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Research Article

Integrative Single-Cell RNA Transcriptomic and Mendelian Randomization Study Identify Key Genetic Determinants Between Systemic Lupus Erythematosus and Alzheimer's Disease Comorbidity

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Abstract

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by aberrant immune activation and multisystem organ damage, whereas Alzheimer's disease (AD) is a neurodegenerative disorder associated with β-amyloid deposition and tau hyperphosphorylation. Although both diseases impose a substantial global health burden, the genetic susceptibility underlying their comorbidity and the molecular mechanisms linking SLE and AD remain insufficiently characterized. In this study, we integrated single-cell RNA transcriptomic data with Mendelian randomization (MR) analysis to identify key genes associated with SLE-AD comorbidity and explore potential mechanistic links. Single-cell transcriptomic analysis identified 361 SLE-associated genes and 1,713 AD-associated genes, including 40 shared genes between the two diseases. Subsequent MR analysis highlighted ENTPD1 and THEMIS2 as key genetic determinants implicated in both conditions. Network-based functional characterization linked these genes to nucleoside phosphate and carbohydrate derivative metabolic processes, while computational disease-drug association analysis suggested shared comorbidities, including autoimmune disorders and neurological dysfunctions, as well as potential therapeutic agents such as Bisphenol A and Entinostat. Single-cell annotation revealed nine major immune cell types in SLE and five in AD, with T cells representing the predominant cell population in both diseases. Pseudotime trajectory analysis further delineated distinct T cells subpopulations and functional trajectories during disease pathogenesis. Overall, this integrative study identifies ENTPD1 and THEMIS2 as key molecular determinants of SLE-AD comorbidity and provides insights into their potential molecular interactions, immune cells dynamics, and therapeutic relevance.

Keywords

Systemic Lupus Erythematosus, Alzheimer's Disease, Comorbidity, Single-cell RNA Data Analysis, Mendelian Randomization Analysis, Key Genes

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1. Introduction

Systemic lupus erythematosus (SLE), is a complicated autoimmune illness that is caused by the interaction of a number of variables, including genetic predisposition, environmental factors, and immunological responses that are deregulated [1, 2]. The skin, joints, kidneys, and central nervous system are the primary organs that are affected by SLE, which is characterised by significant clinical variability and a variable illness history. Over the last several years, there has been a significant improvement in the survival rate of individuals who have SLE as a result of advancements in diagnostic and treatment procedures. In spite of this, chronic inflammation, immunological dysregulation, and vascular damage remain to be factors that contribute to an increase in long-term health risks [3]. The worldwide prevalence of SLE is estimated to vary from around 20 to 70 cases per 100,000 persons, with a significantly higher incidence in females than in men [4, 5]. Furthermore, the prevalence of SLE is much greater in girls than in males. During the course of SLE, neuropsychiatric involvement may show as cognitive impairment, depression, anxiety, and dementia. This suggests that immunological imbalance may have a possible role in the development of neurodegenerative illnesses [6-8].

The aetiology of Alzheimer's disease (AD) involves the accumulation of amyloid-β, aberrant tau hyperphosphorylation, and neuroinflammatory responses [9, 10]. AD is a neurodegenerative condition that is characterised by gradual cognitive decline, memory impairment, and neuropathological abnormalities. The number of people who are afflicted by AD is expected to approach 150 million globally by the year 2050 [11]. This has made AD a significant global public health concern. Epidemiological studies have shown that inflammatory responses, disruptions in the blood-brain barrier, and immunological dysregulation all play significant roles in the pathogenesis of AD, which has a significant amount of similarities with the chronic systemic inflammatory characteristics of SLE [12, 13]. In addition, the frequency of cognitive impairment is greater in patients with SLE compared to the general population. Furthermore, the neuroinflammatory markers that are detected in patients with SLE are similar to those that are shown in patients with AD, which suggests that the two illnesses may share similar molecular pathways and hereditary vulnerability [14, 15].

Despite the fact that the epidemiological connection between SLE and AD has grown more and more obvious, the molecular processes that operate under the surface are still not fully understood. In SLE, the presence of antinuclear antibodies, the accumulation of immune complexes, and abnormal cytokine signalling may hasten the progression of neurodegenerative alterations by disrupting the blood-brain barrier or by having direct effects on neurones [16]. In the meanwhile, persistent neuroinflammation and microglial activation in AD may further worsen neurological impairment that is produced by autoimmune damage [17, 18]. An effective method for systematically identifying putative comorbidity-associated genes that are shared by SLE and AD is provided by the combination of single-cell RNA transcriptomic data with Mendelian randomisation (MR) analysis. This method also reveals the functions that these genes play in inflammatory and neurodegenerative pathways.

In order to find similar molecular traits between SLE and AD at both the cellular heterogeneity and genetic causation levels, the purpose of this work was to combine single-cell data with magnetic resonance in order to detect these features. Our goal was to better explore the possible comorbid processes that connect these two illnesses, especially in the settings of immunological inflammation, neuronal damage, and intercellular communication. This was accomplished using functional enrichment, route analysis, and the development of regulatory networks. In addition, critical targets were screened, and possible therapeutic drugs were anticipated; this resulted in the provision of a unique theoretical framework for the clarification of disease processes and the development of precision intervention techniques.

2. Materials and Methods

2.1. Data Source

The Gene Expression Omnibus (GEO) provided the single-cell sequencing datasets for AD and SLE, with accession codes GSE181279 and GSE135779, respectively. Single-cell data from 40 SLE patients and 16 healthy controls were included in the GSE135779 dataset, whereas data from three AD patients and two healthy controls were included in the GSE181279 dataset. Selected genes were considered exposures for the MR analysis, while SLE and AD were used as independent outcome events. ebi-a-GCST003156 (European populations: 5,201 cases and 9,066 controls) provided genome-wide association study (GWAS) data for SLE, while ebi-a-GCST90027158 (European populations: 39,106 cases and 46,828 controls) provided GWAS data for AD.

2.2. Single-cell Data Processing

For the purpose of managing single-cell data, the Seurat software was used [19]. Initially, a Seurat object was created for each dataset, and then quality control (QC) filtering was applied to each dataset. The quality control criteria were defined as follows: cells that had a mitochondrial gene expression (percent.mt) of less than 20% and a minimum of 300 genes that were identified (nFeature_RNA) were retained for both GSE135779 and GSE181279. Through the use of the harmony technique, we determined the significance of batch effects and made adjustments to account for them. Through the

use of the DoubletFinder function [20, 21], doublets were removed from the data, which had an expected doublet rate of around 1.6%. In order to normalise the data, the NormalizeData function was used, and the FindVariableFeatures function was utilised in order to find genes that exhibited a high degree of variability according to the variance-stabilizing transformation (vst) technique. Next, the top 2,000 genes with fluctuating expression were selected [22]. Before beginning the process of dimensionality reduction, the expression values were scaled according to the ScaleData function. Following the completion of principal component analysis (PCA), the JackStraw and ScoreJackStraw functions were used in order to evaluate the significance of principal components. To determine the optimal number of major components, stree plots and elbow plots were used as data analysis tools. Next, t-distributed stochastic neighbour embedding (tSNE) was used in order to display cellular distributions in the reduced-dimensional space. This was done after cell clustering was performed at a resolution of 0.5 points. In order to classify cell clusters into distinct immunological populations, cell-type annotation was performed with the use of verified marker genes [23, 24]. This was accomplished by using heatmaps to visualize the expression patterns of marker genes over a wide range of cell types. Following this, a comparison was made between the illness group and the control group about the differences in cell-type abundance.

2.3. Identification of Differentially Expressed Genes (DEGs) and Enrichment Analysis

The FindAllMarkers function of the Seurat package was used in order to compare each individual cell cluster to every other cluster in order to identify genes that were expressed differently across a variety of cell types [25, 26]. The statistical and biological trustworthiness of the screening results was verified by classifying differentially expressed genes as those that fulfilled the criterion of an absolute log fold change (logFC) more than 0.5 and a P-value less than 0.05. The genes that were differently expressed in both SLE and AD were then separated and integrated in order to discover the molecular features that were comparable between the two diseases as well as the prospective regulatory elements that were relevant. This was done in order to further investigate the potential common molecular pathways that exist between the two illnesses. After that, the clusterProfiler package was utilised in order to carry out functional enrichment analysis on the selected genes. This analysis included the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis as well as Gene Ontology (GO) annotation, which encompassed biological processes (BP), cellular components (CC), and molecular functions (MF) [27].

2.4. MR Analysis Identifies Key Genes

A two-sample MR analysis was performed in R, and it was carried out with the use of GWAS summary statistics that were available to the public via the IEU OpenGWAS database. MR-Egger, weighted median, inverse variance weighted (IVW), simple mode, and weighted mode are the five complementary analytical tools that were used in order to carefully evaluate the potential causal relationships that exist between exposure components and outcome characteristics. It was determined that a significant causal association between the exposure and the outcome was shown by a P-value that was less than 0.05. The IVW method was the primary analytical technique that was used among them. In order to better identify the direction of impact, odds ratios (OR) were calculated. An OR more than one indicates a risk factor, whereas an OR less than one shows a protective effect. After that, MR analysis was performed on genetic variants that were associated to both multiple sclerosis and Alzheimer's disease in order to identify significant genes that had substantial causal linkages with both diseases. In order to examine the stability and consistency of the instrumental variables, heterogeneity analysis was carried out with the help of the mr_heterogeneity function that is included in the TwoSampleMR package. Horizontal pleiotropy was evaluated with the use of the mr_pleiotropy_test function in order to determine whether or not there was any pleiotropic bias. In addition, the mr_leaveoneout approach was used in order to carry out a leave-one-out sensitivity analysis. This was done in order to determine the influence that certain genetic differences had on the overall results. The MR-Steiger directionality testing was used in order to get a more accurate determination of the direction of causality between the exposure factors and the outcome variables. Consequently, the robustness and reliability of the research were enhanced.

