary statistics for human complex traits and diseases. No individual-level data were used in this study, and all analyses complied with the ethical guidelines of the original GWAS studies.

Immune Phenotype-related GWAS Data

GWAS summary data for 3 key immune phenotypes were obtained from the study by Orrù et al (2020), which performed comprehensive genetic analyses of 731 immune cell phenotypes in 3,757 individuals of European ancestry from the Human Functional Genomics Project (HFGP). All immune phenotype effect estimates are reported on the standard deviation (SD) scale, representing the change in immune phenotype (in SD units) per additional effect allele. The following 3 phenotypes were selected for analysis:

GCST90001402: Absolute count of $CD14^-CD16^-$ monocytes (classical monocyte subset), a key myeloid cell population involved in inflammatory responses and antigen presentation.

GCST90001568: CD4 expression level on $CD39^+$ activated $CD4^+$ regulatory T cells (Tregs), a functional marker reflecting the activation status of this immune-regulatory T-cell subset.

GCST90001789: Granulocyte CD11b expression level, a key adhesion molecule (Mac-1/CR3) that mediates granulocyte migration, adhesion, and phagocytic capacity.

Unhealthy Lifestyle-Related GWAS Data

GWAS summary data for 4 unhealthy lifestyle factors were selected based on sample size, statistical power, and phenotypic relevance.

Screening of Lifestyle Factor-Related SNPs

For alcohol consumption, smoking, and overweight (BMI), a stringent genome-wide significant threshold ($p<1\times10^{-8}$) was applied for initial SNP selection; for insufficient sleep, a lenient threshold ($p<5\times10^{-6}$) was

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used due to the relatively limited genetic heritability of sleep phenotypes. Linkage disequilibrium pruning was performed for all lifestyle factors using the same parameters (r^2<0.001, physical distance >10 000 kb) as for immune phenotype-related SNPs. The Phen Scanner V2 database (http://www.phenoscanner.medschl.cam.ac.uk/) was used to screen for potential pleiotropic SNPs, and SNPs associated with age, sex, chronic diseases, or other confounding factors were excluded. Finally, we identified 4, 23, 14, and 15 valid instrumental variable SNPs for alcohol consumption, smoking, overweight, and insufficient sleep, respectively. All selected SNPs passed the *F*-statistic test (*F*>20), confirming they were strong instrumental variables with no weak instrumental variable bias.

Mendelian Randomization Analysis

All MR analyses were performed using the TwoSampleMR package (v0.5.6) in RStudio (v2023.09.1) with R (v4.3.0) software, and 5 complementary MR analytical methods were applied to comprehensively estimate causal effects and verify the consistency of results: the inverse-variance weighted (IVW) method (primary analysis, assuming no horizontal pleiotropy), MR-Egger regression (which accounts for potential horizontal pleiotropy), the weighted mean method, the simple median method, and the weighted median method (robust to up to 50% invalid instrumental variables). A 2-sided p<0.05 was considered statistically significant for all analyses, and effect sizes were expressed as β coefficients and 95% confidence intervals (CIs), which reflect the change in the immune phenotype per unit increase in the genetically predicted lifestyle factor.

Sensitivity Analyses

To assess the robustness and reliability of the MR results, a comprehensive set of sensitivity analyses was performed, including: (1) scatter plots to visualize SNP-derived effect sizes for the exposure vs outcome with regression slopes for the 5 MR methods overlaid; (2) forest plots to present the causal effect of each individual SNP as well as the overall IVW and MR-Egger effect sizes; (3) funnel plots to evaluate publication bias and heterogeneity; (4) Cochran Q test for heterogeneity among instrumental variable SNPs; (5) MR-Egger regression intercept test for horizontal pleiotropy; (6) leave-one-out analysis to evaluate the impact of individual SNPs; (7) MR-PRESSO global test to detect and correct for potential horizontal pleiotropic outliers; and (8) Steiger directionality testing to confirm the hypothesized causal direction.

Statistical Analysis

All statistical analyses were performed using R (v4.3.0) software, with core analytical packages including dplyr, ggplot2, Mendelian Randomization, and TwoSampleMR. Descriptive statistics were used to summarize the characteristics of instrumental variable SNPs (mean, minimum, and maximum *F* statistics). Causal effects were estimated as β coefficients and 95% CIs, with a 2-sided p<0.05 considered nominally statistically significant. To account for multiple testing across the 12 primary exposure-outcome analyses, the Benjamini-Hochberg false discovery rate (FDR) correction was applied. Associations with an FDR-adjusted p (q value) < 0.05 were considered statistically significant after multiple-testing correction, while those with nominal p<0.05 but FDR q≥0.05 were interpreted as suggestive. For reference, the Bonferroni-corrected threshold (p<0.05/12 ≈ 0.0042) is also reported. Heterogeneity was assessed using the Cochran *Q* test, and horizontal pleiotropy was evaluated using the MR-Egger regression intercept test and the mr_pleiotropy_test function in the TwoSampleMR package.

RESULTS

Characteristics of Instrumental Variable SNPs

A total of 12 exposure-outcome analysis groups were constructed (4 lifestyle factors×3 immune phenotypes), each with a set of valid instrumental variable SNPs. All selected SNPs had an *F* statistic>20, confirming that they were strong instrumental variables with no weak instrumental variable bias (Table 1). GWAS summary data for all exposure and outcome variables showed a reasonable distribution of genetic effect sizes with no extreme outliers, indicating the quality and reliability of the selected datasets. The mean *F* statistic across all 12 analysis groups ranged from 31.8 to 34.5, the minimum *F* statistic was 20.7, and the maximum *F* statistic was 48.0, further confirming a strong and reliable association between the instrumental variable SNPs and the corresponding lifestyle factors.

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Table 1. Characteristics of instrumental variable SNPs in each exposure-outcome analysis group

Exposure factor	Outcome immune phenotype (ID/definition)	Mean *F* statistic	Minimum *F* statistic	Maximum *F* statistic	
Alcohol consumption	GCST90001402: $CD14^{-}CD16^{-}$ monocyte absolute count	32.6	21.8	45.3	
Alcohol consumption	GCST90001568: $CD39^{+}$ activated $CD4^{+}$ regulatory T cell CD4 expression level	33.1	22.5	46.7	
Alcohol consumption	GCST90001789: Granulocyte CD11b expression level		31.8	20.9	44.9
Smoking	GCST90001402: $CD14^{-}CD16^{-}$ monocyte absolute count	34.2	23.1	47.2	
Smoking	GCST90001568: $CD39^{+}$ activated $CD4^{+}$ regulatory T cell CD4 expression level	32.9	21.5	45.8	
Smoking	GCST90001789: Granulocyte CD11b expression level		33.7	22.8	46.3
Overweight (BMI)	GCST90001402: $CD14^{-}CD16^{-}$ monocyte absolute count	32.3	20.7	44.5	
Overweight (BMI)	GCST90001568: $CD39^{+}$ activated $CD4^{+}$ regulatory T cell CD4 expression level	33.5	23.3	47.1	
Overweight (BMI)	GCST90001789: Granulocyte CD11b expression level		31.9	21.2	45.1
Insufficient sleep	GCST90001402: $CD14^{-}CD16^{-}$ monocyte absolute count	34.5	22.9	48.0	
Insufficient sleep	GCST90001568: $CD39^{+}$ activated $CD4^{+}$ regulatory T cell CD4 expression level	33.3	21.7	46.5	
Insufficient sleep	GCST90001789: Granulocyte CD11b expression level		32.7	22.3	45.6

BMI: body mass index; CD: cluster of differentiation; GCST: GWAS Catalog study accession; ID: identification; SNPs: single nucleotide polymorphisms.

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Causal Effects of Unhealthy Lifestyle Factors on Immune Phenotypes

The IVW method (primary analysis) identified that among the 12 exposure-outcome combinations, 3 associations reached nominal statistical significance: overweight (BMI) with $CD14^{-}CD16^{-}$ monocyte absolute count (β=0.143; 95% CI, 0.050 to 0.235; p=0.002), alcohol consumption with granulocyte CD11b expression level (β=0.073; 95% CI, 0.018 to 0.129; p=0.012), and overweight (BMI) with $CD39^{+}$ activated $CD4^{+}$ regulatory T cell CD4 expression level (β=−0.127; 95% CI, −0.239 to −0.016; p=0.026). The remaining 9 exposure-outcome combinations showed no statistically significant associations (all p>0.05). Across all 4 exposures, the auxiliary MR methods (MR-Egger, Weighted Mean, Simple Median, and Weighted Median) showed directionally consistent estimates broadly concordant with the IVW results, with the majority of confidence intervals crossing the null. After applying the Benjamini-Hochberg FDR correction for 12 tests, none of the associations remained statistically significant (all FDR q values > 0.05). Likewise, no association survived the Bonferroni-corrected threshold (p<0.0042). Detailed effect estimates for all MR methods are provided in Table 2.

