

# Identification of differentially expressed genes and signaling pathways in rheumatoid arthritis by integrated bioinformatics analysis

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## Research

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## Abstract

## Background

The disability rate associated with rheumatoid arthritis (RA) ranks high among inflammatory joint diseases. However, the cause and potential molecular events are as yet not clear. Here, we aimed to identify key genes and pathways involved in RA utilizing integrated bioinformatics analysis and uncover underlying molecular mechanisms.

## Materials and methods

The expression profiles of GSE55235, GSE55457, GSE55584 and GSE77298 were downloaded from the Gene Expression Omnibus database, which contained 76 synovial membrane samples, including 49 RA samples and 27 controls. The microarray datasets were consolidated and differentially expressed genes (DEGs) were acquired and further analyzed by bioinformatics techniques. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses of DEGs were performed using R (version 3.6.1), respectively. The protein-protein interaction (PPI) networks of DEGs were developed utilizing the STRING database.

## Results

A total of 828 DEGs were recognized, with 758 up-regulated and 70 down-regulated. GO and KEGG pathway analyses demonstrated that these DEGs focused primarily on multifactorial binding, transcription activity, cytokin-cytokine receptor interaction and relevant signaling pathways. The 30 most firmly related genes among DEGs were identified from the PPI network.

## Conclusion

This study shows that screening for DEGs and pathways utilizing integrated bioinformatics analyses could aid in the comprehension of the molecular mechanisms involved in RA development. In addition, our study provides valuable data for the effective prevention, diagnosis, treatment and rehabilitation of RA patients as well as providing potential targets for the treatment of RA.

## Introduction

Rheumatoid arthritis (RA) occurs in approximately 5 per 1000 people and can inevitably prompt severe joint damage and disability. Significant progress has been made over the past two decades with respect to the disease pathophysiology, optimal outcome measures, and effective treatment strategies, including the understanding of the comprehension in diagnosing and treating RA in the early stage<sup>[1]</sup>. The disability rate of RA ranks high among the arthritic which occur in multiple-joint on the human body, and the incidence of this kind of arthritis is increasing year by year. The incidence of RA is occult, early diagnosis is difficult, and imaging manifestations occur comparatively late. At the point when RA is identified, the patients are usually at an advanced stage of this disease. RA would lead to multiple-joint dysfunction, disability, lower quality of life, respiratory illness, cardiovascular disease and other comorbidities in patients not receiving intervention<sup>[2]</sup>. This process occurs from activation of endothelial cells and neovascularization is another sign of RA synovitis. Expansion of fibroblast-like and macrophage-like cells in synovial would cause hyper-proliferation and intrusion of synovial tissue. This expanded synovial membrane is the principle explanation for the bony erosion and cartilage destruction, and results in clinical symptoms and signs<sup>[3]</sup>. The etiology of RA is still ambiguous. All things considered, both genetic factors and environmental factors, contribute to the occurrence and development of RA<sup>[4]</sup>.

At present, the frequently used methods for early detection and diagnosis of RA are magnetic resonance imaging, ultrasound and serological examination (including rheumatoid factor, anti-cyclic citrullinated peptide, etc). Be that as it may, there are confinements of these techniques, so the exactness and accuracy is not high<sup>[5,6]</sup>. Computed tomography and X-rays can only detect lesions in its advanced stage, but do not detect early impairment. At the moment, the treatment of RA incorporates drug treatment, immunologic purging, functional training, surgical operation, and complementary and/or alternative medicine, and so forth<sup>[7,8]</sup>. Therefore, it is critical to study the potential molecular mechanisms of RA synovial membrane and consequently identify more valid diagnostic techniques and more reliable molecular markers for detecting occurrence and evaluating prognosis, as well as to investigate more valid methods to control and prevent RA. Gene expression microarrays have been generally applied in studying gene expression profiles which provides a moderately new way for exploring genes and offers broad application prospects for drug-based molecular targeting and molecular therapy. At present, a large amounts of data have been published on Gene Expression Omnibus (GEO)<sup>[9]</sup> furthermore, integrating these databases can permit a more profound study of molecular mechanisms.

In this study, we downloaded four original microarray datasets (including GSE55235, GSE55457, GSE55584 and GSE77298) from the GEO database which incorporated a total of 76 samples, with 27 healthy controls and 49 RA samples. Differentially expressed genes (DEGs) in RA samples and control group (CG) were screened utilizing packages in R (version 3.6.1), and gene ontology (GO) pathway and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways enrichment analysis of DEGs were additionally performed. Finally, protein-protein interaction (PPI) network was used to analyze the anticipated associations for a particular group of proteins through the STRING online database.

## Materials And Methods

## Microarray data information

We used the keyword "Rheumatoid arthritis" to search the GEO database, and there were a sum of 5543 results for "Rheumatoid arthritis" in the GEO database from their inception up to November 7, 2019. By restricting the entry type (series), study type (expression profiling by array) and tissue sources (homo sapiens), 5386 pieces of items that were not related to the purpose of this study were excluded. After further selection with title, summary and samples, we discovered absence of required data in 153 items. Finally, 4 series from 2 platforms were included, and gene expression profiles of GSE55235, GSE55457, GSE55584 and GSE77298 were downloaded. Figure 1 depicts the details of the selection process. GSE55235, GSE55457 and GSE55584 are three multi-center genome-wide transcriptomic data sets (Affymetrix HG- U133 A) from a total of 79 individuals, including 20 healthy controls, as well as 26 osteoarthritis patients and 33 RA patients. The platform for GSE77298 is GPL570, [HG-U133\_Plus\_2] Affymetrix Human Genome U133 Plus 2.0 Array, which includes 7 synovial tissue from healthy joints and 16 synovial tissue from rheumatoid arthritis joints. Platform and series matrix file(s) were downloaded from the GEO and saved as TXT files. R software (version 3.6.1) was used to process the downloaded files.