2.5. Regulatory Network Analysis of Key Genes and Drug Prediction

A gene-gene interaction (GGI) network was developed with the use of the GeneMANIA platform (https://genemania.org) in order to investigate the functional connections that exist between the significant genes. Seven significantly enriched pathways were found to be connected to the top twenty genes that had the greatest relationships with the genes that were considered to be relevant. Furthermore, in order to get a better understanding of the post-transcriptional regulatory mechanisms, microRNA (miRNA) prediction was performed by employing the miRWALK (http://mirwalk.umm.uni-heidelberg.de/) and miRDB (http://www.mirdb.org/) databases. It was determined that common microRNAs that target the essential genes were investigated in order to shed light on the probable regulatory activities that they may play in the development and progression of sickness. Visualisation of the interaction network was accomplished via the use of the Cytoscape program (https://cytoscape.org). In addition, the Comparative Toxicogenomics Database (CTD) (https://ctdbase.org) was used in order to conduct an exhaustive investigation into the links between genes and diseases. In addition, diseases that were

closely related with the primary genes were screened and visualised. Following the prediction of important disease linkages by CTD, additional research was conducted to evaluate the possible use of these links as therapeutic targets in the creation of new drugs.

2.6. Identification of Candidate Cell Types and Pseudotime Trajectory Analysis

For each and every sample that was included in the single-cell datasets GSE135779 and GSE181279, the Wilcoxon rank sum test was used in order to assess the degree to which the expression of significant genes differed across the various cellular populations considered. The levels of key gene expression within each identified cell type were compared between the groups who were afflicted with the disease and the control group. Cell types that exhibited significant differences in key gene expression ($P < 0.05$) were designated as suspected relevant cell types. Pseudotime analysis was then performed on these possibly important cell types in order to investigate the changes in cellular dynamics that occurred over the course of the disease. The use of tSNE allowed for the further refinement of the classification of potentially significant cell types into distinct subpopulations. After that, the FindAllMarkers function in Seurat was used with the parameters min.pct = 0.2 and only in order to locate specific marker genes for each subpopulation.pos is equal to TRUE. In order to mimic the temporal evolution of cellular states, Branched expression analysis modelling (BEAM) was used inside the Monocle2 program [28, 29]. This allowed for the inference of cell-state transitions along the pseudotime axis. Lastly, the plot_pseudotime_heatmap function was used in order to display dynamic gene expression patterns. This provided insights into the temporal regulatory characteristics of significant prognostic genes during the progression of the illness.

2.7. Statistical Analysis

Using relevant bioinformatics programs, all data analysis were carried out in R (version 4.4.1). After that, each figure was imported into Adobe Illustrator 2023 for further processing. A P-value of less than 0.05 was deemed statistically significant.

3. Results

3.1. SLE Single-cell Data Analysis

The process of quality control was used to exclude cells of poor quality and duplicate genes by using the criteria of $200 < nFeature_RNA < 5{,}000$ and $percent.mt < 15$. An expression matrix consisting of 21,065 genes and 334,382 cells was produced as a consequence of this. The expression matrix included 216,332 cells from SLE patients and 118,050 cells from the control group (Figure S1A). After using the FindVariableFeatures tool to locate highly variable genes based on expression variance in the filtered cells, the top 2,000 genes with the highest level of variability were selected for further analysis. Among these, the top 10 genes with the highest degree of variability were highlighted (Figure 1A). Following the use of principle component analysis (PCA) to reduce the linear dimension of the data, the findings were presented in the form of scatter images. It was discovered that the principal components (PCs) of both the control samples and the SLE samples were completely integrated (Figure S1B). Through the use of dimensionality reduction and clustering analysis, we were able to identify eighteen cell groupings and define the marker gene expression features of each cluster (Figure 1B and C). Following that, cell annotation was performed by merging data from the online CellMarker database with the identification of known lineage-specific marker genes that were published in the literature. There were nine different kinds of cells that were discovered, including T cells, B cells, NK cells, monocytes, megakaryocytes (Mgk), erythrocytes, plasmacytoid dendritic cells (pDCs), dendritic cells (DCs), and plasma cells (PCs) (Figure 1D). NK cells made up a relatively big proportion in the control group, monocytes were more abundant in the SLE samples, and T cells made up a considerable part in both groups, according to a study of cell-type abundance (Figure 1E). NK cells were the most prevalent kind of cell in the control group. Between the SLE and control groups, 794 genes were upregulated and 1,820 genes were downregulated in T cells, 358 genes were upregulated and 1,420 genes were downregulated in B cells, 635 genes were upregulated and 1,470 genes were downregulated in NK cells, 678 genes were upregulated and 1,814 genes were downregulated in monocytes, 353 genes were upregulated and 692 genes were downregulated in Mgk cells, 712 genes were upregulated and 212 genes were downregulated in erythrocytes, 468 genes were upregulated and 2,787 genes were downregulated in pDCs, 209 genes were upregulated and 959 genes were downregulated in DCs, and 902 genes were upregulated and 2,917 genes were downregulated in PCs. The upregulated DEGs shared across all cell types were designated as SLE genes, yielding a total of 361 genes for subsequent analysis (Figure 1F).

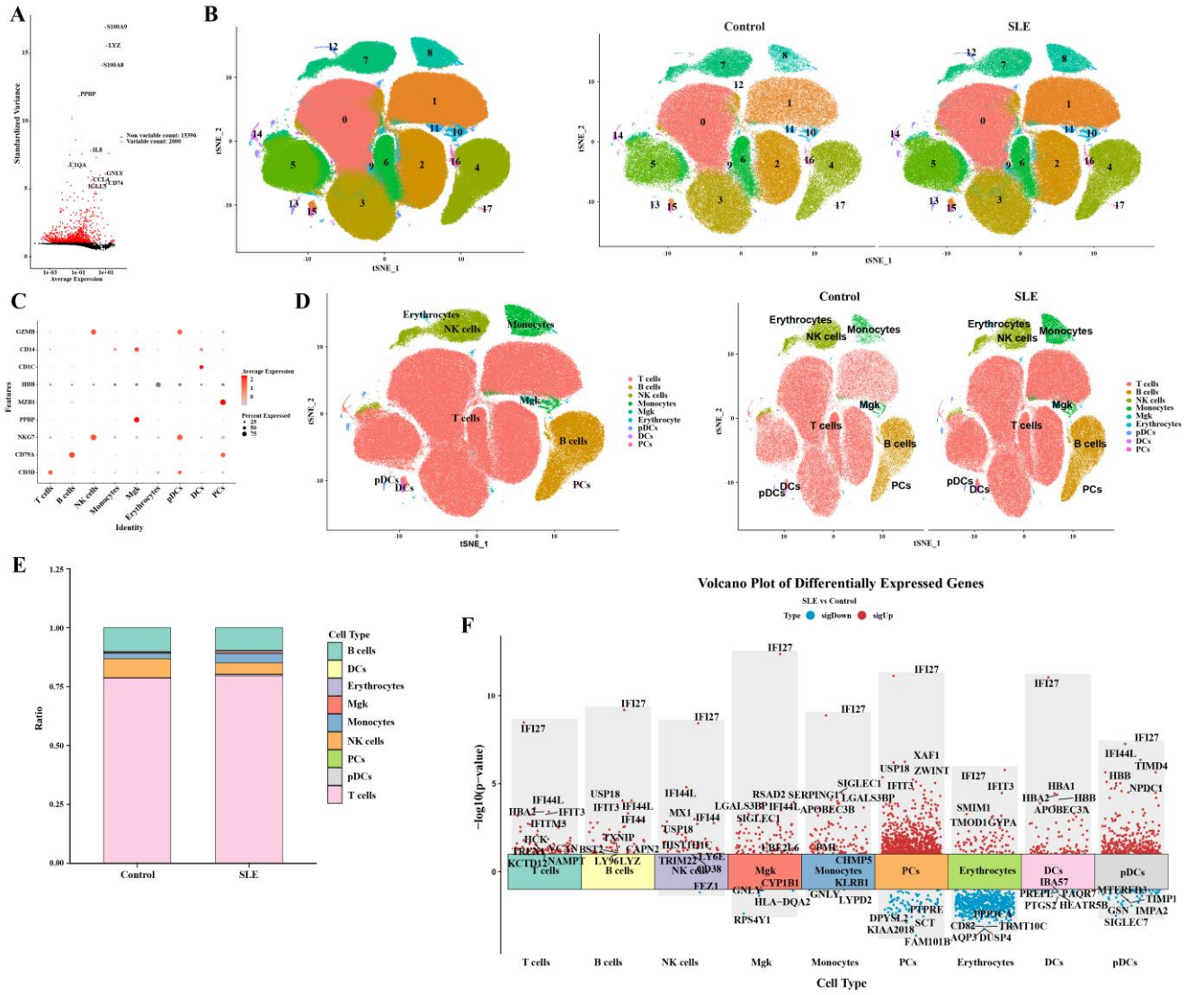


Figure 1. *Single-cell transcriptomic profiling reveals alterations in immune cell composition and transcriptional features between SLE and control samples. A Identification of highly variable genes in the single-cell dataset. B t-SNE visualization of all cells and their distributions in the control and SLE groups, identifying 18 distinct cell clusters. C Dot plot showing the expression of representative marker genes used for cell-type annotation. D t-SNE projection of annotated major cell types and their distributions in the control and SLE groups, including T cells, B cells, NK cells, monocytes, Mgk, erythrocytes, DCs, pDCs, and PCs. E Relative proportions of major cell types in the control and SLE groups. F Volcano plots showing differentially expressed genes in each cell type between the SLE and control groups; red dots indicate upregulated genes, blue dots indicate downregulated genes.*