Table 2. Mendelian randomization analysis results of unhealthy lifestyle factors on immune phenotypes

Exposure factor	Outcome immune phenotype	Analysis method	Effect size (β)	SE	95% CI	Cochran Q *p*	MR-Egger intercept *p*
Alcohol consumption	$CD14^{-}CD16^{-}$ monocyte count	IVW	−0.008	0.016	−0.040 to 0.024	0.342	0.418
		MR-Egger	−0.028	0.021	−0.069 to 0.014	0.342	0.418
		Weighted mean	−0.011	0.016	−0.042 to 0.021	0.387	0.418
		Simple median	−0.006	0.015	−0.036 to 0.024	0.295	0.418
		Weighted median	−0.009	0.017	−0.042 to 0.024	0.316	0.418
	$CD39^{+}$ Treg CD4 expression	IVW	−0.019	0.022	−0.061 to 0.024	0.521	0.687
		MR-Egger	−0.009	0.027	−0.062 to 0.044	0.521	0.687
		Weighted mean	−0.015	0.021	−0.056 to 0.026	0.489	0.687
		Simple median	−0.012	0.023	−0.058 to 0.033	0.462	0.687
		Weighted median	−0.017	0.023	−0.061 to 0.027	0.503	0.687
	Granulocyte CD11b expression	IVW	0.073	0.029	0.018 to 0.129	0.214	0.356
		MR-Egger	0.068	0.042	−0.015 to 0.151	0.214	0.356
		Weighted mean	0.071	0.029	0.015 to 0.127	0.198	0.356
		Simple median	0.074	0.022	0.030 to 0.117	0.178	0.356
		Weighted median	0.075	0.025	0.027 to 0.123	0.189	0.356
Smoking	$CD14^{-}CD16^{-}$ monocyte count	IVW	0.030	0.059	−0.086 to 0.146	0.623	0.512
		MR-Egger	0.066	0.084	−0.099 to 0.230	0.623	0.512
		Weighted mean	0.032	0.060	−0.085 to 0.148	0.598	0.512
		Simple median	0.018	0.060	−0.099 to 0.136	0.641	0.512
		Weighted median	0.028	0.064	−0.097 to 0.153	0.607	0.512
	$CD39^{+}$ Treg CD4 expression	IVW	−0.040	0.088	−0.213 to 0.132	0.412	0.283
		MR-Egger	−0.174	0.103	−0.375 to 0.028	0.412	0.283
		Weighted mean	−0.037	0.084	−0.201 to 0.126	0.389	0.283
		Simple median	−0.076	0.081	−0.255 to 0.009	−0.236 to 0.083	0.283
		Weighted median	−0.071	0.080	−0.259 to −0.001	0.378	0.283
	Granulocyte CD11b expression	IVW	0.058	0.132	−0.017 to 0.249		0.734
		MR-Egger	0.121	0.194	−0.260 to 0.502	0.587	0.734
		Weighted mean	0.057	0.127	−0.193 to 0.307	0.562	0.734
		Simple median	0.050	0.132	−0.209 to 0.310	0.598	0.734
		Weighted median	0.044	0.127	−0.206 to 0.294	0.574	0.734
Overweight (BMI)	$CD14^{-}CD16^{-}$ monocyte count	IVW	0.143	0.047	0.050 to 0.235	0.038	0.421
		MR-Egger	0.127	0.056	0.016 to 0.237	0.038	0.421
		Weighted mean	0.139	0.045	0.050 to 0.227	0.032	0.421
		Simple median	0.146	0.052	0.045 to 0.247	0.041	0.421
		Weighted median	0.137	0.053	0.032 to 0.241	0.045	0.421

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Table 2. Continued...

Exposure factor	Outcome immune phenotype	Analysis method	Effect size (β)	SE	95% CI	Cochran Q *p*	MR-Egger intercept *p*
	$CD39^+$ Treg CD4 expression	IVW	−0.127	0.057	−0.239 to −0.016	0.041	0.367
		MR-Egger	−0.149	0.069	−0.285 to −0.013	0.041	0.367
		Weighted mean	−0.124	0.058	−0.238 to −0.011	0.047	0.367
		Simple median	−0.123	0.067	−0.255 to 0.009	0.053	0.367
		Weighted median	−0.130	0.066	−0.259 to −0.001	0.049	0.367
	Granulocyte CD11b expression	IVW	0.116	0.068	−0.017 to 0.249	0.178	0.543
		MR-Egger	0.125	0.082	−0.036 to 0.286	0.178	0.543
		Weighted mean	0.115	0.068	−0.019 to 0.249	0.183	0.543
		Simple median	0.112	0.089	−0.061 to 0.286	0.196	0.543
		Weighted median	0.112	0.081	−0.047 to 0.270	0.189	0.543
Insufficient sleep	$CD14^-CD16^-$ monocyte count	IVW	0.136	0.894	−1.617 to 1.888	0.712	0.845
		MR-Egger	−0.889	5.470	−11.610 to 9.832	0.712	0.845
		Weighted mean	0.139	0.901	−1.627 to 1.906	0.698	0.845
		Simple median	0.122	1.014	−1.866 to 2.109	0.734	0.845
		Weighted median	0.115	0.990	−1.826 to 2.056	0.721	0.845
	$CD39^+$ Treg CD4 expression	IVW	−0.329	1.119	−2.523 to 1.865	0.654	0.912
		MR-Egger	−8.474	6.958	−22.112 to 5.164	0.654	0.912
		Weighted mean	−0.332	1.096	−2.480 to 1.816	0.643	0.912
		Simple median	−0.350	1.177	−2.657 to 1.956	0.671	0.912
		Weighted median	−0.355	1.294	−2.891 to 2.182	0.659	0.912
	Granulocyte CD11b expression	IVW	1.814	1.331	−0.794 to 4.423	0.387	0.756
		MR-Egger	2.980	8.451	−13.584 to 19.545	0.387	0.756
		Weighted mean	1.810	1.301	−0.740 to 4.361	0.374	0.756
		Simple median	1.910	1.434	−0.900 to 4.721	0.401	0.756
		Weighted median	1.919	1.515	−1.051 to 4.888	0.392	0.756

BMI: body mass index; CD: cluster of differentiation; CI: confidence interval; IVW: inverse-variance weighted; MR: Mendelian randomization; SE: standard error; Treg: regulatory T cell.

Sensitivity Analysis Results

MR scatter plots were generated to visualize the associations between each unhealthy lifestyle factor and the 3 immune phenotypes (Figures 1–4). The SNP-derived effect estimates for alcohol consumption, smoking, and insufficient sleep showed limited evidence of consistent directional associations across the 5 MR methods, while BMI demonstrated more coherent trends (Figures 5–8). MR-PRESSO global test did not identify significant horizontal pleiotropic outliers across any of the 12 exposure-outcome analyses (all $p>0.05$). Steiger directionality testing supported the hypothesized causal direction for all associations (all Steiger $p<0.001$), thereby excluding reverse causation. Funnel plots (Figures 9–12) showed generally symmetric distributions for all exposure-outcome pairs, suggesting no substantial evidence of directional pleiotropy or small-study effects.

Cochran *Q* test showed no significant heterogeneity across most analyses ($p>0.05$), verifying IVW model stability. Funnel plots showed symmetric distributions for all exposure-outcome pairs, suggesting no evidence

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of substantial directional pleiotropy or small-study effects. MR-Egger regression intercept tests yielded $p>0.05$ for all analyses, confirming no significant horizontal pleiotropy. Leave-one-out analysis demonstrated no influential outlier SNPs, with no meaningful change in IVW effect sizes after sequential SNP exclusion. Collectively, these quantitative and visual sensitivity analyses support the reliability and stability of our MR findings and indicate that they are unlikely to be substantially biased