## Integration of microarray data and DEGs

These four raw datasets were incorporated for the analysis. The coordinated microarray datasets were batch-normalized by R software utilizing limma packet analysis and then saved as a TXT file. The operating instruction codes, by means of the R software, were processed automatically and the DEGs in CG and RA samples were analyzed by the limma package. Using R software to run the instruction code. Up or down-regulated genes were obtained independently and utilized for further analysis. The downloaded files (including platform and series of matrix) were converted annotation package utilizing the R software. The ID associated with the probe name was converted into gene symbol using perl programming language (version 5.30.0) and then saved in a TXT file. Adjust  $P$ -value  $\leq 0.05$  and log fold change (logFC)  $\geq 2$  were considered as DEGs.

## GO and KEGG pathway enrichment analyses of DEGs

The functional and pathway enrichment of the proteins encoded by candidate genes were analyzed, and these genes were annotated using the R software. GO and KEGG pathway analysis of DEGs was performed utilizing the appropriate clusterprofiler packages. In this study, we analyzed the DEGs that were significantly up and down-regulated as determined from integrated microarray RA data, and an adjust  $P$ -value of  $\leq 0.05$  was considered statistically significant.

## PPI network integration

The database STRING (version 11) is a precomputed worldwide resource for the exploration and analysis of interactions between known and predicted protein-protein interaction. With regard to a specific group of proteins, the network view analyzes the predicted associations. Each network node represents different protein and the association between these nodes represents the interaction of biological molecules, which can be used for identifying interactions and associated pathways between these proteins encoded by DEGs in RA. The central nodes which are closed related with other corresponding proteins may be the core or key proteins and exert significant physiological functions.

## Results

An aggregate of 828 DEGs were acquired, of which 758 were up-regulated and 70 down-regulated respectively (Fig. 2). Top of the 50 up and down-DEGs from the integrated data are shown in Table 1 separately. The smaller is its adjust  $P$ -value, the greater possibility of DEG and higher ranking in this experiment. R-heatmap software was utilized to draw a heatmap of the 50 up and 50 down-regulated DEGs, as shown in Figure 3.

### GO term and KEGG pathway enrichment analysis of DEGs

The GO term and KEGG pathway enrichment analyses of up and down-regulated genes with an adjust  $P$ -value of  $\leq 0.05$  were obtained respectively. The results of the GO term in RA are shown in table 2 and figure 4 (a and b). The visual analysis results of the KEGG enrichment of DEGs in RA are shown in table 3 and figure 4 (a and d). The up-regulated genes were mainly enriched in cytokine receptor activity, G protein-coupled chemoattractant receptor activity, chemokine receptor activity, MHC protein complex binding, chemokine binding, chemokine receptor binding and cytokine activity. The down-regulated genes were mostly amassed in peroxidase activity and oxidoreductase activity, acting on peroxide as acceptor. In the KEGG analysis, the up-regulated genes were mainly enriched in the chemokine signaling pathway, hematopoietic cell lineage, cytokin-cytokin receptor interaction, viral protein interaction with cytokine and cytokine receptor, primary immunodeficiency, leishmaniasis, osteoclast differentiation, rheumatoid arthritis, cell adhesion molecules (CAMs). The down-regulated enriched KEGG pathways of DEGs included PPAR signaling pathway, regulation of lipolysis in adipocytes, adipocytokine signaling pathway, glucagon signaling pathway, AMPK signaling pathway, calcium signaling pathway, thyroid hormone synthesis, apelin signaling, cGMP-PKG signaling pathway. Besides, the pathway map for targeted RA (Figure 5) was described using in KEGG pathway enrichment. The significantly enriched terms and pathways may enlighten our mind and assist us in further study of the role of DEGs in RA.

### Analyzing DEGs in RA using a PPI network

The DEG expression products in RA were constructed by way of the STRING database to construct PPI networks (minimum required interaction score: 0.990). After deleting all isolated and partially disconnected nodes, an integrated network was built, as shown in figure 6a. The 30 most significant genes (Fig. 6b) which had been displaying statistical significant interaction were CDK1, KIF11, CDC20, CCNB1, CCNB2, MAD2L1, BUB1B, NDC80, AURKA, CCNA2, ISG15, NCAPG, TTK, DLGAP5, LCP2, TPX2, CD247, CKS2, LCK, VAV1, CCL5, CD3E, FOXM1, KIF20A, MX1, NUSAP1, SYK, ZAP70, ZWINT and ASPM.

## Discussion

The characteristics of RA is synovitis, systemic inflammation, and the arrival of autoantibodies [2]. As a result, synovial membrane break down body's immune system, causing chronic inflammation, destruction of cartilage and bone, and dysfunction to other essential organs [1012]. It is reported that 50% of the risk for occurrence and development of RA is related to genetic factors. At the same time, smoking is an environmental risk factor for RA. The early onset of RA is not easy to identify, and in the meantime, cartilage and bone disintegration are frequently found in the end stages of this disease. RA occurrence and development can occur at any age, gender, and nationality for complex biological processes, and the positive rate of serum examination is low as well as non-specific. Consequently, it is miles critical to observe and study the mechanisms and development of RA at molecular level. On the basis of this, differentially expressed genes (DEGs) have been efficaciously used to predict the response of therapeutic approaches for RA patients. As an example, the capability of certain genes (type I interferon-responsive) to predict nonresponders of rituximab [13] and anti-tumor necrosis factor [14].

