

Research Article

Exploration of Hub Genes and Pathogenetic Pathways in Systemic Lupus Erythematosus Complicated with Early Onset Atherosclerosis

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Background. Notwithstanding the mounting evidence to suggest that systemic lupus erythematosus (SLE) accelerates the progression of atherosclerosis, the mechanisms underlying this phenomenon are yet to be completely understood. This research examined the molecular mechanism behind this vascular complication. **Methods.** The Gene Expression Omnibus database was retrieved to acquire the gene expression datasets for SLE (GSE109248) and atherosclerosis (GSE100927). The shared differentially expressed genes (DEGs) of SLE and atherosclerosis were screened with the help of the “limma” package in R software, followed by function enrichment analysis, protein–protein interaction (PPI) network construction, key module analysis, hub gene selection, and coexpression analysis. **Results.** In GSE109248 and GSE100927, 1195 and 418 DEGs in totals were identified, respectively. Subsequently, we acquired 78 common DEGs (70 upregulated genes and eight downregulated genes) with the same expression trends by using the Venn diagram. Finally, 12 hub genes, including PTPRC, TYROBP, FCGR3A, ITGAX, LCP2, IL1B, IRF8, LILRB2, CD68, C1QB, CCR7, and C1QA were identified by using seven different algorithms in Cytohubba. The functional analysis illustrates that these genes were predominantly enriched in immune and inflammation response, lipid and atherosclerosis, and osteoporosis. These results indicate an important role of SLE in inducing excessive inflammation, which may be mediate by these hub genes and can induce osteoporosis and imbalance of the normal mineral balance in the body as well as lipid abnormalities, which eventually leads to premature onset of atherosclerosis. In total, nine transcription factors (TFs) that may participate in regulating the function of these genes were identified. All hub genes and four TFs were validated successfully. **Conclusion.** The results of our research show that SLE and atherosclerosis have common DEGs, pathophysiology, and hub genes. These findings can provide fresh evidence and insights into a further investigation into the mechanisms at play.

1. Introduction

According to accumulating research data, the incidence and prevalence of systemic lupus erythematosus (SLE) is increasing year by year [1, 2]. Patients diagnosed with SLE have an elevated risk of suffering from cardiovascular disease (CVD) as well as atherosclerosis [3]. Nevertheless, among SLE patients, the early occurrence of atherosclerosis in the low-risk population (mostly younger women) cannot be adequately elucidated by conventional cardiovascular risk factors such as smoking, hypertension, and hyperlipidemia [4]. In individuals diagnosed with SLE, the risk of developing CVD and atherosclerosis is up to 50 times higher than that in age-

and gender-matched controls [5, 6]. Atherosclerosis is a comorbidity and the major contributor to death among SLE patients [7]. Both atherosclerosis and SLE share many proinflammatory environmental markers, including systemic and local immune responses and proinflammatory chemokines and cytokines (TNF- α , type I interferons (IFNs), transforming growth factor β , vascular endothelial growth factor, and IL-1, etc.) [8].

Although SLE is considered to be a risk marker for promoting the atherosclerosis process, the underlying mechanisms of these two disorders are still unclear. Neutrophils and neutrophil extracellular traps (NETs)-related cascades are probably one of the most essential pathways.

SLE and atherosclerosis have shared mechanisms in their respective pathogenesis pathways. In SLE, the function of neutrophils is impaired, and the level of NETs is increased [9]. NETs could accelerate the inflammatory response by activating inflammatory factors and other inflammatory cells [10]. In atherosclerosis, local inflammation and propagated arterial intimal injury and thrombosis could be exacerbated and amplified by NETs [11]. In addition, NETs not only reduce the efflux capacity of beneficial cholesterol by inducing oxidative stress and oxidizing high-density lipoprotein particles [12] but also activate NF- κ B signaling in macrophages, leading to the aggravation of atherosclerosis [13].

Common transcriptional features provide new clues to studying the overlapping pathogenic processes of SLE and atherosclerosis. The fundamental objective of this research is to discover hub genes that play a role in the pathogenic mechanism of SLE complicated with atherosclerosis. Using comprehensive bioinformatics and enrichment analysis, we examined the differentially expressed genes (DEGs) that are shared between SLE and atherosclerosis. These genes were found by downloading two sets of gene expression data from the Gene Expression Omnibus (GEO) database: GSE109248 and GSE100927. Subsequently, protein–protein interaction (PPI) networks were constructed, gene modules were evaluated, and hub genes were found by retrieving the Search Tool for the Retrieval of Interacting Genes database and the Cytoscape program. Finally, we narrowed it down to 12 hub genes, after which we conducted additional research on the transcription factors (TFs) associated with these genes and effectively validated their expression in two additional gene expression datasets (GSE43292 and GSE112943). These findings suggest a shared pathological pathway and offer a fresh perspective on how the molecular processes underlying these two illnesses are investigated further.

2. Materials and Methods

2.1. Data Acquisition. GEO (<http://www.ncbi.nlm.nih.gov/geo>) [14] is a publicly available portal that provides comprehensive microarray and high-throughput sequencing datasets for free download. Using SLE and atherosclerosis as keywords to screen for eligible gene expression datasets. Four datasets, GSE109248, GSE100927, GSE112943, and GSE43292, were acquired from the GEO database. We used GSE109248 and GSE100927 to identify hub genes, while GSE112943 and GSE43292 as external validation datasets. The GSE109248 dataset contains 25 cutaneous lupus and 14 control skin. GSE100927 consists of 69 atherosclerotic samples and 35 control arteries without atherosclerotic lesions samples from deceased organ donors. GSE112943 involved 16 cutaneous lupus and 10 control skin. GSE43292 consists of 32 atheroma plaques and the same number of intact tissues. We then downloaded a series of matrix files and data-sheets for the microarray platform, including GPL10558, GPL17077, and GPL6244. This study involved no human or animal subjects.

2.2. Identification of DEGs. We used the data tables of GPL10558, GPL17077, and GPL6244 to annotate the series

matrix files of GSE109248, GSE100927, GSE112943, and GSE43292. Then, we identified the DEGs between the disease group and the control group by performing the “limma” R package. The online Venn diagram tool was utilized to detect the shared DEGs. Such DEGs were determined to be any genes whose adjusted p -value was <0.05 and $|\log_{2}FC$ (fold change) $|\geq 1$.

2.3. Enrichment Analyses of DEGs. The “clusterProfiler” program in the R package and Metascape (<http://metascape.org>), an online analysis platform [15], were used to carry out the analyses of gene ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. $p < 0.05$ suggesting a significant difference.

2.4. Development and Module Analysis of a PPI Network. The Search Tool for the Retrieval of Interacting Genes (STRING) database was utilized to build a PPI network of common DEGs, with a confidence score of >0.4 serving as the cutoff value. Then, the PPI network was entered into Cytoscape for visual representation. Molecular complex detection technology (MCODE), a plugin of Cytoscape, was adopted to evaluate the key functional modules. The K -core value was adjusted to 2, the node score cutoff was established at 0.2, the maximum depth was selected as 100, and the degree cutoff was adjusted to 2. Subsequently, involved modular genes were performed in the KEGG and GO analysis using “clusterProfiler” within the R package.

2.5. Detection and Characterization of Hub Genes. To analyze and determine which genes function as hub genes, we made use of Cytoscape’s cytoHubba plugin and seven standard algorithms (EPC, MNC, Stress, Radiality, Closeness, Degree, and MCC). Afterward, the hub genes that had been identified were entered into the database known as GeneMANIA (<http://www.genemania.org/>) [16], which was utilized to identify internal associations in gene sets to construct a co-expression network.

2.6. Verification of the Expression of Hub Genes in External Data Sets. The GSE112943 and GSE43292 datasets were used as the validation dataset. Wilcoxon-test was performed to compare these two datasets. p -Value < 0.05 was established as the criterion for statistical significance.

2.7. Prediction and Validation of TFs. The Transcriptional Regulatory Relationships Unraveled by Sentence-based Text mining (TRRUST) database is used for predicting the transcriptional regulatory networks and provides information on the target genes corresponding to TFs as well as the regulatory connections between them [17]. By employing the TRRUST database, we obtained the TFs that modulate the hub genes. The significance level was determined to be an adjusted p -value < 0.05 . After that, the Wilcoxon test was utilized to validate the levels of expression of these TFs in GSE109248 and GSE100927.