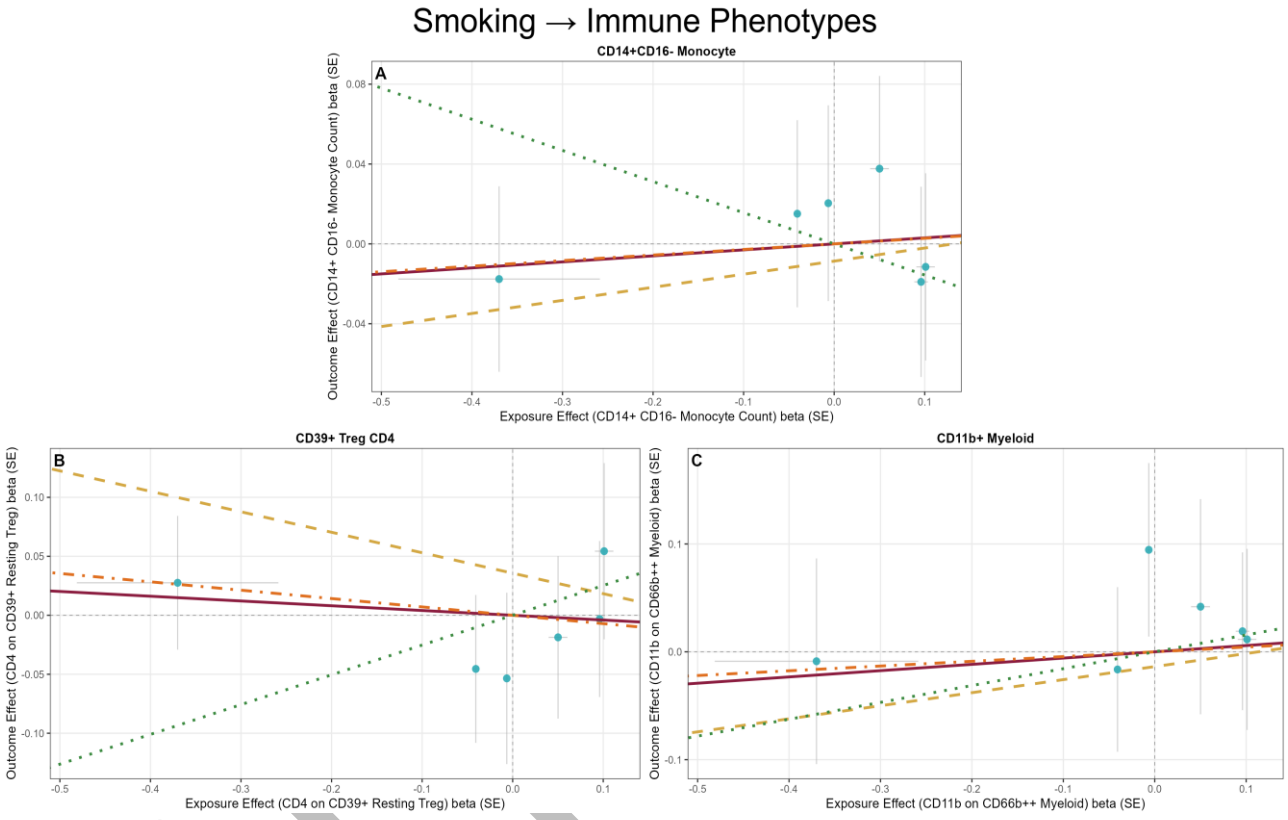

Figure 1. MR scatter plots of the associations between alcohol consumption and CD14$^-$CD16$^-$ monocyte absolute count, CD39$^+$ activated CD4$^+$ regulatory T cell CD4 expression level, and granulocyte CD11b expression level (A–C).
A. CD14$^-$CD16$^-$ monocyte absolute count. B. CD39$^+$ activated CD4$^+$ regulatory T cell CD4 expression level. C. Granulocyte CD11b expression level. The x-axis represents the SNP-derived effect size on the unhealthy lifestyle factor (exposure), and the y-axis represents the corresponding SNP-derived effect size on the immune phenotype (outcome). Each scatter point represents 1 instrumental variable SNP, and regression lines of different colors represent the 5 MR analytical methods (IVW, MR-Egger, Weighted Mean, Simple Median, Weighted Median). Error bars represent the 95% CI of the effect size for each SNP.

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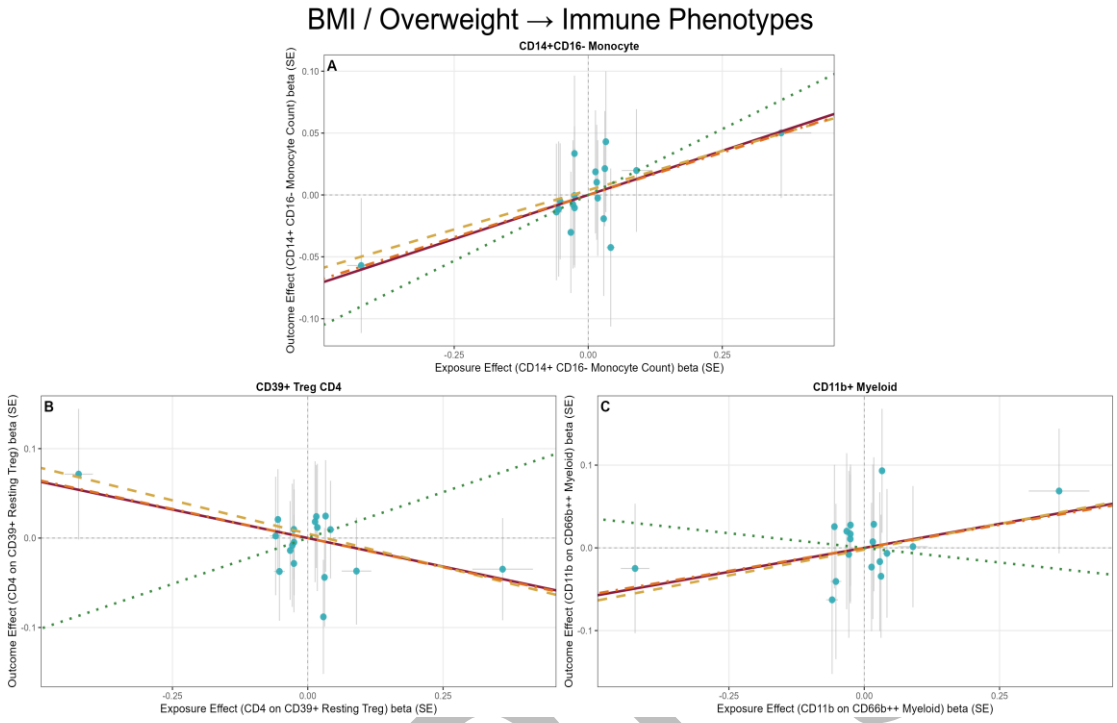

Figure 2. MR scatter plots of the associations between smoking and CD14^{-}CD16^{-} monocyte absolute count, CD39^{+} activated CD4^{+} regulatory T cell CD4 expression level, and granulocyte CD11b expression level (A–C).

A. CD14^{-}CD16^{-} monocyte absolute count. B. CD39^{+} activated CD4^{+} regulatory T cell CD4 expression level. C. Granulocyte CD11b expression level. The x-axis represents the SNP-derived effect size on the unhealthy lifestyle factor (exposure), and the y-axis represents the corresponding SNP-derived effect size on the immune phenotype (outcome). Each scatter point represents 1 instrumental variable SNP, and regression lines of different colors represent the 5 MR analytical methods (IVW, MR-Egger, Weighted Mean, Simple Median, Weighted Median). Error bars represent the 95% CI of the effect size for each SNP.

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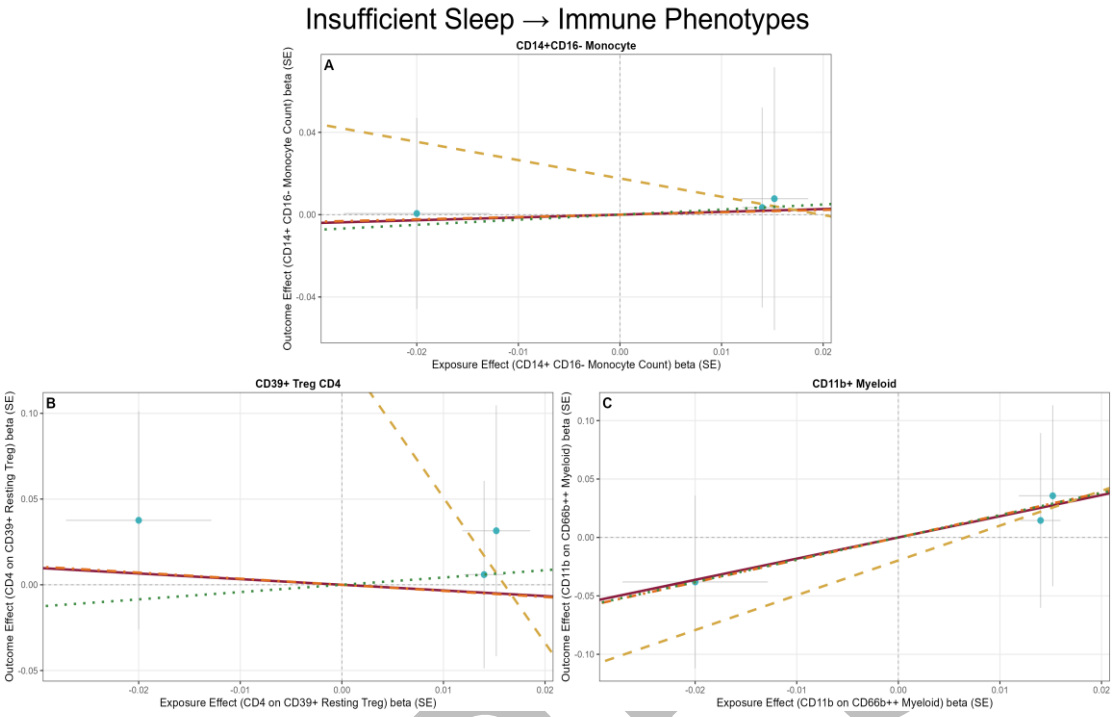

Figure 3. MR scatter plots showing the associations between overweight (BMI) and $CD14^{-}CD16^{-}$ monocyte absolute count, $CD39^{+}$ activated $CD4^{+}$ regulatory T cell CD4 expression level, and granulocyte CD11b expression level (A–C).
A. $CD14^{-}CD16^{-}$ monocyte absolute count. B. $CD39^{+}$ activated $CD4^{+}$ regulatory T cell CD4 expression level. C. Granulocyte CD11b expression level. The x-axis represents the SNP-derived effect size on the unhealthy lifestyle factor (exposure), and the y-axis represents the corresponding SNP-derived effect size on the immune phenotype (outcome). Each scatter point represents 1 instrumental variable SNP, and regression lines of different colors represent the 5 MR analytical methods (IVW, MR-Egger, Weighted Mean, Simple Median, Weighted Median). Error bars represent the 95% CI of the effect size for each SNP.