In this study, we integrated gene expression profile datasets from four specific groups (GSE55235, GSE55457, GSE55584 and GSE77298) and used R (version 3.6.1) to analyze these datasets. A total of 828 DEGs were identified using the limma package, consisting of 758 up-regulated genes and 70 down-regulated genes. The pinnacle 20 most significantly up-regulated genes were ADAMDEC1, IGHM, IGJ, IGKC, IGLL3, IGLV1-44, IGLC1, TNFRSF17, IGLL5, CRTAM, CXCL9, IGLJ3, TRAT1, SDC1, TPD52, IGK, CD27, CXCL10, IL21R and IGHG1. The pinnacle 20 most significantly down-regulated genes were SLC19A2, PLIN1, KLF9, ADCY2, PPAP2B, EBF2, ADH1B, KLF4, PPARGC1A, GABARAPL1, TRHDE, PHKA1, FBXW12, TCEAL2, PCK1, PCDH9, MAFF, LEP, RERGL and SGCA. Constructing a PPI network (minimum required interaction score = 0.990) of DEG-encoding proteins from STRING database and screening the 30 most significant related genes. The enriched GO of DEGs in RA were analyzed by R software, and correlation analysis confirmed that the up-regulated genes have been specifically involved in cytokine receptor activity, G protein-coupled chemoattractant receptor activity, chemokine receptor activity, MHC protein complex binding, chemokine binding, chemokine receptor binding and cytokine activity, and that the down-regulated DEGs were mainly involved in peroxidase activity and oxidoreductase activity, acting on peroxide as acceptor. This finding is consistent with the knowledge that cytokine, chemokine and peroxidase activity play crucial roles in the RA occurrence and progression.

The detection of auto-antibodies (including RA, A-CCP, CRP) in RA patients is identification that distinguishes the disease from other inflammatory arthritis, such as psoriatic arthritis, reactive arthritis and osteoarthritis. In addition to the clinical symptoms and signs arising from arthritis processes in the joints, muscles weakness around joints are also commonly reported by RA patients [1517]. Takashi Yamada et al. [17] discovered that altered Ca<sup>2+</sup> and free radical signaling (such as reactive oxygen and reactive nitrogen species) can result to RA-based muscle weakness. In a general way, RA with CCP + RF + subjects had excessively high citrulline-specific IgG binding, and CCP + RF- and CCP-RF + subjects had modest binding to array peptides [18]. As a systemic autoimmune disease, RA is characterized by inflammation and angiogenesis in synovium. Many cytokines and inflammatory medium are observed in synovial tissues and synovial fluids, whose function is to display angiogenic properties. Inhibitor of DNA binding 1, one of transcription factors, is a marker of cellular self-renewal. This factor within the bone marrow causes the significant reduction of endothelial progenitor cell association with tumor-related vasculogenesis [19, 20]. Amélie Simon et al [21] observed that microscopic polyangiitis is vasculitides typical of necrotizing inflammation for small-sized vessels and is usually connected with serum positivity for those anti-neutrophil cytoplasmic antibodies. In most conditions, anti-neutrophil cytoplasmic antibodies are directed against two constituents of neutrophil primary granules as well as monocyte lysosomes: myeloperoxidase or proteinase 3.

Furthermore, the up-regulated enriched Kyoto Encyclopedia of Genes and (KEGG) pathways of DEGs included the chemokine signaling pathway, hematopoietic cell lineage, cytokin-cytokin receptor interaction, viral protein interaction with cytokine and cytokine receptor, primary immunodeficiency, leishmaniasis, osteoclast differentiation, rheumatoid arthritis, cell adhesion molecules (CAMs). The down-regulated enriched KEGG pathways of DEGs included PPAR signaling pathway, regulation of lipolysis in adipocytes, adipocytokine signaling pathway, glucagon signaling pathway, AMPK signaling pathway, calcium signaling pathway, thyroid hormone synthesis, apelin signaling, cGMP-PKG signaling pathway. Relative studies have demonstrated that fibroblast-like synoviocytes play a crucial role by producing cytokines in all stages of RA. Once fibroblast-like synoviocytes are activated during the course of RA, a series of inflammatory factors and proteases will be produced involved in the inflammatory response, causing progressive destruction of bone and cartilage [22].

RA is associated with an increase in mortality. Previous research displayed that the occurrence rate of the malignancies in RA patients has been reported to be high [7]. A review of scientific studies compiled in Romania demonstrated findings that anaemia and other chronic disease manifestations are relatively common in approximately 6–10% RA patients, and are all related to worse outcomes in particular functional impairment and mortality [23, 24]. The adaptive immune system is closely connected with the generation of anti-tumor immune response. For that reason, RA patients with gastrointestinal cancer history must be carefully monitored while receiving the treatment of disease-modifying antirheumatic drugs [25]. However in many factors, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) is recognized as performing biological functions association with the pathogenesis of RA [26]. Its capabilities include: chemokine amplification, endothelial cell activation, leukocyte accumulation [27], experiencing cardiovascular comorbidity [28], acceleration destruction of osteoclast and chondrocyte, and demonstrating metabolic syndrome [29]. Related studies have reported that PPAR- $\gamma$  may additionally induce activation Wnt/ $\beta$ -catenin signaling [30]. Numerous studies have indicated that decreased expression of adipocyte genes such as nuclear receptors PPAR $\gamma$  in the RA synovial tissue [31, 32], and PPAR $\gamma$  mediates mesenchymal stem cells as well as fibroblast-like synovial cells differentiation into adipocytes [33]. As for the gene expression of AMPK in those newly diagnosed RA patients, a master regulator of metabolic process was decreased in the peripheral blood leukocytes and elevated levels of TGF- $\beta$ 1 in plasma accounts for the occurrence of RA pathogenesis [34]. Recent data evidence suggests that S100A8/A9 is member of the Ca<sup>2+</sup> binding S100 protein family and has become a hot topic as a critical alarmin modulating the inflammatory response. Using small molecule inhibitors that block off S100A8/A9 activity can exhibits beneficial functions on disease relative activities in animal models of autoimmune diseases such as RA [35, 36].

