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$CD28^+$ $CD45RA^-$ CD8br AC mediated the effects of Interleukin- 6 on Alzheimer's disease: A Mendelian Randomization Study

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Abstract

Background IL-6 has garnered significant attention as a potential factor in AD pathogenesis. The association between peripheral immune cells and IL-6 is evident, yet how peripheral immune cells mediate IL-6's effects on AD remains enigmatic regarding the precise pathophysiological processes. To address these uncertainties, we employed genetic evidence to investigate their impact. Our current study explores further the intricate relationship between IL-6, peripheral immune cells, and AD by using extensive publicly available genetic data, aiming to provide novel insights into this critical area of medical research.

Methods The relevant data regarding IL-6, 731 peripheral immune cells, and AD were screened and retrieved from the GWAS database. Subsequently, we predominantly utilized the IVW approach to carry out bi-directional MR analyses between IL-6, 731 peripheral immune cells, and AD. We utilized two-step, two-sample MR analyses to determine three key factors: (*i*) IL-6 exhibits associations with both AD and specific peripheral immune cells, respectively, and there is an absence of inverse causality. (*ii*) Specific peripheral immune cells exhibit associations with AD, and there is an absence of inverse causality. (*iii*) to identify which peripheral immune cells mediate the effects of IL-6 on AD. Then we employed the MVMR approach to verify whether the mediating relationships obtained from the two-step, two-sample MR analyses remained valid. Furthermore, we calculated their respective mediating effects, the combined mediating effects, and the proportions of their mediating effect shares. All of the aforementioned steps were utilized to verify the reliability of causality employing sensitivity analysis, heterogeneity analysis, and horizontal pleiotropy analysis.

Results Our findings indicate a significant correlation between increased IL-6 levels and a reduced risk of AD ($P = 0.009$, OR = 0.941, 95%CI = 0.899- 0.985), along with elevated levels of $CD28^+$ $CD45RA^-$ CD8br AC ($P = 0.007$, OR = 1.159, 95%CI = 1.007- 1.333). Also indicates a significant correlation between increased $CD28^+$ $CD45RA^-$ CD8br AC ($P = 0.005$, OR = 0.983, 95%CI = 0.971- 0.995) levels and a reduced risk of AD. Therefore, through MVMR analysis, the effect of IL-6 on AD increased from -0.061 to -0.046 (95% CI: -0.090, -0.002) after genetic adjustment for $CD28^+$ $CD45RA^-$ CD8br AC.

Conclusions Increased $CD28^+$ $CD45RA^-$ CD8br AC levels appear to partially mediate the effect of IL-6 on reducing AD risk.

Keywords Interleukin- 6, Peripheral immune cells, Alzheimer's disease, Mendelian randomization, Genome-wide association study

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Introduction

Alzheimer's disease (AD) is a specific disorder that's characterized by age-related cognitive and functional decline, ultimately resulting in death [1]. The estimated number of people with dementia caused by all possible reasons is expected to rise from 50 million in 2010 to 113 million worldwide by 2050 [2]. The pathology of AD is characterized by inflammatory response [3–6], oxidative stress [7], synaptic loss [8], abnormal mitochondrial structure and function [9], dysregulation of microRNAs [10], neurodegeneration [11], and neurofibrillary tangles [12]. Accumulation of beta-amyloid [13] and phosphorylated tau protein [14] accumulation are also hallmarks of AD pathology.

Interleukin- 6 (IL- 6) has garnered significant attention in recent years among researchers studying AD, emerging as one of the most debated inflammatory proteins. This interest in IL- 6 stems from its potential role in the pathogenesis of AD, where it is believed to play a crucial role in the inflammation process that leads to neuronal death and cognitive decline [15–17]. Laboratory-based investigations have ascertained that the levels of IL- 6 among AD patients exceed those of healthy individuals [4, 6, 18, 19] and display a positive correlation with the ratio of amyloid-beta 42 to amyloid-beta 40 (Aβ42/Aβ40), phosphorylated tau 181 (p-tau181), as well as Neurofilament Light (NfLight) [20]. Nonetheless, certain studies have put forward that IL- 6 is negatively correlated with Aβ40, Aβ42, and total tau [20]. Consequently, the precise role that IL- 6 assumes within the pathophysiological framework of AD remains unsettled. Furthermore, given its status as a pro-inflammatory factor, the function of IL- 6 in peripheral immune cells is obvious and needs no elaboration. However, the infiltration of immune cells from the periphery into the brain is a notable feature of aging and various neurodegenerative diseases, including AD [21]. Some findings [22–24] collectively indicate that systemic inflammation of the peripheral immune system may exacerbate neurodegenerative progression by inducing persistent chronic neuroinflammation by breaching the blood–brain barrier by inflammatory mediators. Under non-autoimmune conditions, changes can occur in the immune environment, leading to significant modifications in the innate and adaptive immune systems within the brain parenchyma of individuals with AD due to local environmental alterations and the infiltration of immune cells from the blood and boundary regions [25]. The central and peripheral immune systems play a crucial role in clearing the Aβ peptide, and any immune dysfunction can lead to AD development [26, 27].

Genetic factors are essential in AD development, accounting for approximately 70% of the risk. Multiple large-scale genome-wide association studies (GWASs) and meta-analyses have identified over 40 genetic risk loci associated with AD [28–30]. Consequently, we opt to employ the Mendelian randomization (MR) approach to dissect the causal relationship between IL- 6 and AD at the genetic level, and to investigate the role of peripheral immune cells within this process. MR utilizes gene variations (such as single nucleotide polymorphisms (SNPs)) that follow Mendelian laws of inheritance as instrumental variables (IVs) which are characterized by randomness and stability and are not affected by various postnatal factors. Similar to natural randomized controlled trials, it can overcome the biases of traditional observational studies and is applicable in scenarios where conducting real randomized controlled trials is difficult. Moreover, it effectively reduces confounding bias to make causal inference conclusions more reliable. The analysis principle includes selecting IVs that meet the conditions of correlation, independence, and exclusivity, applying the two-stage least squares method to build a model for analyzing the causal relationships between them and the exposure and outcome variables, and conducting pleiotropy and sensitivity analyses to correct related issues and ensure the robustness of results [31].

