Bioinformatics Analysis to Identify Key Genes and Pathways Associated with Sex Differences in Rheumatoid Arthritis

Siwei Su

Shandong First Medical University - Tai'an Campus

Wenjun Jiang

Shandong First Medical University - Tai'an Campus

Xiaoying Wang

Shandong First Medical University - Tai'an Campus

Sen Du

Shandong First Medical University - Tai'an Campus

Lu Zhou

Shandong First Medical University - Tai'an Campus

Jianhong Qi

Shandong First Medical University - Tai'an Campus

hongqiang song (✉ sydydyx@163.com)

Shandong First Medical University - Tai'an Campus <https://orcid.org/0000-0003-0975-571X>

Research

Keywords: Rheumatoid Arthritis, differentially expressed genes, Synovial tissue, Protein-protein interaction networks, Sex differences

Posted Date: July 13th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-687988/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Objective

This study aims to explore the key genes and investigated the different signaling pathways of rheumatoid arthritis (RA) between males and females.

Data and Methods

The gene expression data of GSE55457, GSE55584, and GSE12021 were obtained from Gene Expression Omnibus (GEO) database. Differentially expressed genes (DEGs) were identified using R software. Then, the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways enrichment analysis of DEGs were conducted via Database for Annotation, Visualization, and Integrated Discovery (DAVID). The protein-protein interaction (PPI) networks of DEGs were constructed by Cytoscape 3.6.0.

Results

A total of 416 upregulated DEGs and 336 downregulated DEGs were identified in males, and 744 upregulated DEGs and 309 downregulated DEGs were identified in females. IL6, MYC, EGFR, FOS and JUN were considered as hub genes in RA pathogenesis in males, while IL6, ALB, PTPRC, CXCL8 and CCR5 were considered as hub genes in RA pathogenesis in females.

Conclusion

Identified DEG may be involved in the different mechanisms of RA disease progression between males and females, and they are treated as prognostic markers or therapeutic targets for males and females. The pathogenesis mechanism of RA is sex-dependent.

Introduction

Rheumatoid arthritis (RA) is a serious chronic autoimmune disease characterized by persistent synovitis and systemic inflammation. Global patients with RA account for 0.5-1.0% of [1]. RA is one of the important factors leading to severe physical disability, which brings a heavy financial burden to patients. Reports indicated that RA can significantly shorten the life expectancy of patients [2]. At present, there is no cure for RA, which mainly depends on early detection and aggressive treatment. Therefore, explore the molecular mechanism of RA and analyze related signaling pathways are helpful to discover more effective therapeutic targets.

Sex differences in the incidence of RA cannot be ignored. Research indicated that the probability of females suffering from RA is three times that of males [3]. Although sex differences play a complex role in causing autoimmune disorders, but so far, the link between sex and the incidence of RA is still unknown [4]. In recent years, microarray technology based on high-throughput platforms have been widely used in gene expression profiling and great efforts have been made to identify key genes and pathways

for the progression of RA. However, the majority of studies is focused on the comparison of gene expression data between RA patients and healthy subjects. But there is less information about the differences in gene expression data between sexes. Therefore, the purpose of this study is to explore the key genes and pathways of RA between males and females through comprehensive bioinformatics analysis. It will help to further understanding of sex-specific incidence of RA and providing potential biomarkers and therapeutic targets for RA.

Microarray Data

The data process flowchart is shown in Fig. 1. In this study, we downloaded the gene expression profile GSE55457, GSE55584 and GSE12021 from the Gene Expression Omnibus (GEO) database (<https://www.ncbi.nlm.nih.gov/geo/>) which based on Affymetrix Human Genome U133A Array.

Differential Expression Analysis

Software R and related packages were used to identify differentially expressed genes (DEGs) in males and females in GSE55457, GSE55584 and GSE12021 after integrated. Three datasets were first background adjusted and normalized by $\log_2$ transformation. $P \leq 0.05$ and $|\log_2 FC| \geq 2$ was selected as criteria for identified DEGs. Then, the ggplot2 package in the R software was used to generate DEGs volcano maps.