3.2. AD Single-cell Data Analysis

The process of quality control was used to exclude cells of poor quality and duplicate genes by using the criteria of 200 < nFeature_RNA < 5,000 and percent.mt < 15. An expression matrix consisting of 17,732 genes and 33,885 cells was produced as a consequence of this. The expression matrix included 20,722 cells from Alzheimer's disease patients and 13,163 cells from the control group (Figure S2A). After using the FindVariableFeatures tool to locate highly variable genes based on expression variance in the filtered cells, the top 2,000 genes with the highest level of variability were selected for further analysis. Among these, the top 10 genes with the highest degree of variability were highlighted (Figure 2A). PCA was used for the purpose of linear dimensionality reduction, and the results were presented in the form of scatter plots. Based on the data, it was evident that there was a high level of integration between the PCs of the AD samples and the control samples (Figure S2B). Through the use of dimensionality reduction and clustering analysis, we were able to identify twelve cell groupings and explain the marker gene expression features of each cluster (Figure 2B and C). Following that, cell annotation was performed by merging data from the online CellMarker database with the identification of known lineage-specific marker genes that were published in the literature. Figure 2D illustrates the five different kinds of cells that were

discovered. These cells include T cells, B cells, NK cells, monocytes, and erythrocytes. According to an examination of cell-type abundance (Figure 2E), nodal lymphocytes (NK cells) comprised up a relatively substantial fraction of the control group, but T cells were more frequent in the samples taken from Alzheimer's disease patients. There were 2,150 genes that were upregulated and 1,558 genes that were downregulated in T cells, 1,869 genes that were upregulated and 628 genes that were downregulated in B cells, 2,094 genes that were upregulated and 700 genes that were downregulated in NK cells, 860 genes that were upregulated and 357 genes that were downregulated in monocytes, and 138 genes that were upregulated and 232 genes that were downregulated in erythrocytes between the AD group and the control group. It was determined that AD genes were included among the increased DEGs that were shared by all cell types, which led to the identification of 1,713 genes for future investigation (Figure 2F).

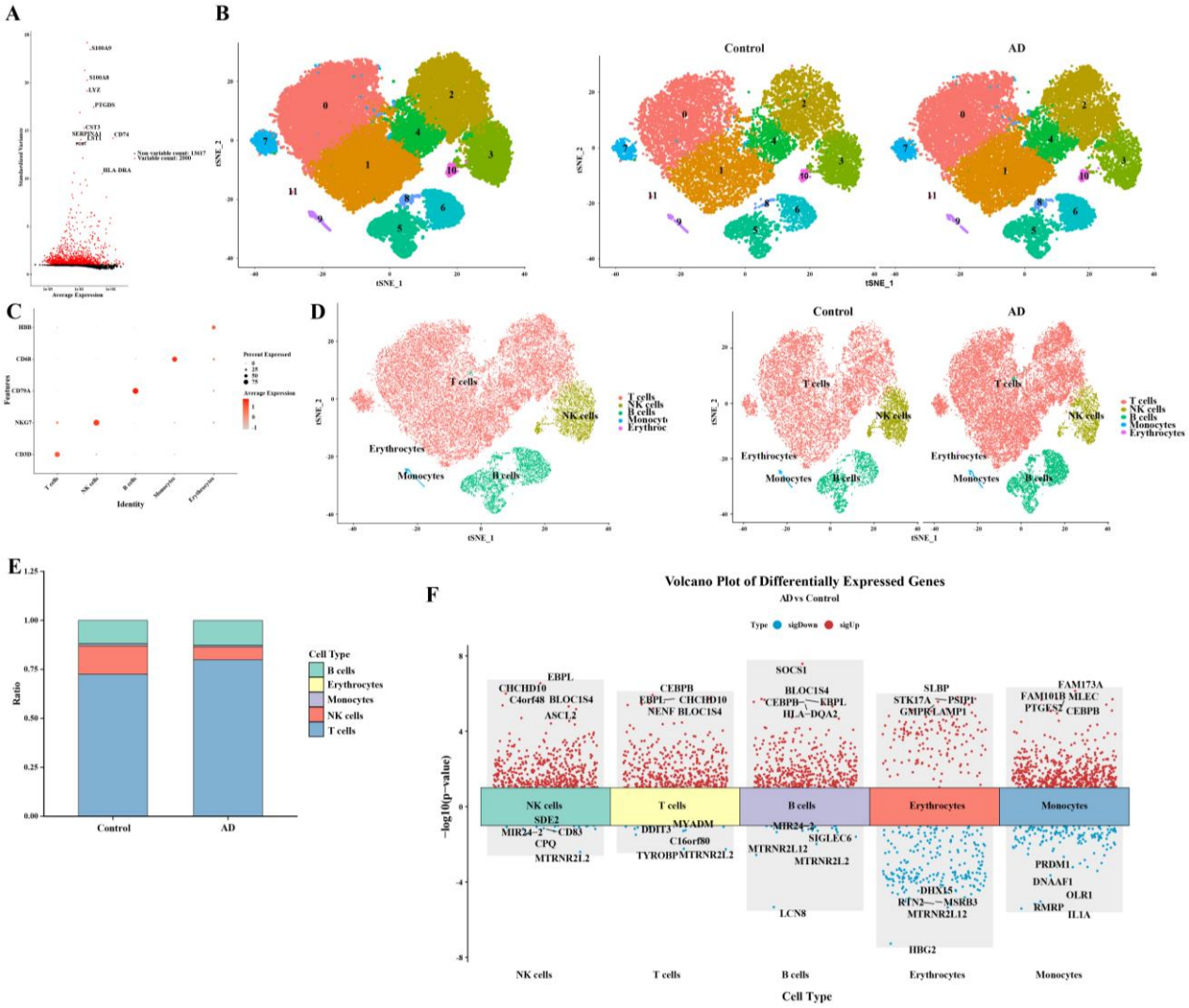
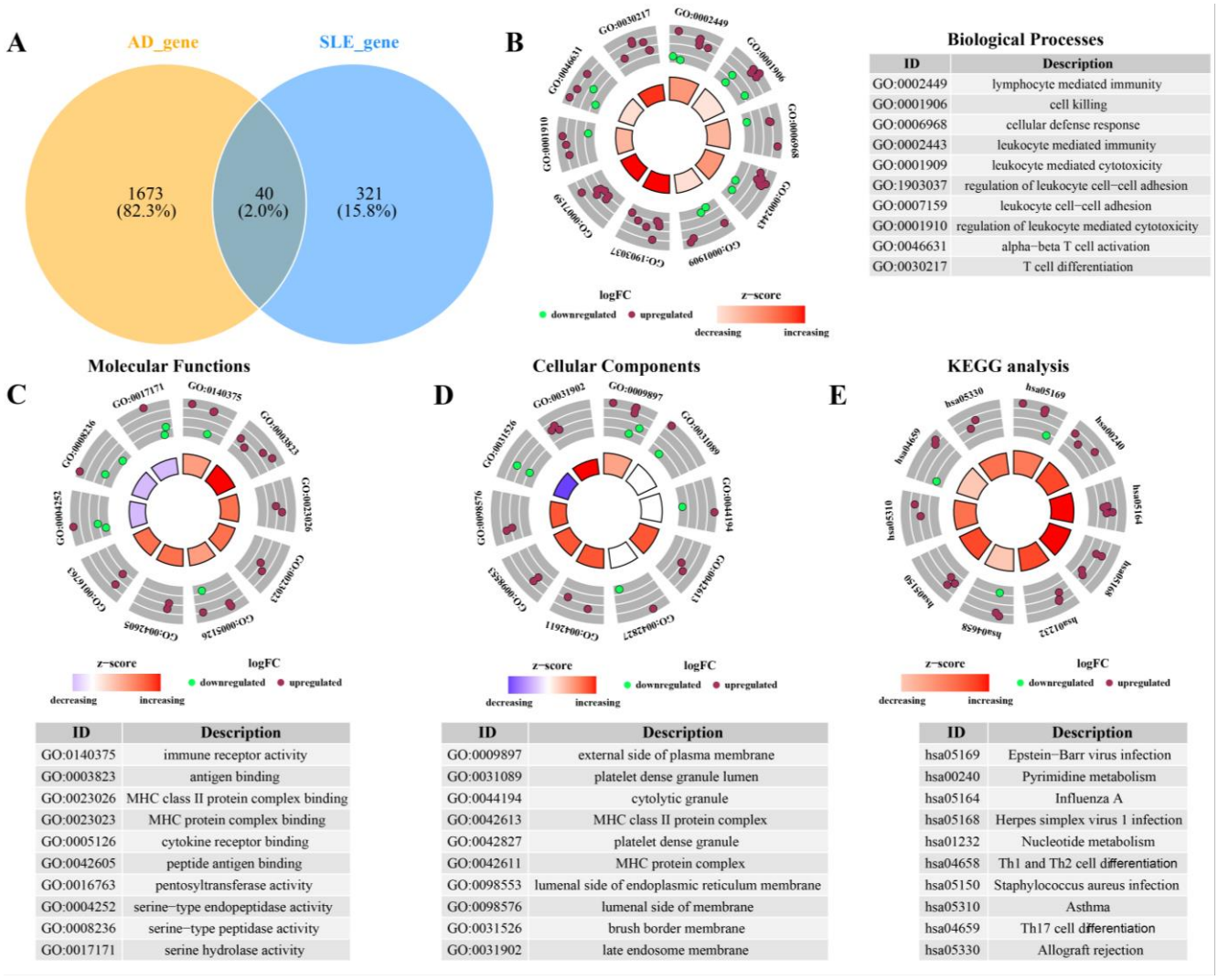


