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ORIGINAL RESEARCH

Bidirectional Mendelian Randomization Suggests Causal Effects of 731 Immunophenotypes on Vitiligo Pathogenesis

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Purpose: This two-sample Mendelian randomization (MR) study aimed to investigate the causal relationship between 731 immune cell traits and vitiligo.

Patients and Methods: Genetic variants with genome-wide suggestive significance ($P < 1\times10^{-5}$) were used as instrumental variables (IVs). Immune phenotype data were sourced from 3757 European individuals (SardiNIA study), while vitiligo data included 385,538 samples (292 cases, 385,509 controls) from FinnGen (Release 9). Primary causal inference utilized inverse-variance weighted (IVW) regression, supplemented by weighted median, mode-based, and MR-Egger methods for sensitivity analyses. Horizontal pleiotropy and heterogeneity were assessed via MR-Egger intercept and Cochran's Q tests. False discovery rate (FDR) correction was applied, with PFDR < 0.05 considered significant and PFDR < 0.20 considered suggestive.

Results: The onset of vitiligo was significantly associated with reduced levels of TD $CD4^+$ %T cells (OR = 0.63, 95% CI: 0.51–0.78; PFDR = 0.015, significant) and showed a suggestive association with decreased CD4 expression on HLA DR^+ $CD4^+$ cells (OR = 0.65, 95% CI: 0.50–0.85; PFDR = 0.166, suggestive). Conversely, suggestive evidence was found for an association between vitiligo and increased CD28 expression on $CD28^+$ $CD45RA^+$ CD8br cells (OR = 1.37, 95% CI: 1.13–1.65; PFDR = 0.166, suggestive) and with elevated CD8 expression on EM CD8br cells (OR = 1.46, 95% CI: 1.19–1.79; PFDR = 0.091, suggestive). Sensitivity analyses confirmed robustness ($P_{pleiotropy} > 0.05$).

Conclusion: We suggest novel causal links between vitiligo and specific immune cell dysregulation, highlighting the pivotal role of adaptive immunity in pathogenesis and informing potential therapeutic targets such as TD $CD4^+$ T cells and $CD8^+$ T cell subsets.

Keywords: vitiligo, mendelian randomization, immune phenotyping, autoimmunity, causal inference

Introduction

Vitiligo is a chronic autoimmune dermatosis characterized by progressive loss of functional melanocytes, resulting in clinically distinctive depigmented macules.[1] With a global prevalence of 0.5–2%, this condition affects millions worldwide and typically manifests before age 20.[2] Beyond its cutaneous manifestations, vitiligo imposes profound psychosocial burdens, including stigmatization, diminished self-esteem, and impaired quality of life.[3] Consequently, effective management requires integrated approaches addressing both pathological mechanisms and psychosocial sequelae.

Although the precise pathogenesis remains incompletely defined, compelling evidence implicates immune dysregulation as a pivotal driver. Aberrant secretion of proinflammatory cytokines – including TNF-α, IL-6, and interferon-gamma (IFN-γ) – establishes a cytotoxic microenvironment that compromises melanocyte viability.[4] Concurrently, activated monocytes and autoreactive T cells infiltrate perilesional skin, directly targeting melanocyte antigens through HLA (Human Leukocyte Antigen)-mediated recognition. These observations position immunomodulation as a promising therapeutic axis.

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The disease exhibits complex genetic architecture, where acquired genetic and epigenetic variations significantly influence susceptibility heterogeneity.[5] Familial aggregation studies have identified candidate risk loci (eg, NLRP1, PTPN22, TYR), though only a fraction of its polygenic basis has been elucidated.[6] Pharmacogenomic approaches seek to overcome treatment refractoriness by personalizing interventions according to genetic profiles.[7] Genome-wide association studies (GWAS) have accelerated the discovery of susceptibility loci and pathogenic pathways, yet cannot establish causal directionality between immune phenotypes and disease initiation.[8]

Mendelian randomization (MR) overcomes this limitation by leveraging genetic variants as instrumental variables to infer causality while minimizing confounding.[9,10] Prior observational studies report associations between immune cell alterations and vitiligo,[11] and a previous MR study examined a limited set of immune cells, but our bidirectional design systematically tests causality in both directions using independent, non-overlapping cohorts (SardiNIA for immune traits, FinnGen for vitiligo). To address this knowledge gap, we conducted a bidirectional two-sample MR analysis interrogating causal links between 731 immunophenotypes and vitiligo pathogenesis.

Materials and Methods

Study Design

We employed a bidirectional two-sample Mendelian randomization (MR) framework to investigate causal relationships between 731 immune cell traits and generalized vitiligo. MR utilizes genetic variants as instrumental variables (IVs) that must satisfy three core assumptions: (1) Relevance assumption: Strong association between IVs and exposures (F-statistic > 10); (2) Exchangeability assumption: No unmeasured confounding between IVs and outcome; (3) Exclusion restriction: IVs affect outcome solely through the exposure pathway (Figure 1). The study protocol received institutional review board approval, with all participants providing informed consent.

Data Source

Immune phenotype data were derived from publicly accessible GWAS summary statistics (accessions GCST0001391-GCST0002121) encompassing 3757 individuals of Sardinian ancestry.[12] Genotyping employed a high-density array imputed via Sardinian reference panels (~22 million SNPs), with analyses adjusted for age, age^2, and sex covariates.[13] The 731 immunophenotypes comprised four categories: relative cell counts (RC, n = 192), absolute cell counts (AC, n = 118), median fluorescence intensity (MFI, n = 389; indicating surface antigen density), and morphological parameters (MP, n = 32; including CDC/TBNK panels characterizing cell size/granularity).