Functional Enrichment Analysis

The Database for Annotation, Visualization, and Integrated Discovery (DAVID, version 6.8, <https://david.ncifcrf.gov/>) [5] is a functional classification online tool that provides comprehensive information for genes. Gene Ontology (GO) [6] provides a common approach for functional studies transcriptomic data. Kyoto Encyclopedia of Genes and Genomes (KEGG) [7] was a database which used for classification correlating gene sets into their respective pathway. In order to obtain the biological functions and signaling pathways of DEGs, GO enrichment and KEGG pathway analyses were used for DEGs through DAVID. $P \leq 0.05$ was considered threshold values.

Protein-Protein Interaction (PPI) Network Analysis

Search Tool for the Retrieval of Interacting Genes (STRING, <http://www.string-db.org>) was an online biological database used to predict the interaction between known and unknown proteins, and was commonly used to construct protein-protein interaction (PPI) networks [8]. In order to identify functional interactions between the DEGs, the DEGs of males and females were uploaded to the STRING database. Then, we performed Cytoscape software (version 3.6.0) [9] to visualize the PPI network. In addition, nodes in the network on behalf of the PPI protein, and the edges represents interactions. The genes with high degree in the network were considered as the hub genes[10, 11].

Module Analysis

Molecular Complex Detection (MCODE) clustering algorithm [12] was used to discover densely connected areas in PPI networks. In this study, we used MCODE to select important modules from the constructed PPI network. The criteria settings were as follows: node score cutoff = 0.2, K-core = 2, degree cutoff = 2. In addition, the functional and pathway enrichment analyses of DEGs in each module were performed using DAVID, $P \leq 0.05$ was considered as the cutoff criterion.

Results

Identification of DEGs

Datasets of GSE55457, GSE55584 and GSE12021 were obtained from GEO. There were 9 Rheumatoid Arthritis cases and 15 normal cases in the male group and 26 Rheumatoid Arthritis cases and 4 normal cases in the female group after screening and collating. 416 upregulated DEGs and 336 downregulated DEGs were identified in males, and 744 upregulated DEGs and 309 downregulated DEGs were identified in females. The volcano plot was shown in Fig. 2, which represents the upregulated genes in red, blue represents the downregulated genes.

GO Enrichment Analysis

GO enrichment analysis results showed that male DEGs were mainly enriched in immune response, cell-cell signaling, positive regulation of transcription from RNA polymerase II, inflammatory response and adaptive immune response in biological process, in cellular composition DEGs were mainly enriched in extracellular region, external side of plasma membrane, integral component of plasma membrane, extracellular space and plasma membrane. In molecular functions DEGs were mainly enriched in cytokine activity, transcriptional activator activity, type endopeptidase activity, chemokine activity, RNA polymerase II core promoter proximal region sequence-specific DNA binding. The female DEGs were significantly enriched in immune response, inflammatory response, chemokine-mediated signaling pathway, adaptive immune response and chemotaxis in biological process, in cellular composition DEGs were mainly enriched in integral component of plasma membrane, plasma membrane, extracellular region, external side of plasma membrane and extracellular space. In molecular functions DEGs were mainly enriched in chemokine activity, C-C chemokine receptor activity, heparin binding, receptor binding and chemokine receptor activity (Table 1–2, Fig. 3).

Table 1
The top 5 GO enrichment analysis of the DEGs in females.

Term	Name	Count	PValue
Biological processes			
GO:0006955	immune response	87	1.78E-26
GO:0006954	inflammatory response	70	1.39E-18
GO:0070098	chemokine-mediated signaling pathway	30	2.19E-18
GO:0002250	adaptive immune response	40	1.49E-16
GO:0006935	chemotaxis	33	1.06E-13
Cellular component			
GO:0005887	integral component of plasma membrane	160	1.62E-20
GO:0005886	plasma membrane	341	2.85E-20
GO:0005576	extracellular region	160	4.99E-15
GO:0009897	external side of plasma membrane	43	1.13E-13
GO:0005615	extracellular space	133	2.70E-12
Molecular Functions			
GO:0008009	chemokine activity	18	3.67E-10
GO:0016493	C-C chemokine receptor activity	8	8.82E-07
GO:0008201	heparin binding	26	1.91E-06
GO:0005102	receptor binding	41	9.97E-06
GO:0004950	chemokine receptor activity	8	1.70E-05

Table 2
The top 5 GO enrichment analysis of the DEGs in males.