2.8. Assessment of Immune Cell Infiltration in SLE and Atherosclerosis. The single sample gene set enrichment analysis (ssGSEA) technique was applied to measure the relative levels of infiltration of 28 different immune cells using the

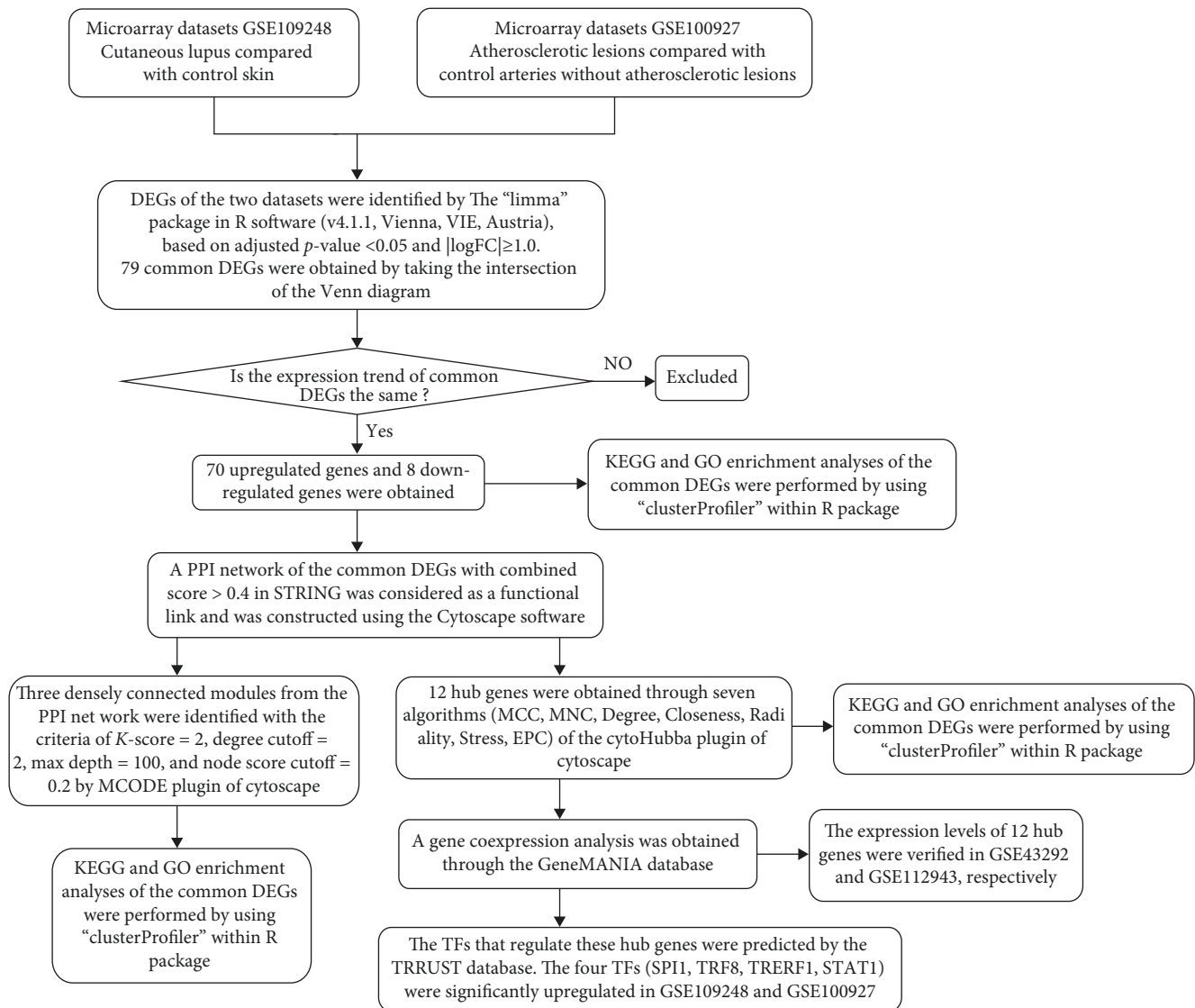


FIGURE 1: Flowchart for research design.

GSE109248 and GSE100927 datasets [18]. R software was used to generate violin plots for demonstrating the differential expression levels of 28 infiltrating cells.

2.9. Statistical Analysis. R software version 4.1.3 was used to perform the Wilcoxon test to identify the expression levels of hub genes and the TFs. p -Value < 0.05 was considered significant.

3. Results

3.1. Identification of DEGs. Figure 1 depicts the study's workflow. The relevant epidemiological statistical information is presented in Figure S1.

In total, 1,195 DEGs in GSE109248 and 418 DEGs in GSE100927 were discovered. The volcano map of DEGs is shown in Figures 2(a) and 2(b). In total, 79 common DEGs in total were obtained through the intersection of the Venn diagram (Figure 2(c)).

Subsequently, after eliminating genes with opposite patterns of expression in GSE109248 and GSE100927, 78 DEGs were retained.

3.2. Analysis of the Functional Enrichment of the Overlapping DEGs. Following the conversion to gene IDs, GO and KEGG enrichment analyses were performed to examine the biological activities and pathways linked to the 94 common DEGs. The analysis of the functional enrichment of DEGs made use of the three components of the GO annotation of DEGs. The findings of GO analysis illustrated a predominant enrichment of these genes in biological activities of immune cells, including regulation of leukocyte activation, positive regulation of cytokine production, T-cell activation, etc. (Figure 3 (a)). In terms of the KEGG pathway, strong enrichment of DEGs was observed in osteoclast differentiation, rheumatoid arthritis, SLE, and immune cell-related signaling pathways, such as Lipid and atherosclerosis, IL-17 signaling pathway, and cytokine–cytokine receptor interaction (Figure 3(b)).

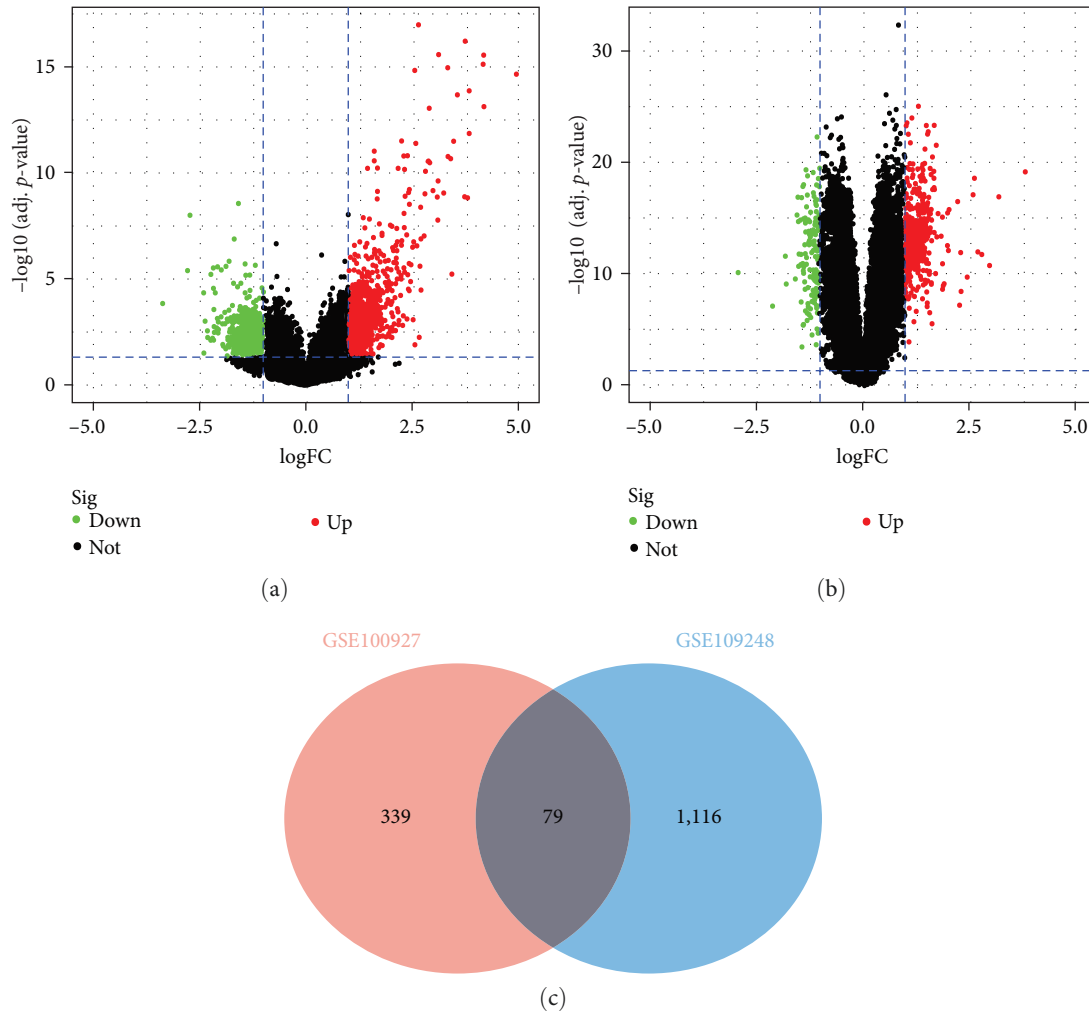


FIGURE 2: The volcano and Venn diagrams of DEGs: (a) GSE109248 dataset represented in a volcano map; (b) GSE100927 dataset depicted in a volcano map; (c) 79 DEGs were shared across both datasets.