Method

Study design

To explore the interplay between IL- 6, 731 peripheral immune cells, and AD, we utilized a two-step, two-sample MR approach. We obtained summary statistics, including effect estimates and standard errors, for the relationships between the exposure and outcome from separate datasets. We conducted an MR analysis using five different approaches and sensitivity analyses. Two-sample MR utilizes distinct datasets to assess the gene-risk factor and gene-outcome relationships. We utilized two-step, two-sample MR analyses to determine three key factors: (*i*) IL- 6 exhibits associations with both AD and specific peripheral immune cells ($P < 0.05$), respectively, and there is an absence of inverse causality ($P > 0.05$). (*ii*) Specific peripheral immune cells exhibit associations with AD ($P < 0.05$), and there is an absence of inverse causality ($P > 0.05$). (*iii*) to identify which specific peripheral immune cells mediate the effects of IL- 6 on AD. Then we employed the Multivariable Mendelian Randomization (MVMR) approach to verify whether the mediating relationships obtained from the two-step, two-sample MR analyses remained valid. Furthermore, we calculated their respective mediating effects, the combined mediating effects, and the proportions of their mediating effect shares. All of the aforementioned steps were utilized to verify the reliability of causality employing sensitivity analysis, heterogeneity analysis,

and horizontal pleiotropy analysis. Three hypotheses should be carefully considered when designing MR [31]: (*i*) genetic variation is strongly associated with the exposure of interest, (*ii*) genetic variation is not associated with potential risk factors for the outcome at a significant level of $P < 10^{-5}$, and (*iii*) genetic variation only affects the outcome through the exposure. A graphical representation of these MR analyses is provided in Fig. 1.

Data sources for IL- 6, 731 peripheral immune cells, and AD patients

We selected SNPs that serve as proxies for IL- 6, 731 peripheral immune cells, and AD from separate datasets. All the data mentioned below can be accessed through the website: http://ftp.ebi.ac.uk/pub/databases/gwas/summary_statistics/.

Genetic IVs for IL- 6: Full per-protein GWAS summary statistics were used to analyze IL- 6 measured using the Olink Target platform in 11 cohorts with a total of 14,824 European participants. The study is carried out using methods such as genomic analysis (performing genome-wide pqtl mapping on 91 plasma proteins and conducting a meta-analysis) and multi-omics data integration (combining pqtl data with expression quantity data). 180 pQTLs (59 cis, 121 trans) were identified for download (https://www.phpc.cam.uk/ceu/proteins), and the EBI GWAS Catalog was used (accession numbers GCST90274781). The specific eligibility criteria for participants, as well as the sources and methods of their selection, have not been described in detail, and it has not been indicated whether any power or sample size calculations have been carried out. Additionally, when validating the results, data from 1,585 participants in the ARISTOTLE cohort were used; in another validation, data from 35,556 Icelanders from the deCODE study were utilized. The data on IL- 6 is derived from a study published in 2023 by Zhao JH et al. [32]. To extract IL- 6 IVs, we used the suggested significance level $P < 10^{-6}$ as only a few inflammatory protein loci identified by GWAS reached genome-wide significance levels. Additionally, linkage disequilibrium testing, MR-PRESSO testing, and *F*-statistic validation were conducted on 25 SNPs.

Potential mediators: The data on peripheral immune cells examined 731 peripheral immune cell features. The GWAS Catalog provides publicly available GWAS summary statistics for each immune trait, with accession numbers ranging from GCST90001391 to GCST90002121. The data on 731 peripheral immune cells are derived from a study published in 2022 by Orru V et al. [33]. The participants were from the general population of the central-east coast of Sardinia, Italy. A total of 6,602 individuals from the general population were genotyped in the study, and 3,757 of them had immune profiling. These 3,757 individuals constituted the main sample group used to assess 731 immune cell traits. The participants were between 18 and 102 years old, the specific selection method was not described in detail, and all signed informed consent forms for the study protocols approved by the Sardinian Regional Ethics Committee (protocol no. 2171/CE). The article did not mention whether power or sample size calculations were performed prior to the main analysis. This comprehensive analysis encompasses a total of 731 immunophenotypes, including absolute cell (AC) counts ($n = 118$), median fluorescence intensities (MFI) representing surface antigen levels ($n = 389$), morphological parameters (MP) ($n = 32$), and relative cell (RC) counts ($n = 192$). Specifically, the features of MFI, AC, and RC encompassed a wide range of immune cell types, including B cells, CDCs, mature stages of T cells, monocytes, myeloid cells, TBNK (T cells, B cells, natural killer cells), and Treg panels. On the other hand, the morphological parameters (MP) feature focused on CDCs and TBNK panels. The initial GWAS, focused on immune traits, was executed utilizing data from 3,757 unrelated European individuals. To ensure unbiased results, there was no overlap among the study cohorts. Approximately 22 million SNPs were genotyped with high-density arrays and credited with a Sardinian sequence-based reference panel [34]. The statistical analysis controlled for variables like sex, age, and age [2] when assessing the associations. We employed a suggested significance level of $P < 10^{-6}$ to identify specific peripheral immune cell IVs associated with AD. Additionally, we conducted linkage disequilibrium testing, MR-PRESSO testing, and *F*-statistic validation on multiple SNPs ranging from 10 to 17.