Figure 2. *Single-cell RNA transcriptomic analysis reveals alterations in cellular composition and differential gene expression patterns between AD and control samples. A Identification of highly variable genes in the single-cell dataset. B t-SNE visualization of all cells and their distributions in the control and AD groups, identifying 12 distinct cell clusters. C Dot plot showing the expression of representative marker genes used for cell-type annotation. D t-SNE projection of annotated major cell types and their distributions in the control and AD groups, including T cells, NK cells, B cells, monocytes, and erythrocytes. E Relative proportions of major cell types in the control and AD groups. F Volcano plots showing differentially expressed genes in each major cell type between the AD and control groups; red dots indicate upregulated genes, blue dots indicate downregulated genes.*

3.3. Identification of Key Genes

The Venn diagram-based intersection analysis indicated that there are a total of 40 DEGs that are shared across SLE and AD (Figure 3A). This is in addition to the 321 DEGs that are special to SLE (15.8% of total DEGs) and the 1,673 DEGs that are specific to AD (82.3% of total DEGs). There were

thirty highly enriched functional words discovered by the use of GO enrichment analysis (Figure 3B-D). These words included ten BP keywords, ten MF phrases, and ten CC terms. There were a number of BP keywords that were heavily enriched, including lymphocyte-mediated immunity, cell death, cellular defense response, leukocyte-mediated cytotoxicity, and T-cell differentiation. There were many essential MF keywords, the most important of which were immune receptor activity, antigen binding, MHC class II protein complex binding, and cytokine receptor binding. The principal places for the most enriched CC keywords were found on the outer side of the plasma membrane, the lumen of the platelet dense granule, the MHC protein complexes, and the lumenal side of the endoplasmic reticulum membrane. Through the use of KEGG pathway enrichment analysis, it was shown that ten other biological pathways had been considerably enriched. According to Figure 3E, these pathways were primarily associated with infectious diseases, such as an infection caused by the Epstein-Barr virus, influenza A, an infection caused by herpes simplex virus type 1, and an infection caused by Staphylococcus aureus. Additionally, these pathways were associated with immune regulatory pathways, such as the differentiation of Th1 and Th2 cells, the differentiation of Th17 cells, and allograft rejection.



ID	Description
GO:0002449	lymphocyte mediated immunity
GO:0001906	cell killing
GO:0006968	cellular defense response
GO:0002443	leukocyte mediated immunity
GO:0001909	leukocyte mediated cytotoxicity
GO:1903037	regulation of leukocyte cell−cell adhesion
GO:0007159	leukocyte cell−cell adhesion
GO:0001910	regulation of leukocyte mediated cytotoxicity
GO:0046631	alpha−beta T cell activation
GO:0030217	T cell differentiation

ID	Description
GO:0140375	immune receptor activity
GO:0003823	antigen binding
GO:0023026	MHC class II protein complex binding
GO:0023023	MHC protein complex binding
GO:0005126	cytokine receptor binding
GO:0042605	peptide antigen binding
GO:0016763	pentosyltransferase activity
GO:0004252	serine−type endopeptidase activity
GO:0008236	serine−type peptidase activity
GO:0017171	serine hydrolase activity

ID	Description
GO:0009897	external side of plasma membrane
GO:0031089	platelet dense granule lumen
GO:0044194	cytolytic granule
GO:0042613	MHC class II protein complex
GO:0042827	platelet dense granule
GO:0042611	MHC protein complex
GO:0098553	lumenal side of endoplasmic reticulum membrane
GO:0098576	lumenal side of membrane
GO:0031526	brush border membrane
GO:0031902	late endosome membrane

ID	Description
hsa05169	Epstein−Barr virus infection
hsa00240	Pyrimidine metabolism
hsa05164	Influenza A
hsa05168	Herpes simplex virus 1 infection
hsa01232	Nucleotide metabolism
hsa04658	Th1 and Th2 cell differentiation
hsa05150	Staphylococcus aureus infection
hsa05310	Asthma
hsa04659	Th17 cell differentiation
hsa05330	Allograft rejection

Figure 3. *Functional enrichment analysis of shared differentially expressed genes between AD and SLE. A Venn diagram showing the overlap of differentially expressed genes between AD and SLE, with 40 shared genes identified. B-D GO enrichment analysis of the shared differentially expressed genes, showing the enriched terms in BP (B), MF (C), and CC (D), respectively. In the circular plots, each sector represents a significantly enriched term; the inner color scale indicates the z-score, and the outer dots indicate gene expression changes within each term, with green representing downregulated genes and magenta representing upregulated genes. The tables summarize representative enriched terms and their IDs. E KEGG pathway enrichment analysis of the shared DEGs, showing significantly enriched pathways and their associated gene expression patterns.*

3.4. Identification of Key Causal Genes for SLE by MR Analysis

Using the IVW approach, which used the previously revealed DEGs shared between SLE and AD as exposure variables and SLE as the outcome in the MR analysis, seven genes were shown to have considerable causal linkages with the risk of SLE. These genes were found to be associated with the potential for SLE. CCR2, CD247, CYBRD1, SIRPB1, and WARS were identified as protective factors (OR < 1). On the other hand, ENTPD1 and THEMIS2 were discovered to be risk factors for systemic lupus erythematosus (OR > 1), as seen in Figure 4A. Using ENTPD1 as a sample gene, an intuitive visualisation of the causative association between gene expression levels and SLE risk was developed by merging the effects of single-nucleotide polymorphisms (SNPs) on gene expression with their impacts on the outcome of SLE. This allowed for the determination of the causal relationship between the two. The fitted regression lines from five different MR techniques, including inverse variance weighted, MR-Egger, weighted median, weighted mode, and simple mode, were shown concurrently in Figure 4B. This was done while these regression lines were being displayed. Forest plots were used to further visualise the individual causal impact estimates of each instrumental SNP on the relationship between ENTPD1 and SLE, as well as the overall pooled effect estimates derived from the IVW and MR-Egger approaches (Figure 4C). These estimates were generated by combining the results of the two methodologies. For the purpose of measuring horizontal pleiotropy, a funnel plot was used. According to the data, the estimations of the SNPs' impacts were distributed in a symmetrical manner, which suggests that this research did not exhibit a significant amount of horizontal pleiotropic bias (Figure 4D). A leave-one-out sensitivity analysis was performed, which demonstrated that the sequential removal of individual SNPs did not result in substantial alterations in the pooled impact estimates of the remaining variants (Figure 4E). This further supported the reliability and stability of the MR findings, which provided additional confirmation of their reliability and stability. The seven genes that were discovered by MR analysis were subsequently identified as significant genes that have substantial causal relationships with SLE susceptibility as a result of these results. These genes have the potential to be significant prospective targets for additional investigation into the molecular pathways that are responsible for the comorbidity of SLE and AD.