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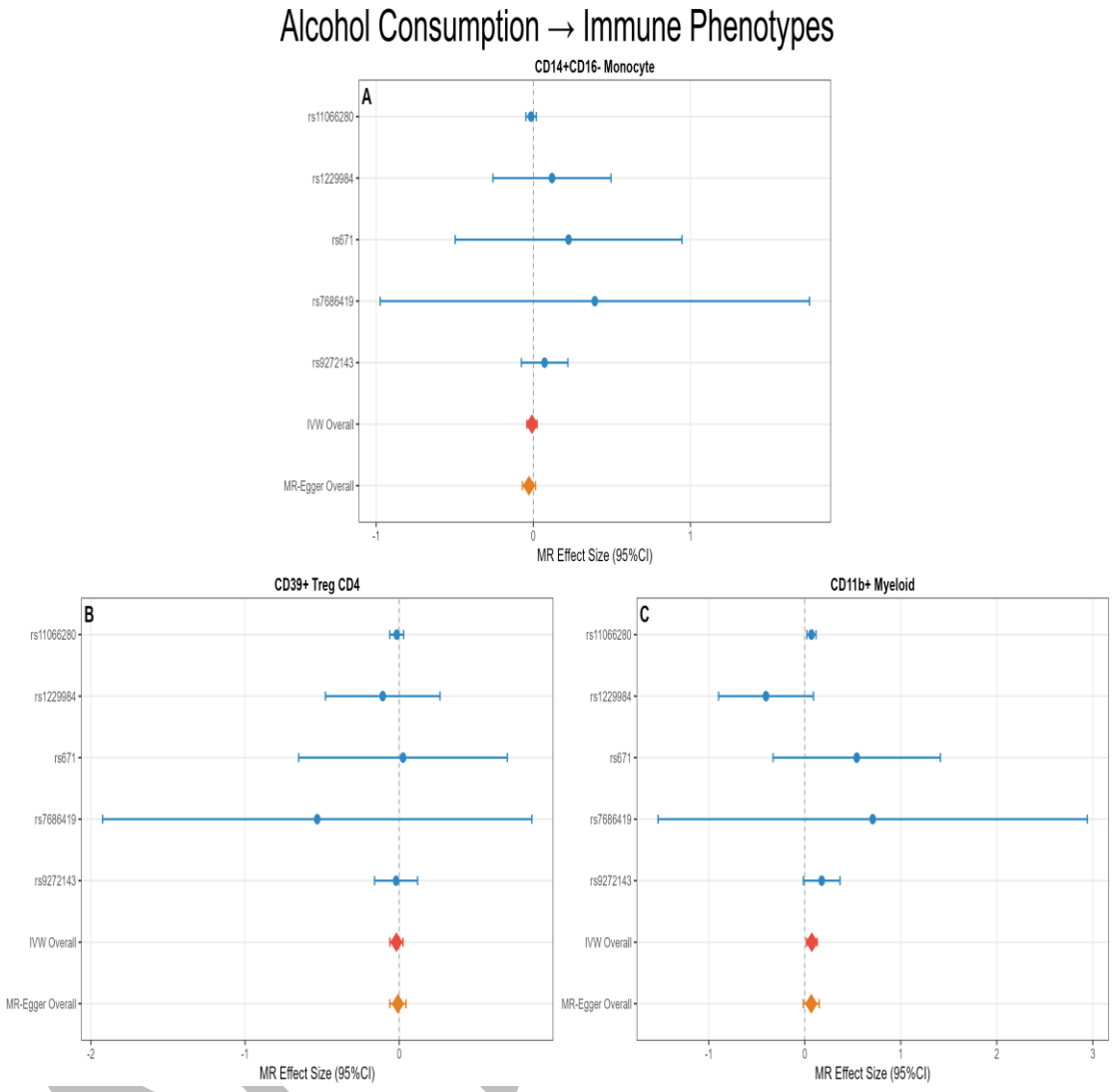

Figure 4. MR scatter plots of the associations between insufficient sleep and CD14⁻CD16⁻ monocyte absolute count, CD39⁺ activated CD4⁺ regulatory T cell CD4 expression level, and granulocyte CD11b expression level (A–C).

A. CD14⁻CD16⁻ monocyte absolute count. B. CD39⁺ activated CD4⁺ regulatory T cell CD4 expression level. C. Granulocyte CD11b expression level. The x-axis represents the SNP-derived effect size on the unhealthy lifestyle factor (exposure), and the y-axis represents the corresponding SNP-derived effect size on the immune phenotype (outcome). Each scatter point represents 1 instrumental variable SNP, and regression lines of different colors represent the 5 MR analytical methods (IVW, MR-Egger, Weighted Mean, Simple Median, Weighted Median). Error bars represent the 95% CI of the effect size for each SNP.

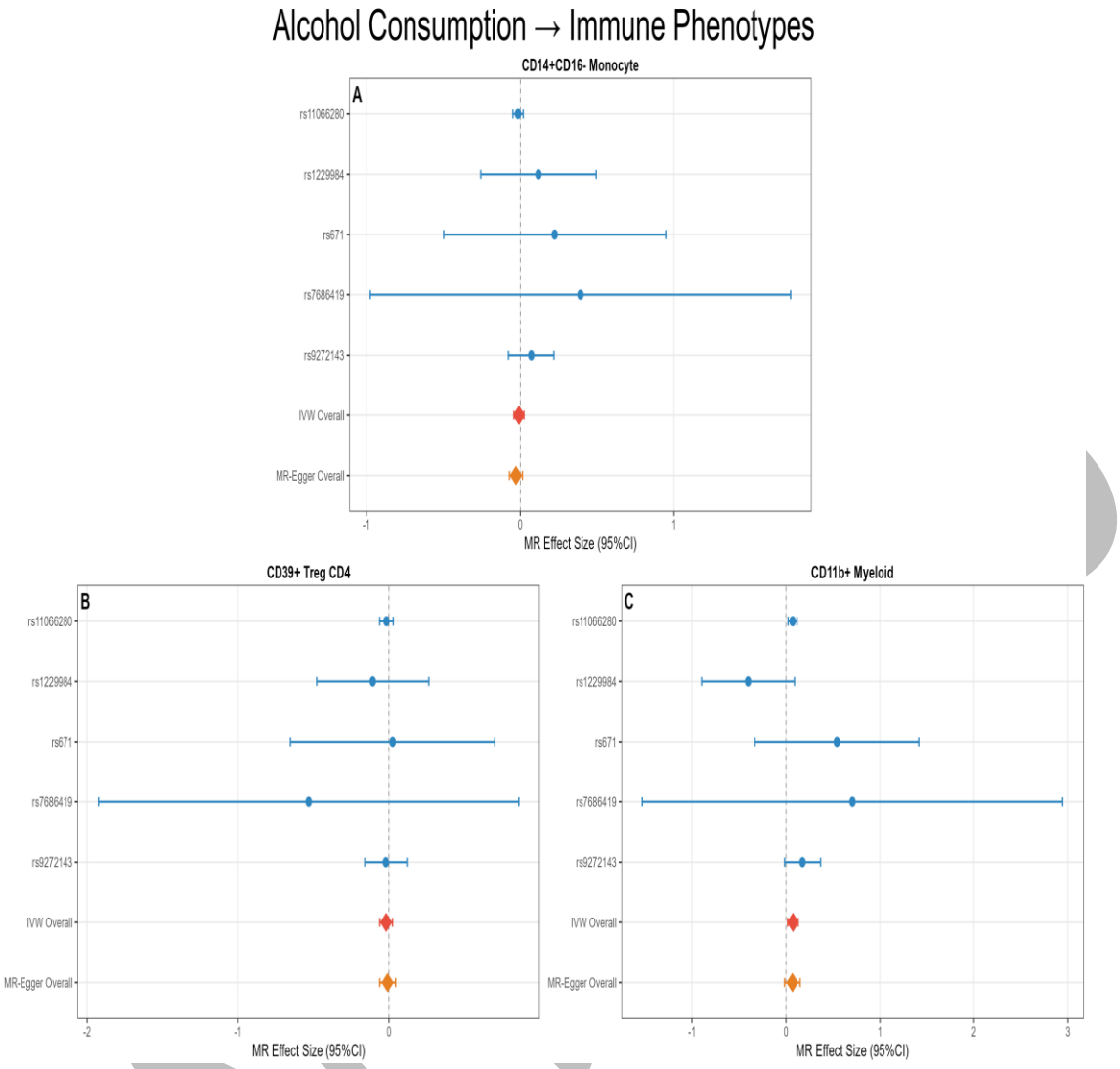

Figure 5. MR forest plots of the associations between alcohol consumption and CD14$^-$CD16$^-$ monocyte absolute count, CD39$^+$activated CD4$^+$regulatory T cell CD4 expression level, and granulocyte CD11b expression level (A-C)

A. CD14$^-$CD16$^-$ monocyte absolute count. B. CD39$^+$ activated CD4$^+$ regulatory T cell CD4 expression level. C. Granulocyte CD11b expression level. The left panel lists the rs-numbers of instrumental variable SNPs associated with the unhealthy lifestyle factor. The horizontal lines represent the 95% CI of the MR effect for each individual SNP, and the square points represent the point estimate of the effect size (the size of the square is proportional to the weight of the SNP). The right panel presents the overall MR effect sizes, 95% CIs, and *p* values for the IVW and MR-Egger regression methods. All analyses are for the corresponding immune phenotype.