Term	Name	Count	PValue
Biological processes			
GO:0006955	immune response	57	8.26E-16
GO:0007267	cell-cell signaling	34	1.46E-09
GO:0045944	positive regulation of transcription from RNA polymerase II promoter	72	3.79E-07
GO:0006954	inflammatory response	37	9.15E-07
GO:0002250	adaptive immune response	20	5.34E-06
Cellular component			
GO:0005576	extracellular region	126	8.01E-15
GO:0009897	external side of plasma membrane	32	1.54E-10
GO:0005887	integral component of plasma membrane	100	2.54E-09
GO:0005615	extracellular space	94	1.59E-08
GO:0005886	plasma membrane	205	1.91E-05
Molecular Functions			
GO:0005125	cytokine activity	21	1.61E-05
GO:0001077	transcriptional activator activity	24	4.68E-05
GO:0004252	serine-type endopeptidase activity	25	5.65E-05
GO:0008009	chemokine activity	10	9.42E-05
GO:0000978	RNA polymerase II core promoter proximal region sequence-specific DNA binding	30	1.31E-04

KEGG Pathway Analysis

In this study, KEGG pathway enrichment analysis was used to understand the signaling pathway enrichment of the DEGs. $P \leq 0.05$ was considered as the cutoff criterion. KEGG pathway analysis revealed that the DEGs in male were mainly enriched in Cytokine-cytokine receptor interaction, HTLV-I infection, Hematopoietic cell lineage, Primary immunodeficiency and Pathways in cancer. KEGG pathway analysis revealed that the DEGs in female were mainly enriched in Cytokine-cytokine receptor interaction, Chemokine signaling pathway, Primary immunodeficiency, Hematopoietic cell lineage and Rheumatoid arthritis (Table 3–4, Fig. 4).

Table 3
The top 5 KEGG pathway analysis of the DEGs in females.

Term	Description	Gene count	PValue
hsa04060	Cytokine-cytokine receptor interaction	57	6.60E-17
hsa0406	Chemokine signaling pathway	37	6.22E-09
hsa05340	Primary immunodeficiency	15	1.44E-08
hsa04640	Hematopoietic cell lineage	22	2.12E-07
hsa05323	Rheumatoid arthritis	20	4.87E-06

Table 4
The top 5 KEGG pathway analysis of the DEGs in males.

Term	Description	Gene count	PValue
hsa04060	Cytokine-cytokine receptor interaction	35	3.02E-09
hsa05166	HTLV-I infection	30	3.65E-06
hsa04640	Hematopoietic cell lineage	15	3.24E-05
hsa05340	Primary immunodeficiency	9	1.08E-04
hsa05200	Pathways in cancer	33	8.81E-04

PPI Network Construction

In order to understand the interaction between the DEGs, STRING database was used to construct a PPI network. The network file was then imported into Cytoscape 3.6.0. As shown in Fig. 5, the genes with higher scores were usually considered as hub genes, indicating higher degree may be associated with RA. IL6, MYC, EGFR, FOS and JUN were considered as hub genes in male. IL6, ALB, PTPRC, CXCL8 and CCR5 were considered as hub genes in females.

Module Analysis

In this study, the first clusters of males and females were selected, and the genes contained in the first clusters were subjected to functional annotation analysis. KEGG enrichment analysis showed that the genes of males were mainly associated with Chemokine signaling pathway, Cytokine-cytokine receptor interaction and Toll-like receptor signaling pathway. KEGG enrichment analysis showed that the genes of females were mainly associated with Chemokine signaling pathway, Cytokine-cytokine receptor interaction and Neuroactive ligand-receptor interaction. The results of MCODE analysis were shown in Fig. 6.

Discussion

RA is the most common immune-mediated arthritis in adults. The inflammatory process of rheumatoid arthritis can lead to the destruction of joint bone and promote the progress of atherosclerosis (11). RA is dominated by women, and the incidence is usually three to four times that of men. At present, the underlying mechanism leading to sex differences in RA is still unclear. Therefore, studying the molecular mechanisms of sex differences in RA is of great significance for the treatment of RA. To the best of our knowledge, this study is the first to identify key genes and pathways in the synovium membrane of RA males and RA females through bioinformatics analysis. In this study, bioinformatics was used to analysis GEO datasets of genes expression profiles to explore the biological mechanism involved in the pathogenesis of sexes differences in RA.