3.3. Development of the PPI Network and an Analysis of the Modules. We generated the PPI network of the common DEGs utilizing Cytoscape with combined scores <0.4 (Figure 4(a)). With the help of the MCODE plug-in of Cytoscape, three closely connected gene modules, including 31 common DEGs were, obtained (Figure 4(b)–4(d)).

The findings of the GO analysis demonstrated a link between 31 genes of these three modules and the immune response and inflammation (Figure 5(a)). According to the findings of the KEGG pathway analysis, these genes are mostly associated with tuberculosis, Chagas disease, and cytokine–cytokine receptor interaction (Figure 5(b)).

From these results, we can easily find that immune response and inflammation might perform a critical function in SLE and atherosclerosis.

3.4. Identification and Analysis of Hub Genes. Seven algorithms of plug-in cytoHubba were utilized to calculate the top 20 hub genes (Table 1).

Subsequently, we found 12 common hub genes (PTPRC, TYROBP, FCGR3A, ITGAX, LCP2, IL1B, IRF8, LILRB2, CD68, C1QB, CCR7, and C1QA) by using the intersection

of the Venn diagrams (Figure 6(a)). Table 2 displays their respective names as well as their corresponding functions.

Next, the GeneMANIA database was utilized to conduct an analysis of the coexpression network as well as the functions linked to these genes. The findings illustrated that these genes had a complex PPI network, with coexpression accounting for 64.77% of the network, physical interactions accounting for 14.78%, colocalization accounting for 14.51%, genetic interactions accounting for 1.41%, pathways accounting for 1.27%, and shared protein domains accounting for 0.56% (Figure 6(b)).

As shown in GO analysis, these genes are predominantly enriched in various immune and inflammatory responses (Figure 7(a)). Additionally, the findings of the KEGG pathway illustrated a significant enrichment of these genes in osteoclast differentiation, infectious diseases, and complement and coagulation cascades (Figure 7(b)).

3.5. Validation of the Expression of Hub Genes in External Datasets. The reliability of the expression levels of these genes was evaluated using two different external datasets containing SLE and atherosclerotic plaques. As shown in

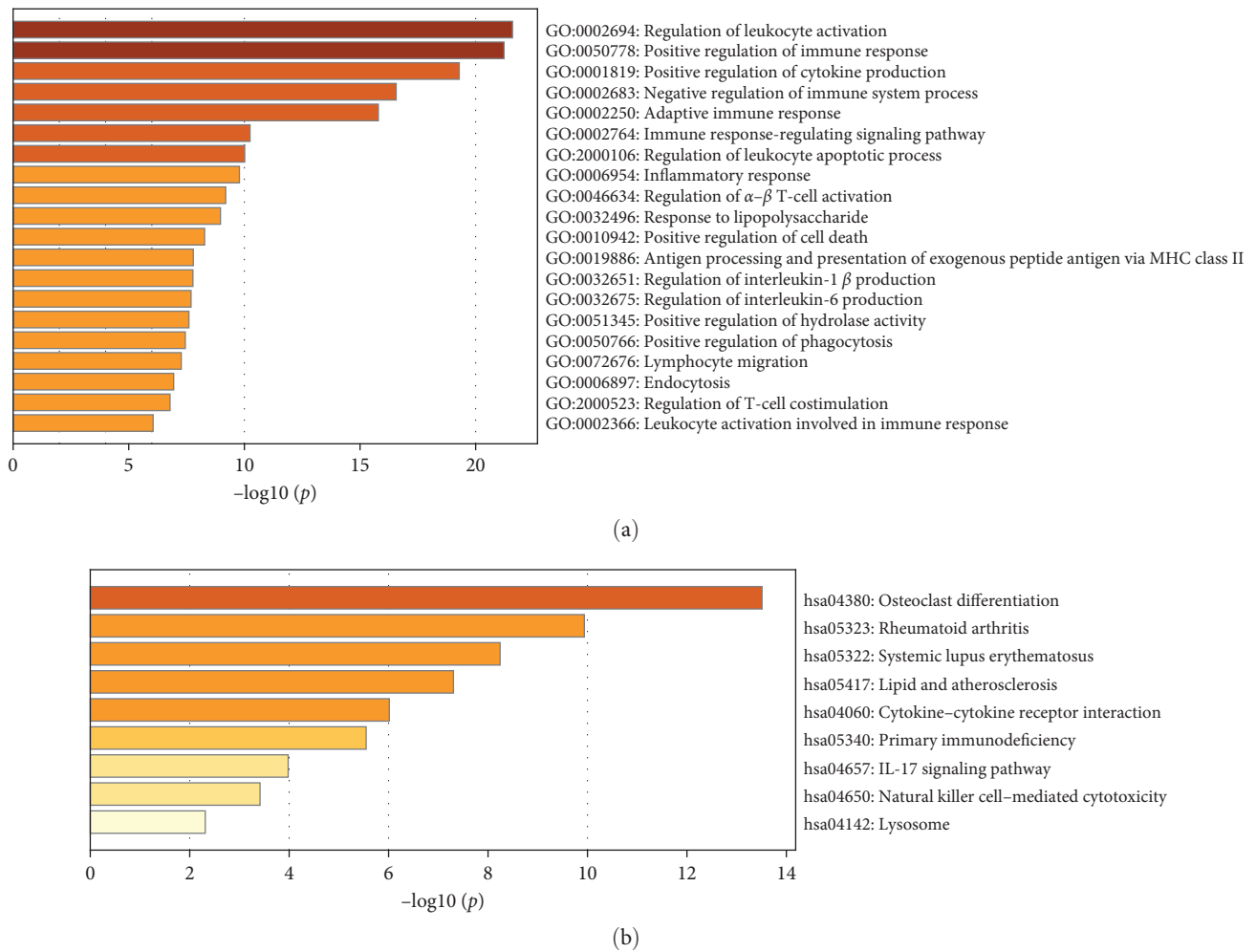


FIGURE 3: Analysis results for common DEG enrichment: (a) the GO pathway enrichment analysis findings; (b) results of KEGG pathway-related enrichment analysis. The significance threshold value was determined to be p -value < 0.05 .

Figure 8(a), all hub genes were substantially expressed at a high level in cutaneous lupus lesions compared with control skin. The levels of expression of all genes were likewise considerably elevated in atherosclerotic plaques relative to vascular tissues that served as the controls (Figure 8(b)).

3.6. Prediction and Validation of TFs. We discovered nine TFs that might be implicated in modulating the expression of these genes based on the data acquired from the TRRUST database (Figure 9(a) and Table 3).

Subsequently, four TFs that were upregulated in cutaneous lupus lesions and atherosclerotic plaques were identified by validation (Figures 9(b) and 9(c)). They have a function coordinately in the modulation of six hub genes, namely, IL1B, CD68, ITGAX, CCR7, LILRB2, and IRF8.

3.7. Immune Infiltration Analysis in SLE and Atherosclerosis. Investigation into the difference in the infiltration levels of immune cells in different samples was carried out utilizing the ssGSEA algorithm. The immune system-associated 28 cells were visualized regarding their distribution in the GSE109248 and GSE100927 by employing violin plots (Figures 10(a) and 10(b)). In comparison with normal samples, the SLE samples

showed enhanced infiltration of B cells, T cells, macrophages, neutrophils, dendritic cells (DCs), natural killer cells, and mast cells (Figure 10(a)). The atherosclerosis samples also demonstrated enhanced infiltration of B cells, T cells, macrophages, neutrophils, DCs, natural killer cells, and mast cells (Figure 10(b)).

4. Discussion

This is the first investigation in the field of bioinformatics that examines SLE and atherosclerosis concurrently. The main purpose is to screen and identify the common DEGs and mechanisms in SLE and atherosclerosis to provide new methods to prevent and treat SLE complicated with atherosclerosis.