Outcome of the study: We obtained summary statistics for the AD association from the original GWAS on AD, which included 111,326 clinically diagnosed/'proxy'AD cases and 677,663 controls, all of European descent. These data are available for download from the EBI GWAS Catalog with the accession number GCST90027158. Sources are the EADB collecting data from 15 European countries and the UKBB providing proxy AD data. The method is likely through collaborations with various institutions, hospitals, and communities following certain inclusion and exclusion criteria. The proxy-AD designation is based on questionnaire data in which individuals are asked whether their parents had dementia. Thus, we will refer to these cases as proxy AD and related dementia (proxy-AD). This method has been used successfully in the past [35] but is less specific than a clinical or pathological AD diagnosis. Clinically-diagnosed cases are presumed to meet international diagnostic criteria for AD and related dementias. A total of 111,326 clinically-diagnosed/proxy AD cases and 677,663

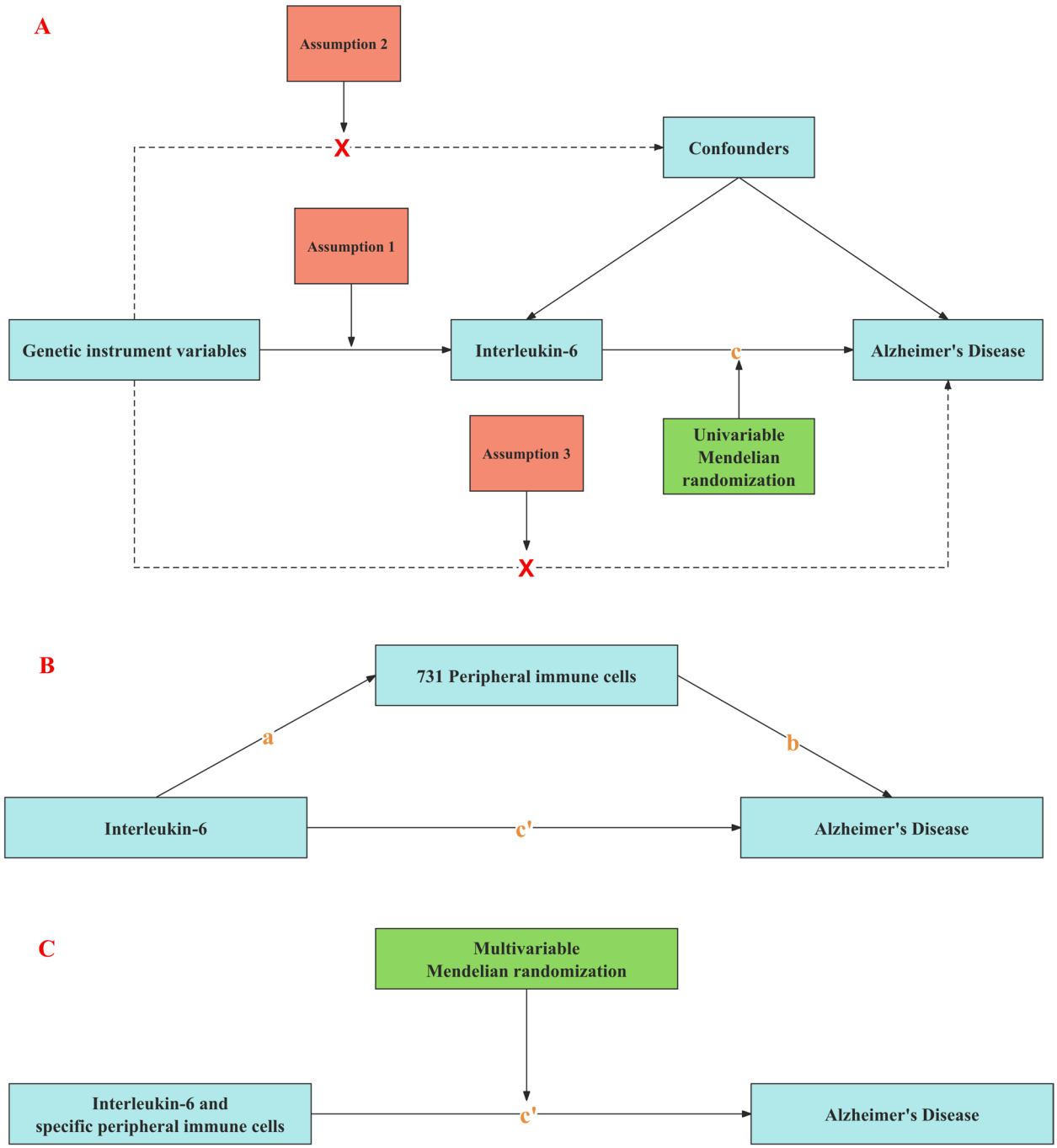

Fig. 1 Explanation of study design and workflow. A: The total effect of IL- 6 on AD, c, was derived using Two-sample Mendelian randomization (i.e., genetically predicted IL- 6 as exposure and AD as outcome). Assumption 1: genetic variation is strongly correlated with the exposure of interest. Assumption 2: genetic variation is not associated with potential risk factors for the outcome ($P < 10^{-5}$). Assumption 3: genetic variation influences only the outcome through exposure. B: The total effect was divided into two components: (*i*) an indirect effect determined through a two-step process (where'a'represents the overall influence of IL- 6 on peripheral immune cells, and'b'represents the impact of peripheral immune cells on AD, adjusted for IL- 6), calculated using the product method (a*b), and (*ii*) a direct effect (c'—a*b). C: For the mediation of specific peripheral immune cells combined, the indirect effect was derived using the difference method (c—c'). The proportion mediated was then calculated by dividing the indirect effect by the total effect

controls. The first stage has 39,106 clinically-diagnosed AD cases, 46,828 proxy ADD cases, and 401,577 controls; the second stage has 25,392 AD cases and 276,086 controls. The data on AD is derived from a study published in 2022 by Bellenguez C et al. [36]. The original literature does not mention whether power or sample-size calculations were done before the main analysis.