Vitiligo genetic data were obtained from the FinnGen consortium (Release R9; https://www.finngen.fi/en covering 385,538 Finnish individuals (292 cases, 385,509 controls). The cohort exhibited a median vitiligo onset age of 45.91 years (females: 42.35; males: 48.21), with genome-wide analysis identifying >21.31 million independent SNPs across 470,000 phenotypic assessments.

Crucially, for each direction of the bidirectional MR analysis, the exposure and outcome datasets were sourced from two completely independent, non-overlapping cohorts (SardiNIA and FinnGen). Therefore, there is zero sample overlap between the immune phenotype GWAS and the vitiligo GWAS in either analytical model. This strict independence satisfies a critical assumption of the MR framework and eliminates bias due to sample overlap.

Instrumental Variable Selection

Instrumental variables were selected using the following sequential criteria: (1) genome-wide significance ($P < 1\times10^{-5}$), (2) linkage disequilibrium pruning (LD $r^2 < 0.1$ within a 500 kb window using the 1000 Genomes Phase 3 European reference panel,[14] and (3) elimination of weak instruments (F-statistic > 10 calculated as $F = \beta^2/SE^2$ for each SNP. Palindromic SNPs with intermediate allele frequencies were aligned using the reference allele frequency information from the 1000 Genomes Project.

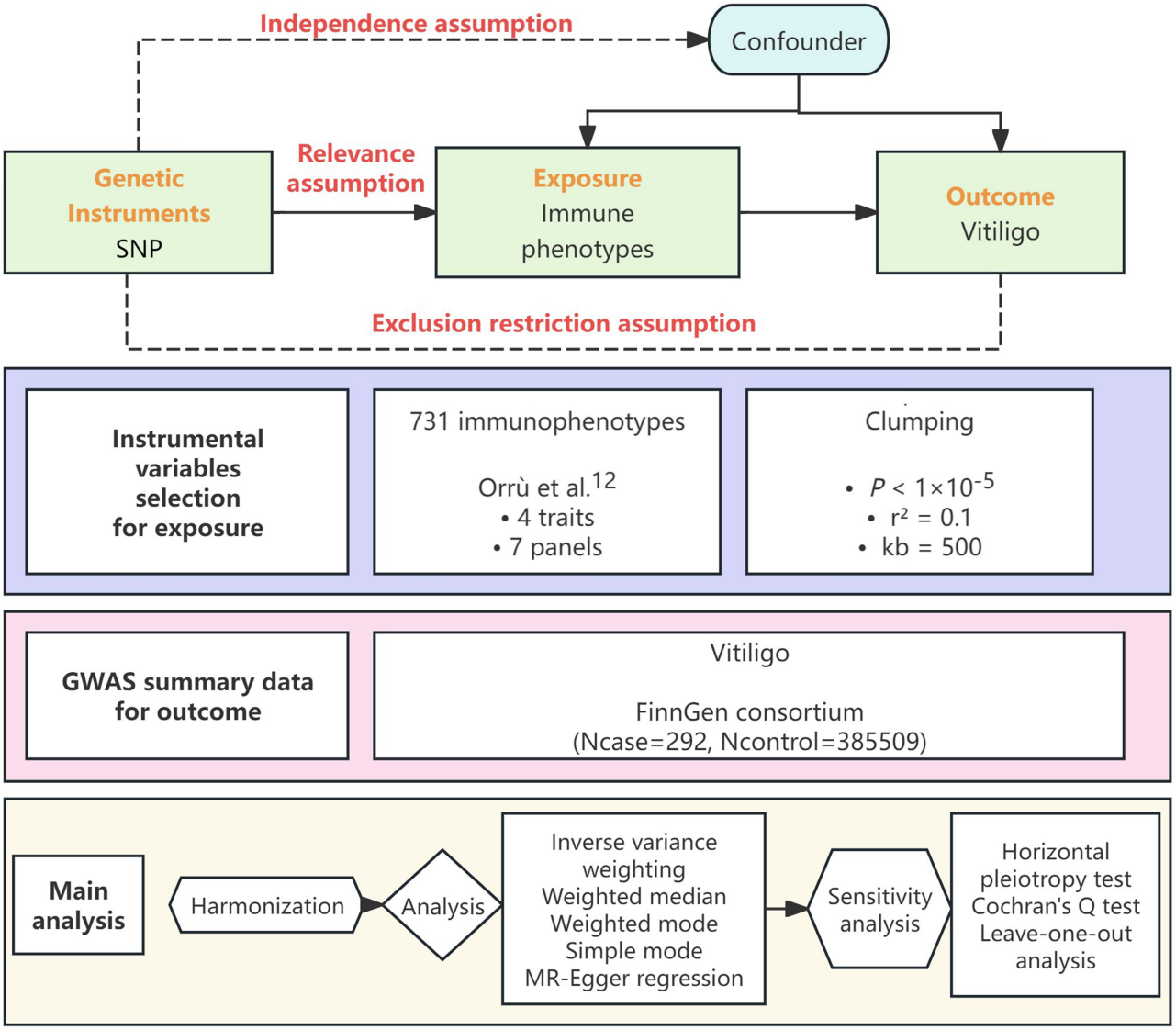

Figure 1 Overview of this bidirectional MR study design.