In this study, 78 common DEGs were identified in both two diseases, 12 of which were determined to be the hub genes and included PTPRC, TYROBP, FCGR3A, ITGAX, LCP2, IL1B, IRF8, LILRB2, CD68, C1QB, CCR7, and C1QA. The GO and KEGG pathway enrichment analyses both found that these genes have a strong enrichment in immunological and inflammatory pathways. The regulation of cytokine, leukocyte, and chemokines production and activation, including TNF- α , type I IFNs, MCP-1, IL-1, S100A8/9,

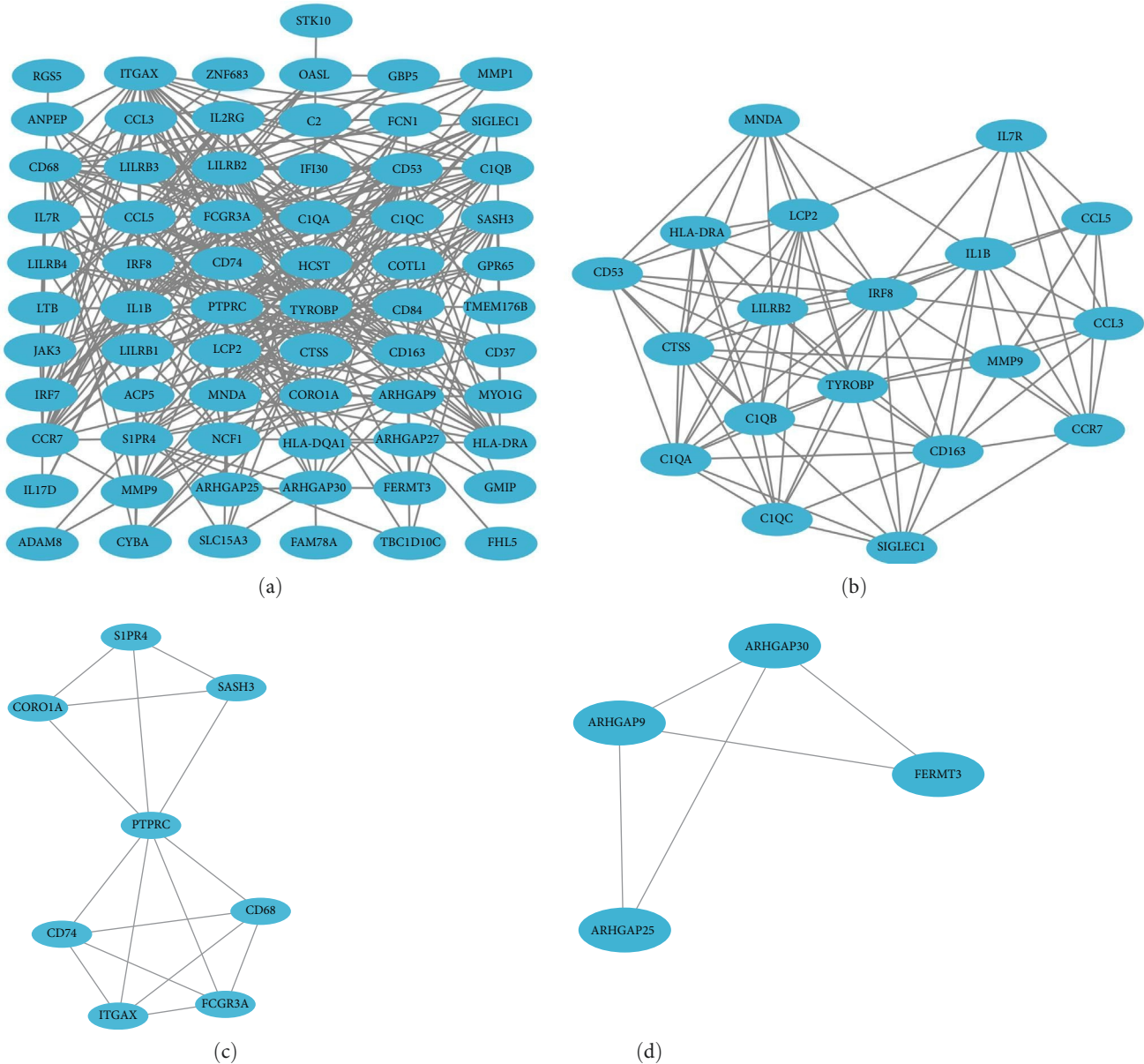


FIGURE 4: PPI network and significant gene modules: (a) PPI network diagram; (b)–(d) three significant gene clustering modules.

and Nets, which exert a synergistic effect in both the onset and progression of these two inflammatory illnesses [9, 11]. GO analysis suggests that the response to lipopolysaccharides also assumes a crucial function in both illnesses. The lipopolysaccharide can not only mediate nuclear transduction of NF- κ B but also induce the activation of Toll-like receptor 4, causing the release of inflammatory factors, which ultimately results in the onset as well as the advancement of SLE and atherosclerosis [19, 20]. Enrichment of these genes was found in Osteoclast differentiation, Complement and coagulation cascades, and Infection-related diseases in the KEGG pathway. Some studies have shown that imbalanced normal mineral homeostasis within the body provides the condition for the formation of vascular calcification and atherosclerotic plaques [21]. The onset of both SLE and atherosclerosis is associated with infection [22, 23]. The autoantibodies and

circulating immune complexes of SLE patients can promote the activation of complement and coagulation cascades, which impairs the endothelium, induces pro-adhesive and proinflammatory endothelial cell phenotypes, and changes the metabolism of lipoproteins that participate in atherogenesis to worsen the development of atherosclerosis [24]. In addition, our results showed that nine TFs may be involved in the regulation of these genes. Following subsequent validation, we discovered TFs upregulated in SLE and atherosclerotic plaques, which included IRF8, TRERF1, STAT1, and SPI1. They contributed in a coordinated manner to the modulation of six hub genes comprising IL1B, CD68, ITGAX, CCR7, LILRB2, and IRF8.

Inflammation and vitamins also play an important role in both two diseases. The inflammation and deficiency of vitamins that occur in SLE could cause osteoporosis, which

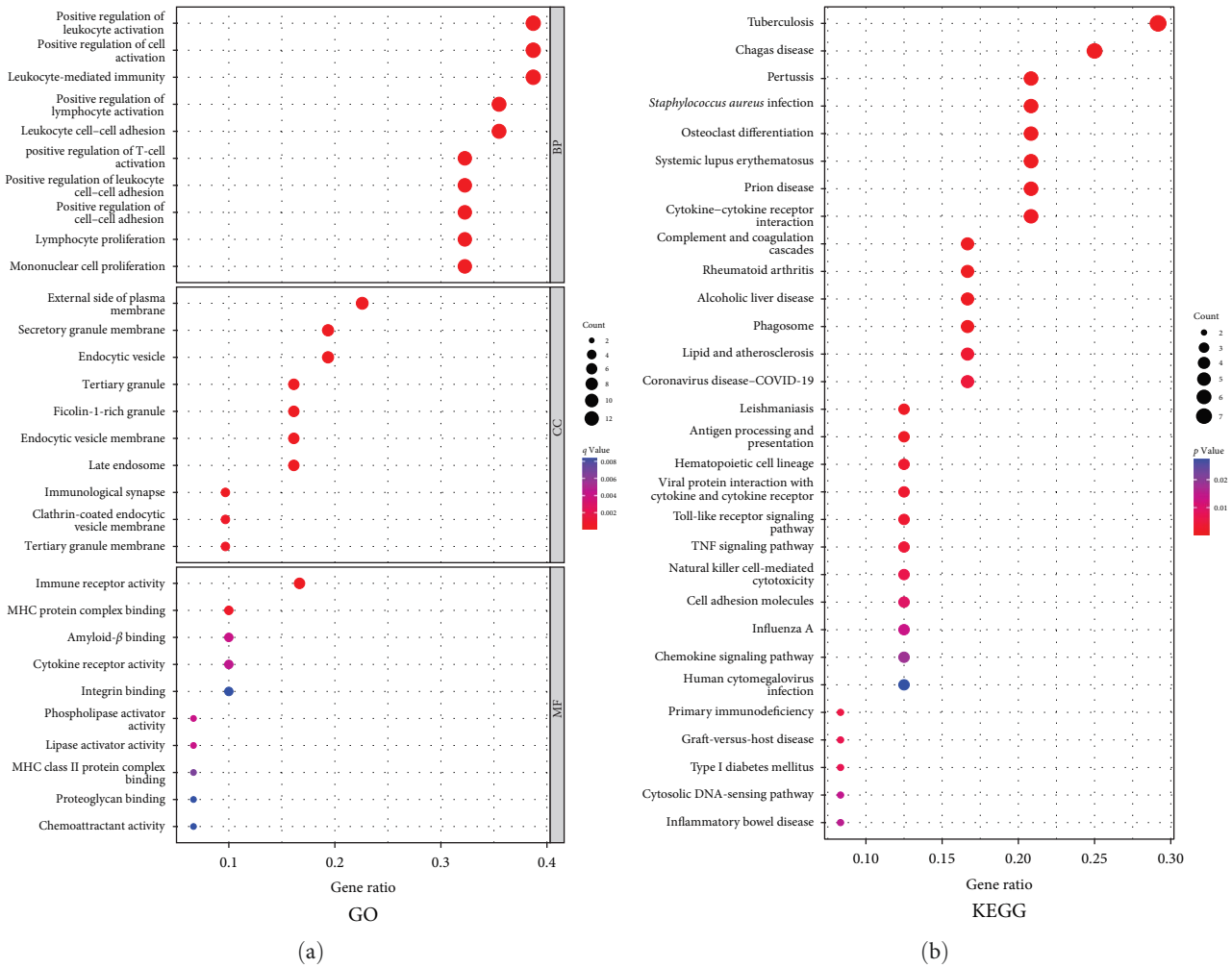


FIGURE 5: The modular genes enrichment analysis results: (a, b) enrichment study for GO and KEGG of the modular genes. The number of genes involved is represented by the size of the circle, and the frequency of the genes involved in the term total genes is shown by the abscissa.

