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ORIGINAL ARTICLE

Mediation Mendelian Randomization Analysis of the Effect of Immune Cells on Autism Spectrum Disorder Mediated by Inflammatory Factors

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Abstract

BACKGROUND: Autism Spectrum Disorder (ASD) is a neurodevelopmental condition that impairs communication. Increasing research indicates that maternal immune activation (MIA) is one of the most important environmental factors that increase the risk of autism spectrum disorder (ASD) in offspring. Maternal immune activation produces elevated cytokine levels that cross the placental barrier and disrupt fetal neurodevelopment, increasing ASD risk. However, the specific causal pathways and mediating mechanisms remain unclear, limiting our understanding.

METHODS: This mediation Mendelian randomization study examined causal pathways linking immune cell traits (exposures) and inflammatory factors (mediators) to ASD risk.The research merged immune data (731 phenotypes + 48 cytokines) and ASD data from a cohort comprising 18,382 cases and 27,969 controls. Various MR approaches were used to reduce potential biases, along with thorough descriptions of statistical procedures and instrumental variable selection.

RESULTS: The study's findings propose potential causal relationships among cytokines representing inflammatory factors, immune cells, and ASD through mediation Mendelian randomization. Reverse MR was then employed to investigate the possibility of reverse causality. $CD8^+$ T cell %leukocyte (OR = 1.099, 95% CI: 1.039–1.163, p = 0.001), $CCR2^+$ $CD62L^+$ myeloid dendritic cells

(OR = 0.933, $p = 0.029$), and $CD45^+$ immature myeloid-derived suppressor cells (OR = 1.056, $p = 0.001$) showed evidence of causal association with ASD risk. Furthermore, reduced Artemin levels and elevated FLT3L and 2B4 levels were significantly linked to ASD risk, indicating that abnormalities in immunomodulatory factors may play a crucial role in the pathogenesis of ASD. Additionally, ASD occurrence may result in alterations in Natural Killer cell receptor 2B4 levels.

CONCLUSION: This mediation Mendelian randomization study provides evidence that immune dysfunction is associated with ASD pathophysiology through inflammatory mediators, requiring functional validation before clinical application.Cytokines act as mediators in the pathogenesis of ASD, providing a theoretical basis for understanding its immunoinflammatory pathogenesis and offering insight into treatment.

INTRODUCTION

Autism Spectrum Disorder (ASD) is recognized as a persistent neurological disorder with lifelong effects on individuals, significantly impacting their learning, communication, and daily functioning abilities (Shin *et al.* 2025). ASD core deficits: social deficits and RRBs (Tian *et al.* 2022; Liu *et al.* 2022). It usually emerges around the age of 3 and persists into adulthood (van 't Hof *et al.* 2021; Zhang *et al.* 2022). Early diagnosis and intervention are regarded as the key to improving the prognosis of patients with ASD (Rossignol & Frye, 2025). However, the pathogenic mechanism of ASD is complex and variable and is not yet fully understood at present, which brings challenges to accurate diagnosis and effective treatment. At present, the prevalence of ASD worldwide has exceeded 1%, with a male-to-female ratio of 3:1 to 4:1 (Maenner *et al.* 2021). A recent survey indicates that among Chinese children aged 6 to 12, approximately one in every 143 is an ASD patient (Zhou *et al.* 2020).

The clinical manifestations of the core symptoms of ASD patients are: difficulty in social common attention, eye contact disorders, lack of social desire and behavior, and obsession with stereotyped and repetitive sensory stimuli (such as rotating fans, etc.) (Liu *et al.* 2025). Rubenstein and Merzenich proposed the etiological hypothesis of ASD: There is an excitation-inhibition imbalance in the neural circuits of the brain of ASD patients, and this imbalance is the basis of abnormal social, behavioral, emotional, cognitive, sensory, and motor control in ASD patients (Rubenstein & Merzenich, 2003).

The current treatment methods for ASD mainly include behavioral intervention, occupational and speech therapy, music therapy, and dietary therapy, and there is also antipsychotic drug treatment for non-core symptoms (Hodis, *et al.* 2025). There are also physical therapies such as hyperbaric oxygen and repetitive transcranial magnetic stimulation (Tu *et al.* 2025; Tian *et al.* 2025). Although extensive research has been conducted in the medical field on the diagnosis of ASD, ASD cannot be diagnosed through a single examination at present. Clinically, it is subjectively evaluated based on symptomological characteristics, medical history data, and scales of social function. Given the severe situation that The incidence of autism spectrum disorder demonstrates a consistent upward trend annually at present, it is urgent to carry out research and analysis on ASD (Wang & Wang, 2024).