Sample Overlap Assessment

Despite the immune phenotypes and vitiligo data being sourced from different consortia, we assessed potential sample overlap that could bias MR estimates. We estimated the genetic correlation (*rg*) using LD score regression (LDSC) and calculated the correlation between Z-statistics from independent, LD-pruned SNPs ($r^2 < 0.1$) for each exposure-outcome pair. A significant genetic or Z-statistic correlation might indicate sample overlap or shared genetic architectures.

Statistical Analysis

Analyses were performed in R v4.4.1 using the "TwoSampleMR" package. Primary causal inference employed inverse-variance weighted (IVW) regression. Sensitivity analyses included: weighted median, mode-based estimation, and MR-Egger regression (testing horizontal pleiotropy via intercept term significance). Robustness was evaluated through Cochran's Q test (heterogeneity assessment), leave-one-out analysis (influential variant detection), and visualization via funnel/scatter plots. Multiple testing correction used false discovery rate (FDR) with significance thresholds defined as PFDR < 0.05 (significant) and PFDR < 0.20 (suggestive). The threshold of $P < 1\times10^{5}$ for instrumental variable selection was chosen to retain sufficient genetic instruments given the large number of immune phenotypes (731),

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following prior MR studies of immunophenotypes.[12] *F*-statistics for all exposures exceeded 10, confirming the absence of weak instrument bias (data available upon request). The reverse MR analysis had limited statistical power due to the small number of vitiligo cases (n = 292), whereas the forward MR was adequately powered given the large sample size for immune traits (n = 3757).

Results

Causal Effects of Immune Phenotypes on Vitiligo (Forward MR)

By FDR correction (PFDR < 0.05), we found one immunophenotype to be protective against vitiligo: TD (T Helper Cell) $CD4^+$ %T cell (Figure 2). Using the IVW method, a genetically predicted one-standard-deviation (SD) increase in TD $CD4^+$ %T cell level was associated with a lower odds of vitiligo (OR = 0.63, 95% CI = 0.51–0.78, PFDR = 0.015,

Exposure	Method	nSNP	PFDR	OR(95%CI)	P−value
TD CD4+ %T cell	MR Egger	47	0.015*	0.35 (0.17−0.76)	0.010
	Weighted median	47		0.59 (0.42−0.82)	0.002
	Inverse variance weighted	47		0.63 (0.51−0.78)	2.02e−05
	Simple mode	47		0.59 (0.29−1.20)	0.150
	Weighted mode	47		0.55 (0.34−0.87)	0.015
CD8 on EM CD8br	MR Egger	40	0.091	1.68 (1.04−2.71)	0.041
	Weighted median	40		1.30 (0.98−1.72)	0.066
	Inverse variance weighted	40		1.46 (1.19−1.79)	2.50e−04
	Simple mode	40		1.20 (0.65−2.21)	0.570
	Weighted mode	40		1.15 (0.76−1.73)	0.516
CCR2 on monocyte	MR Egger	40	0.114	0.78 (0.52−1.17)	0.240
	Weighted median	40		0.74 (0.58−0.94)	0.014
	Inverse variance weighted	40		0.75 (0.64−0.88)	4.69e−04
	Simple mode	40		0.77 (0.49−1.21)	0.265
	Weighted mode	40		0.74 (0.52−1.07)	0.115
FSC−A on NK	MR Egger	22	0.142	0.32 (0.13−0.80)	0.025
	Weighted median	22		0.53 (0.35−0.81)	0.003
	Inverse variance weighted	22		0.62 (0.47−0.82)	0.001
	Simple mode	22		0.84 (0.39−1.81)	0.655
	Weighted mode	22		0.47 (0.25−0.90)	0.034
CD28 on CD28+ CD45RA+ CD8br	MR Egger	29	0.166	1.32 (0.80−2.18)	0.288
	Weighted median	29		1.33 (1.00−1.77)	0.053
	Inverse variance weighted	29		1.37 (1.13−1.65)	0.002
	Simple mode	29		1.37 (0.85−2.19)	0.202
	Weighted mode	29		1.40 (1.04−1.88)	0.033
CD4 on HLA DR+ CD4+	MR Egger	36	0.166	0.93 (0.46−1.86)	0.836
	Weighted median	36		0.76 (0.54−1.06)	0.109
	Inverse variance weighted	36		0.65 (0.50−0.85)	0.002
	Simple mode	36		0.75 (0.40−1.42)	0.380
	Weighted mode	36		0.77 (0.46−1.30)	0.339
CD25 on CD39+ CD4+	MR Egger	98	0.166	0.89 (0.74−1.07)	0.204
	Weighted median	98		0.87 (0.75−1.02)	0.083
	Inverse variance weighted	98		0.86 (0.78−0.94)	0.001
	Simple mode	98		0.89 (0.68−1.18)	0.432
	Weighted mode	98		0.85 (0.72−1.00)	0.051