can then progress to atherosclerosis [25]. According to the findings of certain studies, the active form of vitamin D has an anti-inflammatory effect. Vitamin D deficiency is significantly correlated with the increased incidence, or aggravation, of SLE [26]. By increasing the serum level of vitamin D, the inflammatory response in SLE patients was significantly reduced [27]. Vitamin D plays a key role in the regulation of cardiovascular system function. Vitamin D, which plays a protective role in atherosclerosis formation, can reduce the release of inflammatory factors and the formation of foam cells [28]. According to clinical data, the serum active vitamin D level of patients with atherosclerosis is significantly lower than that of healthy control patients [29]. These evidences show that SLE is closely related to atherosclerosis. Individuals with SLE have a variety of inflammatory mediators that may be detected in their blood, proving that the body was experiencing a systemic inflammation at the time when SLE symptoms first appeared, which caused osteoporosis and an imbalance of normal mineral homeostasis within the body to provide an environmental foundation for the onset of vascular calcification and atherosclerotic plaques [30, 31]. Research findings have also illustrated

that dyslipidemia, which is another comorbidity of SLE, is linked to atherosclerosis and is characterized by abnormally high levels of total cholesterol, triglycerides, and low-density lipoprotein, as well as abnormally low levels of high-density lipoprotein [32]. Low-density granulocyte (LDG), which belongs to the neutrophil subset, has been shown to be elevated in SLE [33] and is in connection with vascular dysfunction within the body and inflammation and coronary plaque [34]. The LDGs of SLE might promote vascular injury, which is the primary mechanism that leads to neutrophil infiltration in individuals who have concurrent SLE and atherosclerosis, and in an inflammatory setting, the LDG that disrupts high-density lipoprotein function might be an important link between SLE and atherosclerosis [34].

There is some evidence that SLE and atherosclerosis share common inflammatory and immunological regulatory mechanisms. One of them is the IL23/Th17 axis, which is related to the pathophysiology of both disorders [35, 36]. In addition, a research report that investigated single nucleotide polymorphisms that are linked to CVD in SLE discovered that two novel putative risk loci are implicated in increasing the risk for CVD in SLE [37]. In addition, apoptotic cells

TABLE 1: The top 20 hub genes rank in cytoHubba.

MCC	MNC	EPC	Degree	Radiality	Stress	Closeness
PTPRC	PTPRC	PTPRC	PTPRC	PTPRC	PTPRC	PTPRC
FCGR3A	TYROBP	TYROBP	TYROBP	TYROBP	TYROBP	TYROBP
TYROBP	FCGR3A	FCGR3A	FCGR3A	ITGAX	IL1B	FCGR3A
IRF8	ITGAX	ITGAX	ITGAX	FCGR3A	LILRB2	ITGAX
CTSS	LCP2	IRF8	LCP2	LCP2	IRF8	LCP2
C1QB	IL1B	LCP2	IL1B	IL1B	FCGR3A	IL1B
C1QA	IRF8	IL1B	IRF8	LILRB2	LCP2	IRF8
LCP2	LILRB2	LILRB2	LILRB2	IRF8	CORO1A	LILRB2
HLA-DRA	CTSS	CD74	CTSS	CD68	NCF1	CTSS
CD53	CD68	CTSS	CD68	CTSS	ITGAX	CD68
CD74	CD74	CD68	CD74	CD53	CCR7	CD53
LILRB2	CD53	C1QA	CD53	CD74	C1QB	CD74
C1QC	C1QB	C1QB	C1QB	CD163	C1QA	C1QB
ITGAX	CCR7	CD53	CCR7	C1QB	CD68	CCR7
CD163	C1QA	CCR7	C1QA	CCR7	ARHGAP9	C1QA
MNDA	CCL5	CD163	CCL5	C1QA	S1PR4	CD163
CD68	CD163	CCL5	CD163	IL2RG	MMP9	CCL5
IL1B	IL2RG	C1QC	IL2RG	CORO1A	OASL	IL2RG
CCR7	CORO1A	HLA-DRA	CORO1A	MMP9	C1QC	CORO1A
CCL5	C1QC	IL2RG	C1QC	CCL5	IL2RG	MMP9

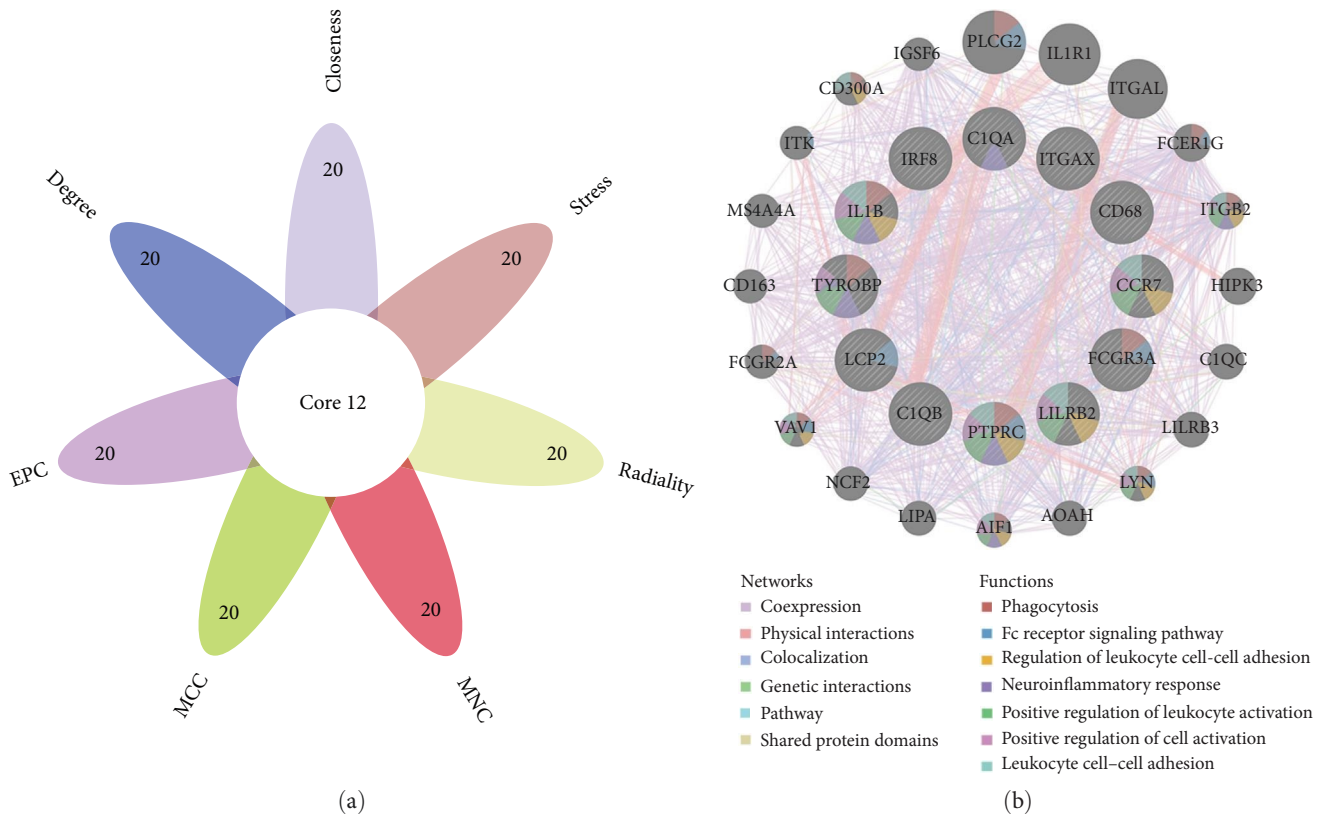


FIGURE 6: Hub gene coexpression network and Venn diagram: (a) seven algorithms were used to screen 12 common hub genes, as shown in the Venn diagram; (b) GeneMANIA was used to analyze hub genes and related coexpression genes.

TABLE 2: The details of the hub genes.