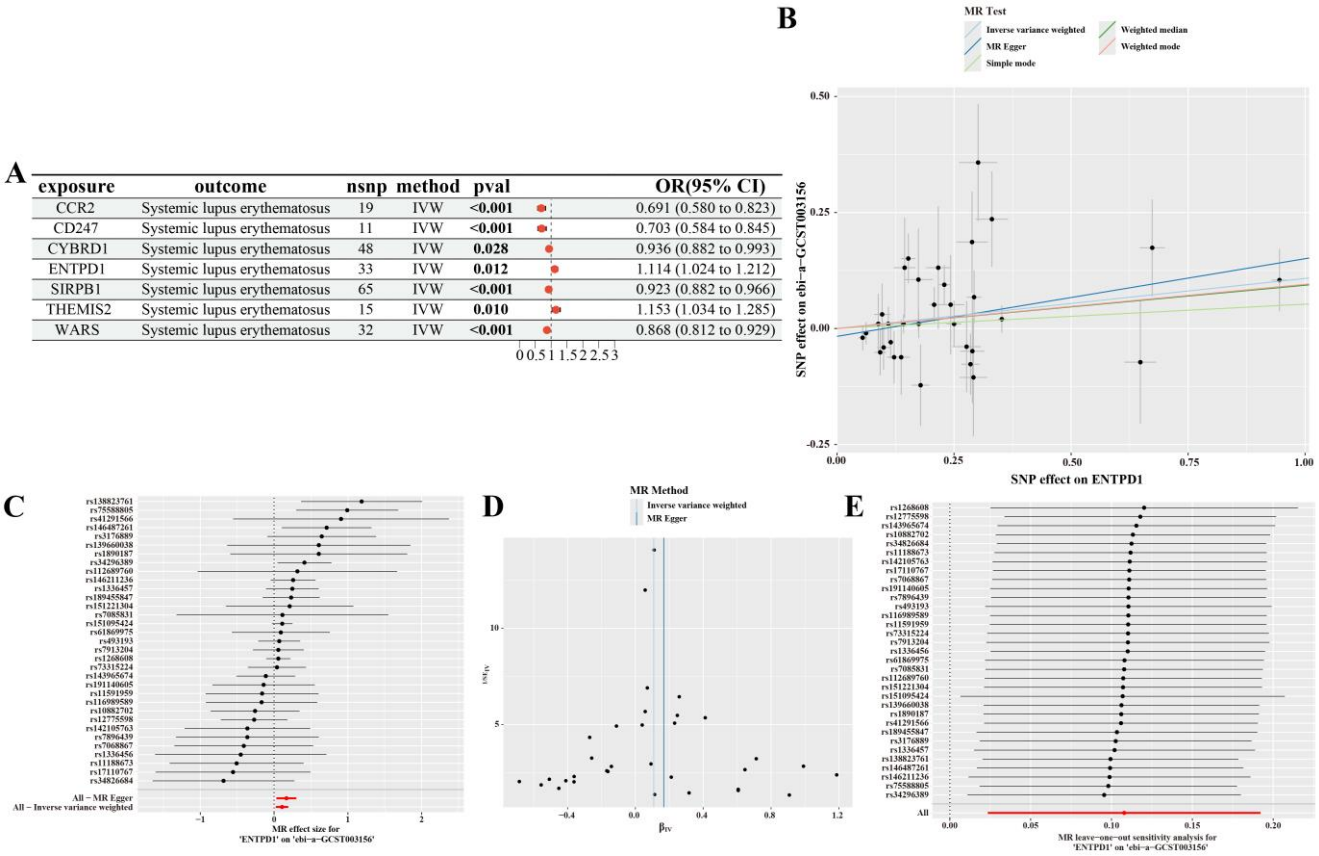


Figure 4. *MR analysis identifies candidate genes associated with SLE and supports a potential causal effect of ENTPD1. A Summary of MR results for candidate genes associated with SLE. B Scatter plot showing the causal association between ENTPD1 and systemic lupus erythematosus. C Forest plot showing the single-SNP and overall MR effect estimates for ENTPD1 on SLE, with corresponding confidence intervals. D Funnel plot for ENTPD1. E Leave-one-out sensitivity analysis for ENTPD1, showing the stability of the overall causal estimate after sequential exclusion of each instrumental SNP.*

3.5. Identification of Key Causal Genes for AD by MR Analysis

Using the previously found DEGs that are shared across SLE and AD as exposure variables and AD as the outcome, MR analysis using the IVW approach showed four genes that have considerable causal linkages with the risk of AD. In the group of factors, PARP12 and TYROBP were classified as protective factors (OR < 1), whereas THEMIS2 and ENTPD1 were found to be risk factors for AD (OR > 1), as seen in Figure 5A. The effects of SNPs on the exposure gene were combined with their effects on the AD outcome, and a scatter plot was produced as a consequence of this combination. A representative gene, ENTPD1, was selected for the purpose of conducting a direct investigation into the causal link that exists between the exposure genes and the result. Figure 5B displays fitted regression lines that were created by five different MR techniques: inverse variance weighted, MR-Egger, weighted median, weighted mode, and simple mode. These all produced fitted regression lines simultaneously. A forest plot was generated to demonstrate the individual estimated impacts of each instrumental SNP on the causal relationship between ENTPD1 and AD (Figure 5C). This was done in addition to the overall pooled impact estimates that were obtained using the IVW and MR-Egger techniques. Testing for horizontal pleiotropy showed that the SNP impact estimates of the genes that were found were distributed in a manner that was fairly symmetrical (Figure 5D). This finding provided further evidence that this method does not exhibit a significant amount of horizontal pleiotropic bias of any kind. The reliability and robustness of the MR results were further validated by leave-one-out sensitivity analysis, which shown that the removal of any one SNP had very little influence on the pooled estimates of the remaining variants (Figure 5E). This further demonstrated that the MR observations were reliable and robust. These findings led to the classification of the four genes discovered by MR analysis as AD_MR genes. These genes have significant causal links with AD and have the potential to be key opportunities for future study into the molecular mechanisms that are responsible for the comorbidity between AD and systemic lupus erythematosus.

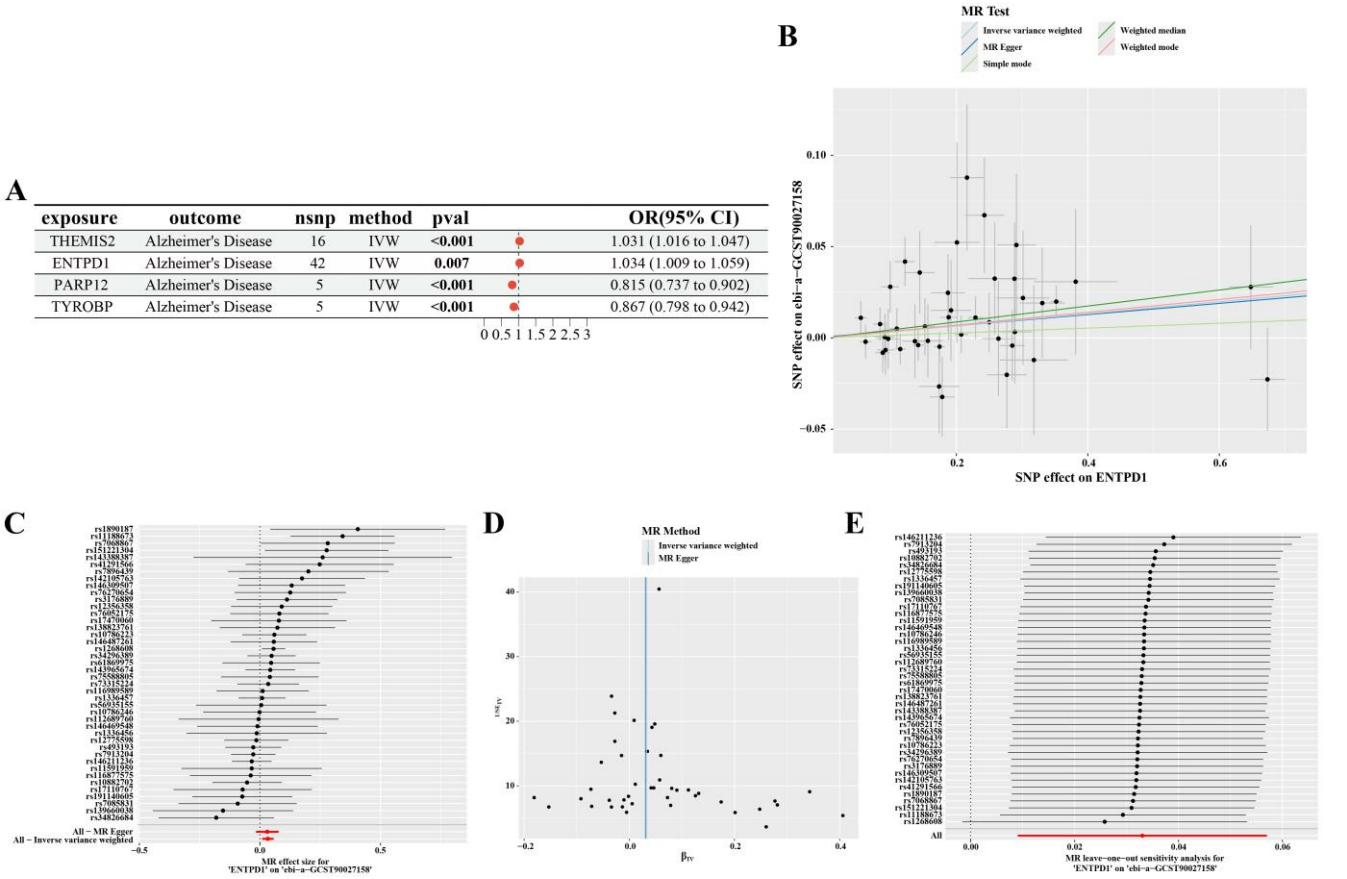


Figure 5. *MR analysis identifies candidate genes associated with AD and supports a potential causal effect of ENTPD1. A Summary of MR results for candidate genes associated with AD. B Scatter plot showing the causal association between ENTPD1 and AD. C Forest plot showing the single-SNP and overall MR effect estimates for ENTPD1 on AD, with corresponding confidence intervals. D Funnel plot for ENTPD1. E Leave-one-out sensitivity analysis for ENTPD1, showing the stability of the overall causal estimate after sequential exclusion of each instrumental SNP.*

3.6. Regulatory Network Analysis of Key Genes and Drug Prediction

Intersection examination of genes with beneficial impacts (OR > 1) between the AD_MR and SLE_MR gene sets led to the discovery of two shared key pathogenic genes, ENTPD1 and THEMIS2 (Figure 6A). These genes were revealed to be responsible for many diseases. A gene-gene interaction (GGI) network analysis was carried out in order to understand the functional links that exist between these groups of genes. It has been shown that a number of genes, such as FAS, CYBC1, ENTPD3, and ENTPD4, are closely associated with these significant genes. Among the several types of interactions that were included into the interaction patterns were co-expression, genetic interactions, physical interactions, and pathway-based correlations. In addition, the nucleoside diphosphatase activity, the nucleoside phosphate catabolic processes, and the organophosphate ester catabolic processes were among the enhanced functions that were associated with these genes (Figure 6B). Integrated miRNA prediction using miRWALK and miRDB identified 42 candidate miRNAs predicted to target THEMIS2 and 58 candidate miRNAs predicted to target ENTPD1, thereby forming a putative miRNA-gene post-transcriptional regulatory network (Figure 6C). These predicted interactions were derived from computational databases and therefore represent candidate regulatory relationships rather than experimentally validated interactions. ENTPD1 was connected to numerous illnesses, including learning disorders, neurotoxicity syndromes, AD, and SLE (Figure 6D). On the other hand, THEMIS2 was strongly associated with neurobehavioral symptoms, learning disorders, neurotoxicity syndromes, and SLE (Figure 6E). Furthermore, according to Figure 6F, these significant genes exhibited potential drug-chemical interactions with Entinostat, Tetrachlorodibenzodioxin, and Bisphenol A.