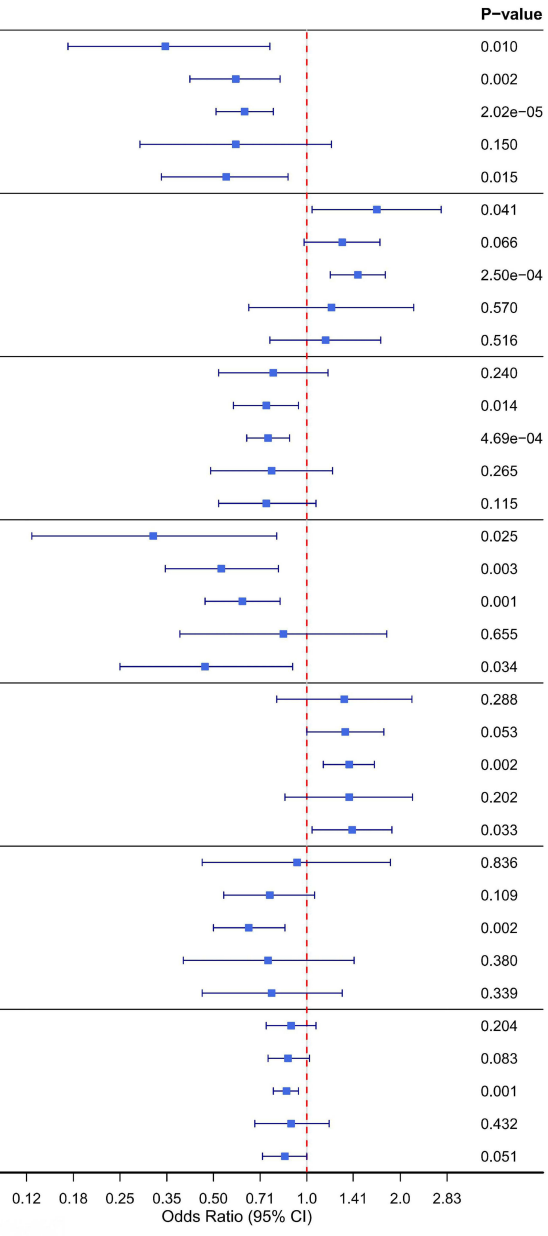

Figure 2 Forest plots showed the causal effects of immune cell phenotypes on vitiligo risk (forward MR analysis).
Notes: This plot includes all immune phenotypes with PFDR < 0.2 (1 significant, 6 suggestive associations). *p < 0.05 was considered statistically significant.

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$P = 2.02\times10^{-5}$). Four other methods yielded similar results: weighted mode (OR = 0.55, 95% CI = 0.34–0.87, $P = 0.015$); weighted median (OR = 0.59, 95% CI = 0.42–0.82, $P = 0.002$); simple mode (OR = 0.59, 95% CI = 0.29–1.20, $P = 0.015$); and MR-Egger (OR = 0.35, 95% CI = 0.17–0.76, $P = 0.010$). In addition, for all five associations, the MR-Egger intercept excluded the notion of collapsed products (Supplementary Table 1). The MR-PRESSO test indicated potential horizontal pleiotropy for TD $CD4^+$ %T cell ($P < 0.001$; outlier SNPs: rs115625939, rs12210947, rs1632969, rs33964890). After their removal, the global test was no longer significant ($P = 0.089$), supporting absence of residual pleiotropy for this exposure (Supplementary Table 2).

By FDR correction ($0.05 < \text{PFDR} < 0.2$), we found six immune phenotypes suggestively associated with vitiligo: CD8 on EM (Effector Memory) CD8br (CD8-bright) (OR = 1.46, 95% CI = 1.19–1.79, $P = 0.091$), CCR2 on monocyte (OR = 0.75, 95% CI = 0.64–0.88, $P = 0.114$), FSC-A on NK (OR = 0.62, 95% CI = 0.47–0.82, $P = 0.142$), CD28 on $CD28^+$ $CD45RA^+$ CD8br (OR = 1.37, 95% CI = 1.13–1.65, $P = 0.166$), CD4 on HLA DR^+ $CD4^+$ (OR = 0.65, 95% CI = 0.50–0.85, $P = 0.166$) and CD25 on $CD39^+$ $CD4^+$ (OR = 0.86, 95% CI = 0.78–0.94, $P = 0.166$) (Supplementary Table 1). Among these, CD4 on HLA DR^+ $CD4^+$ retained residual horizontal pleiotropy after outlier removal (MR-PRESSO global test $P = 0.027$); its estimate should therefore be regarded as exploratory and interpreted cautiously (Supplementary Table 2). Overall, the association between these immune cells and vitiligo remains suggestive rather than conclusive.

Sensitivity Analyses Robustly Support a Forward MR Effect of Immunophenotypes on Vitiligo

The robustness of the forward Mendelian randomization causal inferences from immunophenotypes to vitiligo was rigorously evaluated through a suite of sensitivity analyses (Table 1). The linear trends observed in the scatter plots (Supplementary Figure 1) supported the primary MR model assumptions. For the significant associations, including $CD4^+$ %T cell, CCR2 on monocyte, FSC-A on NK, and CD28 on $CD28^+$ $CD45RA^+$ CD8br, complementary methods (weighted median, MR-Egger) yielded directionally consistent effect estimates. Critically, these associations exhibited no evidence of horizontal pleiotropy (MR-Egger intercept $P > 0.05$) or instrumental heterogeneity (Cochran's Q $P > 0.05$). The symmetrical patterns of the funnel plots (Supplementary Figure 2) for these exposures indicated an absence of directional bias. The leave-one-out analysis (Supplementary Figure 3) further confirmed the stability of these results, demonstrating that no single SNP disproportionately drove the causal estimates. In contrast, the suggestive association for CD4 on HLA-DR^+ $CD4^+$ exhibited residual horizontal pleiotropy after outlier removal (MR-PRESSO global $P = 0.027$; Cochran's Q $P = 0.021$) and should be treated as exploratory.