Statistical analysis

We reassessed the data obtained from the EBI GWAS catalog to guarantee the precision of the outcomes. We then established $R^2 < 0.001$ and clustering distance = 10,000 kb to guarantee no linkage disequilibrium between the genetic tools. Palindromic SNPs and SNPs that were absent from the outcomes were subsequently eliminated from the IVs. As a measure of the strength of the association between genetic markers and phenotypic expressions, we present the proportion of variation in IL- 6 and other potential mediators explained by their respective genetic instruments, as well as the *F*-statistic for the regression of IL- 6 and these mediators on their genetic instruments. We generated *F* and R^2 values for each SNP to assess the impact of exposure using the following formula: $F = [R^2 \times (N- 2)]/(1-R^2)$, $R^2 = [2 \times \beta^2 \times EAF \times (1-EAF)]/[2 \times \beta^2 \times EAF \times (1-EAF) + 2 \times SE^2 \times N \times EAF \times (1-EAF)]$. N and EAF denote sample size and effector allele frequency, respectively, whereas β and SE denote the estimated impact size and standard error of SNPs on exposure. SNPs with *F*-statistics less than ten were eliminated because they lacked adequate validity. After MR-PRESSO testing, any SNPs identified as outliers (global test $P < 0.05$) were excluded from the analysis [37]. Subsequently, a leave-one-out sensitivity analysis was conducted by sequentially removing each SNP one at a time to evaluate the potential of individual SNPs in driving the association between exposure and outcome. Additionally, the MR-Egger regression test was utilized to distinguish horizontal pleiotropy in MR analysis, highlighting the statistical significance of the intercept term [38]. Lastly, we calculated the Cochran Q statistic to detect heterogeneity, establishing the significance threshold at $P = 0.05$ [39]. It is important to note that if these mediators are interrelated, estimating the proportion of the IL- 6 on AD effect mediated by a group of mediators may be biased. Furthermore, if the outcome affects the mediator (indicating reverse causality) and the instrument impacts the mediators through the outcome, this could introduce additional bias into the estimation. Therefore, we employed the Inverse Variance Weighted (IVW) approach to examine bidirectional causal relationships between IL- 6, potential mediators, and AD. This approach considers the estimates from multiple studies and provides a weighted average based on the inverse variance of each study's estimate. Using this approach, we aim to obtain a more robust and precise estimation of these variables'potential bidirectional causal effects. To accurately evaluate the impact of IL- 6 on AD, it is crucial to consider the genetic determinants of possible mediators. We utilized the IVW approach, adjusting for SNP-potential mediator effects [40]. Subsequently, we used the False Discovery Rate (FDR) correction to adjust the *P*-values and prevent the occurrence of false positives. We visualized the results for each positive survival outcome, such as scatter plots, funnel plots, forest plots, and leave-one-out plots.

Ultimately, we use MVMR to verify the mediating relationship from two-step MR. We construct a regression equation considering instrumental variables'impact on exposure factors, then incorporate exposure factors into the equation with outcome variables. Using specific algorithms and estimation methods (e.g., instrumental variable estimation), we estimate independent causal effect coefficients while controlling other exposure factors. Given gene pleiotropy complexity with multiple IVs, we apply MVMR-Egger regression to detect and correct it, followed by sensitivity analysis to ensure reliable results by observing result stability via altering parameters and including/excluding data. This advanced statistical technique allows us to assess the degree to which any potential mediators mediate the overall genetic effect of IL- 6 on AD risk [31, 41–43]. All the detailed analysis regarding MR can be found in Supplementary Table 4, the"STROBE-MR-checklist".

The complete analysis was conducted using R (version 4.3.2) statistical software developed by the R Foundation for Statistical Computing. MR analysis was implemented through the'TwoSampleMR'and'MVMR'packages.

Results

Effects of IL- 6 on 731 Peripheral immune cells and AD

Table 1 provides the bi-directional MR results of IL-6, potential mediators, and AD. Our findings indicate a significant correlation between increased IL- 6 levels and a reduced risk of AD ($P = 0.009$, OR = 0.941, 95%CI = 0.899- 0.985), along with elevated levels of $CD28^+$ $CD45RA^-$ CD8br AC ($P = 0.005$, OR = 1.159, 95%CI = 1.007- 1.333), detailed in Table 2. The sensitivity analyses demonstrated robust results. We compared the mr_pleiotropy_test function with the mr_presso function to ensure accurate and focused research outcomes. The MR-PRESSO global test evaluates the overall horizontal pleiotropy among all IVs in a single MR test. It compares the observed distance of all variants to the regression line (residual sum of squares) with the expected distance based on the null hypothesis of no horizontal pleiotropy [37]. The intercept represents the

Table 1 Results of bi-directional MR for IL- 6, peripheral immune cells and AD

Outcome	Exposure		
	IL- 6	$CD28^+$ $CD45RA^-$ CD8br AC	AD
IL- 6	/	0.001(0.010)	0.007(0.020)
$CD28^+CD45RA^-$ CD8br AC	0.147(0.071)*	/	− 0.043(0.047)
AD	− 0.061(0.023)**	− 0.017(0.006)**	/

All results are from two-sample MR utilizing the IVW approach and are presented in the format of β (SE).

β and SE denote the estimated impact size and standard error of exposure on outcome. AC: Absolute Count. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

average pleiotropic effect across the genetic variants, which is the average direct effect of a variant on the outcome. If the intercept differs from zero, as indicated by the MR-Egger test, it provides evidence of directional pleiotropy [44]. Both the MR-Egger intercept test and the global test of MR-PRESSO were utilized to detect the presence of pleiotropy; if any of the functions mentioned above exhibit a *P*-value that is not statistically significant at the 0.05 level, it is reasonable to postulate the absence of horizontal pleiotropy [45, 46].

None of the approaches supported a causal effect of IL- 6 on the rest of the peripheral immune cells, as detailed in Supplementary Table 1. Furthermore, we conducted a weighted median analysis and demonstrated the directionality of the lesions using the IVW method in Fig. 2. In addition, we visualized the MR results. There is the scatter plot (Supplementary Fig. 1), funnel plot (Supplementary Fig. 2), forest plot (Supplementary Fig. 3), and leave-one-out plot (Supplementary Fig. 4) regarding IL- 6 and AD. There is the scatter plot (Supplementary Fig. 5), funnel plot (Supplementary Fig. 6), forest plot (Supplementary Fig. 7), and leave-one-out plot (Supplementary Fig. 8) regarding IL- 6 and $CD28^+$ $CD45RA^-$ CD8br AC. All the MR analyses regarding IL- 6 and peripheral immune cells are detailed in Supplementary Table 1. All the SNPs used for IL- 6 are shown in Supplementary Table 2.