The specific etiology of autism spectrum disorders (ASDs) remains unclear. Maternal immune activation (MIA) elicits elevated cytokine production; these inflammatory mediators cross the placental barrier and disrupt fetal neuroimmune development, increasing ASD risk. (Vasistha & Sawa, 2025; Suprunowicz *et al.* 2024; Montalvo-Martínez *et al.* 2023). This leads to the production of inflammatory mediators by the maternal immune system. These substances may have a negative effect on the developing fetal brain (Griego *et al.* 2022; Velloso *et al.* 2022). Some patients with ASD show immune dysregulation, with dysfunction of lymphocytes and dendritic cells in the central and peripheral systems (Nadeem *et al.* 2020; Nour-Eldine *et al.* 2022). There is also evidence that neuroinflammation plays a central role in the pathogenesis of ASD and can be involved in ASD-associated pathological changes through glial cell integration in the brain(Meng *et al.* 2024).Glial cells cause neuroinflammation by secreting many inflammatory cytokines after being pathologically stimulated (Ashwood, 2025). A vicious cycle is formed through glial cell integration, jointly leading to pathological changes related to ASD (Liu *et al.* 2025). In addition, mast cells in the middle process can be jointly stimulated by corticotropin-releasing factor and neurotensin and can release inflammatory mediators to disrupt the blood-cerebrospinal fluid barrier, thereby activating microglia and causing inflammation (Huang *et al.* 2024; Priego-González *et al.* 2025). Within the CNS of autistic individuals, the inflammatory response can activate microglia, inducing release of cytokines including IL-1β, IL-6, TNF-α, and IL-10 (Tan *et al.* 2024). Conversely, pro-inflammatory cytokines worsen neuroinflammation and spread, causing healthy neuronal cells to degenerate and damage brain function (Mallick *et al.* 2025). The clinical heterogeneity of ASD is extremely high, which may be related to the complexity of its immunopathological mechanisms.

It is vital to clarify how immune cell functions and inflammatory factors contribute to the development of ASD, as this knowledge is key to designing effective treatment methods. In this study, we employed Mendelian randomization (MR) to investigate these relationships and inferred causality via genetic instruments, minimizing the confusion effect to the greatest extent.This two-sample mediation MR study examined: (1) causal effects of immune cell traits on ASD,

(2) whether inflammatory factors mediate this association, and (3) reverse causality (bidirectionality).

MATERIALS AND METHODS

Study design

This two-sample MR study assessed causal relationships between immune traits and ASD. Integrated immunome GWAS stats, 48 inflammatory cytokines, and ASD (18382 cases/27969 controls). We selected instrumental variables with a significance threshold of $p < 1\times10^{-5}$ and then pruned them to ensure independence ($r^2 < 0.001$ and distance >10 kb). Multi-SNP analyses were conducted using the inverse-variance weighted and weighted median methods, while single SNP effects were estimated by the Wald ratio method. Sensitivity analyses involving MR-Egger regression and weighted median estimation were conducted to assess the robustness of the findings.

GWAS data sources

Immune cell data

Immune cell phenotypes (n = 731) were obtained from the Integrated Immunome GWAS study (GWAS Catalog ID: GCST90002121 from https://www.ebi.ac.uk/gwas/studies/GCST90002121). Phenotypes encompassed four categories: absolute counts (n = 118), median fluorescence intensity (n = 389), morphological parameters (n = 32), and relative cell counts (n = 192), derived from flow cytometry across 14,936 European individuals.

Inflammatory factor data

The GWAS data for 48 cytokines representing inflammatory factors was obtained from the European Bioinformatics Institute GWAS Catalog Database (EBI GWAS Catalog Database), which combined 11 cohorts totaling 14,824 European resident participants.

ASD data

ASD GWAS data (IEU ID: ieu-a-1185, iPSYCH-PGC 2017) comprised 18,382 European-ancestry ASD cases and 27,969 controls, with genome-wide genotyping of 9,112,386 SNPs (HG19/GRCh37 reference). This meta-analysis excluded populations with non-European ancestry.