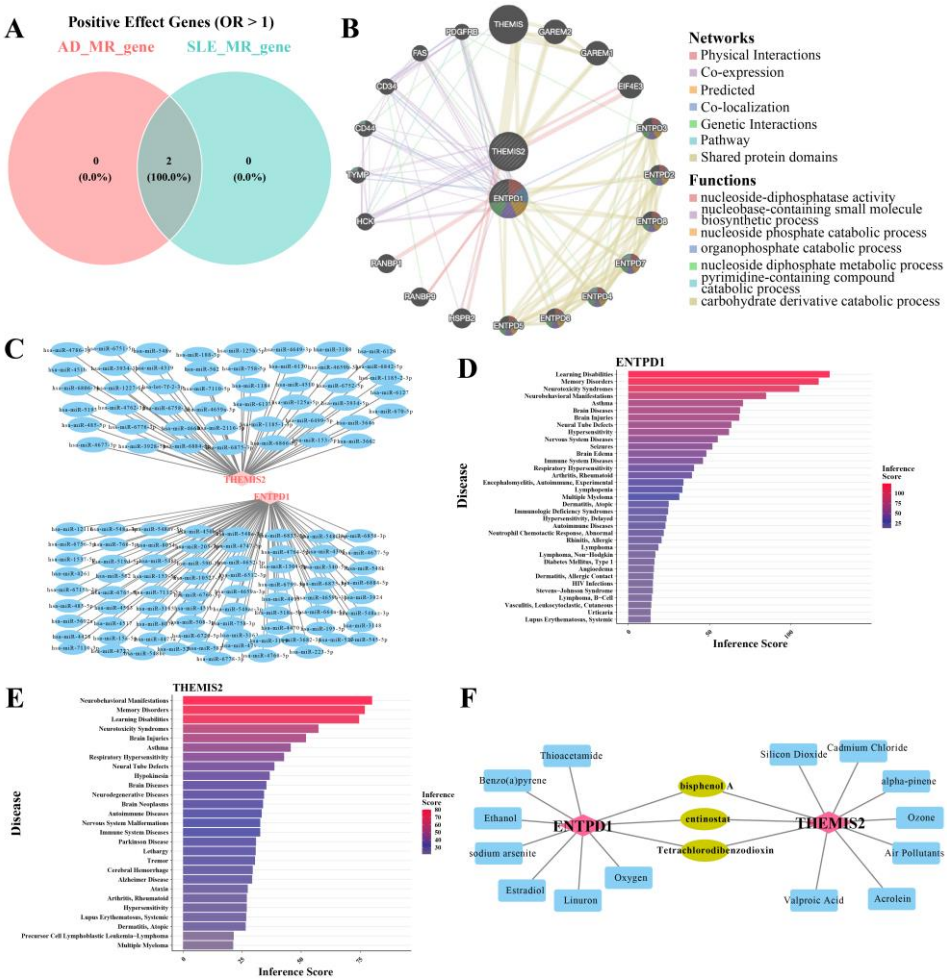


Figure 6. *Network and functional characterization of shared positive-effect genes between AD and SLE. A Venn diagram showing the overlap of positive-effect genes (OR > 1) identified by MR analysis in AD and SLE, revealing two shared genes, ENTPD1 and THEMIS2. B Gene interaction network of the shared genes. C miRNA-gene regulatory network of ENTPD1 and THEMIS2. D-E Disease association analysis of ENTPD1 and THEMIS2 based on in ference databases. Bar plots show the inference scores for disease terms. F Chemical-gene interaction network of ENTPD1 and THEMIS2, showing the potential associations of these genes with multiple environmental exposures or chemical factors.*

3.7. Identification of Putative Relevant Cell Types and Pseudotime Analysis

We examined the single-cell transcriptome dataset from SLE patients (GSE135779) to find distinct cell groups linked to SLE. According to our research, T cells and monocytes were identified as potentially relevant cell types in SLE because they both demonstrated notable differential expression of the two important genes, ENTPD1 and THEMIS2 (Figure 7A). All immune cells were subsequently separated into five different groups by further cell clustering analysis (Figure 7B).

We looked at prognostic gene expression at several phases of the T-cell developmental continuum to investigate the dynamic expression patterns of the important genes during differentiation. Based on their stage-specific expression patterns, the analysis revealed that these two genes could be divided into two groups, suggesting different expression properties (Figure 7C). The developmental trajectory of immune cells was further disclosed using pseudotime analysis, where cluster 0 was designated as the initial cell state and the trajectory's beginning point. This subset progressively changed into diverse immune cell types throughout pseudotime (Figure 7D).

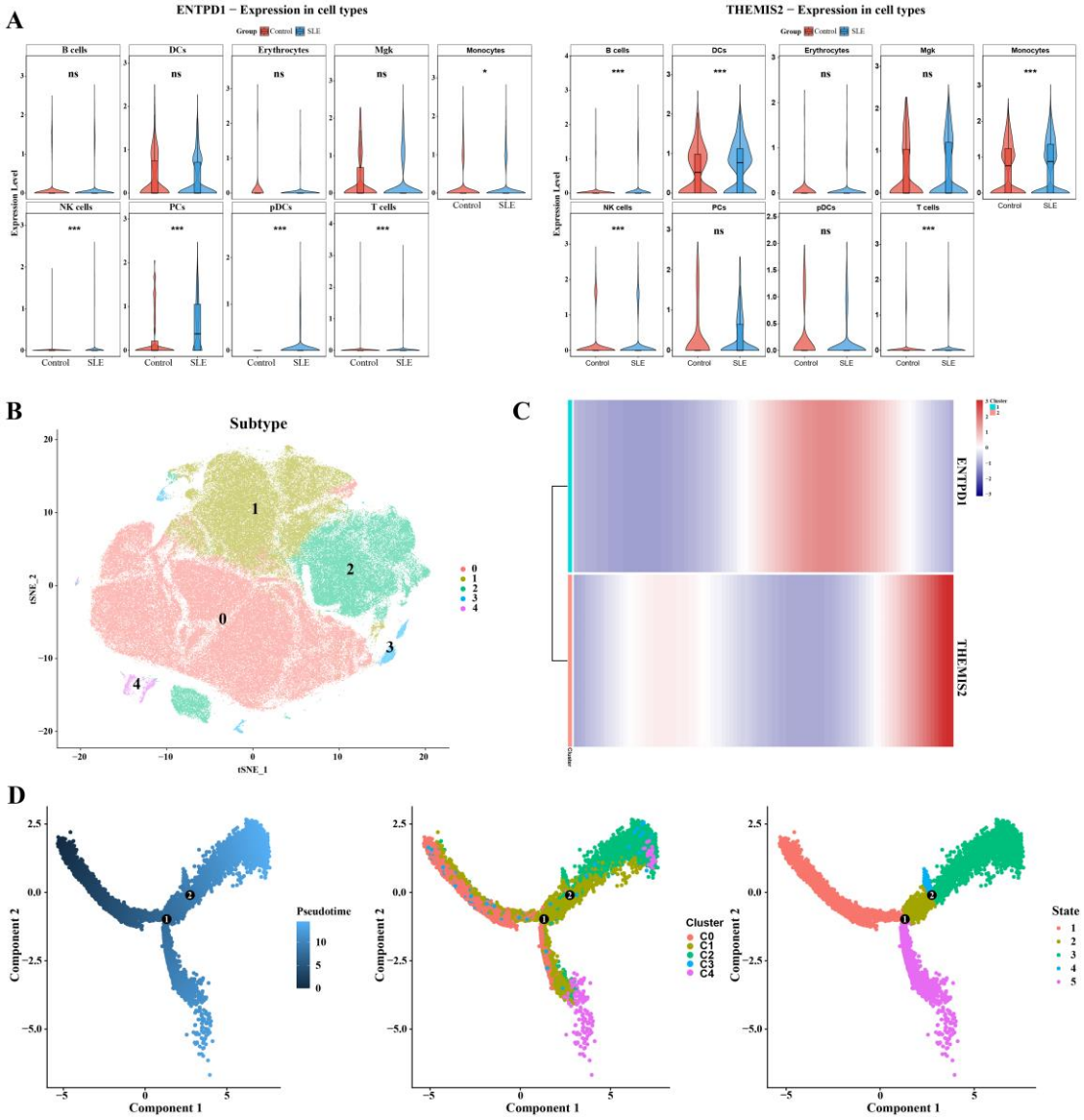


Figure 7. *Cell type-specific expression patterns of ENTPD1 and THEMIS2 and pseudotime trajectory analysis of cellular subtypes. A Violin plots showing the expression of ENTPD1 and THEMIS2 across major cell types in the control and SLE groups. ENTPD1 showed significant differences in monocytes, NK cells, PCs, pDCs, and T cells, whereas THEMIS2 showed significant differences in B cells, DCs, monocytes, NK cells, and T cells. B t-SNE visualization of the selected cell population, identifying five distinct subtypes. C Heatmap showing the expression patterns of ENTPD1 and THEMIS2 across different subtypes, illustrating their dynamic expression under cellular heterogeneity. D Pseudotime trajectory analysis of the selected cell population. The left panel shows cells ordered along pseudotime, the middle panel shows the distribution of clusters (C0-C4) along the trajectory, and the right panel shows the distribution of cell states (State 1-5), indicating a branched developmental process and state transition.*

We examined the single-cell transcriptome dataset from AD patients in order to search for distinct cell types linked to AD (GSE181279). Due to their extremely substantial differential expression of both important genes, T cells were identified by the data as the potentially relevant cell group in AD (Figure 8A). All immune cells were subsequently separated into six unique groups by cell clustering analysis (Figure 8B). We assessed the expression of ENTPD1 and THEMIS2 at various phases of immune cell development in the dataset in order to describe the dynamic expression patterns of important genes during differentiation. According to stage-specific expression patterns, heatmap analysis revealed that these two genes could be divided into two groups, suggesting different expression properties (Figure 8C). The developmental and differentiation trajectory of immune cells was further defined using pseudotime analysis, which indicated cluster 1 as the first cell state and the beginning of differentiation. This subpopulation progressively changed into distinct immune cell subpopulations as pseudotime went on (Figure 8D).