Effects of potential mediators on AD

Our findings indicate a significant correlation between increased $CD28^+$ $CD45RA^-$ CD8br AC ($P = 0.005$, OR = 0.983, 95%CI = 0.971- 0.995) levels and a reduced risk of AD, detailed in Table 3. The bidirectional MR results of potential mediators and AD can be obtained in Table 1. Furthermore, we conducted a weighted median analysis and demonstrated the directionality of the lesions using the IVW method in Fig. 3. There is the scatter plot (Supplementary Fig. 9), funnel plot (Supplementary Fig. 10), forest plot (Supplementary Fig. 11), and leave-one-out plot (Supplementary Fig. 12) regarding $CD28^+$ $CD45RA^-$ CD8br AC and AD.

Mediating effects of mediators on IL- 6—AD effects

To mitigate the risk of false-positive results, we employed the FDR correction for all positive findings. All the FDR-corrected results are presented in Supplementary Table 3. We undertook a rigorous exploration of potential mediators, supported by causal evidence from MR, that were influenced by IL- 6 (step 1) and subsequently impacted AD (step 2). This comprehensive analysis aimed to identify critical mediators that played a significant role in the relationship between IL- 6 and AD. Our findings suggest that $CD28^+$ $CD45RA^-$ CD8br AC is crucial mediators that contribute significantly to the mediation of IL- 6 on AD, as detailed in Table 4.

Discussion

IL- 6 is considered an inflammatory protein in previous research with multiple roles and is expressed by macrophages, dendritic cells, lymphocytes, endothelial cells, osteoclasts, and hepatocytes, which has a wide range of functions in a variety of physiological processes, including metabolism, aging, development, exercise, angiogenesis, osteoclastogenesis, cell differentiation, trauma, and acute and chronic inflammation. IL- 6 has also been linked to a range of illnesses, including cancer, autoimmune diseases, cognitive dysfunction, and mental health conditions[15–17]. Our research discovered that IL- 6 serves as an upstream protective factor for AD. Some studies support our view that IL- 6 performs neuroprotection during neuroinflammation. Specifically, IL- 6 protects neurons from inflammation-induced poptosis [47]. It does so by inhibiting cleaved caspase- 3 expression in neurons and maintaining intracellular Ca^{2+} homeostasis [20]. Adding IL- 6—enriched solutions to inflammation—stimulated neurons significantly reduces the rate of neuronal apoptosis. Conversely, treating with anti-IL- 6 tralizing antibodies abolishes this protective effect, leading to a marked increase in neuronal apoptosis rate. Moreover, certain studies have put forward that IL- 6 is negatively correlated with biomarkers of AD (e.g., Aβ40, Aβ42, and total tau20) [20]. Based on the conclusions of the aforementioned research, we speculate that IL- 6 may delay the development of AD through several mechanisms. Primarily, upon binding to its receptors, IL- 6 activates the JAK-STAT pathway, which regulates gene expression, inhibits BACE1 activity or synthesis, and reduces the cleavage of amyloid precursor protein through the β-secretase pathway, thereby decreasing the production of Aβ40 and Aβ42 [48, 49]. In parallel, the