Instrumental variable (IV) selection

SNPs with $p < 1 \times 10^{-5}$ were selected as instruments (Fadista *et al.* 2016). Linkage disequilibrium (LD) was pruned ($r^2 < 0.001$, 10 Mb window) to ensure instrument independence (Auton *et al.* 2015). F-statistics were calculated ($F = r^2(N-k)/(1-r^2)$); $F > 10$ indicates robust instruments free of weak instrument bias. Additionally, the r2 value for each single-nucleotide polymorphism (SNP) was employed to ascertain the proportion of exposed variants. The strength of the tool was measured by the F-value, F = r2(N-2)/(1-r2) (Burgess & Thompson, 2011), N: sample size; F < 10 SNPs excluded against weak instruments.

MR analysis

This MR uses genetic instruments to examine the effects of causal exposure on outcomes.We combine multiple methods to ensure reliable causal reasoning. For datasets with multiple SNPs, analyses were conducted using inverse variance weighting (IVW), while single SNP effects were assessed via the Wald ratio method. To enhance result reliability, additional approaches such as MR-Egger regression, weighted median, and weighted pattern-based methods were also applied. Cochran's Q test ($p < 0.05$) was utilized to evaluate heterogeneity among SNP-specific causal estimates. Observed heterogeneity prompted sensitivity analyses for robustness assessment. Assessment of pleiotropy involved multiple techniques, including MR-Egger regression where the intercept tested for directional pleiotropy ($p < 0.05$), Pleiotropy correction via MR-PRESSO. All statistical procedures were performed using the TwoSampleMR package in R version 4.4.2.

RESULTS

Screening of instrumental variables

A detailed investigation has revealed a potential connection between 32 distinct immune cell types and ASD. Furthermore, a comprehensive analysis identified 10 cytokines factors that exhibited a potential causal relationship with ASD, with a *p*-value less than 0.05. The F-statistics for all instrumental variables (IV) were found to exceed 10, indicating an absence of slight instrumental bias. Detailed findings are provided in the Supplementary material 1.

Immune cells' causal impact on ASD

Mediation MR identified immune cell associations with ASD (n = 32 of 731 phenotypes, $p < 0.05$). Significant findings included: $CCR2^+$ $CD62L^+$ myeloid dendritic cells (weighted median: OR = 0.933, 95% CI: 0.878–0.991, $p = 0.029$) and $CD28^-$ $CD8^+$ T cells (IVW: OR = 1.070, 95% CI: 1.008–1.133, $p = 0.026$), consistent with altered immune regulation in ASD.A slight increase in absolute CD45RA+ CD28- CD8+ T cell Absolute Count (OR = 1.000013, $p = 0.030$) also showed a potential association. CD45 on Immature Myeloid-Derived Suppressor Cells (OR = 1.056, $p = 0.001$) were significantly and positively correlated with the risk of ASD, suggesting that T-cell depletion and abnormal myeloid immunosuppressive function may be involved in the disease; HLA-DR in CD33+ myeloid cells showed the risk factor (OR = 1.031, $p = 0.038$), suggesting a disturbed immunoregulatory network. Most immune cell subsets (e.g., CD20+ B cells, CD4+ Treg cells) showed no significant association. $CD8^+$ T cell %leukocyte demonstrated the strongest association with ASD (IVW: OR = 1.099, 95% CI: 1.039–1.163, $p = 0.001$)