We constructed a PPI network of protein encoded by DEGs and identified the subsequent top 10 closely related genes: CDK1, KIF11, CDC20, CCNB1, CCNB2, MAD2L1, BUB1B, NDC80, AURKA and CCNA2. These genes are key nodes for construction a PPI network and play a distinct role in the pathogenesis of RA. In accordance with the proinflammatory CDK signaling, p16<sup>INK4A</sup> protein as a Cyclin-Dependent Kinases inhibitor in synovial fibroblasts also demonstrates an

inhibitory action in the development of RA<sup>[37]</sup>. Ectopic expression of p16<sup>INK4A</sup> protein can also suppress LPS-induced IL-6 expression in macrophages<sup>[38]</sup>, and simultaneously enhance the observations that CDK inhibitory proteins relative features to counteract inflammation<sup>[39]</sup>. Interleukin-6 (IL-6) signaling is a critical target in inflammatory pathways<sup>[40]</sup>. In patients with RA, the high level of IL-6 and IL-6R are found in both serum and synovial fluid of related joints effected by the disease. IL-6 is a cytokine serving several biological and biochemical functions that affect the immune and vasculature system. Generally speaking, conventional IL-6 signaling is in charge of the anti-inflammatory capabilities of IL-6, conversely, trans-signaling is in charge of the pro-inflammatory properties of IL-6. Consequently, disorders of the IL-6 axis can result in the onset or progression of disease states, especially in autoimmune and inflammatory dysregulation<sup>[41]</sup>. Activation of epidermal growth factor receptor (EGFR) signaling leads to propagation and metabolism of synovial fibroblast in RA. Beyond that, in addition to its function in propagation and metabolism, EGFR can generate cytokine in synovial tissues during the pathogenesis of RA. Some animal experiments have yielded potentially prospective results aiming at target EGFR involving RA. As a result, pharmacologic modulations or its ligands targeting EGFR may reveal undiscovered methods for the treatment of RA<sup>[42]</sup>. EGF receptor is a tyrosine kinase. At present, only NEK6 and CDK1 kinases can phosphorylate KIF11 at Ser1033 and Thr926 respectively, causing the combination of microtubules and KIF11 in the process of mitotic spindle assembly<sup>[43,45]</sup>. Some inflammatory cytokines are controlled by the expression of the c-Fos. Both IL-1 $\beta$  and c-Fos are interacted with each other, including its gene expression and activities, and causing cross-link effect that is vital mechanism to arthritic joint destruction. As a result, the blockade of IL-1 $\beta$ , c-Fos or link between both can be an effective therapeutically as a treatment method for RA patients joint destruction<sup>[46]</sup>. Researchers at the University of Chicago, found through mice experiments that the inhibition of c-Myc or c-Raf-1 can significantly decreased the invasiveness of RA synovial fibroblasts. Besides, dominant-negative mutants c-Raf-1 reduced the expression of phosphorylated c-Jun in vivo as well as the expression of disease-relevant MMPs<sup>[47]</sup>.

## Conclusions

It is beneficial for the research community to study this network to further examine and understand the interaction between RA relevant DEGs. These findings may help us to enhance our general understanding of the pathogenesis of RA. Our study has an important clinical meaning for the effective prevention, diagnosis, treatment and rehabilitation of RA in addition to providing targeted goals for the treatment of RA. However, further relevant molecular biological experiments are required to affirm the function of the identified genes associated with RA.

## Abbreviations

DEGs: differentially expressed genes; RA:rheumatoid arthritis; GO:gene ontology; KEGG:Kyoto Encyclopedia of Genes and Genomes; logFC:log fold change; PPI:protein-protein interaction.

## Declarations

## Competing interests

The authors declare that they have no competing interests.

### Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

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## Authors' contributions

Yanzhi Ge, Li Zhou and Jinying Yang analyzed and extracted the data, contributed analysis tools. Zuxiang Chen and Ting Li prepared figures and tables. Yanzhi Ge and Letian shan wrote the main protocol and prepared the manuscript. Peijian Tong conceived and designed the study, approved the final draft.