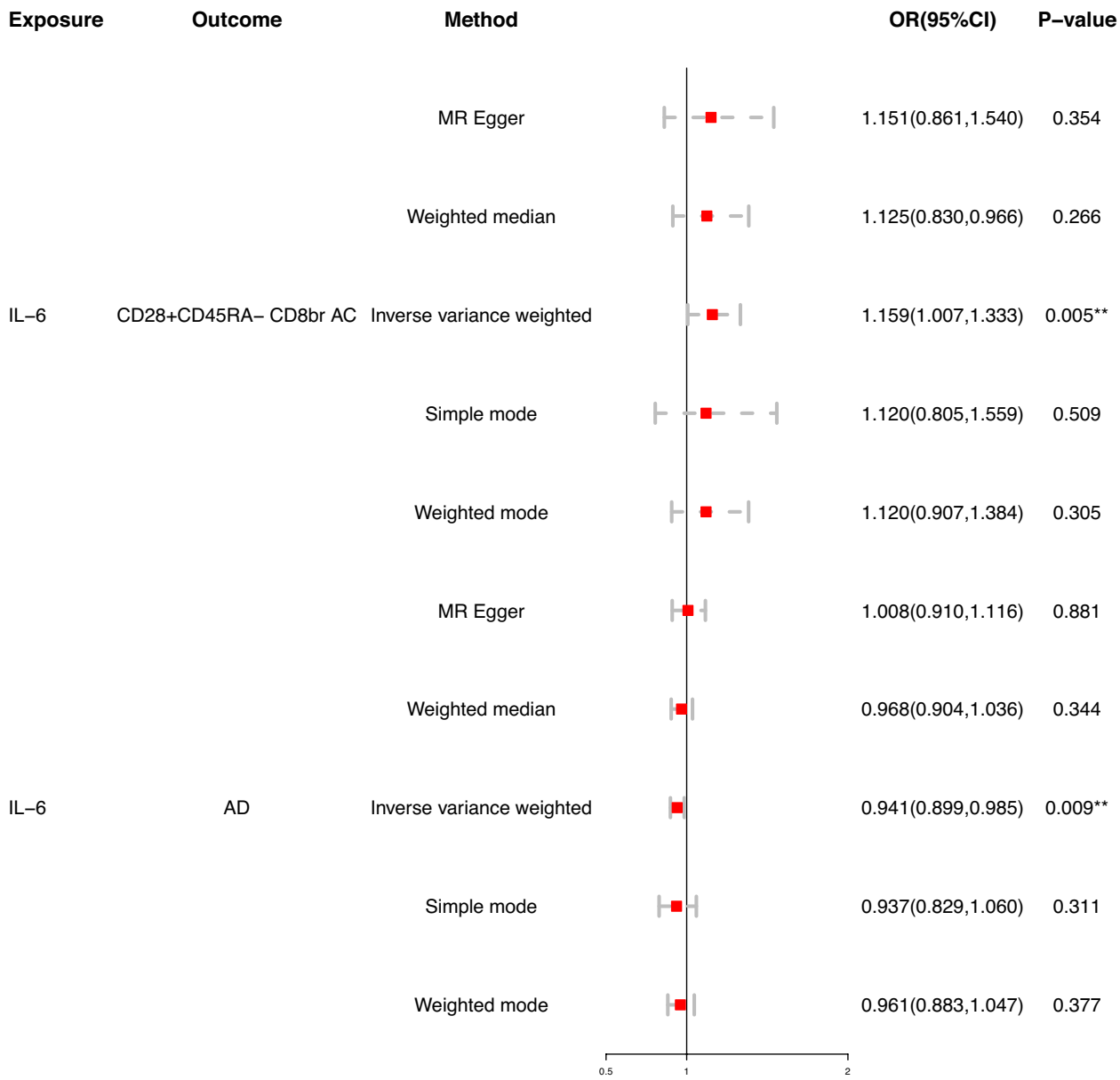

Fig. 2 Forest plot for the causal effect of IL- 6 on potential mediators and AD

Table 2 MR analyses by IVW approach and sensitivity analyses of IL- 6 on potential mediators and AD

Classification		nSNP	IVW			Heterogeneilty		Pleiotropy			MR-PRESSO
Exposure	Outcome		SE	*P* value	OR (95%CI)	Q	*P* value	Egger intercept	SE	*P* value	Global Test *P*-value
IL- 6	$CD28^{+}$ $CD45RA^{-}$ CD8br AC	22	0.071	0.005	1.159 (1.007–1.333)	18.339	0.627	0.001	0.016	0.961	0.657
	AD	22	0.023	0.009	0.941 (0.899–0.985)	20.105	0.515	– 0.008	0.006	0.156	0.529

OR odds ratios, *CI* confidence interval.

Table 3 MR analyses by IVW approach and sensitivity analyses of potential mediators on AD

Classification		nSNP	IVW			Heterogeneilty		Pleiotropy			MR-PRESSO
Exposure	Outcome		SE	*P* value	OR (95%CI)	Q	*P* value	Egger intercept	SE	*P* value	Global Test *P*-value
$CD28^{+}$ $CD45RA^{-}$ CD8br AC	AD	17	0.006	0.005	0.983 (0.971–0.995)	15.148	0.514	0.002	0.004	0.591	0.579

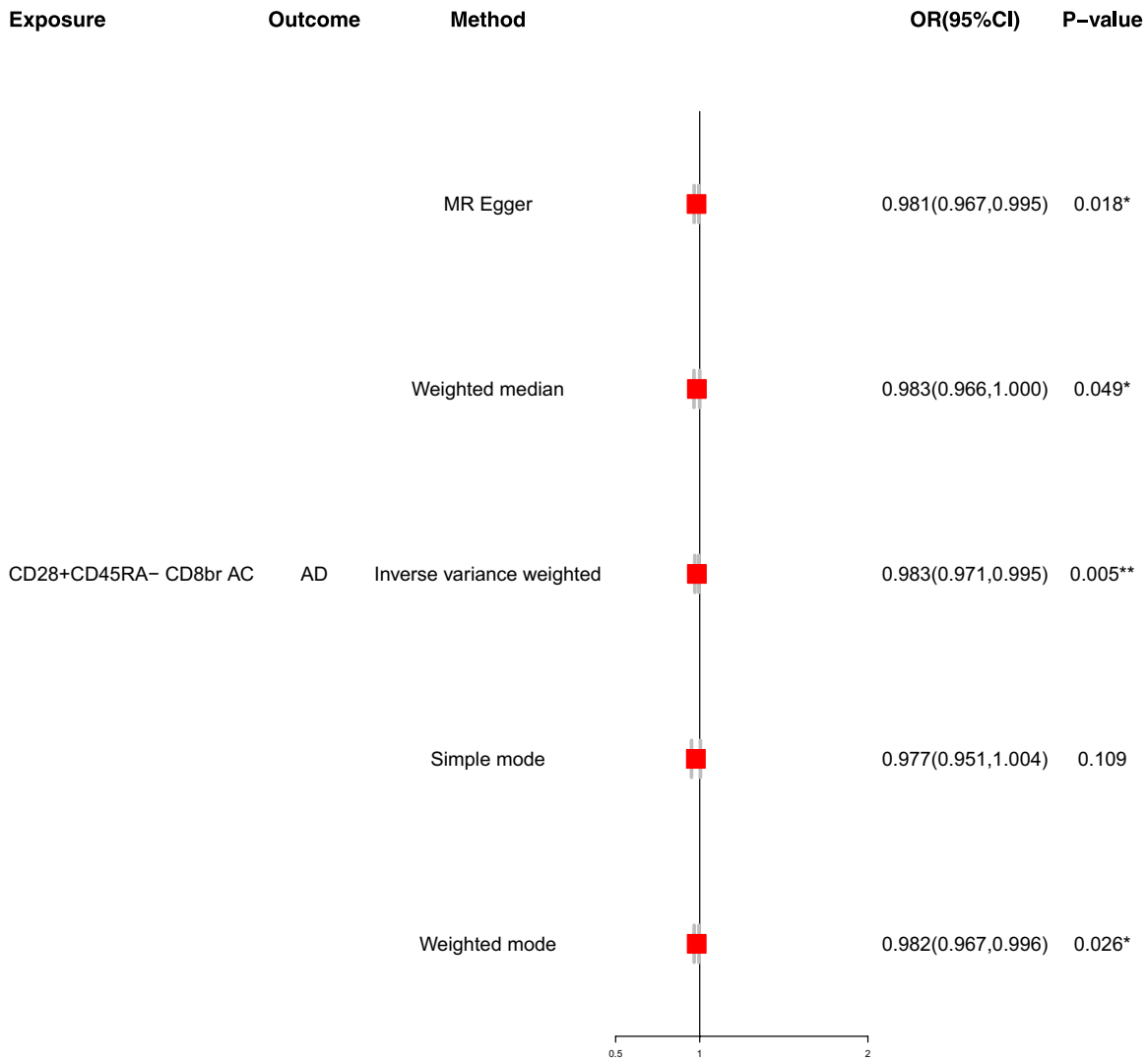

Fig. 3 Forest plot for the causal effect of potential mediators on AD

Table 4 Multivariate separate-sample MR analysis of the effect of IL- 6 on AD

	Method	Estimate (95% CI)	*P*-value	Mediation effect (%)
Univariate Model				
IL- 6	IVW	− 0.061 (− 0.106, − 0.015)	0.009	
Multivariate Model				
Adjusted for $CD28^{+}$ $CD45RA^{-}$ CD8br AC	IVW	− 0.046 (− 0.090, − 0.002)	0.040	24.7