No.	Gene symbol	Full name	Function
1	PTPRC	Encoding protein-tyrosine phosphatase receptor-type C	PTPRC is one of the most abundant leukocyte cell surface glycoproteins and is expressed exclusively upon cells of the hematopoietic system
2	TYROBP	Transmembrane immune signaling adaptor TYROBP	The encoded protein may associate with the killer-cell inhibitory receptor (KIR) family of membrane glycoproteins and may act as an activating signal transduction element
3	FCGR3A	Fc gamma receptor IIIa	This gene encodes a receptor for the Fc portion of immunoglobulin G, and it is involved in the removal of antigen–antibody complexes from the circulation
4	ITGAX	Integrin subunit alpha X	This gene encodes the integrin alpha X chain protein
5	LCP2	Lymphocyte cytosolic protein 2	Participates in immune response and intracellular signal transduction
6	IL1B	Interleukin 1 beta	This cytokine is an important mediator of the inflammatory response and is involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis
7	IRF8	Interferon regulatory factor 8	Plays critical roles in interferon (IFN) signaling pathways governing the establishment of innate immune responses by myeloid and dendritic cells
8	LILRB2	Leukocyte immunoglobulin-like receptor B2	Control inflammatory responses and cytotoxicity to help focus the immune response and limit autoreactivity
9	CD68	CD68 molecule	Clear cellular debris, promote phagocytosis, and mediate the recruitment and activation of macrophages
10	C1QB	Complement C1q B chain	Plays an important role in maintaining the stability of the body's environment, oxidative stress, glucose and lipid metabolism, and other processes
11	CCR7	C–C motif chemokine receptor 7	This receptor was identified as a gene induced by the Epstein–Barr virus (EBV), controls the migration of memory T cells to inflamed tissues, as well as stimulates dendritic cell maturation
12	C1QA	Complement C1q A chain	Plays an important role in maintaining the stability of the body's environment, oxidative stress, glucose and lipid metabolism, and other processes

gather in various tissues of SLE patients and cause inflammation and necrosis [38]. There are also a large number of apoptotic cells in advanced atherosclerotic plaque, which induce secondary necrosis and inflammation [39]. The experiment shows that the related pathways of phagocyte clearing necrotic tissue in SLE patients are impaired, and these pathways also exist in atherosclerosis [6]. Endothelial dysfunction caused by endothelial cell damage is an early sign of atherosclerosis [40]. SLE patients have an autoimmune response against endothelial cells, resulting in endothelial dysfunction. This undoubtedly increases the risk of atherosclerosis [41].

IL-1B is one of the members of the interleukin 1 cytokine family, which is a crucial regulator of inflammation and is associated with diverse cellular activities, including proliferation, differentiation, and apoptosis of cells, and associated with the development of CVDs [42]. Studies have proved that the levels of IL1B are significantly higher in SLE patients [43]. In SLE, IL-1B is also involved in the platelet-mediated activation of endothelial cells, which may take part in the pathogenesis of CVD in patients with SLE [44]. In addition, IL-1B can induce NETs formation [45], which performs an integral function in SLE and atherosclerosis [9, 11]. CD68 is mainly expressed by human tissue macrophages and monocytes and is often used as a marker of macrophage infiltration.

Both infiltrating and resident macrophages via producing proinflammatory cytokines that contribute to ongoing injury of lupus nephritis, which is a common complication of SLE [46]. In atherosclerosis, macrophages contribute to preserve the local inflammatory response and accelerate and promote plaque development and thrombosis formation [47]. ITGAX is also known as CD11c. In SLE, CD11c is highly expressed in DCs, presenting an upregulation of Toll-like receptors 7 and 9 responses with enhanced expression of C-X-C motif chemokine ligand 13 and interleukin 10, which increased immune and inflammatory response [48]. In atherosclerosis, the majority of the immune cells that expressed CD11c were macrophages, and the expression of ITGAX/CD11c is involved in the index of vulnerability, levels of proinflammatory plaque cytokine, area of the necrotic core, and with each other [49]. The CCR7 is implicated in both immunity and tolerance by leading T cells and antigen-presenting DCs to and retaining them in lymph organs. Increased expression of CCR7 was associated with active SLE [50]. In addition to this, it participates in triggering the adaptive immune response by directing the migration, positioning, and interaction of naïve T cells and DCs within secondary lymphoid organs. The loss of CCR7 receptor in murine atherosclerosis not only leads to atherosclerotic plaque content reduction but also leads to disruption of T cell entry and exit within the inflamed vessel wall [51]. IRF8

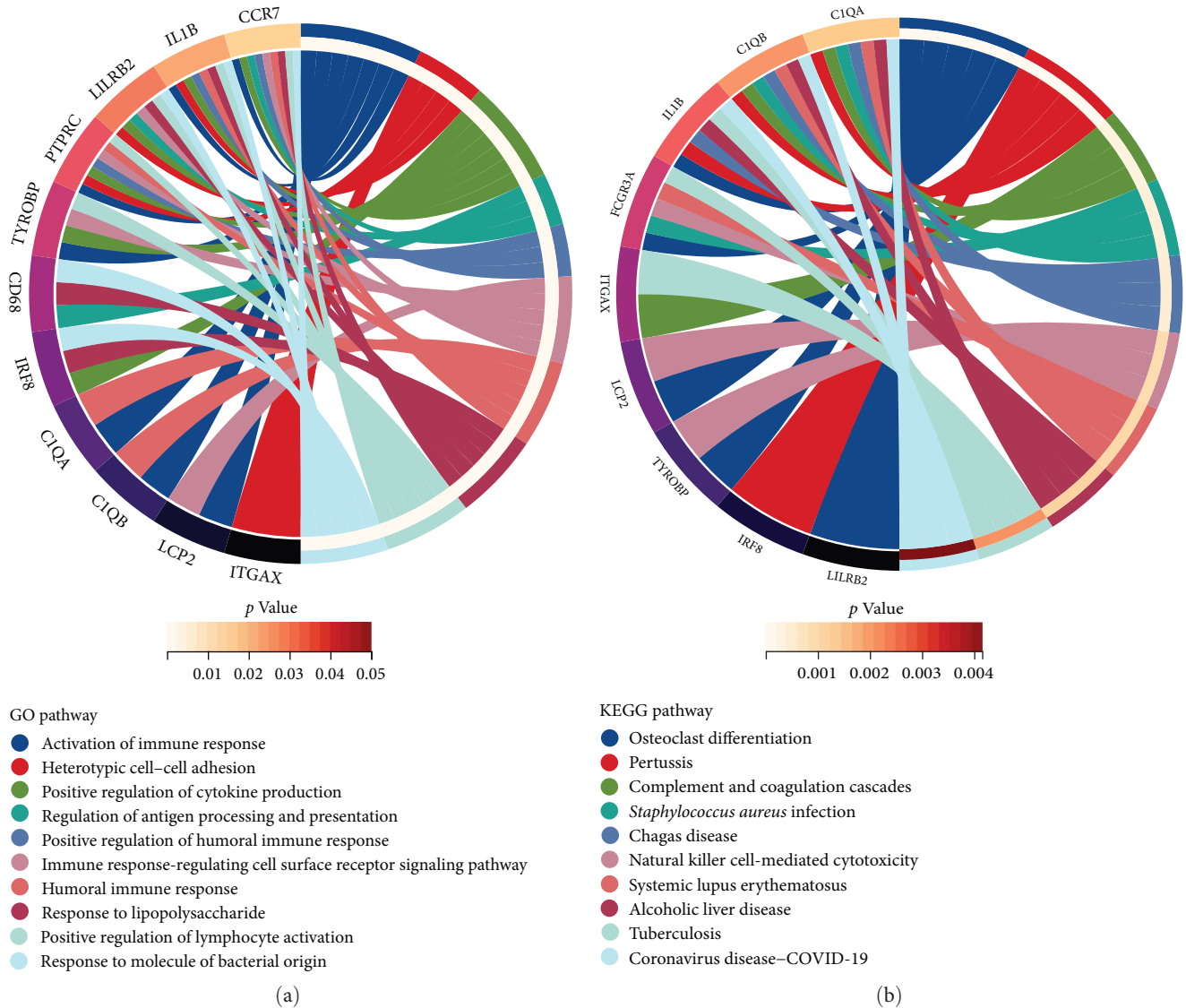


FIGURE 7: Hub gene function enrichment analysis: (a, b) analysis of hub gene-related GO and KEGG enrichment. The external circle on the left is the gene symbol, and the external circle on the right is the pathway involved that can indicate which functions the genes are involved in. The significant p -value of the pathway is represented by the internal circle on the right; the darker the color, the smaller the p -value.

is a TF of the IFN regulatory factor (IRF) family. In SLE, the recognition of circulating immunological complexes by endosomal Toll-like receptors leads to the activation of downstream IRF8 proteins, which affects the IFN pathway [52]. Studies have also shown that the deletion of IRF8 in DCs significantly decreases the development of atherosclerosis [53]. LILRB2 is one of the members of the leukocyte immunoglobulin-like receptor family. The receptor is subjected to the expression on immune cells, where it combines with major histocompatibility complex class I molecules that are present on antigen-presenting cells. This combination then transmits a negative signal that suppresses the activation of an immune response [54]. However, few studies explore the effects of LILRB2 in SLE and atherosclerosis, which may be a new direction to study the pathogenesis of both diseases in the future.