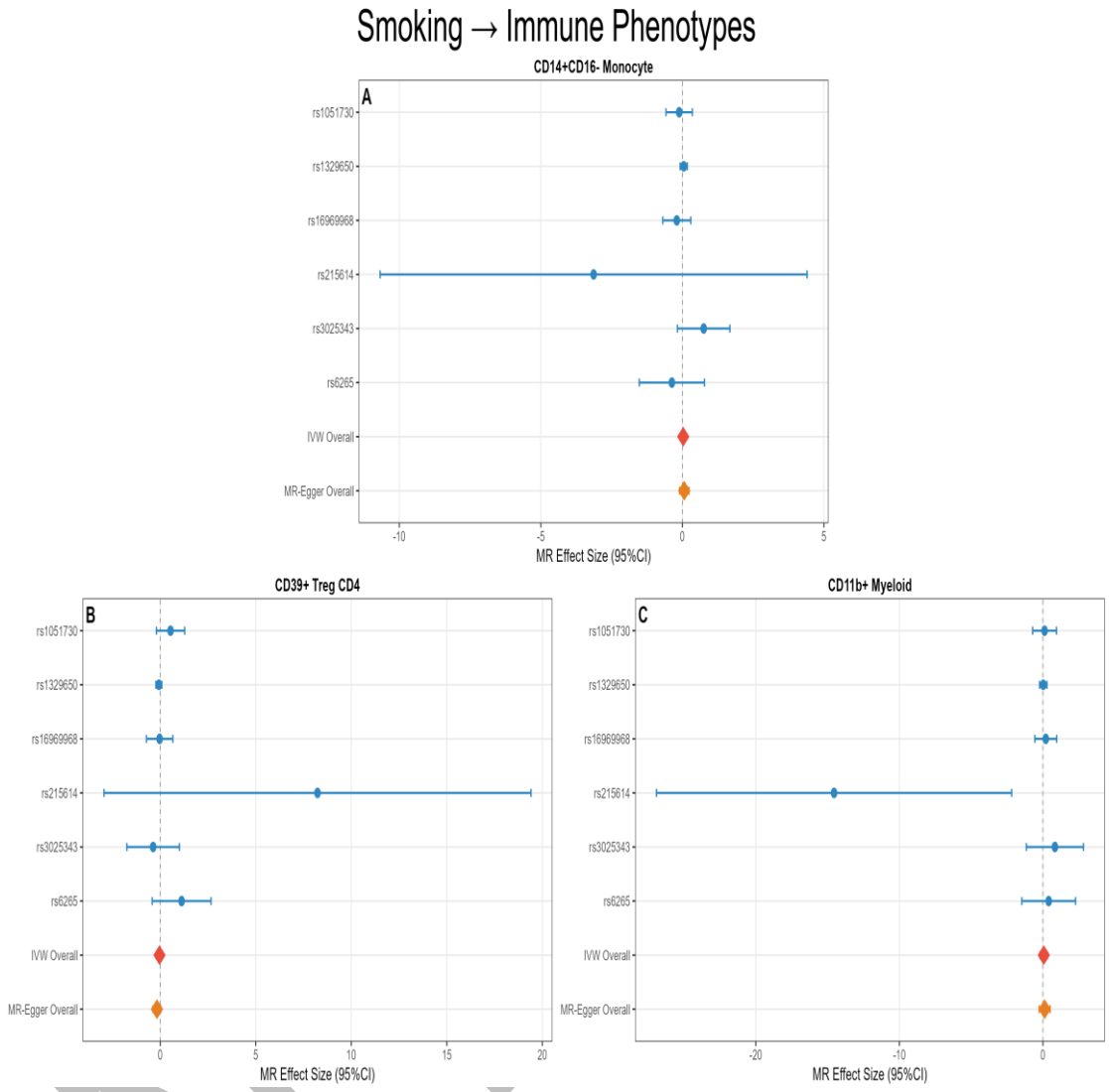

Figure 6. MR forest plots of the associations between smoking and CD14$^-$CD16$^-$ monocyte absolute count, CD39$^+$ activated CD4$^+$regulatory T cell CD4 expression level, and granulocyte CD11b expression level (A-C)

A. CD14$^-$CD16$^-$ monocyte absolute count. B. CD39$^+$ activated CD4$^+$ regulatory T cell CD4 expression level. C. Granulocyte CD11b expression level. The left panel lists the rs-numbers of instrumental variable SNPs associated with the unhealthy lifestyle factor. The horizontal lines represent the 95% CI of the MR effect for each individual SNP, and the square points represent the point estimate of the effect size (the size of the square is proportional to the weight of the SNP). The right panel presents the overall MR effect sizes, 95% CIs, and *p* values for the IVW and MR-Egger regression methods. All analyses are for the corresponding immune phenotype.

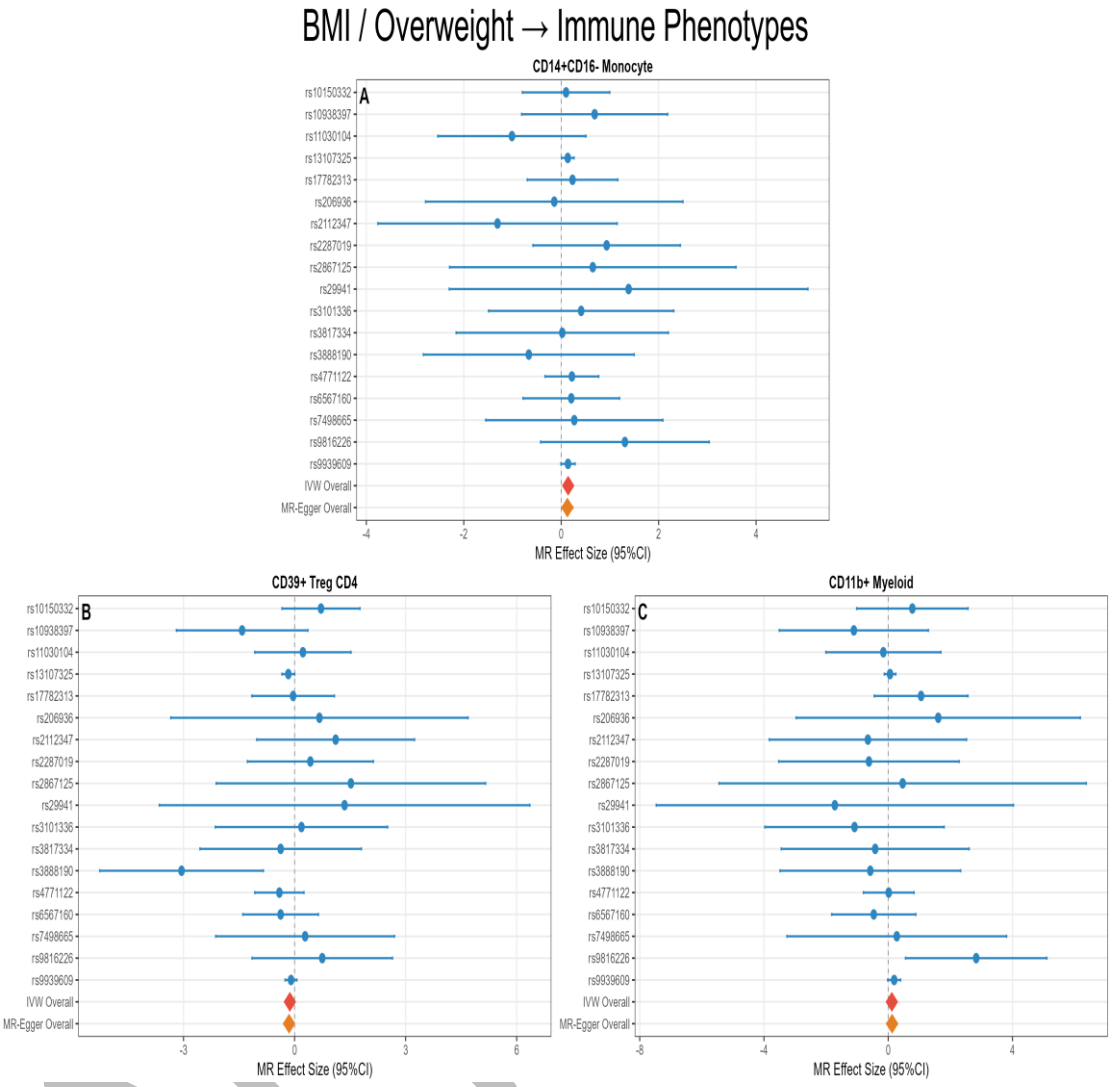

Figure 7. MR forest plots of the associations between overweight (BMI) and CD14$^-$CD16$^-$ monocyte absolute count, CD39$^+$activated CD4$^+$regulatory T cell CD4 expression level, and granulocyte CD11b expression level (A-C)

A. CD14$^-$CD16$^-$ monocyte absolute count. B. CD39$^+$ activated CD4$^+$ regulatory T cell CD4 expression level. C. Granulocyte CD11b expression level. The left panel lists the rs-numbers of instrumental variable SNPs associated with the unhealthy lifestyle factor. The horizontal lines represent the 95% CI of the MR effect for each individual SNP, and the square points represent the point estimate of the effect size (the size of the square is proportional to the weight of the SNP). The right panel presents the overall MR effect sizes, 95% CIs, and *p* values for the IVW and MR-Egger regression methods. All analyses are for the corresponding immune phenotype.