IL- 6 signaling pathway modulates the subunits of the gamma secretase complex, altering its activity and cleavage specificity, and thus reducing the production of Aβ42 [20]. Moreover, IL- 6 activates signaling pathways related to microglia and astrocytes, enhancing their phagocytic and degradative capabilities towards Aβ and reducing Aβ deposition in the brain. Another aspect is that the IL- 6 signaling pathway inhibits the activity of protein kinases such as GSK- 3β and CDK5, which in turn reduces tau protein phosphorylation. Also importantly, IL- 6 upregulates the activity or expression of protein phosphatases like PP2 A, promoting tau protein dephosphorylation and inhibiting its aggregation [50].

Conversely, a controlled trial, through the analysis of serum Aβ42 levels in patients with AD, found that these levels were not associated with IL- 6 levels [51]. Another meta-analysis also concluded that there is insufficient evidence to prove that serum IL- 6 is a specific serum biomarker for AD [52]. It has also been observed that the levels of IL- 6 are elevated in the serum of AD patients

[53, 54], or that IL- 6 is positively correlated with AD biomarkers, such as the Aβ42/Aβ40 ratio, p-tau181, and NfLight [20]. It's important to note that our findings do not contradict previous research. This is because, despite changes in IL- 6 levels (whether increased or decreased), it is not feasible to establish a causal relationship between IL- 6 and AD, given the limitations of the experiments designed in previous studies.

Genes and immune cells linked to innate immunity, particularly those in the peripheral regions, not only play a positive role in AD's neurodegenerative mechanisms but also exhibit pathological effects. These cells can cross the blood–brain barrier, thereby influencing the neurodegenerative processes associated with AD [55]. A comprehensive analysis has revealed characteristic changes in the proportions and gene expression patterns of peripheral immune cell subgroups, including B cells, $CD4^+$ T cells, $CD8^+$ T cells, Hematopoietic Stem Cells, monocytes, and NK cells, among patients with AD compared to healthy individuals, as confirmed by single-cell RNA-seq analysis [1, 56, 57]. Meanwhile accumulating evidence indicates that IL- 6 promotes $CD8^+$ T cell activation, proliferation, and differentiation. It may also indirectly affect neurodegenerative disease development by regulating $CD8^+$ T cell function [58, 59].

$CD28^+$ $CD45RA^-$ CD8br AC refers to a subset of $CD8^+$ T cells that express the CD28 molecule but do not express the CD45RA molecule. It is noteworthy that $CD8^+$ T cells, which are notably elevated in the blood of AD patients [60]. It is generally accepted that $CD8^+$ T cells, which have been linked to cognitive decline and memory impairment in AD patients [61], demonstrate neurotoxicity by inducing significant neuronal death through mechanisms such as Fas ligand, lymphocyte function-associated antigen- 1, and CD40-dependent intercellular contact [62]. Emerging evidence suggests that $CD8^+$ T cells play a crucial role in restricting the pathological progression of AD [63]. Researchers generated TCRα-deficient 5xFAD mice (lacking $CD8^+$ and $CD4^+$ T cells) and 5xFAD mice deficient in B2 m (specifically losing $CD8^+$ T cells). Analysis of these models showed that $CD8^+$ T cell deficiency increases Aβ deposition and impairs cognitive function. In-depth studies on the CXCR6-CXCL16 axis in $CD8^+$ T cell—microglia interactions, using techniques like single-cell RNA sequencing, verified that $CD8^+$ T cells can inhibit microglial pro-inflammatory cytokine expression, attenuating neuroinflammation. In vitro co-culture experiments further confirmed $CD8^+$ T cells'ability to suppress microglial pro-inflammatory activity. Our results are further bolstered by these conclusions.

It has been shown that limiting Treg activity in both the peripheral and central nervous systems accelerates the harmful effects of Aβ-Teffs [19]. The crucial pathogenic process in AD involves the direct involvement of systemic inflammatory responses induced by Aβ-Teff in neuroinflammatory cascades and amyloid deposition [64].Furthermore, research evidence shows that the temporary disruption of immune tolerance through Treg depletion is linked to the removal of amyloid and the restoration of neuroinflammation by recruiting immunoregulatory Treg and monocyte-derived macrophages to the central nervous system. Other studies have shown the neuroprotective potential of polyclonal Treg adoptive transfer or expansion in animal models of Alzheimer's disease [65, 66]. They are indirectly proving our results that the impact of IL- 6 on reducing AD risk was partially mediated by increased levels of $CD28^+$ $CD45RA^-$ CD8br AC from the Treg panel. Certain immune cell types and genetic susceptibility can potentially be biomarkers for AD risk, paving the way for earlier diagnosis and more effective treatment options. Therefore, it is meaningful to investigate the mechanistic link between immune cells and the pathogenesis of AD at the genetic level. Given the complexity of this process, the current research findings require additional evidence to support the observed conclusions firmly. Future studies are warranted to understand better the mechanisms underlying this phenotypic switching and its potential impact on cellular function and disease pathogenesis.

Strengths and limitations

This study possesses several strengths. Firstly, our extensive study employs genetic variants from the most significant sample in GWAS studies to overcome some of the primary constraints of conventional multivariable regression methods in mediation. Horizontal pleiotropy poses a considerable challenge to MR studies, but we utilized various MR techniques with distinct assumptions to investigate its potential impact. We evaluated the consistency among these estimators. The IVW results were validated through sensitivity analyses utilizing MR-PRESSO, leave-one-out, MR-Egger, and Cochran Q tests. The mediators we included in our MR-based mediation analyses exhibited consistent causal effects across all methods for both steps. Before our primary two-step MR analyses, we tested causal relationships between potential mediators, which did not suggest any causal effects. Nevertheless, MR studies cannot definitively rule out a causal relationship between potential mediators. Moreover, using germline genetic variants as IVs for exposure achieved random allocation, eliminating the potential for reverse causation and confounding factors without posing ethical risks.