Table 1 Summary of Sensitivity Results of Forward MR: Causal Effects of Immune Phenotypes on Vitiligo Risk

Exposure	Q	Q *P* value	MR-PRESSO Global Test *P*value	MR-Egger Intercept	*P* value	Outlier SNPs
TD $CD4^+$ %T cell	58.63	0.100	< 0.001	0.104	0.123	rs115625939, rs12210947, rs1632969, rs33964890
CD8 on EM CD8br	60.36	0.016	0.021	−0.038	0.532	NA
CCR2 on monocyte	31.16	0.810	0.828	−0.012	0.835	NA
FSC-A on NK	26.49	0.188	0.197	0.133	0.151	NA
CD28 on $CD28^+$ $CD45RA^+$ CD8br	30.54	0.338	0.393	0.009	0.889	NA
CD4 on HLA DR^+ $CD4^+$	54.05	0.021	0.001	−0.070	0.285	rs2596464
CD25 on $CD39^+$ $CD4^+$	87.46	0.746	0.769	−0.012	0.673	NA

Notes: An MR-Egger intercept *P* value > 0.05 was interpreted as no evidence of directional pleiotropy. Outlier SNPs indicates the outlier SNPs identified and removed by MR-PRESSO; NA denotes no outlier SNPs detected. After removing outliers, the causal effect direction of all phenotypes remained consistent, and the heterogeneity (Cochran's Q test *P* value) was reduced, confirming the robustness of the primary MR results.

Causal Effects of Vitiligo on Immune Phenotypes (Reverse MR)

Among the 731 immune-cell phenotypes, only five passed Steiger's directionality test (correct causal direction = true), indicating that genetically predicted vitiligo risk temporally preceded changes in immune-cell levels. However, none of the IVW estimates for these five phenotypes reached the significance threshold of PFDR < 0.05 (Table 2).

Per-standard-deviation increase in genetically predicted vitiligo risk was associated with a modest, non-significant reduction in CD3 expression on $CD4^+$ regulatory T cell (OR = 0.97, 95% CI = 0.87–1.07, PFDR = 0.90).

A parallel protective pattern was observed for BAFF-R (B-cell activating factor receptor) expression on IgD^- CD38^dim^ B cell (OR = 0.97, 95% CI = 0.88–1.07, PFDR = 0.91).

CD24 levels on both IgD^- CD38^dim^ B cell (OR = 1.02, 95% CI = 0.93–1.12, PFDR = 0.94) and $CD24^+$ $CD27^+$ B cell (OR = 1.00, 95% CI = 0.91–1.10, PFDR = 1.00) subsets remained unchanged, and CD45RA expression on $CD39^+$ resting T cells showed no measurable association (OR = 1.00, 95% CI = 0.90–1.12, PFDR = 0.99).

Sensitivity analyses (Q-test, MR-Egger intercept) revealed neither heterogeneity nor directional pleiotropy (all $P > 0.05$) (Table 3).

Collectively, the reverse-direction MR yielded no evidence for a causal effect of vitiligo on any of the investigated immune-cell phenotypes, implying that previous observational links may be attributable to confounding or reverse-measurement error, and reinforcing our primary inference that the causal pathway runs from immune traits to vitiligo.

Additional Robustness Check: Sample Overlap Bias

The sample overlap assessment revealed minimal genetic correlation (eg, for TD $CD4^+$ %T cell vs vitiligo, $rg = 0.02$, SE = 0.05) and non-significant Z-score correlations ($P > 0.05$) for all significant exposures, suggesting that sample overlap is unlikely to substantially bias our causal estimates.

Table 2 Results of Reverse MR Analysis: Causal Effects of Vitiligo on Immune Phenotypes

Outcome	No. SNPs	Method	*P* value	OR	95% CI	Outlier SNPs
CD3 on CD4 regulatory T cell	3	IVW	0.55	0.97	0.87, 1.07	NA
		MR-Egger	0.72	0.89	0.55, 1.45	
		Weighted Median	0.63	0.97	0.86, 1.10	
		Weighted Mode	0.75	0.98	0.86, 1.11	
		Simple Mode	0.62	0.95	0.80, 1.13	
BAFF-R on IgD^- CD38^dim^ B cell	3	IVW	0.59	0.91	0.88, 1.07	NA
		MR-Egger	0.92	1.03	0.65, 1.62	
		Weighted Median	0.60	0.97	0.87, 1.08	
		Weighted Mode	0.63	0.97	0.87, 1.08	
		Simple Mode	0.66	0.97	0.85, 1.10	
CD24 on IgD- CD38^dim^ B cell	3	IVW	0.67	1.02	0.93, 1.12	NA
		MR-Egger	0.84	0.94	0.60, 1.47	
		Weighted Median	0.70	1.02	0.92, 1.14	
		Weighted Mode	0.62	1.03	0.93, 1.15	
		Simple Mode	0.89	0.99	0.86, 1.14	
CD45RA on $CD39^+$ resting CD4 regulatory T cell	3	IVW	0.96	1.00	0.90, 1.12	NA
		MR-Egger	0.63	1.19	0.71, 1.97	
		Weighted Median	0.95	1.00	0.88, 1.13	
		Weighted Mode	0.86	0.99	0.87, 1.12	
		Simple Mode	0.90	1.01	0.86, 1.19	
CD24 on $CD24^+$ $CD27^+$ B cell	3	IVW	1.00	1.00	0.91, 1.10	NA
		MR-Egger	0.68	0.88	0.57, 1.37	
		Weighted Median	0.90	1.01	0.90, 1.12	
		Weighted Mode	0.84	1.01	0.91, 1.13	
		Simple Mode	0.77	1.02	0.89, 1.18	

Table 3 Summary of Sensitivity Results of Reverse MR: Causal Effects of Vitiligo on Immune Phenotypes