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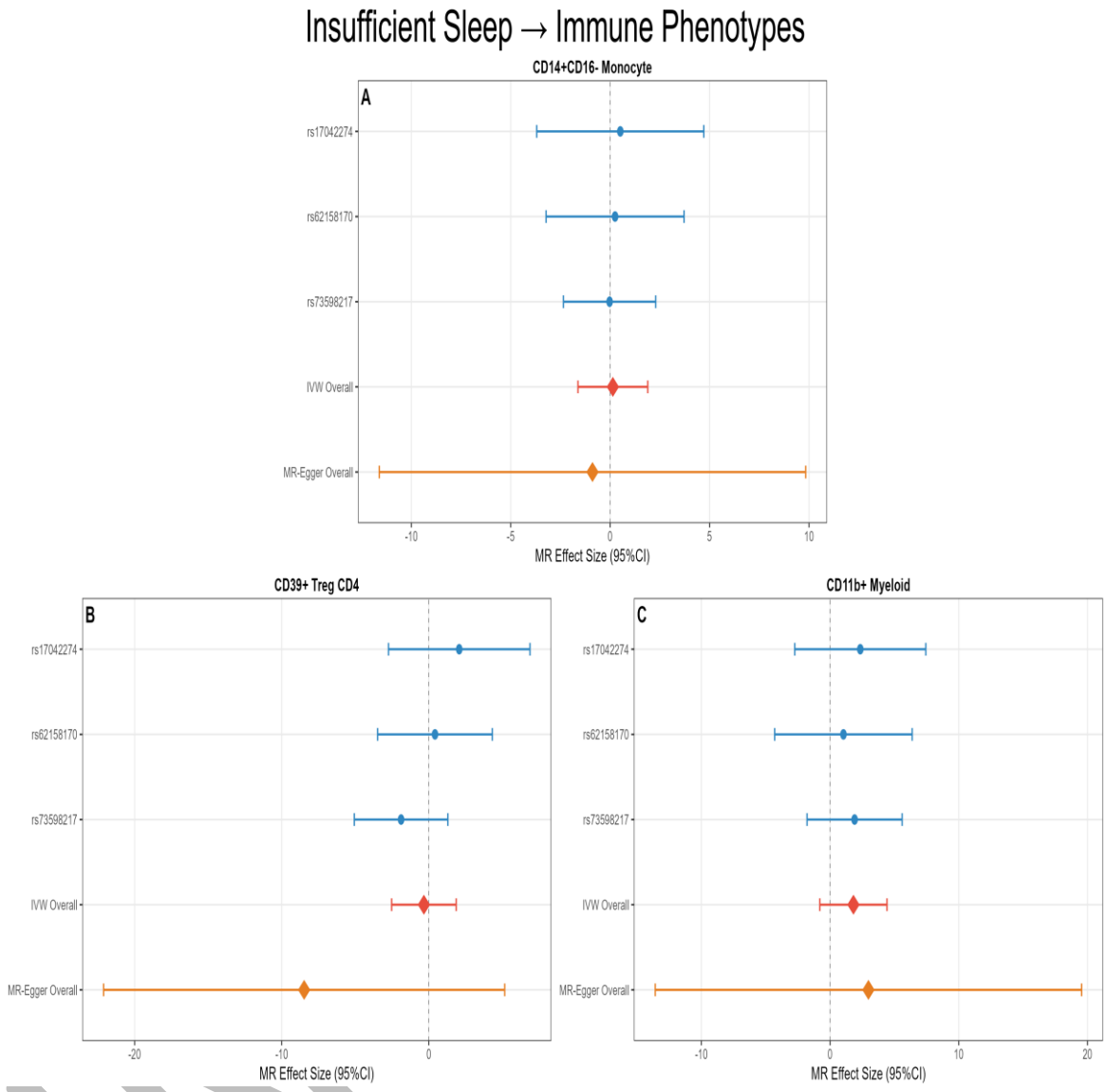

Figure 8. MR forest plots of the associations between insufficient sleep and $CD14^{-}CD16^{-}$ monocyte absolute count, $CD39^{+}$activated $CD4^{+}$regulatory T cell CD4 expression level, and granulocyte CD11b expression level (A-C)

A. $CD14^{-}CD16^{-}$ monocyte absolute count. B. $CD39^{+}$ activated $CD4^{+}$ regulatory T cell CD4 expression level. C. Granulocyte CD11b expression level. The left panel lists the rs-numbers of instrumental variable SNPs associated with the unhealthy lifestyle factor. The horizontal lines represent the 95% CI of the MR effect for each individual SNP, and the square points represent the point estimate of the effect size (the size of the square is proportional to the weight of the SNP). The right panel presents the overall MR effect sizes, 95% CIs, and *p* values for the IVW and MR-Egger regression methods. All analyses are for the corresponding immune phenotype.

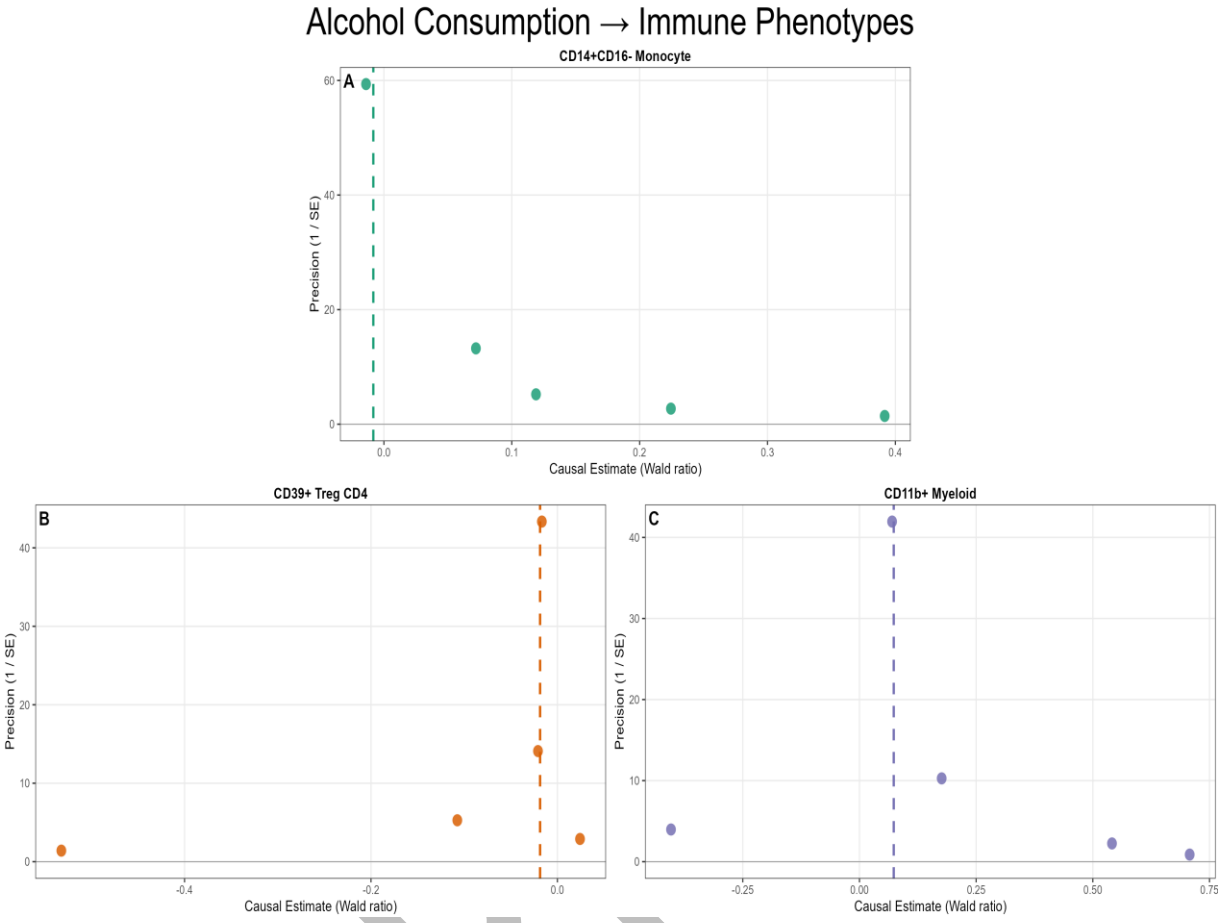

Figure 9. MR funnel plots of the associations between alcohol consumption and CD14^{-}CD16^{-} monocyte absolute count, CD39^{+}activated CD4^{+}regulatory T cell CD4 expression level, and granulocyte CD11b expression level (A-C)

A. CD14^{-}CD16^{-} monocyte absolute count. B. CD39^{+} activated CD4^{+} regulatory T cell CD4 expression level. C. Granulocyte CD11b expression level. The x-axis represents the MR effect size of instrumental variable SNPs on the immune phenotype (outcome), and the y-axis represents the standard error of the effect size. Each scatter point represents 1 instrumental variable SNP. The vertical solid line represents the overall IVW effect size, and the dashed lines on both sides represent the 95% CI of the IVW effect size. A symmetric distribution of scatter points indicates no significant publication bias or heterogeneity.