However, this study also has its limitations. Firstly, our understanding of the distribution of peripheral blood

immune cells in AD patients is mainly based on flow cytometry studies. Nevertheless, immune cells'specific functional states (primarily T and B cells) remain a mystery. Although this study detected 731 features of peripheral immune cells, it mainly focused on a subset, and this subset might not cover all aspects of immune involvement in AD. Such selective attention may overlook other relevant immune mediators. While focusing on specific immune cells can be explored to a certain depth, excluding other potentially relevant cell types might lead to an incomplete understanding of the immune involvement in AD. Future studies should consider conducting a more comprehensive exploration of immune mediators.

Although previous observational studies have already established the associations between specific immune cells and AD, these studies are limited by potential confounding factors, selection bias, and small sample sizes. Moreover, in the GWAS database of AD patients used in this paper, a portion of AD cases were classified as"proxy Alzheimer's disease"based on questionnaire data rather than clinical or pathological diagnoses. This approach may introduce misclassification bias, which may subsequently affect the validity of the study. The use of proxy indicators for AD will bring uncertainty to the accuracy of case identification. Consequently, misclassification may weaken the true associations or create false associations, thus undermining the conclusions drawn from the study.

It is worth noting that among the three cell phenotypes in the conclusions of this study, we focused on the P-values of the IVW method. This indicates that the study results may have limitations. Some possible reasons involve the existence of multicollinearity, which may introduce biases to the IVW method. Although sensitivity analyses have been conducted, the existence of horizontal pleiotropy still cannot be completely ruled out. This may confound the causal inferences among interleukin- 6, immune cells, and Alzheimer's disease. In MR studies, horizontal pleiotropy remains a critical issue because it can lead to biases in causal estimations. Although sensitivity analyses can reduce this risk, the inability to eliminate the pleiotropy effect still calls into question the robustness of causal inferences. More studies are needed to confirm the results, such as expanding the sample size, increasing the number of instrumental variables, and adopting more robust analytical techniques to verify the study results.

Furthermore, this study solely encompassed individuals of European descent, potentially introducing ethnic bias and restricting the generalizability of the findings. Lastly, while this method allows for a preliminary assessment of the causal relationship between IL- 6 mediated by peripheral immune cells and AD, the underlying biological mechanisms between the two still need to be completed. Consequently, further research is imperative to establish a definitive link between them.

Conclusion

This study applies MR method to infer the causal relationship between IL- 6 mediated by peripheral immune cells and AD. Our results support a causal effect of higher IL- 6 on reducing AD risk that is partially mediated by increased levels of $CD28^{+}$ $CD45RA^{-}$ CD8br AC.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12883-025-04194-5.

Supplementary Material 1.
Supplementary Material 2.
Supplementary Material 3.
Supplementary Material 4.
Supplementary Material 5.
Supplementary Material 6.
Supplementary Material 7.
Supplementary Material 8.
Supplementary Material 9.
Supplementary Material 10.
Supplementary Material 11.
Supplementary Material 12.
Supplementary Material 13.
Supplementary Material 14.
Supplementary Material 15.
Supplementary Material 16.

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Authors' contributions
Authors'contributions Yongjun Gao, Rui Xu: Conceptualization: Ideas; formulation or evolution of overarching research goals and aims. Yongjun Gao, Rui Xu: Writing—Review & Editing: Preparing, creating, and presenting the published work by those from the original research group, specifically critical review, commentary, or revision—including pre- or postpublication stages. Yongjun Gao: Supervise： Oversee and take leadership responsibility for the research activity planning and execution, including mentorship external to the core team. Rui Xu: Methodology： Development or design of methodology; creation of models. Rui Xu: Software： Programming, software development, designing computer programs, implementing the computer code. Rui Xu: Formal analysis： Application of statistical, mathematical, computational, or other formal techniques to analyze or synthesize study data. Rui Xu: Data Curation： Management activities to annotate (produce metadata), scrub data, and maintain research data (including software code, where it is necessary for interpreting the data itself) for initial use and later reuse. Rui Xu: Writing—Original Draft： Preparing, creating, and presenting the published work, explicitly writing the initial draft (including substantive translation). Rui Xu: Visualization： Preparing, creating, and presenting the published work, specifically visualization/data presentation.

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Data availability
Full per-protein GWAS summary statistics were used to analyze 91 circulating inflammatory proteins, including IL- 6, measured using the Olink Target platform in 14,824 European participants across 11 cohorts. 180 pQTLs (59 cis, 121 trans) were identified, with the EBI GWAS Catalog (accession numbers GCST90274758—GCST90274848, and GCST90274781 specifically for IL- 6) employed. Validation of IL- 6—related results utilized data from 1,585 participants in the ARISTOTLE cohort and 35,556 Icelanders from the deCODE study. The data on 91 circulating inflammatory proteins, including IL- 6, is derived from a study published in 2023 by Zhao JH et al.[32] The data on 731 peripheral immune cell features are publicly available through the GWAS Catalog, with accession numbers ranging from GCST90001391 to GCST90002121. These data are derived from a study published in 2022 by Orru V et al.[33]. We obtained summary statistics for the AD association from the original GWAS on AD, which included 111,326 clinically diagnosed/'proxy'AD cases and 677,663 controls, all of European descent. These data are available for download from the EBI GWAS Catalog with the accession number GCST90027158. The proxy—AD designation is based on questionnaire data in which individuals are asked whether their parents had dementia. This method has been used successfully in the past29 but is less specific than a clinical or pathological diagnosis of AD; hence, we will refer to these cases as proxy AD and related dementia (proxy—AD). The data on AD is derived from a study published in 2022 by Bellenguez C et al.[36].

Declarations

Ethics approval and Consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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