In this study, after data screening and collating of GSE55457, GSE55584, and GSE12021, we compared 26 females with RA and 4 normal females and 9 males with RA and 15 normal males. In total, 309 downregulated DEGs and 744 upregulated DEGs were identified in females, and 336 downregulated and 416 upregulated DEGs were identified in males. In this study, KEGG pathway analysis of DEGs shows that those were mainly enriched in males were Cytokine-cytokine receptor interaction, HTLV-I infection, Hematopoietic cell lineage, Primary immunodeficiency and Pathways in cancer. KEGG pathway analysis of DEGs shows that those were mainly enriched in females were Cytokine-cytokine receptor interaction, Chemokine signaling pathway, Primary immunodeficiency, Hematopoietic cell lineage and Rheumatoid arthritis. The HTLV-I infection and Chemokine signaling pathway have been previously shown to participate in the pathogenesis of rheumatoid arthritis[13–16]. HTLV-I is a human retrovirus, and the HTLV-I Tax may induced NF- κ B activation of host cells. Furthermore, Tax-mediated NF- κ B activation involves resistance toward apoptotic stimuli in HTLV-I infected cells. These modulatory effects of HTLV-I Tax are believed to be important in promoting autoimmune disorders such as RA inn HTLV-I-seropositive subjects[17]. In addition, the connection of sex in RA-related pathways has not been studied. However, there are pathways enriched in both males and females. such as cytokine-cytokine receptor interaction, Hematopoietic cell lineage and Primary immunodeficiency, However, there is currently no literature study the relationship between these pathways of RA.

In the analysis of PPI network construction, IL6, MYC, EGFR, FOS and JUN were considered as hub genes in male, IL6, ALB, PTPRC, CXCL8 and CCR5 were considered as hub genes in females, and IL6 were identified as hub genes both in females and males. However, other hub genes are completely different between different males and females. The epidermal growth factor receptor (EGFR) plays an important role in tumor growth[18]. Similar to tumor growth, the upregulation of EGFR and its ligands in RA leads to hyperproliferation and invasiveness of the synovial membrane in rheumatoid arthritis[19]. Animal experiments had shown that the pharmacological regulation of EGFR or its ligands may be an effective method for the treatment of RA[20, 21]. The dimeric complex AP-1 composed of JUN protein and FOS protein is an important transcription factor in the nucleus. c-Fos/AP-1 can control the expression of inflammatory cytokine IL-1 β and matrix-degrading matrix metalloproteinases (MMPs)[22]. IL-1 β is the most important inducer of a variety of matrix metalloproteinases, mainly responsible for cartilage destruction and osteoclastogenesis[23]. In addition, IL-1 β and c-Fos/AP-1 affect each other's gene expression and activity, and aggravate the progression of rheumatoid arthritis [24].

PTPRC, also known as CD45, classical protein tyrosine phosphatase, expressed in all nucleated cells of hematopoietic origin[25]. PTPRC binds to dipeptidyl peptidase-4 acts as a T cell activating factor. Protein tyrosine protein phosphatase is necessary for the activation of T cells by T cell antigen receptors[26]. Several risk alleles have been identified that are associated with increased susceptibility to RA, and upregulation of PTPRC may be beneficial to maintaining normal synovial function[27]. Chemokines are the main regulators of cell adhesion and cell trafficking, and they have the ability to induce directed chemotaxis of nearby responding cells[28]. As an inflammatory mediator, CXCL8 is expressed in cells such as fibroblasts and monocytes, and as a chemoattractant for neutrophils, causing neutrophils to accumulate in inflammatory sites such as the inflamed joints of patients with RA[29]. CCR5 is a receptor for intracellular β -chemokine, which regulates the migration, proliferation and immunity of T cells and monocytes/macrophages, CCR5 is mainly expressed on the cell membranes of monocytes and immature dendritic cells[30]. In addition to being a co-receptor necessary for early HIV1 infection, CCR5 and its ligands also play an important role in rheumatoid arthritis and systemic lupus erythematosus[31, 32].

In addition, a modular analysis of the PPI network revealed that the differences between males were mainly related to Chemokine signaling pathway, Cytokine-cytokine receptor interaction, and Toll-like receptor signaling pathway; and between females were mainly related to Chemokine signaling pathway, Cytokine-cytokine receptor interaction, and Neuroactive ligand-receptor interaction.