Outcomes	Q	Q *P* value	MR-PRESSO Global Test *P* value	MR-Egger Intercept	*P* value
CD3 on CD4 regulatory T cell	0.126	0.939	Not testable	0.042	0.783
BAFF-R on IgD^- CD38^dim^ B cell	0.078	0.962	Not testable	−0.027	0.846
CD24 on IgD^- CD38^dim^ B cell	0.189	0.910	Not testable	0.039	0.783
CD45RA on $CD39^+$ resting CD4 regulatory T cell	0.441	0.802	< 3 SNPs	−0.082	0.627
CD24 on $CD24^+$ $CD27^+$ B cell	0.506	0.776	< 3 SNPs	0.061	0.674

Interpretation of Non-Significant Findings

Among the 731 immunophenotypes tested, only one reached statistical significance (PFDR < 0.05) and six showed suggestive associations (PFDR < 0.20). The remaining 724 immunophenotypes did not show evidence of causal association with vitiligo. Several factors may contribute to this observation. First, the reverse MR analysis had limited statistical power due to the small number of vitiligo cases (n = 292), which may have failed to detect weak effects. Second, the high correlations among immune phenotypes (e.g., within the same lineage) impose a stringent multiple-testing burden, potentially masking true but modest associations. Third, vitiligo pathogenesis may be driven by specific immune pathways rather than widespread immune dysregulation, making null findings for many phenotypes biologically plausible. Readers should therefore not interpret the absence of association for undiscussed phenotypes as evidence of no effect; replication in larger, independent cohorts is warranted.

Discussion

This study uses bidirectional, two-sample Mendelian randomization to screen 731 immunophenotypes for potential causal links to vitiligo. Forward analyses suggest that dysregulation of adaptive—rather than innate—immune cells may contribute to disease susceptibility, while reverse analyses had limited statistical power due to the small number of vitiligo cases (n = 292) and therefore found no evidence of a meaningful effect of vitiligo on these traits. These findings provide new genetic clues to the immune mechanisms underlying vitiligo.

TD $CD4^+$ % T Cell: A Central Protective Role

In our Mendelian randomization analysis, we observed that genetically predicted higher levels of TD $CD4^+$ % T cell were significantly associated with a reduced risk of vitiligo (OR = 0.63, PFDR = 0.015). By "TD $CD4^+$ % T cell" we refer to the proportion of terminally differentiated $CD4^+$ T cells, a subset often synonymous with $CD4^+$ TEMRA (T effector memory RA^+) cells. $CD4^+$ T cells include several functionally distinct subsets such as Th1, Th2, Th17 and regulatory T-cells (Tregs, commonly $CD4^+CD25^+FOXP3^+$) that coordinate immune responses and maintain peripheral tolerance. In the context of vitiligo — a depigmenting autoimmune skin disorder characterized by destruction of melanocytes — numerous immunological studies have implicated T-cell dysregulation. For example, patients with generalized vitiligo had significantly decreased $CD4^+/CD8^+$ ratios and lower peripheral Treg cell percentages,[15] suggesting a loss of $CD4^+$ helper/regulatory T-cell capacity in disease onset and progression. Another study described a dominant type-1 cytokine polarization in both $CD4^+$ and $CD8^+$ T cells in vitiligo patients, supporting the idea that $CD4^+$ helper T-cells (alongside $CD8^+$) play a role in melanocyte loss.[16] More recently, a meta-analysis[17] focused on Tregs in vitiligo reported that patients exhibit significantly lower counts and impaired suppressive function of $CD4^+FOXP3^+$ regulatory T cells compared with controls, consistent with impaired $CD4^+$-mediated immune regulation. Taken together, our MR results show that genetically higher $CD4^+$ TEMRA (T effector memory RA-positive) % and surface CD4 levels on $HLA\text{-}DR^+$ $CD4^+$ cells are causally linked to lower vitiligo risk. $HLA\text{-}DR^+$ $CD4^+$ T cells represent a subset with potent antigen-presenting and helper capacity; their reduction suggests a weakened $CD4^+$ helper arm. Although the current immunophenotype panel includes Treg subsets characterized by CD25hi expression, a comprehensive investigation of their role was beyond the scope of this initial analysis. Therefore, future studies should prioritize analyzing these and other

classical Treg markers (e.g., $CD25^{+}CD127^{-}$) to determine if regulatory T-cell loss independently contributes to vitiligo susceptibility.

CD8 on EM CD8br: $CD8^{+}$ T Cells Drive Pathogenesis

Our MR analysis provided suggestive evidence that genetically predicted higher CD8 levels on EM CD8br cells may be causally associated with increased vitiligo risk (OR = 1.46, PFDR = 0.091), suggesting a key role for $CD8^{+}$ effector memory T cells in the disease process. $CD8^{+}$ Effector memory T cells ($CD8^{+}$ T cells) are antigen-experienced cytotoxic T cells that circulate peripherally and rapidly respond to previously encountered antigen without requiring homing to secondary lymphoid organs; they are distinguished phenotypically by low expression of CCR7 and CD62L and by functional readiness for cytokine production and cytolytic activity. In the context of vitiligo, accumulating evidence implicates memory $CD8^{+}$ T cells in melanocyte destruction and disease persistence: For example, CXCL9/10-mediated recruitment of $CD8^{+}$ T cells to the dermal–epidermal junction facilitates melanocyte killing, and their differentiation into tissue-resident memory $CD8^{+}$ T cells supports relapse at previous lesion sites.[18] Similarly, Jacquemin et al[19] identify a subset of $NKG2D^{+}$ $CD8^{+}$ T cells in vitiligo skin with elevated IFN-γ and TNF-α production, suggesting these cells are operative effectors in melanocyte loss. Taken together, our MR finding aligns mechanistically with these observational and mechanistic studies by suggesting that enhanced $CD8^{+}$ T cells activity may be a causal driver of vitiligo risk rather than simply a consequence. This supports a model in which genetically predisposed melanocyte-directed autoimmunity fosters expansion or maintenance of cytotoxic $CD8^{+}$ effector memory T cells, which in turn promote melanocyte destruction and clinical disease, thereby raising the possibility that therapeutic targeting of the $CD8^{+}$ T cell compartment (or their recruitment/activation pathways) may mitigate risk or progression of vitiligo. These suggestive findings warrant replication in larger cohorts.