In addition, the ssGSEA algorithm was used to determine the infiltration of immune cells in SLE and atherosclerosis.

It is noteworthy that the infiltration level of T cells, macrophages, and neutrophils is significantly higher in SLE and atherosclerosis. $CD4^+$ T cells can differentiate into multiple cell types, such as Th1 cells, Th17 cells, and Tregs. The imbalance of T helper cells subsets is closely related to the formation and severity of SLE [55]. The infiltration of Treg cells is generally considered beneficial in both SLE and atherosclerosis [56, 57]. However, with the loss of the protective function of Treg cells in SLE and atherosclerosis, its inhibition of inflammatory response disappeared and can converted to proinflammatory Th cells [58, 59]. Macrophages are crucial to the occurrence and development of SLE [60]. The number of M1-like inflammatory macrophages in peripheral blood determines the severity of SLE [61]. Macrophages can not only form foam cells, which are early markers of atherosclerotic plaque formation [62] but also can derive pro-inflammation cytokines that accelerate arteriosclerosis,

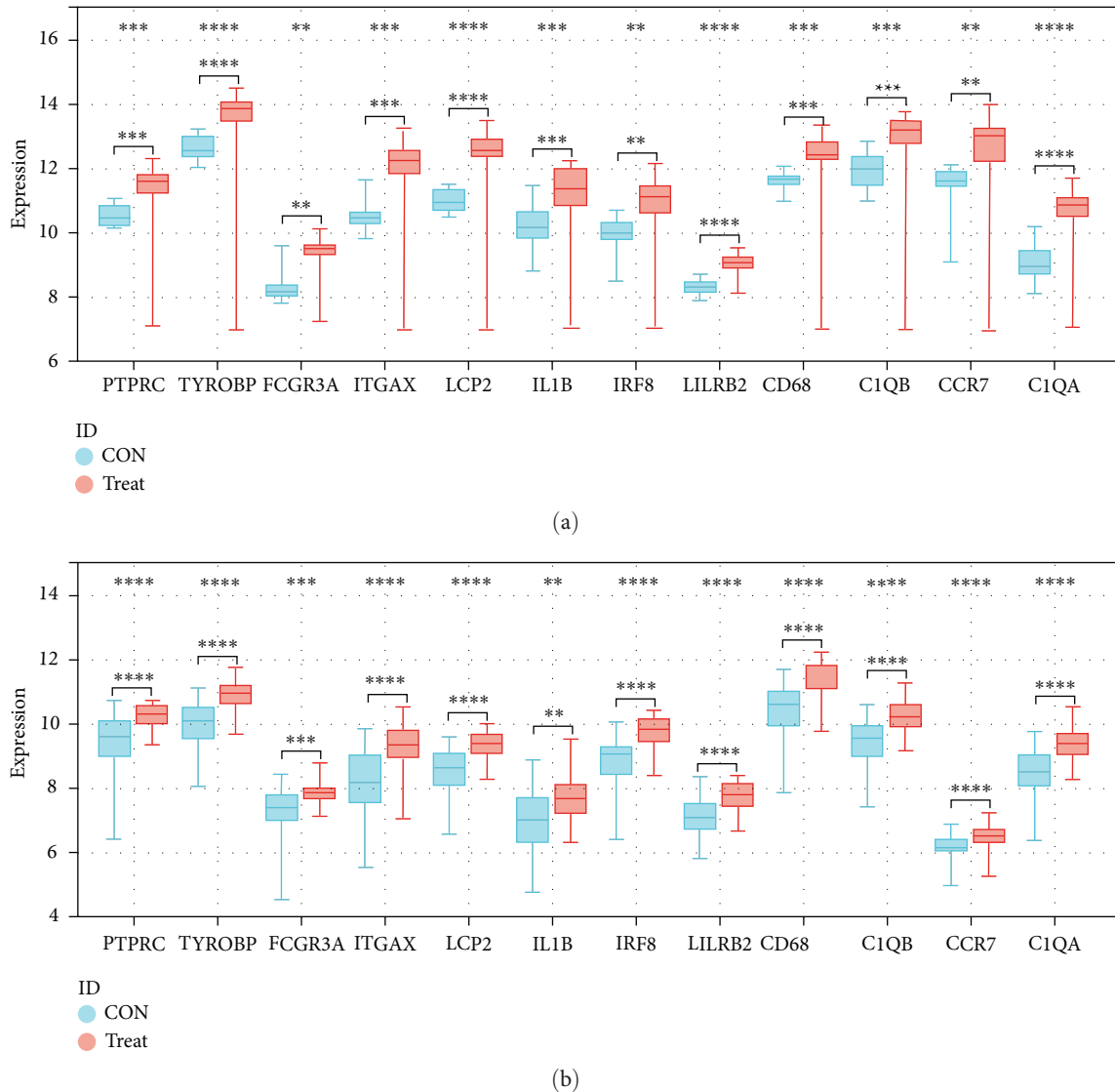


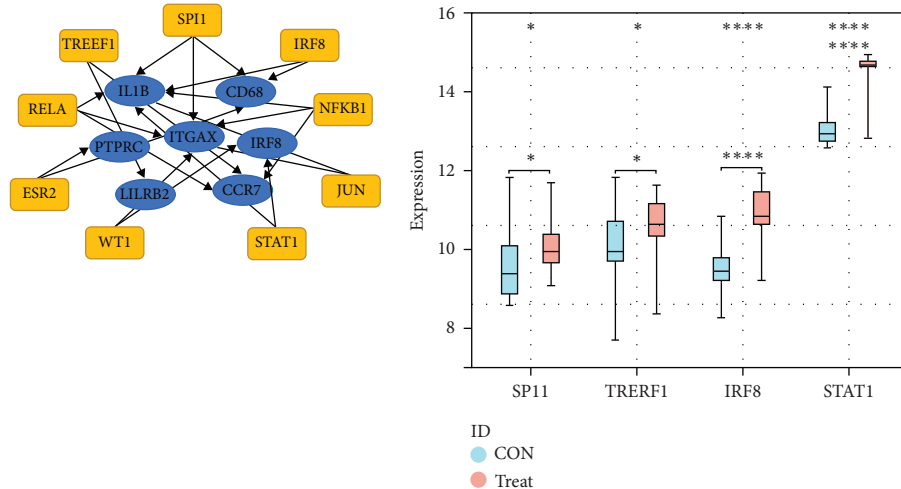
FIGURE 8: Validation of the expression of hub genes: (a) the expression level of hub gene in GSE112943.CON, control skin; Treat, cutaneous lupus lesions; (b) the expression levels of hub genes in GSE43292. CON, control vascular tissue; Treat, atherosclerotic plaques. The Wilcoxon test is employed to compare the two datasets. p -Value < 0.05 was selected as a criterion of statistical significance. ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

which are also found in SLE patients with atherosclerosis [63]. Neutrophils, key cells in the aseptic inflammation response, exert their functional effects in SLE via secreting NETs and producing reactive oxygen species (ROS) [64]. Similarly, neutrophils also promote the infiltration of immune cells and the release of inflammatory cytokines through the formation of NETs and ROS to accelerate atherosclerosis [65]. These results indicate that the immune microenvironment of SLE patients provides an advantageous condition for the formation of atherosclerosis. This provides a favorable entry point for us to explore the mechanism and new treatment strategy of SLE complicated with early atherosclerosis.

While earlier research evaluated the hub genes linked to SLE [66] and atherosclerosis separately [67], our study uses

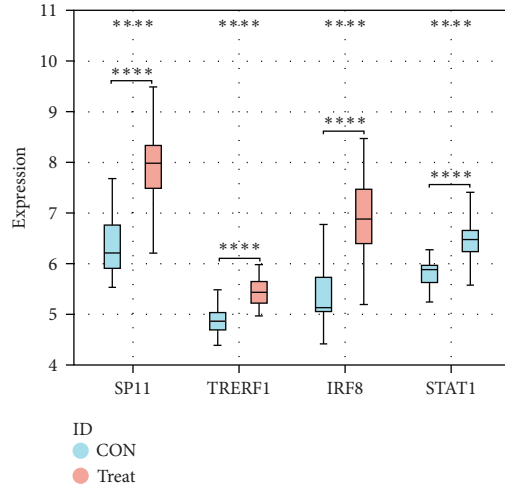
viable bioinformatics to investigate the similarities in the underlying molecular pathways that both of these diseases share. Because of the high rate of comorbidity between SLE and atherosclerosis, as well as the fact that SLE accelerates the course of atherosclerosis, leading to CVDs, atherosclerosis is one of the primary causes of mortality among SLE patients. Precisely for this reason, we hope to further elucidate the mechanisms of SLE and atherosclerosis by identifying common DEGs, TFs, and hub genes between the two diseases, which will help improve the prognosis of patients with SLE combined with atherosclerosis.