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## Tables

Table 1. Up an down-regulated DEGs in RA by integrated data	
DEGs	Gene symbol
Up-regulated	ADAMDEC1 IGHM IGJ IGKC IGLL3P IGLV1-44 IGLC1 TNFRSF17 IGLL5 CRTAM CXCL9 IGLJ3 TRAT1 SDC1 TPD52 IGK CD27 CXCL10 IL21R IGHG1 RRM2 MZB1 DAZL PNOC GUSBP11 CD79A AIM2 ALOX5 TRBC1 SNX10 LOC102723479 CCL18 MMP1 NKG7 SEL1L3 LOC101929272 CYTIP HLA-DOB TNFSF11 GGH CXCL6 PLXNC1 LCK BLNK APOBEC3B IGHD CCL5 RASGRP1 LOC100293211 SLAMF8
Down-regulated	SLC19A2 PLIN1 KLF9 ADCY2 PPAP2B EBF2 ADH1B KLF4 PPARGC1A GABARAPL1 TRHDE PHKA1 FBXW12 TCEAL2 PCK1 PCDH9 MAFF LEP RERGL SGCA ADH1C TMOD1 EDNRB ANGPTL7 ADIPOQ C6 C7 JUN ABCA8 NFIL3 CES1P1 SLC47A1 ACADL NPY1R GADD45B ATP1A2 FGF13 KCNK3 DDX3Y CLSTN2 GPC5 PODXL2 SERPINA3 TOX3 CYR61 LDB3 CNN1 CLIC5 DUOX2 FABP4
DEGs - differentially expressed genes; RA - rheumatoid arthritis.	

Table 2. GO analysis of up-regulated and down-regulated DEGs			
ID (Up-regulated)	Description	Adjusted P-values	Gene symbol
GO:0004896	cytokine receptor activity	8.17E-09	IL21R/IL7R/IL2RG/CCR5/CCR2/CSF2RB/CCR7/CXCR4/CXCR6/CXCR3/CD4/CCR1/IL2RA/IL15RA/CX3CR1/
GO:0001637	G protein-coupled chemoattractant receptor activity	1.30E-07	CCR5/CCR2/CCR7/CXCR4/CXCR6/CXCR3/CCR1/CX3CR1/CCRL2/CXCR5/CCR6
GO:0004950	chemokine receptor activity	1.30E-07	CCR5/CCR2/CCR7/CXCR4/CXCR6/CXCR3/CCR1/CX3CR1/CCRL2/CXCR5/CCR6
GO:0023023	MHC protein complex binding	1.30E-07	HLA-DOB/MS4A1/CD8A/HLA-DMB/HLA-DMA/KLRD1/LILRB2/LILRB1/HLA-DRA/TAPBPL/CD74
GO:0019956	chemokine binding	7.20E-07	ITGA4/CCR5/CCR2/CCR7/CXCR4/CXCR6/CXCR3/CCR1/CX3CR1/CCR6

Table 2. Continued				
ID	Description	Adjusted P-values	Gene symbol	Count
<b>(Down-regulated)</b>				
GO:0004601	peroxidase activity	0.044092206	DUOX2/GPX3/PTGS2	3
GO:0016684	oxidoreductase activity, acting on peroxide as acceptor	0.044092206	DUOX2/GPX3/PTGS2	3

GO - gene ontology; DEGs - differently expressed genes.

Table 3. KEGG pathway of up-regulated and down-regulated DEGs				
ID	Description	Adjusted P-values	Gene symbol	
<b>(Up-regulated)</b>				
hsa04062	Chemokine signaling pathway	1.90E-14	CXCL9/CXCL10/CCL18/CXCL6/CCL5/CCR5/STAT1/CCR2/ITK/PRKCB/NCF1/CXCL13/RAC1	
hsa04640	Hematopoietic cell lineage	8.51E-13	HLA-DOB/MS4A1/IL7R/ITGA4/CD38/CD2/CD8A/CD3D/HLA-DMB/CSF1R/CD19/3ADCY7/CD11B	
hsa04060	Cytokine-cytokine receptor interaction	4.76E-12	TNFRSF17/CXCL9/CD27/CXCL10/IL21R/CCL18/TNFSF11/CXCL6/CCL5/IL7R/IL2RG/CCR5	
hsa04061	Viral protein interaction with cytokine and cytokine receptor	6.00E-12	CXCL9/CXCL10/CCL18/CXCL6/CCL5/IL2RG/CCR5/CCR2/CXCL13/CSF1R/CCR7/CXCL5/CCL1	
hsa05340	Primary immunodeficiency	7.15E-11	CD79A/LCK/BLNK/IL7R/IL2RG/PTPRC/CD8A/CD3D/CD19/CD3E/TAP1/RFX5/ZAP70/CD28	
hsa05140	Leishmaniasis	1.16E-10	HLA-DOB/ITGA4/STAT1/HLA-DMB/PRKCB/NCF1/ITGB2/3ADCY7/HLA-DMA/HLA-DPB1/CD11B	
hsa04380	Osteoclast differentiation	4.24E-10	TNFSF11/LCK/BLNK/STAT1/NCF1/CSF1R/FCGR2B/PLCG2/SYK/LILRB2/CYBA/LILRB4/LILRB3	
hsa05323	Rheumatoid arthritis	1.27E-09	MMP1/HLA-DOB/TNFSF11/CXCL6/CCL5/HLA-DMB/MMP3/ITGB2/ITGAL/3ADCY7/IL15/IL13	

Table 3. Continued				
ID (Down-regulated)	Description	Adjusted P-values	Gene symbol	
hsa03320	PPAR signaling pathway	0.000189284	PLIN1/PCK1/PPAR	
hsa04923	Regulation of lipolysis in adipocytes	0.000398572	PLIN1/ADCY2/PPAR	
hsa04920	Adipocytokine signaling pathway	0.000818815	ADCY291/PCK1/PPAR	
hsa04922	Glucagon signaling pathway	0.004887657	ADCY2/ADCY1/PPAR	
hsa04152	AMPK signaling pathway	0.006451484	ADCY291/PCK1/PPAR	
hsa04020	Calcium signaling pathway	0.006451484	ADCY2/PHKA1/PLN	
hsa04918	Thyroid hormone synthesis	0.008113772	ADCY2/ATP1A1/PLN	
hsa04371	Apelin signaling pathway	0.008113772	PLIN1/ADCY2/PPAR	
hsa04022	cGMP-PKG signaling pathway	0.016012172	ADCY2/EDNRI/PLN	
hsa04925	Aldosterone synthesis and secretion	0.016012172	ADCY2/ATP1A1/PLN	

KEGG - Kyoto Encyclopedia of Genes and Genomes; DEGs - differently expressed genes.

