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**Abstract**
Polycystic ovary syndrome (PCOS) is the most common hormonal and metabolic disorder among women of reproductive age. Although the clinical features and pathology of PCOS have been well documented, the molecular mechanisms underlying this unique pathogenesis remain largely unknown. The present study was therefore designed to identify candidate genes, along with their potential targeting pathways, which are involved in the occurrence and development of PCOS, using bioinformatics analysis. The gene expression profiles of GSE34526 from 7 PCOS and 3 controls were downloaded from Gene Expression Omnibus (GEO) database. Differentially expressed genes (DEGs) were then identified using GCBI online tool. Expression levels of candidate genes were finally verified using quantitative RT-PCR (qRT-PCR) and Western blot. There were 426 DEGs identified by GCBI online tool, including 418 up-regulated and 8 down-regulated genes. Function and pathway enrichment analyses showed that these DEGs were significantly enriched in inflammation and immune-related pathways. Additionally, protein–protein interaction (PPI) network and module analysis showed that two modules involved the Toll-like receptor signaling pathway were ranked among the most upregulated modules, and the candidate genes involved in this signaling pathway consisted of TLR1, TLR2, TLR8, and CD14. Finally, the expression levels of TLR2, TLR8 and CD14 were significantly increased in samples from PCOS patients, evidenced by qRT-PCR and Western blot analysis. Collectively, the results suggested that the Toll-like receptor signaling pathway might play an important role in the pathogenesis of PCOS, and TLR2, TLR8 and CD14 may be the key target genes that are regulated by the Toll- like receptor signaling pathway.


Keywords: bioinformatics analysis; PCOS; microarray differentially expressed genes (DEGs); gene ontology (GO)

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# Identification of the key pathways and genes related to polycystic ovary syndrome using bioinformatics analysis

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**Abstract** Polycystic ovary syndrome (PCOS) is the most common hormonal and metabolic disorder among women of reproductive age. Although the clinical features and pathology of PCOS have been well documented, the molecular mechanisms underlying this unique pathogenesis remain largely unknown. The present study was therefore designed to identify candidate genes, along with their potential targeting pathways, which are involved in the occurrence and development of PCOS, using bioinformatics analysis. The gene expression profiles of GSE34526 from 7 PCOS and 3 controls were downloaded from Gene Expression Omnibus (GEO) database. Differentially expressed genes (DEGs) were then identified using GCBI online tool. Expression levels of candidate genes were finally verified using quantitative RT-PCR (qRT-PCR) and Western blot. There were 426 DEGs identified by GCBI online tool, including 418 up-regulated and 8 down-regulated genes. Function and pathway enrichment analyses showed that these DEGs were significantly enriched in inflammation and immune-related pathways. Additionally, protein–protein interaction (PPI) network and module analysis showed that two modules involved the Toll-like receptor signaling pathway were ranked among the most upregulated modules, and the candidate genes involved in this signaling pathway consisted of TLR1, TLR2, TLR8, and CD14. Finally, the expression levels of TLR2, TLR8 and CD14 were significantly increased in samples from PCOS patients, evidenced by qRT-PCR and Western blot analysis. Collectively, the results suggested that the Toll-like receptor signaling pathway might play an important role in the pathogenesis of PCOS, and TLR2, TLR8 and CD14 may be the key target genes that are regulated by the Toll- like receptor signaling pathway.

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## Introduction

Polycystic ovary syndrome (PCOS) is a common cause of anovulatory infertility in 5%-10% reproductive-aged women (Wang and Alvero 2013). It is characterized by hyperandrogenmia, irregular or absent ovulation, and polycystic ovary. Currently, it is believed that both genetic and environmental factors may play important roles in the occurrence and development of PCOS(de Melo et al. 2015). Due to the diversity and complexity of PCOS, its etiologies and mechanisms are largely unknown. Therefore, understanding the molecular mechanism of occurrence and development in PCOS is crucial to develop the more effective diagnostic and therapeutic strategies.

Microarrays technology, as one of the large-scale and efficient techniques to collect biological information, can monitor genome-wide changes in gene expression levels and detect sequence changes of tens of thousands of genes simultaneously(Zhang et al. 2004). At present, microarray technology has been widely employed in studies on many diseases(Duan et al. 2017; Pereira et al. 2017; Zhang et al. 2017). Recently, there are studies using microarrays to identify potentially candidate genes associated with PCOS(Aydos et al. 2016; Lei et al. 2017; Su et al. 2017). However, the involved signaling pathways and related candidate genes in the occurrence and development of PCOS remain to be further defined.