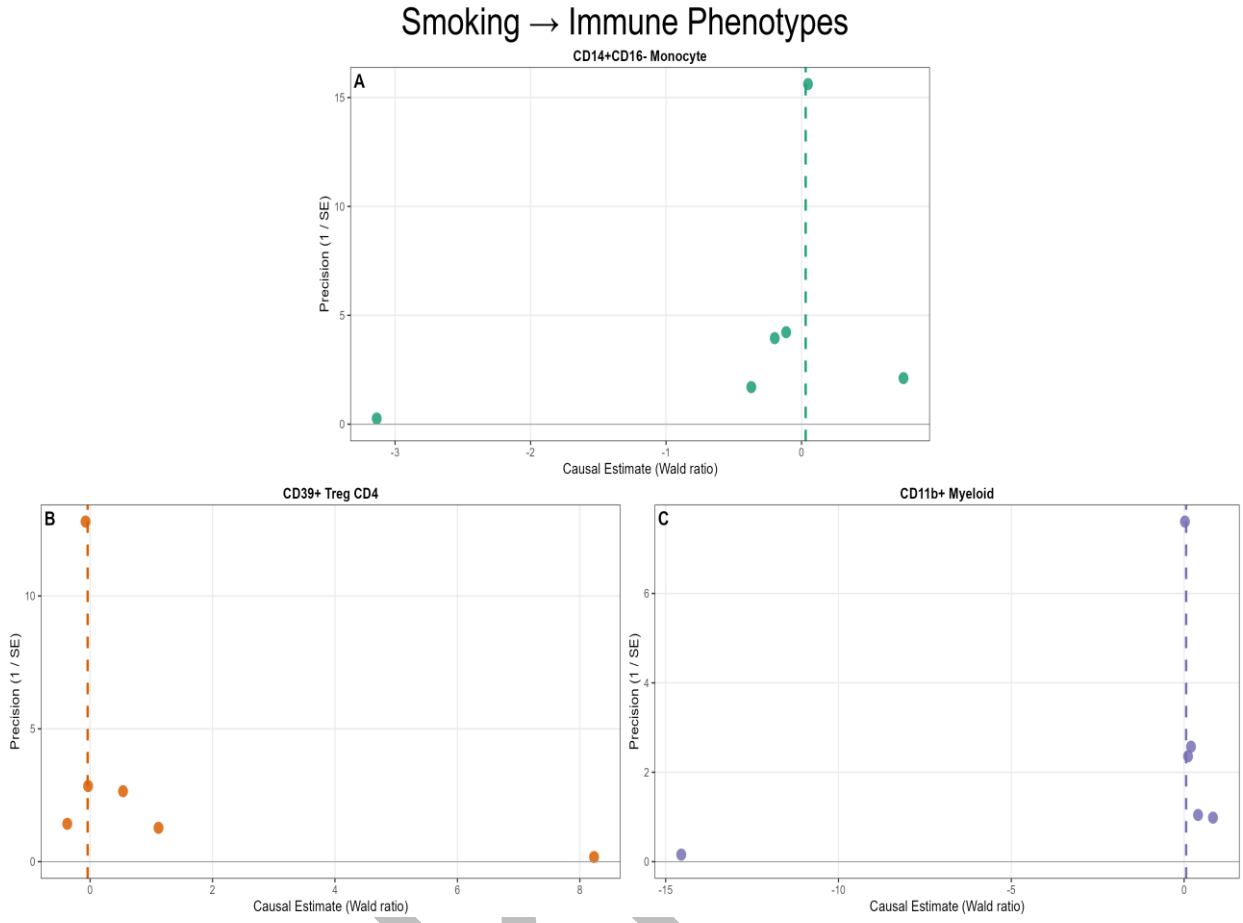

Figure 10. MR funnel plots of the associations between smoking and CD14$^-$CD16$^-$ monocyte absolute count, CD39$^+$activated CD4$^+$regulatory T cell CD4 expression level, and granulocyte CD11b expression level (A-C)

A. CD14$^-$CD16$^-$ monocyte absolute count. B. CD39$^+$ activated CD4$^+$ regulatory T cell CD4 expression level. C. Granulocyte CD11b expression level. The x-axis represents the MR effect size of instrumental variable SNPs on the immune phenotype (outcome), and the y-axis represents the standard error of the effect size. Each scatter point represents 1 instrumental variable SNP. The vertical solid line represents the overall IVW effect size, and the dashed lines on both sides represent the 95% CI of the IVW effect size. A symmetric distribution of scatter points indicates no significant publication bias or heterogeneity.

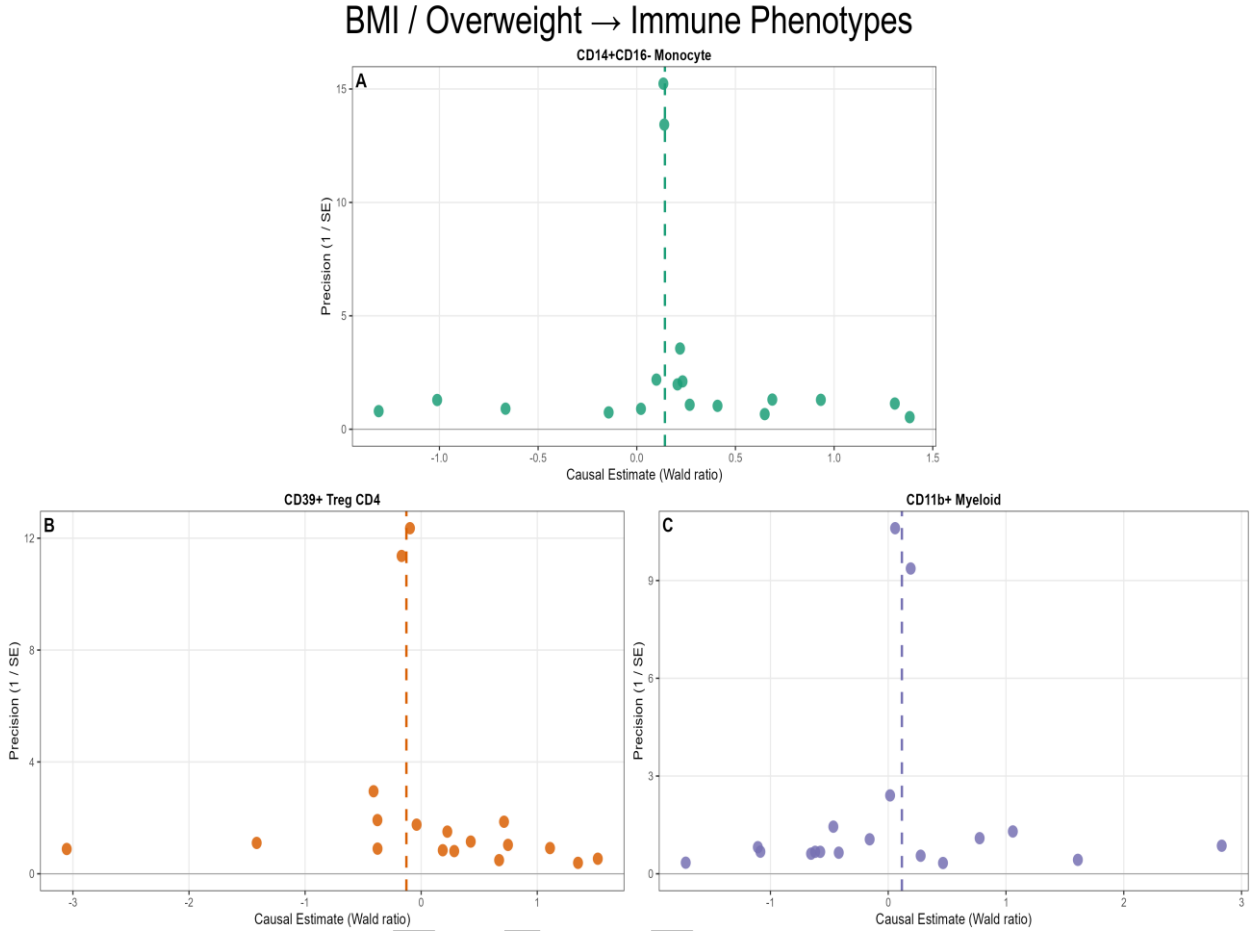

Figure 11. MR funnel plots of the associations between overweight (BMI) and CD14^{-}CD16^{-} monocyte absolute count, CD39^{+}activated CD4^{+}regulatory T cell CD4 expression level, and granulocyte CD11b expression level (A-C)

A. CD14^{-}CD16^{-} monocyte absolute count. B. CD39^{+} activated CD4^{+} regulatory T cell CD4 expression level. C. Granulocyte CD11b expression level. The x-axis represents the MR effect size of instrumental variable SNPs on the immune phenotype (outcome), and the y-axis represents the standard error of the effect size. Each scatter point represents 1 instrumental variable SNP. The vertical solid line represents the overall IVW effect size, and the dashed lines on both sides represent the 95% CI of the IVW effect size. A symmetric distribution of scatter points indicates no significant publication bias or heterogeneity.