## Figures

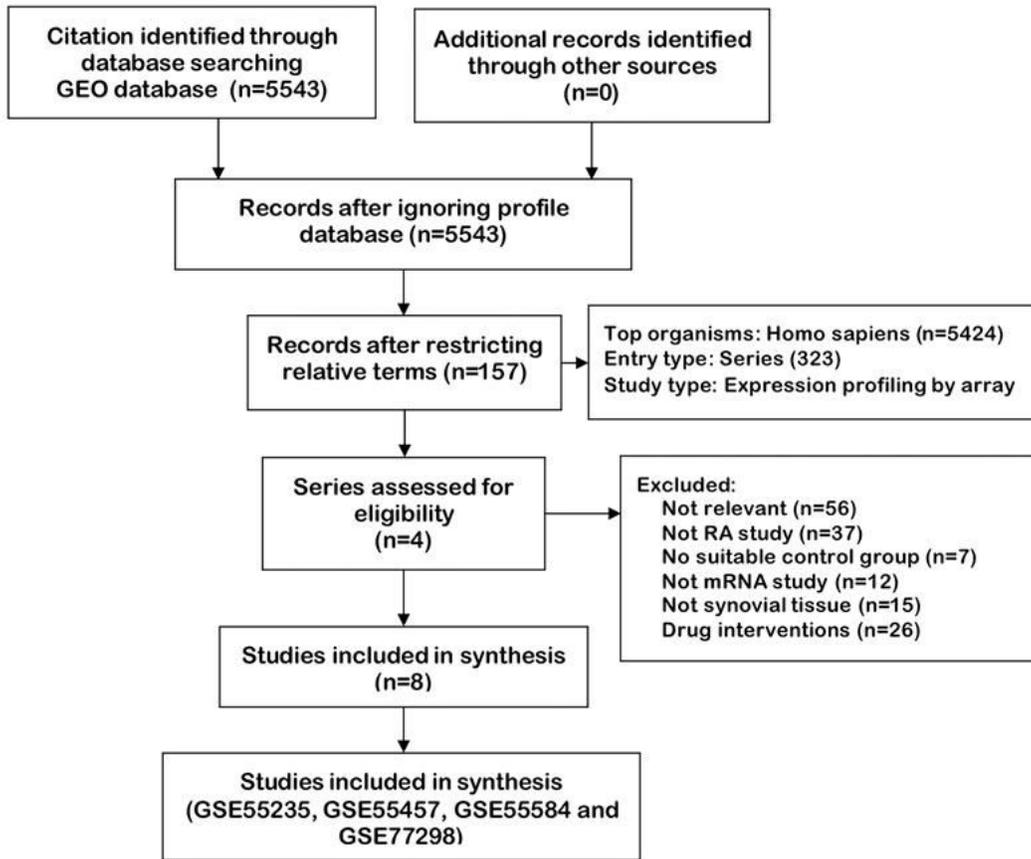
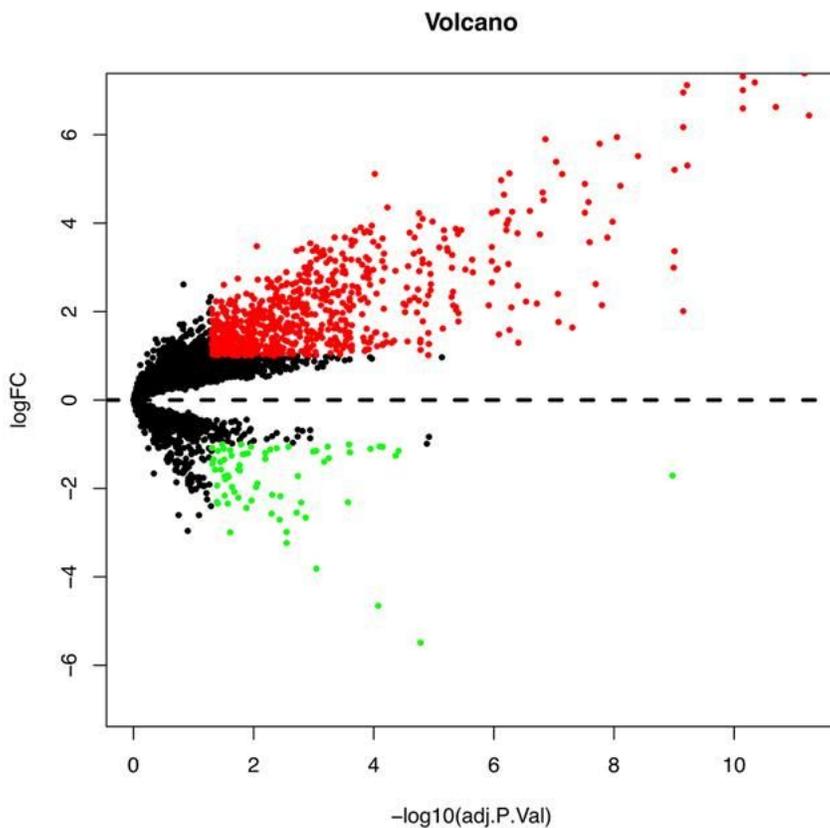


Figure 1  
Serials selection process.