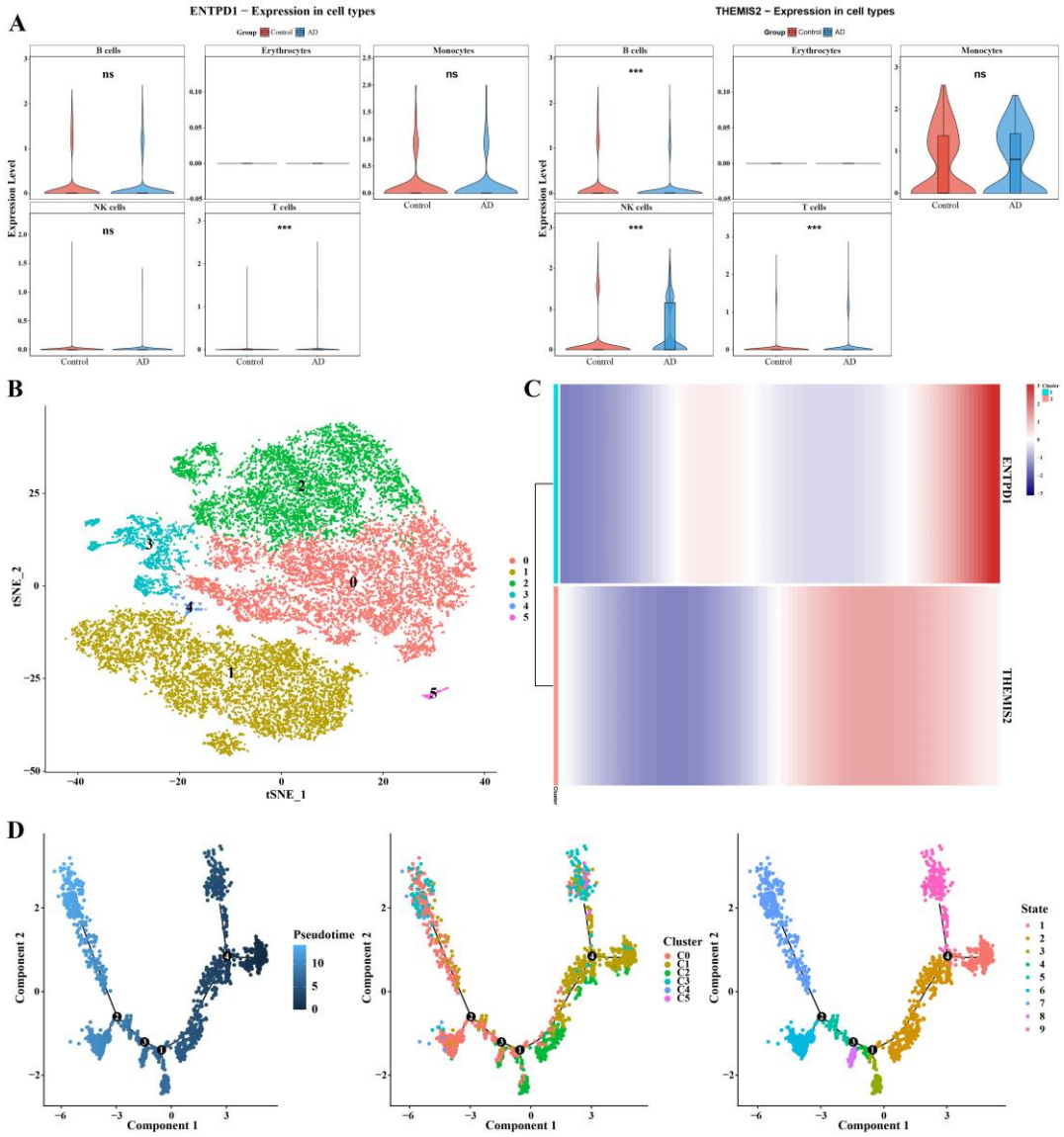


Figure 8. *Cell type-specific expression patterns of ENTPD1 and THEMIS2 and pseudotime trajectory analysis in AD-related cell populations. A Violin plots showing the expression of ENTPD1 and THEMIS2 across major cell types in the control and AD groups. ENTPD1 showed a significant difference in T cells, whereas no significant differences were observed in B cells, monocytes, or NK cells. THEMIS2 showed significant differences in B cells, NK cells, and T cells, whereas no significant difference was observed in monocytes. B t-SNE visualization of the selected cell population, identifying six distinct subtypes. C Heatmap showing the expression patterns of ENTPD1 and THEMIS2 across different subtypes, illustrating their dynamic expression under cellular heterogeneity. D Pseudotime trajectory analysis of the selected cell population. The left panel shows cells ordered along pseudotime, the middle panel shows the distribution of clusters (C0-C5) along the trajectory, and the right panel shows the distribution of cell states (State 1-9), indicating continuous state transitions and a branched developmental process under the disease-related context.*

4. Discussion

This work methodically examined the possible common genetic underpinnings and immune-cell processes connecting SLE and AD by combining peripheral blood single-cell RNA transcriptomic analysis with MR. Initially, 1,713 genes linked to AD and 361 genes linked to SLE were found by single-cell research. 40 of these genes were shared by the two conditions. Further MR analysis revealed that ENTPD1 and THEMIS2 could be important risk genes that are jointly implicated in the onset and progression of AD and SLE. Peripheral immune dysregulation may be a significant molecular basis for SLE-AD comorbidity, according to functional enrichment analysis, which revealed that the shared differentially expressed genes were primarily enriched in immune regulatory processes, including lymphocyte-mediated immunity, cell killing, T-cell differentiation, immune receptor activity, antigen binding, and Th1/Th2 as well as Th17 cell differentiation. According to earlier research, individuals with SLE are more likely to experience dementia and cognitive impairment, whereas the pathophysiology of AD includes changes in the central immune microenvironment, peripheral immune cell activation, and neuroinflammation in addition to neurones themselves [30-32]. Thus, our data supports the idea that SLE-AD comorbidity may have a similar immunoinflammatory base at both the single-cell resolution and genetic causal inference levels.

This research found that one of the major risk genes for SLE-AD comorbidity is ENTPD1. The ectonucleoside triphosphate diphosphohydrolase protein encoded by ENTPD1, also referred to as CD39, is primarily responsible for hydrolysing extracellular ATP and ADP into AMP, which is then transformed into adenosine by CD73. This process contributes to the control of inflammatory reactions, immunosuppression, and purinergic signalling after tissue injury [33, 34]. Adenosine production, which is facilitated by the CD39/CD73 axis, is a key way whereby regulatory T cells dampen the immune system. According to research by Deaglio et al., CD39 and CD73 co-express on the surface of regulatory T cells and may prevent effector T-cell activation by producing adenosine, which preserves immunological homeostasis [34]. Since the breakdown of peripheral immunological tolerance combined with aberrant activation of autoreactive T and B cells is one of the primary clinical hallmarks of SLE, this pathway is very pertinent to the pathogenesis of the disease. Additionally, Loza et al. observed that abnormal ENTPD1/CD39 may contribute to decreased immunosuppressive capability and prolonged inflammation in SLE, since deficient CD39 expression in T cells from SLE patients may be linked to altered regulatory T-cell activity [35]. Accordingly, MR analysis revealed ENTPD1 as a common risk-associated gene for AD and SLE, which is physiologically compatible with its known function in immune modulation. It is also mechanistically probable that ENTPD1 and AD are related. Amyloid-β accumulation, aberrant tau hyperphosphorylation, microglial activation, inflammatory cytokine release, blood-brain barrier failure, and peripheral immune cell involvement are all part of the pathological process of AD. According to earlier research, CD39/ENTPDase1 may affect microglial migration, the balance of purinergic signalling, and microglial responses to ATP and other damage-associated signals [36]. Extracellular ATP typically functions as a danger signal that stimulates inflammatory responses in neuroinflammatory situations, whereas CD39-mediated ATP breakdown may change the ratio of pro-inflammatory ATP to immunosuppressive adenosine. Therefore, by altering purinergic signalling in both peripheral immune cells and central microglia, dysregulation of ENTPD1 may contribute to neuroinflammation in AD. Adaptive immune responses may be involved in the pathophysiology of AD, according to Gate et al.'s finding of clonal proliferation of $CD8^+$ T lymphocytes in the peripheral blood and cerebral fluid of AD patients [37]. These findings, along with our discovery that T cells were the primary location of AD-related cell types, imply that ENTPD1 may act as a shared immunoregulatory node connecting SLE and AD by regulating T-cell activation, adenosine signalling, and the neuroinflammatory microenvironment.