CCR2 on Monocyte: A Potential Modulatory Mechanism

Our analysis suggests a protective effect of genetically predicted higher CCR2 expression on monocytes (OR = 0.75, PFDR = 0.114) in relation to vitiligo risk. CCR2 is a chemokine receptor highly expressed on circulating monocytes and plays a critical role in monocyte egress from the bone marrow and recruitment to peripheral tissues upon ligation by its primary ligand CCL2 (MCP-1). For monocytes, CCR2 engagement facilitates chemotaxis, infiltration into sites of inflammation, and subsequent differentiation into macrophages or dendritic-type cells, thereby influencing immune surveillance and tissue remodelling. Mechanistically, although direct studies of CCR2 expression in monocytes in vitiligo are scarce, the chemokine axis CCL2/CCR2 has been implicated in skin-immune interactions relevant to pigment-cell destruction. For example, Speeckaert et al[20] conducted a meta-analysis of chemokines in vitiligo and noted that increased CCL2 levels (the ligand for CCR2) may contribute to immune cell recruitment in vitiligo lesions, suggesting the CCL2/CCR2 axis is activated in the disease context. Another study by Xin et al[11] in a bidirectional two-sample MR design reported that higher peripheral monocyte counts were causally associated with greater vitiligo risk—supporting the idea that monocyte-lineage cells may drive disease. Taken together, our finding that genetically higher CCR2 expression on monocytes is associated with lower vitiligo risk suggests a somewhat counterintuitive but mechanistically plausible scenario: enhanced CCR2 signalling might promote efficient monocyte homeostasis or clearance of inflammatory cues, thereby restraining persistent monocyte-mediated inflammation or aberrant antigen presentation that could fuel melanocyte destruction. Alternatively, higher CCR2 expression might reflect a more regulated monocyte compartment less prone to pathogenic recruitment or activation in vitiligo. Although our result remains suggestive, it highlights CCR2-expressing monocytes as a potentially protective immune axis in vitiligo and motivates future mechanistic work to determine how monocyte CCR2 expression influences melanocyte-targeted autoimmunity.

FSC-A on NK Cell: Implications for Natural Killer Cell Function

The results from our Mendelian randomization analysis indicate a suggestive protective association between higher FSC-A on NK cells and vitiligo risk (OR = 0.62, PFDR = 0.142). FSC-A is a flow-cytometric parameter reflecting the relative size of the cell event as it passes through the laser beam, and in the context of NK cells may serve as an indirect indicator of cellular activation state or morphological enlargement associated with effector differentiation.[21] NK cells are innate

lymphoid effectors that rapidly respond to stressed or transformed cells, secrete IFN-γ, and engage innate and adaptive immune responses; they have more recently been implicated as early initiators or amplifiers of the cytotoxic cascade in the autoimmune depigmentation disorder vitiligo.[22] In vitiligo, elevated NK cell activity and accumulation of NK and ILC-1 cells in perilesional skin and circulation have been described, as well as increased expression of innate-ligand receptors and chemokines.[23]

Our finding that higher FSC-A on NK cells may be protective is somewhat counterintuitive given the predominant narrative of NK cell contribution to melanocyte destruction. However, this apparent contradiction may be reconciled by the emerging recognition of NK cell functional heterogeneity. Although our data do not allow for direct identification of NK cell subsets (eg, CD56bright vs CD56dim), it is plausible that an increase in FSC-A, indicating cellular enlargement, could reflect a shift toward a distinct functional state. For instance, the well-characterized CD56bright NK subset, known for immunoregulatory functions, often displays different morphological characteristics compared to the highly cytotoxic CD56dim subset.[24,25] Thus, our finding motivates future research using single-cell technologies to directly link NK cell morphological phenotypes with specific functional states in vitiligo.

CD28 on $CD28^+$ $CD45RA^+$ CD8br: Costimulation in Naive-Like T Cells

Our Mendelian randomization study revealed a suggestive link between genetically predicted vitiligo and increased CD28 expression on the $CD28^+$ $CD45RA^+$ CD8br cell subset (OR = 1.37, PFDR = 0.166), indicating that this activated/naïve-marker $CD8^+$ T cells population with CD28 costimulatory expression may play a role in disease susceptibility. CD28 is a key costimulatory receptor on T cells that binds CD80/CD86 on antigen-presenting cells and provides signal-2 required for full T-cell activation, proliferation, survival and IL-2 production; its expression on $CD8^+$ T cells generally denotes a less differentiated, more responsive state compared to $CD28^-$ counterparts. Mechanistically, although few studies in vitiligo have focused specifically on $CD28^+$ $CD45RA^+$ CD8br cells, several lines of evidence implicate $CD8^+$ T cells with costimulatory capacity in melanocyte destruction. For example, Boniface et al[26] documented that skin lesions in vitiligo harbour $CD8^+$ tissue-resident memory T cells (TRM) with potent effector potential. More generally, Orrù et al[12] emphasised the relevance of costimulatory receptor expression (including CD28) in determining immune-cell phenotypes linked to autoimmunity. Taken together, our finding supports a model in which individuals genetically predisposed to vitiligo may harbour $CD8^+$ T cells that retain CD28 expression and thus remain more readily costimulatable; these cells may be poised for activation against melanocyte-derived antigens or tissue stress. Such cells might act as initiators or amplifiers of autoimmunity leading to melanocyte destruction, which is consistent with the increased vitiligo risk associated with higher CD28 expression on this $CD8^+$ subset.