exposure	nsnp	method	pval	OR(95% CI)
CCR2 on CD62L+ myeloid Dendritic Cell || id:ebi-ic731-GCST90002014	11	Weighted median	**0.029**	0.933 (0.876 to 0.993)
	11	Inverse variance weighted	**0.041**	0.943 (0.892 to 0.998)
CD127 on CD28+ CD4+ T cell || id:ebi-ic731-GCST90001924	17	Inverse variance weighted	**0.032**	0.968 (0.939 to 0.997)
	17	Weighted median	0.211	0.975 (0.937 to 1.014)
CD14 on CD33+ HLA DR+ CD14dim || id:ebi-ic731-GCST90002020	18	Inverse variance weighted	**0.012**	0.960 (0.930 to 0.991)
	18	Weighted median	0.147	0.966 (0.923 to 1.012)
CD20 on IgD- CD24- B cell || id:ebi-ic731-GCST90001753	25	Inverse variance weighted	**0.048**	1.030 (1.000 to 1.061)
	25	Weighted median	0.257	1.025 (0.982 to 1.069)
CD20 on IgD+ CD38- B cell || id:ebi-ic731-GCST90001748	22	Inverse variance weighted	**0.007**	1.061 (1.016 to 1.108)
	22	Weighted median	0.342	1.030 (0.969 to 1.094)
CD25 on activated & secreting CD4 regulatory T cell || id:ebi-ic731-GCST90001943	15	Inverse variance weighted	**0.021**	0.970 (0.945 to 0.995)
	15	Weighted median	0.293	0.978 (0.939 to 1.019)
CD25 on CD4 regulatory T cell || id:ebi-ic731-GCST90001936	18	Inverse variance weighted	**0.005**	0.963 (0.939 to 0.989)
	18	Weighted median	0.501	0.987 (0.949 to 1.026)
CD25++ CD8+ T cell %T cell || id:ebi-ic731-GCST90001679	11	Weighted median	**0.041**	0.937 (0.880 to 0.997)
	11	Inverse variance weighted	0.218	0.970 (0.925 to 1.018)
CD25++ CD8+ T cell Absolute Count || id:ebi-ic731-GCST90001681	17	Weighted median	0.050	0.933 (0.870 to 1.000)
	17	Inverse variance weighted	0.423	0.979 (0.928 to 1.032)
CD28- CD8+ T cell %CD8+ T cell || id:ebi-ic731-GCST90001686	18	Inverse variance weighted	**0.026**	1.070 (1.008 to 1.137)
	18	Weighted median	0.100	1.067 (0.988 to 1.153)
CD28- CD8dim T cell %T cell || id:ebi-ic731-GCST90001661	18	Inverse variance weighted	**0.020**	1.053 (1.008 to 1.101)
	18	Weighted median	0.312	1.029 (0.973 to 1.089)
CD3 on CD39+ resting CD4 regulatory T cell || id:ebi-ic731-GCST90001852	11	Inverse variance weighted	**0.016**	1.056 (1.010 to 1.104)
	11	Weighted median	**0.043**	1.066 (1.002 to 1.135)
CD3 on HLA DR+ CD8+ T cell || id:ebi-ic731-GCST90001850	18	Inverse variance weighted	**0.017**	1.048 (1.008 to 1.089)
	18	Weighted median	**0.032**	1.060 (1.005 to 1.118)
CD34 on Hematopoietic Stem Cell || id:ebi-ic731-GCST90001870	9	Inverse variance weighted	**0.012**	0.966 (0.940 to 0.992)
	9	Weighted median	0.123	0.973 (0.940 to 1.007)
CD39 on granulocyte || id:ebi-ic731-GCST90002033	22	Inverse variance weighted	**0.028**	0.966 (0.937 to 0.996)
	22	Weighted median	0.465	0.984 (0.942 to 1.028)
CD4 on activated CD4 regulatory T cell || id:ebi-ic731-GCST90002066	22	Inverse variance weighted	**0.043**	1.027 (1.001 to 1.054)
	22	Weighted median	0.156	1.033 (0.988 to 1.081)
CD4+CD8+ T cell %T cell || id:ebi-ic731-GCST90001595	6	Inverse variance weighted	**0.011**	0.878 (0.795 to 0.971)
	6	Weighted median	**0.027**	0.862 (0.755 to 0.983)
CD45 on CD33+ HLA DR+ CD14- || id:ebi-ic731-GCST90002042	15	Inverse variance weighted	**0.025**	0.956 (0.919 to 0.994)
	15	Weighted median	0.086	0.954 (0.903 to 1.007)
CD45 on HLA DR+ T cell || id:ebi-ic731-GCST90001918	10	Inverse variance weighted	**0.009**	0.945 (0.906 to 0.986)
	10	Weighted median	0.159	0.961 (0.910 to 1.016)
CD45 on Immature Myeloid-Derived Suppressor Cells || id:ebi-ic731-GCST90002052	6	Inverse variance weighted	**0.001**	1.056 (1.021 to 1.093)
	6	Weighted median	**0.016**	1.052 (1.010 to 1.097)
CD45RA- CD28- CD8+ T cell Absolute Count || id:ebi-ic731-GCST90001695	197	Inverse variance weighted	**0.003**	1.000 (1.000 to 1.000)
	197	Weighted median	0.080	1.000 (1.000 to 1.000)
CD45RA+ CD28- CD8+ T cell Absolute Count || id:ebi-ic731-GCST90001698	366	Inverse variance weighted	**0.030**	1.000 (1.000 to 1.000)
	366	Weighted median	0.322	1.000 (1.000 to 1.000)
CD8 on CD28+ CD45RA+ CD8+ T cell || id:ebi-ic731-GCST90002119	21	Inverse variance weighted	**0.015**	1.037 (1.007 to 1.067)
	21	Weighted median	0.171	1.029 (0.988 to 1.073)
CD8 on naive CD8+ T cell || id:ebi-ic731-GCST90002055	11	Inverse variance weighted	**0.023**	0.933 (0.879 to 0.990)
	11	Weighted median	0.197	0.946 (0.870 to 1.029)
CD8+ T cell %leukocyte || id:ebi-ic731-GCST90001607	12	Inverse variance weighted	**0.001**	1.099 (1.039 to 1.163)
	12	Weighted median	0.063	1.081 (0.996 to 1.173)
Central Memory CD4-CD8- T cell %T cell || id:ebi-ic731-GCST90001565	8	Inverse variance weighted	**0.049**	0.956 (0.913 to 1.000)
	8	Weighted median	0.243	0.966 (0.912 to 1.024)
Hematopoietic Stem Cell Absolute Count || id:ebi-ic731-GCST90001514	12	Inverse variance weighted	**0.038**	1.043 (1.002 to 1.086)
	12	Weighted median	0.456	1.021 (0.967 to 1.077)
HLA DR on CD33+ HLA DR+ CD14- || id:ebi-ic731-GCST90002108	18	Weighted median	**0.038**	1.031 (1.002 to 1.061)
	18	Inverse variance weighted	0.205	1.014 (0.992 to 1.037)
IgD- CD38- B cell %B cell || id:ebi-ic731-GCST90001445	8	Inverse variance weighted	**0.045**	0.907 (0.825 to 0.998)
	8	Weighted median	0.336	0.943 (0.836 to 1.063)
SSC-A on HLA DR+ Natural Killer || id:ebi-ic731-GCST90002077	20	Weighted median	**0.040**	0.964 (0.931 to 0.998)
	20	Inverse variance weighted	**0.046**	0.974 (0.948 to 1.000)
SSC-A on monocyte || id:ebi-ic731-GCST90002073	15	Weighted median	**0.011**	0.948 (0.910 to 0.988)
	15	Inverse variance weighted	0.105	0.974 (0.943 to 1.006)