In this work, THEMIS2 was shown to be another important gene that underlies SLE-AD comorbidity. THEMIS2, sometimes referred to as ICB1, is a member of the THEMIS family and is mainly engaged in immune receptor signalling in B cells, macrophages, and other myeloid cells, according to earlier research. According to Peirce et al., THEMIS2/ICB1 acts as a signalling scaffold protein that specifically controls macrophage cytokine production and Toll-like receptor signalling. Cheng et al. also showed that THEMIS2 interacts with molecules including Grb2, Lyn, and PLC-γ2 to improve downstream B-cell receptor signalling and reduce the activation threshold of B cells in response to low-affinity antigens [38]. These results imply that THEMIS2 could affect how innate and adaptive immunity integrate signals. Aberrant B-cell activation, autoantibody synthesis, dysregulated T-cell assistance, and monocyte/macrophage-mediated inflammatory responses are all key components in the development and course of SLE. Therefore, by increasing immune-cell response to self-antigens or inflammatory stimuli, aberrant THEMIS2 expression or function may facilitate autoimmune amplification in SLE. There is still not much direct evidence connecting THEMIS2 to AD. Nonetheless, a reasonable foundation for understanding our results is provided by its function in immune signalling control. Peripheral immune cells, meningeal immune cells, and microglia may interact to affect neuroinflammation, Aβ clearance, and neuronal damage in AD, which is becoming acknowledged as a condition with major neuroimmune aspects [31, 32]. In a three-dimensional human neuroimmune model, Jorfi et al. demonstrated that invading $CD8^+$ T lymphocytes may worsen neuroinflammation and neurodegenerative damage associated with AD [39]. Therefore, THEMIS2

may influence AD-related immunoinflammatory processes indirectly by changing the activation threshold of peripheral immune cells, the degree of antigen responsiveness, and the release of inflammatory mediators rather than directly influencing neuronal pathology. These findings, together with the current discovery that THEMIS2 was shown by MR analysis to be a risk-associated gene for both SLE and AD, imply that it could be a significant candidate gene that connects autoimmune dysregulation with neuroinflammatory reactions. To confirm the expression patterns and functional functions of THEMIS2 in various cellular situations, further research using peripheral blood immune cells, cerebrospinal fluid cells, and brain tissue samples will be necessary.

T cells seem to be a common cell population of special significance in both SLE and AD, according to our single-cell data. Both T cells and monocytes in the SLE sample exhibited significant differential expression of ENTPD1 and THEMIS2. T cells were shown to be the most important cell type in the AD dataset. Widespread T-cell abnormalities, such as dysregulated T-cell receptor signalling, an imbalance in the Th17/Treg ratio, aberrant expansion of T follicular helper (Tfh) cells, and activation of interleukin-17 (IL-17) and interferon (IFN)-related inflammatory pathways, have been found in SLE patients [40-42]. In addition to causing tissue damage and autoantibody formation, these anomalies may also have an impact on the central nervous system via cytokine-mediated neurotoxicity, disruption of the blood-brain barrier, and systemic inflammation. However, single-cell research indicates that peripheral adaptive immunity, specifically T cell-mediated immune responses, may play a role in the development and progression of AD. Additionally, changes in the proportions and clonal expansion states of peripheral blood T-cell subsets have also been documented in AD patients [23]. The idea that T cell-mediated immune dysregulation may serve as a crucial connection between SLE and AD comorbidity is therefore further supported by the current study's identification of T cells as a crucial significant cell type in both disorders. Additionally, special consideration should be given to the function of monocytes in SLE. Monocytes were also found in the current research to have notable differential expression of THEMIS2 and ENTPD1 in SLE. SLE patients' peripheral blood monocytes often show reduced phagocytic clearance, increased production of inflammatory mediators, improved antigen presentation, and overexpression of type I IFN-stimulated genes. Nehar-Belaid et al. showed that high expression of type I IFN-stimulated genes is widely distributed across several immune cell subsets, including monocytes, T cells, NK cells, B cells, and plasma cells, using single-cell sequencing to create a peripheral immune atlas of SLE [24]. This result is in line with the substantial enrichment of T-cell differentiation, immunological response, and infection-related pathways shown in our functional enrichment study. Since monocytes can develop into DCs or macrophages and affect the central nervous system through vascular inflammation, antigen presentation, and cytokine secretion, their aberrant activation may be a significant peripheral immune source that contributes to neuropsychiatric symptoms and cognitive decline in SLE patients.

The important genes or associated disease networks may be connected to Bisphenol A, Entinostat, valproic acid, tetrachlorodibenzodioxin, acrolein, and air pollution, according to our medication and chemical connection predictions. These results should not be seen as direct causal proof, but rather as computational hints. Among them, a common endocrine-disrupting chemical called bisphenol A has been linked to a higher risk of SLE. According to Wang et al.'s 2024 research, patients with SLE had higher urine levels of Bisphenol A and Bisphenol F than healthy controls, and greater exposure levels were positively correlated with a higher chance of developing SLE [43]. It has also been suggested that BPA affects neurodegenerative alterations in AD by means of oxidative stress, neuroinflammation, Aβ deposition, and tau phosphorylation [44]. Furthermore, in animal models of AD, entinostat/MS-275, a histone deacetylase inhibitor, has been shown to improve behavioural performance while reducing microglial activation and Aβ deposition [45]. All of these results point to potential interactions between immunoinflammatory processes, environmental exposure, and epigenetic control. However, as the drug-disease connections in this investigation were mostly obtained from database-based predictions, more validation via cellular studies, animal models, and clinical cohorts is still necessary to determine their true therapeutic potential and safety.

The current study found shared key genes connecting SLE and AD at two complementary levels-cellular heterogeneity and genetic causal inference-by combining single-cell RNA transcriptomic analysis with MR. This approach improved candidate target interpretability compared to differential expression analysis alone. However, it is important to recognize a few limits. First, peripheral blood samples were used to create all single-cell datasets. The transcriptional characteristics of brain tissue, microglia, meningeal immune cells, or cerebrospinal fluid cells cannot be completely captured by these data, despite the fact that they may indicate systemic immunological state. Peripheral blood single-cell data may only capture a portion of the immune-related signals in AD since many important pathogenic processes take place in the central nervous system. Therefore, peripheral blood may not be able to detect brain-specific transcriptional programs-particularly those originating from microglia and other resident cells of the central nervous system-as well as tissue-specific cell-cell interactions. Second, only three AD patients and two healthy controls made up the sample size of GSE181279, which may have limited the robustness of the observed variations in cell proportions and gene expression. Third, the findings of MR analysis are still dependent on the source population of the GWAS data, the strength of the instrumental factors, and the control of horizontal pleiotropy, even though it may lessen confounding and reverse-causation bias. Since the majority of

the GWAS datasets utilised in this research were from European populations, it is still unclear if the results can be applied to other ethnic groups. Fourth, there is currently little direct experimental evidence connecting THEMIS2 and ENTPD1 to SLE-AD comorbidity. Therefore, rather than clearly pinpointing pathogenic targets, our results should be largely viewed as identifying possible mechanistic genes. Due to the limitations of the AD dataset, future research should validate these observations in larger, independent, and more balanced AD cohorts. Meanwhile, to better understand the roles of these two genes in T cells, monocytes, and central immune cell populations, future research should include independent cohorts, validation by quantitative real-time polymerase chain reaction (qPCR) or flow cytometry, gene knockdown and overexpression experiments, and animal models. Notwithstanding these drawbacks, ENTPD1 and THEMIS2 were shown to be possible shared risk-associated genes between AD and SLE. The results also imply that T cell-mediated immunological dysregulation may be a significant biological substrate for the comorbidity of these two conditions. When taken as a whole, these findings give fresh genetic and cellular evidence for comprehending the processes connecting SLE and AD. They also suggest a possible path for the future development of precision treatment approaches that target both neuroinflammation and autoimmune dysregulation.

5. Conclusion

This work methodically identified the possible similar genetic architecture and immune regulatory underpinnings behind the comorbidity between SLE and AD by combining peripheral blood single-cell transcriptomic data with MR analysis. 361 SLE-associated genes and 1,713 AD-associated genes were found using single-cell analysis; 40 of these genes were common to both conditions. Further MR analysis revealed THEMIS2 and ENTPD1 as important comorbidity-associated genes that increased the risk of AD and SLE. While T cells showed a more prominent disease association in AD, cell-type analysis revealed that T cells and monocytes may represent the major immune cell populations associated with aberrant expression of these key genes in SLE. This suggests that T cell-mediated immunoinflammatory responses may constitute an important cellular basis linking SLE and AD. These results provide fresh insights into the molecular connections that underlie SLE-AD comorbidity, and they also imply that ENTPD1 and THEMIS2 might be future targets for combination therapy and precision intervention. More research is still needed to confirm the expression patterns and regulatory mechanisms of these two genes in larger, multiethnic cohorts as well as in brain tissue, cerebrospinal fluid, and peripheral immune cell samples. Functional experiments are also needed to elucidate their precise roles in autoimmune dysregulation, neuroinflammation, and cognitive impairment.

Abbreviations

SLE	Systemic Lupus Erythematosus
AD	Alzheimer's Disease
MR	Mendelian Randomization
GEO	Gene Expression Omnibus
GWAS	Genome-wide Association Study
QC	Quality Control
PCA	Principal Component Analysis
tSNE	T-distributed Stochastic Neighbour Embedding
DEGs	Differentially Expressed Genes
logFC	Log Fold Change
KEGG	Kyoto Encyclopedia of Genes and Genomes
GO	Gene Ontology
BP	Biological Processes
CC	Cellular Components
MF	Molecular Functions
IVW	Inverse Variance Weighted
OR	Odds Ratios
GGI	Gene-gene Interaction
miRNA	microRNA
CTD	Comparative Toxicogenomics Database
BEAM	Branched Expression Analysis Modelling
PCs	Principal Components
Mgk	Megakaryocytes
pDCs	Plasmacytoid Dendritic Cells
DCs	Dendritic Cells
PCs	Plasma Cells
SNPs	Single-nucleotide Polymorphisms
Tfh	T Follicular Helper
IL-17	Interleukin-17
IFN	Interferon
qPCR	Quantitative Real-time Polymerase Chain Reaction

Supplementary Material

The supplementary material can be accessed at https://doi.org/10.11648/j.cnn.20261004.12

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Chen Cai: Formal Analysis, Methodology, Resources, Software

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Rurong Sun: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing

Data Availability Statement

The data that support this article are available in both the paper and its online supple-mental material.

Conflicts of Interest

The authors declare no conflicts of interest.

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