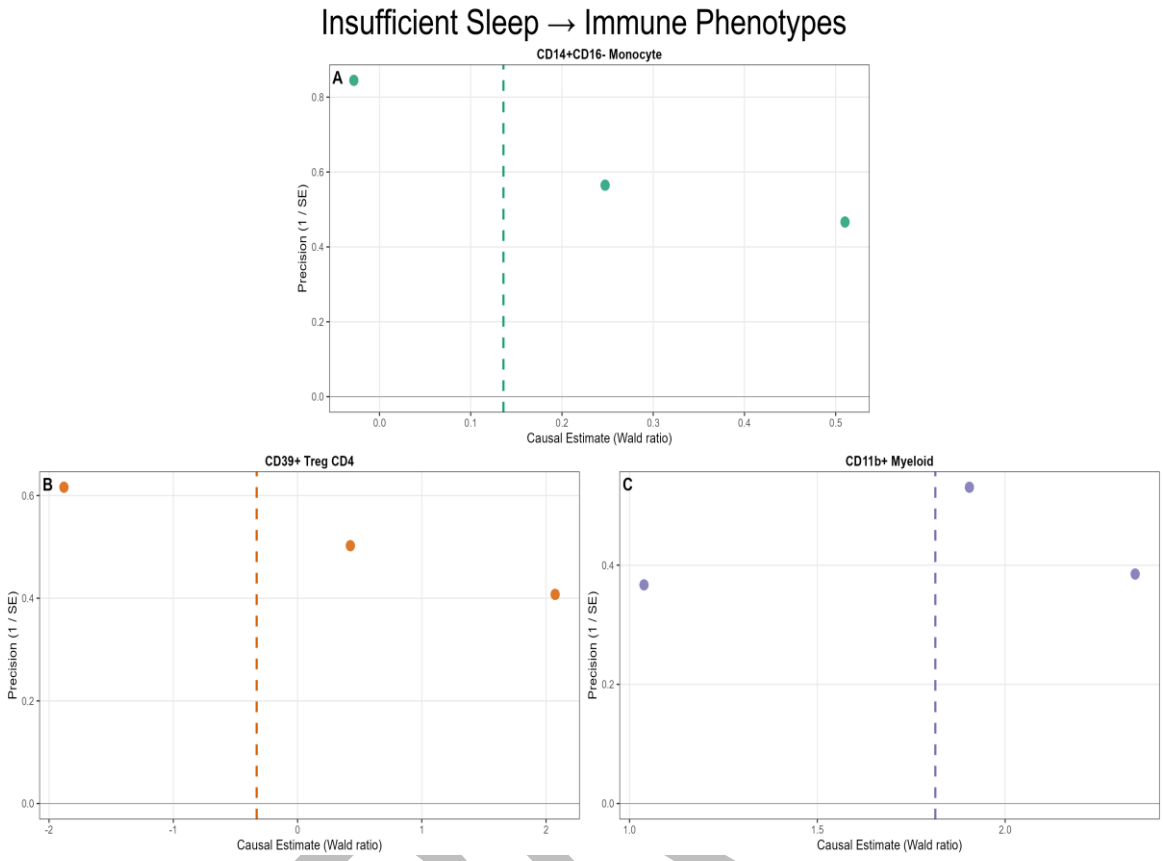

Figure 12. MR funnel plots of the associations between insufficient sleep and $CD14^{-}CD16^{-}$ monocyte absolute count, $CD39^{+}$activated $CD4^{+}$regulatory T cell CD4 expression level, and granulocyte CD11b expression level (A-C)
A. $CD14^{-}CD16^{-}$ monocyte absolute count. B. $CD39^{+}$ activated $CD4^{+}$ regulatory T cell CD4 expression level. C. Granulocyte CD11b expression level. The x-axis represents the MR effect size of instrumental variable SNPs on the immune phenotype (outcome), and the y-axis represents the standard error of the effect size. Each scatter point represents 1 instrumental variable SNP. The vertical solid line represents the overall IVW effect size, and the dashed lines on both sides represent the 95% CI of the IVW effect size. A symmetric distribution of scatter points indicates no significant publication bias or heterogeneity.

DISCUSSION

In this 2-sample Mendelian randomization study, we systematically investigated the associations between 4 common unhealthy lifestyle factors (alcohol consumption, smoking, overweight, and insufficient sleep) and 3 key immune phenotypes ($CD14^{-}CD16^{-}$ monocyte count, $CD39^{+}$ activated $CD4^{+}$ Treg CD4 expression, and granulocyte CD11b expression). Our findings provide preliminary genetic evidence suggesting that, among the 4 exposures examined, only genetically predicted overweight (BMI) showed nominally significant associations with certain immune phenotypes, while the majority of associations did not reach statistical significance after accounting for multiple testing. Compared with traditional observational studies, our MR design reduced the influence of confounding factors and reverse causality; however, the largely null findings underscore the need for larger GWAS datasets with greater statistical power to detect potentially modest causal effects.

Causal Effect of Unhealthy Lifestyles on $CD14^{-}CD16^{-}$ Monocytes

Our findings provide limited genetic support for the hypothesis that lifestyle factors influence chronic inflammatory disease risk through myeloid cell dysregulation. Larger GWAS datasets with greater statistical power are needed to further evaluate this potential mechanism. While our study did not identify statistically significant associations between alcohol consumption, smoking, or insufficient sleep and

$CD14^{-}CD16^{-}$ monocyte count after correction for multiple testing, the observed positive direction of the BMI association (β=0.143; *p*=0.002, nominal significance only) is consistent with previous mechanistic studies of lifestyle-mediated myeloid cell dysregulation. Alcohol-induced acetaldehyde accumulation activates monocytes through the TLR4/NF-κB signaling pathway and promotes their release from the bone marrow into the peripheral circulation.[7] Cigarette smoke components, including nicotine and polycyclic aromatic hydrocarbons, activate nicotinic acetylcholine receptors on monocyte membranes, stimulating monocyte proliferation and activation in both healthy individuals and patients with chronic diseases.[8] Obesity is characterized by chronic low-grade adipose tissue inflammation, with elevated levels of pro-inflammatory cytokines (TNF-α and IL-6) that recruit classic $CD14^{+}CD16^{-}$ monocytes to infiltrate peripheral tissues and exacerbate systemic inflammation.[9] Acute sleep deprivation activates the sympathetic nervous system and the hypothalamic-pituitary-adrenal axis, leading to increased secretion of cortisol and catecholamines, which in turn enhance monocyte production in the bone marrow and their mobilization into the circulation.[10] Notably, an increased count of $CD14^{+}CD16^{-}$ monocytes is closely associated with the development of chronic inflammatory diseases, including cardiovascular diseases, rheumatoid arthritis, and type 2 diabetes.[11] Our findings thus suggest that unhealthy lifestyle factors may increase the risk of chronic inflammatory diseases by expanding the classic monocyte pool and promoting systemic immune activation.

Causal Effect of Unhealthy Lifestyles on $CD39^{+}$ Activated $CD4^{+}$ Treg CD4 Expression

The observed association between BMI and Treg CD4 expression was modest in magnitude and did not survive multiple-testing correction, suggesting that any effect of overweight on Treg CD4 expression is likely subtle. $CD39^{+}$ activated $CD4^{+}$ Tregs are a key subset of regulatory T cells that maintain immune tolerance and prevent excessive immune activation. The observed inverse association between genetically predicted BMI and CD4 expression on $CD39^{+}$ activated $CD4^{+}$ Tregs (β=−0.127; *p*=0.026, nominal significance only), while not surviving multiple-testing correction, is directionally consistent with previous observational studies suggesting that obesity may modulate regulatory T-cell function. This upregulation is likely a compensatory homeostatic response to lifestyle-induced systemic immune activation, as Tregs are known to proliferate and activate in response to chronic inflammation to limit immune-mediated tissue damage.[12] However, recent single-cell transcriptome studies have shown that long-term Treg activation induced by obesity and chronic stress impairs the function of effector T cells, leading to reduced anti-infective and antitumor immune responses and increased susceptibility to infection and malignancy.[13] In contrast, enhanced Treg activity may exert a protective effect in the context of autoimmune diseases by suppressing excessive autoreactive immune responses.[14] The context-dependent role of lifestyle-induced Treg activation (protective vs detrimental) warrants further investigation in large cohort studies with stratified analyses by disease status.

Causal Effect of Unhealthy Lifestyles on Granulocyte CD11b Expression

Granulocyte CD11b is a critical adhesion molecule that mediates granulocyte migration to sites of inflammation and enhances their phagocytic and cytotoxic capacity.[16] The nominally significant positive association between genetically predicted alcohol consumption and granulocyte CD11b expression (β=0.073; *p*=0.012), while not surviving multiple-testing correction, provides suggestive evidence that alcohol may influence granulocyte function. This finding is consistent with a 2023 GWAS-MR study that demonstrated a causal association between sleep disturbance and neutrophil activation, as well as an increased risk of acute respiratory tract infections.[17] Upregulation of granulocyte CD11b expression also plays a key role in tumor-associated neutrophil infiltration and tumor progression, particularly in non-small cell lung cancer.[18] Given that smoking and overweight are well-established risk factors for non-small cell lung cancer,[19,20] our findings suggest that granulocyte CD11b may serve as a key intermediate biomarker linking unhealthy lifestyles to increased cancer risk via immune dysregulation. The suggestive effect of alcohol consumption on granulocyte CD11b expression highlights the potential role of lifestyle factors in regulating granulocyte function and innate immune homeostasis, providing a potential mechanistic explanation for the increased risk of infectious diseases in individuals with chronic alcohol use.

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Future Directions

Based on our findings, several future research directions are proposed: replication in diverse populations to validate the causal associations identified in this study using GWAS data from non-European populations (eg, Asian, African, and Hispanic populations) to assess ethnic heterogeneity; dose-response and longitudinal analyses to investigate the temporal relationships between unhealthy lifestyles and immune dysregulation; multi-omics mechanistic studies to integrate GWAS data with transcriptomics, epigenomics, and single-cell RNA sequencing data to identify key genes and pathways mediating the causal effects; interventional studies to design randomized controlled trials investigating whether targeted lifestyle interventions can reverse immune dysregulation; and biomarker development to evaluate the clinical utility of the 3 immune phenotypes as intermediate biomarkers for risk stratification and monitoring of lifestyle interventions in immune-related diseases.

In conclusion, our 2-sample Mendelian randomization study provides suggestive genetic evidence that overweight (BMI) and alcohol consumption may be nominally associated with select immune phenotypes, including $CD14^{-}CD16^{-}$ monocyte count, $CD39^{+}$ activated $CD4^{+}$ Treg CD4 expression, and granulocyte CD11b expression. However, these associations did not survive correction for multiple testing, and the majority of exposure-outcome combinations examined showed no statistically significant associations. Our findings suggest that any causal effects of unhealthy lifestyle factors on these immune phenotypes are likely to be modest in magnitude. Lifestyle modifications (eg, smoking cessation, moderate alcohol consumption, weight management, and sleep optimization) may still represent potential strategies to support immune health and potentially reduce the risk of immune-related disorders, including chronic inflammatory diseases, infections, and malignant tumors. However, further mechanistic studies and adequately powered randomized controlled trials are needed to validate these findings and evaluate their clinical translation potential. The immune phenotypes studied are cell-surface marker expression levels rather than functional immune assays or clinical endpoints, and the clinical relevance of these findings requires further investigation.

STATEMENT OF ETHICS

Not applicable.

FUNDING

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGMENTS

Not applicable.

DATA AVAILABILITY

All GWAS summary data used in this study are publicly available from the IEU OpenGWAS database (https://gwas.mrcieu.ac.uk/). The R code used for MR analyses is available from the corresponding author upon reasonable request. A completed STROBE-MR checklist is provided as Supplementary Material.

AI ASSISTANCE DISCLOSURE

Not applicable.

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