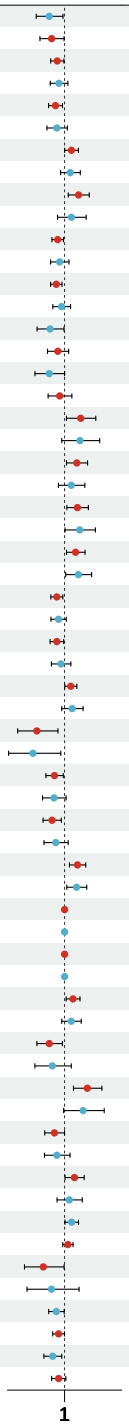

Fig. 1. Forest plot of MR results for different immune cells and autism spectrum disorder.

(Fig 1). Reverse MR analysis found no significant bidirectional association ($p > 0.05$), supporting forward causality.

Causal effects of Inflammatory factor on AD

A comprehensive analysis employing the inverse variance weighting method has revealed a significant association between a series of inflammatory factor and ASD likelihood. This association was deemed to be statistically significant at a p-value less than 0.05. Genetically predicted lower Artemin levels showed increased ASD risk (OR = 0.861, 95% CI: 0.746–0.992, $p = 0.039$), consistent with neuroprotective Artemin signaling in central nervous system development. Conversely, higher levels of the Fms-related tyrosine kinase 3 ligand (FLT3L) were associated with an increased risk of autism spectrum disorder (ASD) (OR = 1.108, 95% CI=1.024–1.199, $p = 0.011$). Similarly,