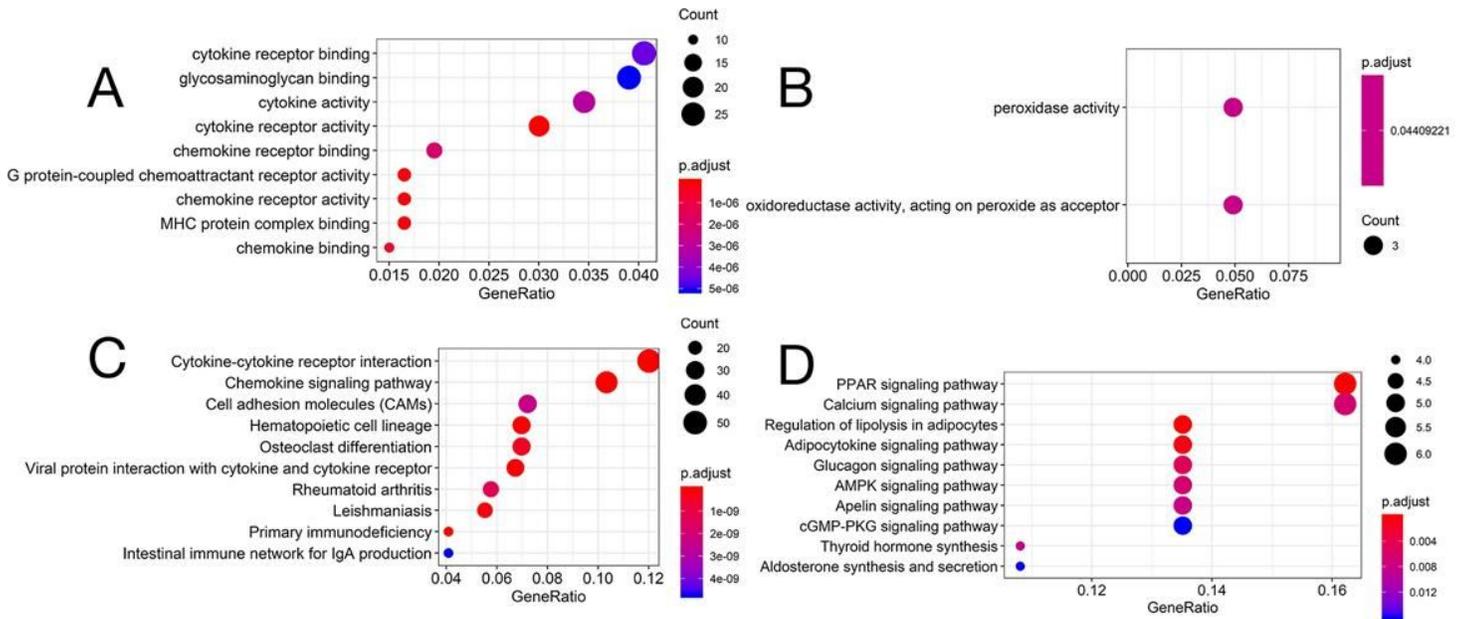


Figure 4

GO and KEGG pathway enrichment analysis of DEGs in GSE55235, GSE55457, GSE55584 and GSE77298. a GO terms in the enrichment analysis of the up-regulated genes. b GO terms in the enrichment analysis of the down-regulated genes. c KEGG terms in the enrichment analysis of the up-regulated genes. d KEGG terms in the enrichment analysis of the down-regulated genes.