However, there are some limitations of this study. On the one hand, the sample was small, included only 26 RA patients and 4 normal individuals among females and 9 RA patients and 15 normal individuals among males. On the other hand, we did not perform a qPCR to verify the screened hub genes.

Conclusions

We separately enriched and analyzed the DEGs of male and female patients with RA and the different hub genes were obtained. We have found relevant evidences that lead to differences in the pathogenesis

of rheumatoid arthritis between male and female, and provide further promising therapeutic targets for rheumatoid arthritis

Declarations

Acknowledgments

We sincerely acknowledge the publicly available GEO database (<https://www.ncbi.nlm.nih.gov/geo/>).

Funding

This study was supported by Shandong Provincial Natural Science Foundation (No. ZR2017MH080) and the Project of Shandong Province Higher Educational Science and Technology Program (No. J17KA247).

Ethics approval and consent to participate

Not necessary.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

SS and WJ and XW are the principal investigator. SS conducted statistical analysis and data management. WJ and XW conducted the data presentation.

SD and LZ conducted the reviews and revises the original research. JQ and HS Oversight and leadership responsibility for the research activity planning and execution.

Availability of data and material

The raw data of this study are derived from GEO database (<https://www.ncbi.nlm.nih.gov/geo/>), which are publicly available databases.

References

1. Fassio A, et al. Inhibition of tumor necrosis factor-alpha (TNF-alpha) in patients with early rheumatoid arthritis results in acute changes of bone modulators. *Int Immunopharmacol.* 2019;67:487–9.

2. Kiadaliri AA, et al. Brief Report: Rheumatoid Arthritis as the Underlying Cause of Death in Thirty-One Countries, 1987–2011: Trend Analysis of World Health Organization Mortality Database. *Arthritis Rheumatol*. 2017;69(8):1560–5.
3. Favalli EG, et al. Sex and Management of Rheumatoid Arthritis. *Clin Rev Allergy Immunol*. 2019;56(3):333–45.
4. Terao C, Raychaudhuri S, Gregersen PK. Recent Advances in Defining the Genetic Basis of Rheumatoid Arthritis. *Annu Rev Genomics Hum Genet*. 2016;17:273–301.
5. Huang DW, et al. DAVID Bioinformatics Resources: expanded annotation database and novel algorithms to better extract biology from large gene lists. *Nucleic Acids Res*. 2007;35(suppl_2):W169–75.
6. Ashburner M, et al. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nat Genet*. 2000;25(1):25–9.
7. Kanehisa M, et al. KEGG as a reference resource for gene and protein annotation. *Nucleic Acids Res*. 2016;44(D1):D457-62.
8. Szklarczyk D, et al. STRING v10: protein-protein interaction networks, integrated over the tree of life. *Nucleic Acids Res*. 2015;43(Database issue):D447-52.
9. Shannon P. Cytoscape: A Software Environment for Integrated Models of Biomolecular Interaction Networks. *Genome Res*. 2003;13(11):2498–504.
10. Zhang J, et al. Single-cell transcriptome-based multilayer network biomarker for predicting prognosis and therapeutic response of gliomas. *Brief Bioinform*. 2020;21(3):1080–97.
11. Gershwin ME, Kruse W, Goldstein G. The effect of thymopoietin32-36 and ubiquitin on spontaneous immunopathology of New Zealand mice. *J Rheumatol*. 1979;6(6):610–20.
12. Bader GD, Hogue CW. An automated method for finding molecular complexes in large protein interaction networks. *BMC Bioinformatics*. 2003;4:2.
13. Yoshiki T, et al. Models of HTLV-I-induced diseases. Infectious transmission of HTLV-I in inbred rats and HTVL-I env-pX transgenic rats. *Leukemia*. 1997;11 **Suppl 3**:245–6.
14. Suzuki T, et al. Brief Report: Attenuated Effectiveness of Tumor Necrosis Factor Inhibitors for Anti-Human T Lymphotropic Virus Type I Antibody-Positive Rheumatoid Arthritis. *Arthritis Rheumatol*. 2018;70(7):1014–21.
15. Zhang L, et al. Chemokine Signaling Pathway Involved in CCL2 Expression in Patients with Rheumatoid Arthritis. *Yonsei Med J*. 2015;56(4):1134–42.
16. Zhang L, et al. Low-level laser irradiation treatment reduces CCL2 expression in rat rheumatoid synovia via a chemokine signaling pathway. *Lasers Med Sci*. 2011;26(5):707–17.
17. Kawakami A, Eguchi K. Role of HTLV-I infection in the pathogenesis of Sjögren's syndrome and rheumatoid arthritis. *Mod Rheumatol*. 2001;11(2):87–90.
18. Liu X, et al. Expression of EGFR in Pituitary Corticotroph Adenomas and Its Relationship With Tumor Behavior. *Front Endocrinol (Lausanne)*. 2019;10:785.