exposure	nsnp	method	pval	OR(95% CI)
Artemin levels || id:ebi-IPs91-GCST90274760	19	Inverse variance weighted	**0.039**	0.861 (0.746 to 0.992)
	19	Weighted median	0.057	0.859 (0.735 to 1.005)
C-X-C motif chemokine 10 levels || id:ebi-IPs91-GCST90274780	26	Weighted median	0.055	1.119 (0.997 to 1.256)
	26	Inverse variance weighted	0.472	1.042 (0.932 to 1.165)
C-X-C motif chemokine 9 levels || id:ebi-IPs91-GCST90274784	32	Inverse variance weighted	0.633	0.980 (0.901 to 1.065)
	32	Weighted median	0.967	0.997 (0.880 to 1.130)
Delta and Notch-like epidermal growth factor-related receptor levels || id:ebi-IPs91-GCST90274785	22	Inverse variance weighted	0.863	1.009 (0.909 to 1.121)
	22	Weighted median	0.938	0.994 (0.860 to 1.149)
Eukaryotic translation initiation factor 4E-binding protein 1 levels || id:ebi-IPs91-GCST90274758	12	Weighted median	**0.011**	0.810 (0.689 to 0.953)
	12	Inverse variance weighted	0.131	0.904 (0.793 to 1.031)
Fibroblast growth factor 23 levels || id:ebi-IPs91-GCST90274789	22	Inverse variance weighted	**0.012**	0.868 (0.778 to 0.970)
	22	Weighted median	0.057	0.861 (0.738 to 1.005)
Fms-related tyrosine kinase 3 ligand levels || id:ebi-IPs91-GCST90274791	35	Inverse variance weighted	**0.011**	1.108 (1.024 to 1.199)
	35	Weighted median	0.152	1.086 (0.970 to 1.217)
Glial cell line-derived neurotrophic factor levels || id:ebi-IPs91-GCST90274792	14	Weighted median	**0.011**	1.161 (1.034 to 1.303)
	14	Inverse variance weighted	0.153	1.098 (0.966 to 1.249)
Interleukin-2 levels || id:ebi-IPs91-GCST90274806	16	Inverse variance weighted	**0.006**	0.850 (0.757 to 0.955)
	16	Weighted median	**0.038**	0.842 (0.715 to 0.991)
Interleukin-2 receptor subunit beta levels || id:ebi-IPs91-GCST90274811	16	Inverse variance weighted	**0.007**	0.857 (0.766 to 0.959)
	16	Weighted median	0.128	0.890 (0.765 to 1.034)
Monocyte chemoattractant protein 2 levels || id:ebi-IPs91-GCST90274822	33	Inverse variance weighted	0.654	0.992 (0.957 to 1.028)
	33	Weighted median	0.856	0.996 (0.956 to 1.039)
Monocyte chemoattractant protein-4 levels || id:ebi-IPs91-GCST90274824	24	Inverse variance weighted	**0.008**	1.097 (1.025 to 1.174)
	24	Weighted median	**0.012**	1.126 (1.027 to 1.235)
Natural killer cell receptor 2B4 levels || id:ebi-IPs91-GCST90274771	24	Inverse variance weighted	**0.003**	1.132 (1.042 to 1.230)
	24	Weighted median	**0.024**	1.143 (1.017 to 1.283)
Neurturin levels || id:ebi-IPs91-GCST90274828	17	Weighted median	0.552	0.954 (0.816 to 1.115)
	17	Inverse variance weighted	0.829	0.986 (0.870 to 1.118)
Sulfotransferase 1A1 levels || id:ebi-IPs91-GCST90274836	27	Inverse variance weighted	**0.039**	1.074 (1.003 to 1.150)
	27	Weighted median	0.247	1.061 (0.960 to 1.172)
T-cell surface glycoprotein CD5 levels || id:ebi-IPs91-GCST90274773	19	Inverse variance weighted	**0.012**	1.137 (1.028 to 1.257)
	19	Weighted median	**0.036**	1.167 (1.010 to 1.347)
Thymic stromal lymphopoietin levels || id:ebi-IPs91-GCST90274845	17	Weighted median	**0.042**	0.856 (0.736 to 0.995)
	17	Inverse variance weighted	0.177	0.909 (0.792 to 1.044)
Tumor necrosis factor ligand superfamily member 14 levels || id:ebi-IPs91-GCST90274842	33	Inverse variance weighted	**0.012**	0.916 (0.855 to 0.981)
	33	Weighted median	0.189	0.934 (0.844 to 1.034)