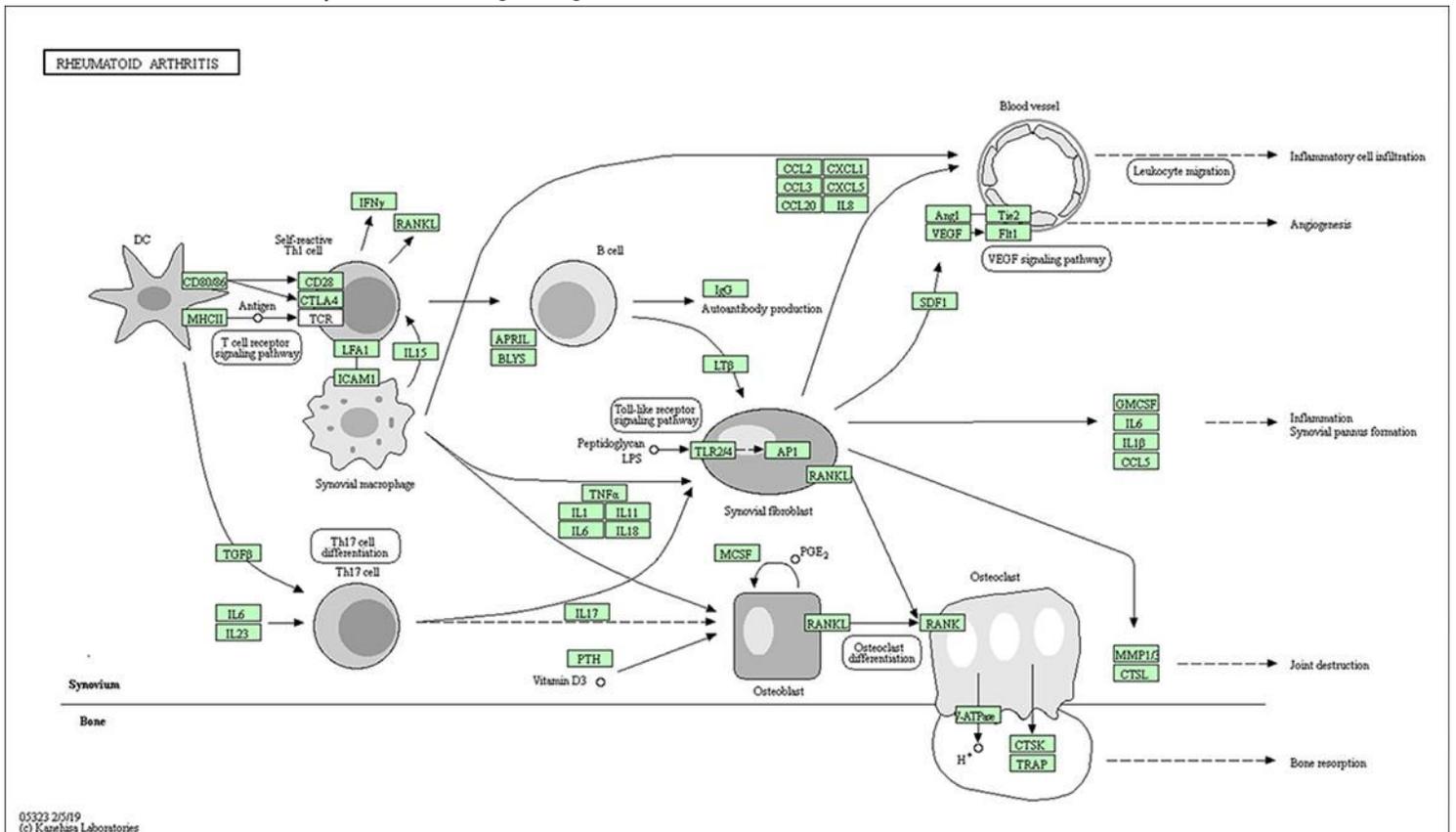
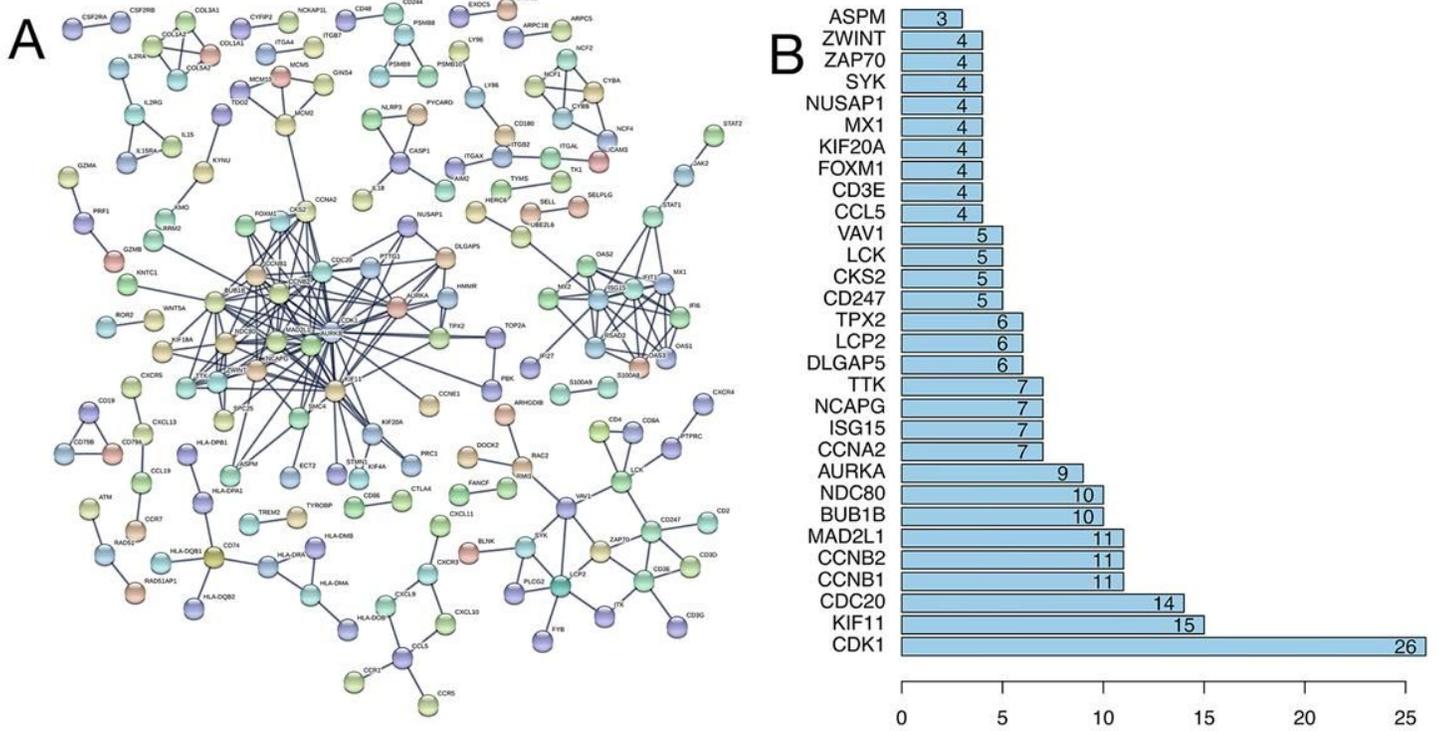


Figure 5

KEGG pathway enrichment analysis and pathway map for RA.



**Figure 6**  
a PPI network (828 DEGs filtered into the PPI network that contained 103 nodes and 168 edges). b The predicted association rank (from low to high) of top 30 genes in PPI network.