19. Yuan FL, et al. Epidermal growth factor receptor (EGFR) as a therapeutic target in rheumatoid arthritis. *Clin Rheumatol*. 2013;32(3):289–92.
20. Sumariwalla PF, et al. Antagonism of the human epidermal growth factor receptor family controls disease severity in murine collagen-induced arthritis. *Arthritis Rheum*. 2008;58(10):3071–80.
21. Chen SY, et al. Amelioration of experimental arthritis by intra-articular injection of an epidermal growth factor receptor tyrosine kinase inhibitor. *Clin Exp Rheumatol*. 2015;33(6):839–43.
22. Mar AC, et al. Interleukin-1 Receptor Type 2 Acts with c-Fos to Enhance the Expression of Interleukin-6 and Vascular Endothelial Growth Factor A in Colon Cancer Cells and Induce Angiogenesis. *J Biol Chem*. 2015;290(36):22212–24.
23. Fujii T, et al. MEF2C regulates osteoclastogenesis and pathologic bone resorption via c-FOS. *Bone Res*. 2021;9(1):4.
24. Kontny E, et al. Modified expression of c-Fos and c-Jun proteins and production of interleukin-1 beta in patients with rheumatoid arthritis. *Clin Exp Rheumatol*. 1995;13(1):51–7.
25. Zhang T, et al. Estradiol regulates the expression of CD45 splicing isoforms in lymphocytes. *Mol Biol Rep*. 2020;47(4):3025–30.
26. Lagou V, et al. Genetic Architecture of Adaptive Immune System Identifies Key Immune Regulators. *Cell Rep*. 2018;25(3):798–810.e6.
27. Ferreiro-Iglesias A, et al. Replication of PTPRC as genetic biomarker of response to TNF inhibitors in patients with rheumatoid arthritis. *Pharmacogenomics J*. 2016;16(2):137–40.
28. Gowhari Shabgah A, et al. Possible Anti-inflammatory Effects of Mesenchymal Stem Cells Transplantation via Changes in CXCL8 Levels in Patients with Refractory Rheumatoid Arthritis. *Int J Mol Cell Med*. 2019;8(3):191–9.
29. Sato H, et al. Resistin upregulates chemokine production by fibroblast-like synoviocytes from patients with rheumatoid arthritis. *Arthritis Res Ther*. 2017;19(1):263.
30. Barbonetti A, et al. Beta-chemokine receptor CCR5 in human spermatozoa and its relationship with seminal parameters. *Hum Reprod*. 2009;24(12):2979–87.
31. Cai P, et al. Comparison of rheumatoid arthritis (RA) and osteoarthritis (OA) based on microarray profiles of human joint fibroblast-like synoviocytes. *Cell Biochem Funct*. 2019;37(1):31–41.
32. Martens HA, Kallenberg CG, Bijl M. *Role of CCR5 Delta32 bp deletion in RA and SLE*. *Autoimmunity*, 2009. 42(4): p. 260–2.

Figures

Figure 1

Data process flowchart of the study.

Figure 2

Volcano plots of DEGs of GSE55457, GSE55584 and GSE12021. (A) females. (B) males.

Figure 3

(A) The top 5 GO enrichment analysis of the DEGs in females. (B) The top 5 GO enrichment analysis of the DEGs in males.

Figure 4

(A) The top 5 KEGG pathway analysis of the DEGs in females. (B) The top 5 KEGG pathway analysis of the DEGs in males.

A

B

Figure 5

Protein-protein interaction network in this study (A)females. (B)males. Red represents up-regulated genes, green represents down-regulated genes.