However, this study still has some limitations. First, the majority of our bioinformatics findings were on the basis of publicly available datasets. second, we did not validate it in vitro by molecular experiments. Deeper studies in the future



(a)

(b)



(c)

FIGURE 9: The network of TFs regulatory and its expression levels in GSE109248 and GSE100927: (a) network of TFs regulatory. The yellow rectangle represents TFs, and the blue oval represents hub genes; (b) the level of TF expression in GSE109248. CON, control skin; Treat, cutaneous lupus lesions; (c) the expression level of TFs in GSE100927. CON, control vascular tissue; Treat, atherosclerotic plaques. The Wilcoxon test is used to compare the two datasets. p -Value < 0.05 denoted a significant difference. * $p < 0.05$; **** $p < 0.0001$.

TABLE 3: Key transcriptional factors (TFs) of hub genes.

Key TF	Description	p -Value	Genes
SPI1	Spleen focus forming virus (SFFV) proviral integration oncogene sp1	7.27E-06	IL1B, CD68, ITGAX
TRERF1	Transcriptional regulating factor 1	7.77E-06	CCR7, LILRB2
IRF8	Interferon regulatory factor 8	2.03E-05	IL1B, CD68
ESR2	Estrogen receptor 2 (ER beta)	6.30E-05	CD68, PTPRC
WT1	Wilms tumor 1	0.00058	ITGAX, IRF8
RELA	v-rel reticuloendotheliosis viral oncogene homolog A (avian)	0.000794	IL1B, CCR7, ITGAX
NFKB1	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1	0.00081	ITGAX, IL1B, CCR7
STAT1	Signal transducer and activator of transcription 1, 91 kDa	0.00126	IL1B, IRF8
JUN	Jun proto-oncogene	0.00388	IL1B, ITGAX

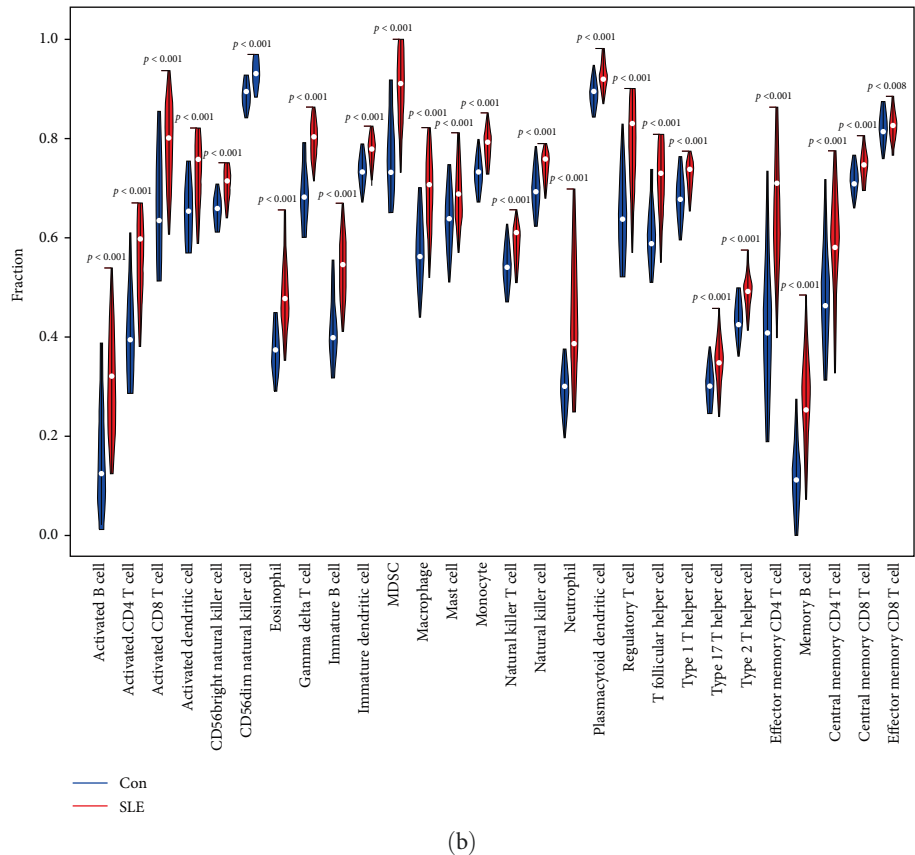
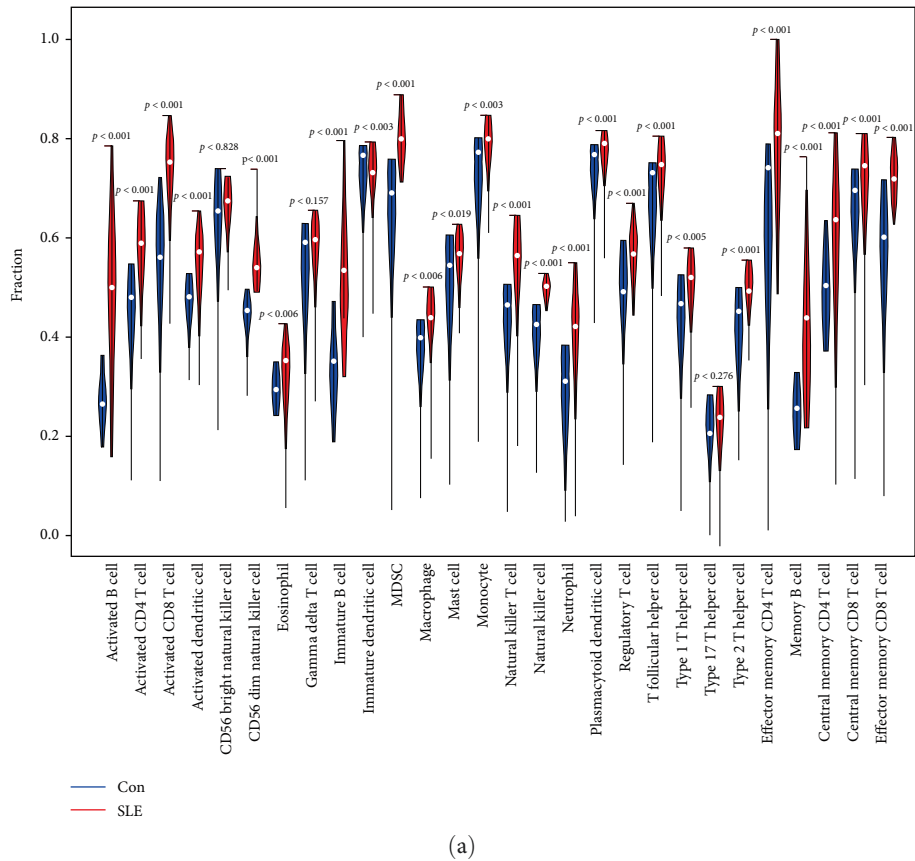


FIGURE 10: Analyzing the immune infiltration in systemic lupus erythematosus and atherosclerosis: (a) violin plot showing the difference of 28 types of immune cells in normal and SLE; (b) violin plot showing the difference of 28 types of immune cells in normal and atherosclerosis. SLE, systemic lupus erythematosus; AS, atherosclerosis.

are indispensable to clarify the fundamental functions and mechanisms of hub genes in these two diseases.

5. Conclusions

With the help of a series of bioinformatics methods, we examined the common DEGs between SLE and atherosclerosis and conducted PPI network and enrichment analyses. We discovered that the onset and advancement of atherosclerosis in SLE may trace a similar mechanism as atherosclerosis despite the young age, which might be regulated by certain hub genes. The current analysis offers direction for future research that aims to investigate the pathogenesis of SLE and atherosclerosis and develop innovative treatment techniques targeting these hub genes.

Abbreviations

SLE:	Systemic lupus erythematosus
GEO:	Gene Expression Omnibus
DEGs:	Differentially expressed genes
PPI:	Protein–protein interaction
CVD:	Cardiovascular disease
NETs:	Neutrophil extracellular traps
MF:	Molecular functions
CC:	Cellular components
BP:	Biological processes
KEGG:	Kyoto Encyclopedia of Genes and Genomes
STING:	The Search Tool for the Retrieval of Interacting Genes
MCODE:	Molecular complex detection technology
TRRUST:	The Transcriptional Regulatory Relationships Unraveled by Sentence-based Text mining
TFs:	Transcription factors
ssGSEA:	single sample gene set Enrichment analysis.

Data Availability

The data are public and can be downloaded from the GEO database.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Supplementary Materials

Figure S1: epidemiological information on systemic lupus erythematosus. (*Supplementary Materials*)

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