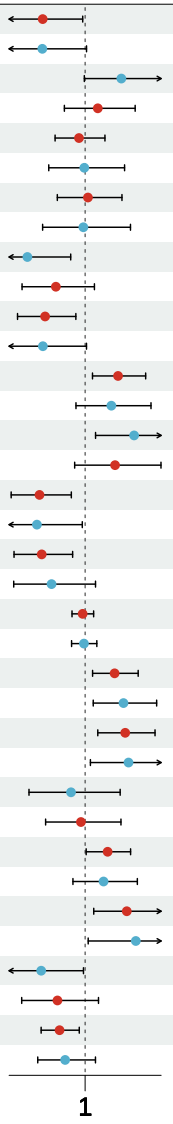

Fig. 2. Forest plot of MR results for different inflammatory factors and ASD.

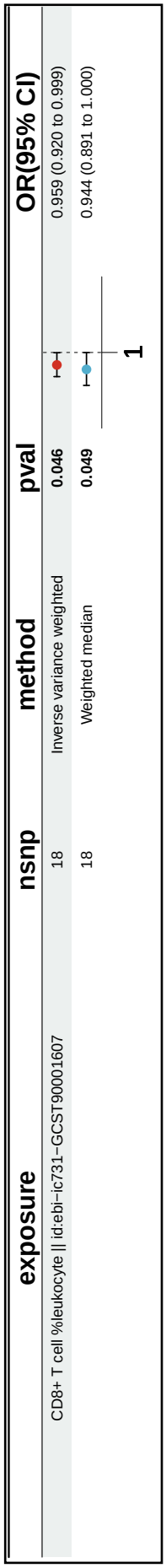

Fig. 3. Forest plot of MR results for inflammatory factors and immune cells.

elevated levels of the natural killer cell receptor 2B4 (2B4) were associated with a higher risk of ASD (OR = 1.132, 95% CI = 1.042–1.230, p = 0.003). Study findings indicate that abnormalities in immunomodulatory factors may be involved in the etiology of the disease. Elevated MCP-4 and SULT1A1 levels showed positive association with ASD risk (OR=1.097, 95% CI: 1.025–1.174, p = 0.008; SULT1A1: OR = 1.07, 95% CI: 1.01–1.15, p = 0.039). Reduced levels of fibroblast growth factor 23 (FGF23) was tied to greater ASD incidence, respectively (OR = 0.868, 95% CI = 0.778-0.970, p = 0.012). The results of the study indicated that decreased levels of IL-2 and its receptor beta subunit (IL-2Rβ) were significantly associated with a reduced likelihood of developing ASD (OR = 0.850, 95% CI= 0.757-0.955, p = 0.006; OR = 0.857, 95% CI = 0.766-0.959). Furthermore, the negative correlation between tumor necrosis factor superfamily member 14 (TNFSF14) (OR = 0.916, 95% CI = 0.855-0.981, p = 0.012) lends further support to the hypothesis that immune pathways may affect the etiology of ASD. These results suggest that abnormalities in immune regulation and the disturbed metabolism of specific cytokines and chemokines may be important molecular mechanisms in the pathogenesis of ASD. (Fig 2).

Mediation analysis revealed an inverse association between $CD8^+$ T cell %leukocyte and NK cell receptor 2B4 levels (OR = 0.959, 95% CI: 0.920-0.999, p = 0.046) (Fig 3). This finding is consistent with immune cell-mediated regulation of NK function; however, GWAS cannot establish mechanistic pathways. Sensitivity analyses (Cochran's Q heterogeneity, MR-Egger intercept test, MR-PRESSO) were performed. All p-values > 0.05, indicating minimal heterogeneity and no evidence of directional horizontal pleiotropy. However, residual pleiotropy cannot be completely excluded.Reverse MR (IVW method) found no significant causal association of ASD with immune cell phenotypes (731 traits) or inflammatory factors (48 cytokines), p > 0.05. This excludes substantial reverse causality for the tested phenotypes.