Methodologically, our two-sample MR design leveraging 385,538 individuals provides robust causal inference while minimizing confounding through genetic instrumental variables. Rigorous sensitivity analyses confirmed absence of horizontal pleiotropy and heterogeneity, with FDR correction controlling false positives. Nevertheless, several limitations merit consideration. First, while we examined a broad spectrum of immune cell phenotypes, data availability constraints precluded the analysis of certain phenotypes, which may have led to an incomplete immunological profile. Second, all genetic data were derived from the European-ancestry populations, which limits the generalizability of our findings to other ethnic groups. Third, the use of summary-level GWAS data restricted our ability to perform stratified analyses (e.g., by age or disease severity) or to explore non-linear relationships. A key methodological consideration is that, although sensitivity analyses were applied, the exclusion restriction assumption for instrumental variables could not be fully verified, and residual horizontal pleiotropy may still bias the causal estimates. Finally, the comparatively limited sample sizes for some autoimmune diseases in current public databases may affect the stability and precision of the estimates. The reverse MR analysis was underpowered due to the small number of vitiligo cases (n = 292), which may have precluded detection of weak causal effects from vitiligo to immune phenotypes. While bidirectional MR could elucidate reverse causation, our PFDR < 0.05 threshold for TD $CD4^+$ %T cell provides strong evidence for unidirectional effects. Future research should not only employ individual-level data from larger, multi-ethnic cohorts to validate these associations and address pleiotropy but also incorporate multi-omics approaches to dissect the underlying tissue-specific immune mechanisms.

Conclusion

In conclusion, our bidirectional Mendelian randomization analysis suggests novel causal links between specific immune phenotypes—particularly reduced HLA-DR$^+$ CD4$^+$ helper subsets and suggestive evidence for expanded cytotoxic CD8$^+$ T cells—and vitiligo pathogenesis, providing genetic evidence for adaptive immunity's central role in melanocyte destruction. By leveraging instrumental variables in large-scale cohorts (n = 385,538), we mitigated confounding biases inherent in observational studies, offering robust mechanistic insights into immune dysregulation. These findings advance the molecular understanding of vitiligo as an immune-mediated disorder and facilitate the development of targeted immunomodulatory strategies for early intervention and prevention. However, our findings require validation in larger, independent cohorts and functional studies.

Abbreviations

MR, Mendelian randomization; GWAS, genome-wide association studies; IVs, instrumental variables; IVW, inverse-variance weighted; HLA, Human Leukocyte Antigen; SNP, single nucleotide polymorphism; *rg*, genetic correlation; LDSC, LD score regression; FDR, false discovery rate; TD, T Helper Cell; SD, standard-deviation; EM, Effector Memory; CD8br, CD8-bright; TD, Terminally Differentiated; CD, Cluster of Differentiation; EM, Effector Memory; CCR2, C-C Chemokine Receptor Type 2; NK, Natural Killer; CD45RA, Leukocyte Common Antigen RA Isoform; DR, D-related; FSC-A, Forward Scatter-Area; OR, Odds Ratio; CI, Confidence Interval; NA, Not Applicable; BAFF-R, B-cell activating factor receptor; IgD, Immunoglobulin D; No., Number; TEMRA, T effector memory RA-positive; TRM, tissue-resident memory T cells.

Data Sharing Statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author. Data are available upon request from the corresponding author, Guangshan Chen (dermac@163.com).

Ethical Declarations

The data for this study were obtained from publicly accessible genome-wide association study (GWAS) summary statistics. Immune phenotype data were derived from the SardiNIA study conducted in Sardinia, Italy, which was performed in accordance with the Italian Data Protection Code (Legislative Decree No. 196/2003, as amended by Legislative Decree No. 101/2018) and the General Data Protection Regulation (GDPR, EU Regulation 2016/679). Vitiligo data were obtained from the FinnGen study (Release 9) conducted in Finland, which was approved by the Coordinating Ethics Committee of the Hospital District of Helsinki and Uusimaa (HUS) and conducted in accordance with the Finnish Biobank Act, the Finnish Data Protection Act (1050/2018), and GDPR. As this study utilized only publicly available, aggregated summary-level data, no additional ethical approval was required.

The design and implementation of this study followed the relevant regulations and ethical guidelines of China. According to Article 32 of the "Ethical Review Measures for Human Life Sciences and Medical Research" jointly issued by relevant Chinese departments on February 18, 2023, the following two types of studies can be exempted from ethical review: (1): Non-intrusive observational studies conducted in public places, and those that do not involve the collection of private behaviors and information that cannot identify individual identities; (2): Studies using legally available database data or information collected through anonymous methods, and those that cannot be traced back to specific individuals and will not pose any risks to the subjects. After assessment, this study falls under the circumstances stipulated in the second item of the above-mentioned provisions. Therefore, this study is exempt from the review and approval of the institutional ethics committee.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agreed to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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