DISCUSSION

This mediation Mendelian randomization study examined causal pathways linking immune cells and inflammatory factors to ASD. We tested three hypotheses: (1) immune cell effects on ASD, (2) inflammatory factor mediation, and (3) bidirectionality via reverse MR.The results of the study showed that CD8+ T cell %leukocyte, CCR2 on CD62L+ myeloid Dendritic Cell, CD45 on Immature Myeloid-Derived Suppressor Cells and ASD risk were significantly correlated. Artemin levels were reduced, elevated levels of FLT3L and 2B4 were significantly associated with the risk of ASD, suggesting that abnormalities in immunomodulatory factors may be one of the key pathogenic mechanisms. Reverse MR suggests potential bidirectional signaling between ASD and NK cell receptor 2B4 (OR = 1.132, 95% CI = 1.042–1.230, p = 0.003, forward direction; reverse p > 0.05). However, incomplete reverse testing (reverse MR conducted only for subset of significant immune traits) limits conclusions regarding true bidirectionality.

Several studies have shown that immune system function is altered in people with ASD (Szabo *et al.* 2024; Li *et al.* 2025). Total monocyte counts, as well as levels of both the classical (CD14++/CD16-) and non-classical (CD14+/CD16++) subpopulations, are significantly higher in children with ASD. These levels correlate with ASD risk and symptom severity (Breece *et al.* 2025; Li *et al.* 2023). Mutations in the ASD risk gene CHD8 can directly affect the stability and function of regulatory T cells (Tregs), resulting in an autoimmune predisposition (Yang *et al.* 2025). Additionally, an imbalance in the Th1/Th17 cell ratio, abnormal distribution of cytotoxic CD8+ T cells and increased γδ T cells in ASD suggest an overactive and unregulated T cell immune response (Davis *et al.* 2025; Zhang *et al.* 2023; Kim *et al.* 2022). Additionally, studies have confirmed that patients with ASD are accompanied by inflammatory responses that affect the changes of immune-related genes and proteins in the body. The reduction of cytokinin-induced gene 6 (MIG-6) is associated with the severity of ASD (Russo, 2014). MIG-6 downregulates the EGFR and MET receptor tyrosine kinases, and the activation of the latter is related to the development and repair of nerve cells (Xu & Li, 2021). Animal experiments, autopsy studies, and clinical evidence all support neuroinflammation as an important pathogenic mechanism of ASD, mainly manifested as the brain glia activation and the increase of inflammatory factors in brain tissue, cerebrospinal

fluid, peripheral blood, etc. A retrospective case-control study included 331 patients with ASD and 698 healthy controls. The results showed that inflammatory cytokines in the amniotic fluid of ASD patients were significantly increased (Abdallah *et al.* 2013). Intraperitoneal injection of lipopolysaccharide (LPS) during pregnancy in rats mimics maternal viral infection or bacterial infection during pregnancy, and ASD model rats are constructed through maternal immune activation and inflammatory cytokines in brain tissue and peripheral blood are found to increase (Boksa, 2010; Knuesel *et al.* 2014). The meta-analysis included 61 studies and analyzed 76 inflammatory cytokines. The results showed that pro-inflammatory cytokines in the peripheral blood of ASD patients were significantly increased while anti-inflammatory cytokines were relatively decreased (Zhao *et al.* 2021). Elevated cytokines/chemokines were observed in ASD patients compared to matched controls (Breece *et al.* 2025). Observational, experimental, and genetic evidence supports immune dysregulation as a significant etiological contributor to ASD. However, prospective birth cohort studies and functional validation are required before immune markers can inform clinical prediction tools or therapeutic targets (Suzuki *et al.* 2011; Chen *et al.* 2025; Breece *et al.* 2025).

In summary, our findings suggest that immune cells may stimulate the secretion of cytokines, thereby disrupting the immune microenvironment in the ASD brain. This finding highlights the significance of immune function in the development of ASD. It also provides a foundation for future ASD research and therapeutic development.

The present study also has some limitations: (1) although some MR results showed a potential causality, whether immune cells and/or inflammatory factors are actually relevant to ASD needs to be clarified by further studies and confirmed clinically or in animal studies; (2) as the population used was European, there may also be limitations in generalizing the results to other ethnic groups. Further follow-up studies may be needed to clarify this. Further studies with larger samples are needed to confirm these results.

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AUTHOR CONTRIBUTIONS

Designed the study: Xueying Zhou, Fangjie Shang, Deyi Xu; Performed data analysis and data interpretation: Fangjie Shang, Xueying Zhou; Results: Xueying Zhou, Fangjie Shang; (Methods) Critical evaluation: Xueying Zhou, Fangjie Shang, Deyi Xu; Supervision and revising the manuscript: Xueying Zhou, Deyi Xu. All authors contributed to the writing and editing of the manuscript.

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