In this study, we downloaded the original data (GSE21815) from Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/). The DEGs of granulosa cells (GCs) from PCOS patients were screened using GCBI online tool. Subsequently, the function and pathways enrichment analysis for DEGs were analyzed. Additionally, we established

PPI network of the DEGs and picked out major signaling pathways and the related candidate genes. Expression levels of these candidate genes were finally verified by qRT-PCR analysis. Overall, our systematic analysis will gain insights into PCOS pathogenesis at molecular level and help to identify the potential candidate biomarkers for diagnosis, prognosis, and drug targets for PCOS.

## Materials and methods

### Microarray data

The gene expression profiles of GSE34526 were downloaded from GEO database (http://www.ncbi.nlm.nih.gov/geo). The GSE34526, which was based on Agilent GPL570 platform ([HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array).

### Differentially expressed genes (DEGs) analysis

The DEGs was analyzed by web-based tool GCBI (https://www.gcbi.com.cn/gclib/html/index), which is a platform that combines a variety of research findings, genetic informations, sample informations, data algorithms and bioinformatics (Xiao et al. 2017). The adjusted $P$ value < 0.05 and fold change |logFC| ≥ 2 were defined as DEGs.

### Enrichment analysis of DEGs

In order to analyze the biological processes involved in the pathogenesis of PCOS, GO enrichment analysis was performed using DAVID (The Database for Annotation, Visualization and Integrated Discovery). $P$ < 0.05 was considered significantly different. To further improve interpretation of the biological significance, we constructed pathway relation network for the DEGs and identified the relationship among the pathways using the ClueGO plug-in of Cytoscape software.

**Protein–protein interaction (PPI) network and module analysis**

To evaluate the functional interactions between DEGs, we carried out PPI network analysis. First, we mapped the DEGs to STRING version 10.5 (http://string-db.org/), and a combined score with values >0.4 was set as the cut off criterion. Then, PPI networks were constructed and visualized using the Cytoscape version 3.6.0. The plug-in Molecular Complex Detection (MCODE) app was used to screen the modules of PPI network in Cytoscape. The criteria was set as follows: MCODE scores≥4 and number of nodes>4. Moreover, the genes of pathway analysis were performed in the top 3 modules by DAVID.

**Subjects**

78 patients were enrolled into the study from Reproductive Medicine Center, Shanxi women and children's hospital. PCOS patients were diagnosed according to 2003 Rotterdam diagnostic criteria and patients with normal ovulatory function due to tubal blockage or male factor infertility were included as controls. The patients, who had undergone IVF/intracytoplasmic sperm injection (ICSI)-embryo transfer (ET) for the first time, were subjected to the same ovulation protocol. The demographic and clinical data were recorded accordingly. Human luteinized granulosa cells (GCs) were obtained during oocyte retrieval and stored at -80°C until further use. The remaining follicular fluid was used for ELISA detection. The study protocol, strictly following the ethical standards of Helsinki Declaration, was approved by the Ethics Committee of Shanxi women and children's hospital, and informed consents were obtained from all participants.

**Validation of the expression levels of candidate genes by qRT-PCR**

Total RNA was extracted from human GCs using RNeasy Mini Kit (Qiagen, Hilden,

Germany), and cDNA was synthetized using a SuperScript® III Kit (Thermo Fisher Scientific, Shanghai, China). Samples were amplified according to the following protocol: an initial denaturation of 3 min at 95°C followed by 40 cycles denaturing for 10 sec at 95°C, annealing for 1 min at 60°C, and extension for 1 min at 72°C. Primer sequences and product size of genes are listed in Table 3. Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) was used as an internal control for quantification. The relative expression levels of target transcripts were calculated using the $2^{-\Delta\Delta Ct}$ method (Dong et al. 2016). Quantification of qRT-PCR results was performed in which the level of a target transcript was normalized against the target transcript level in Ctrl patient #1, which was arbitrarily set at 1.

**Determination of inflammatory biomarkers in follicle fluid (FF) supernatants**

According to the manufacturer's instructions, inflammatory biomarkers (TNF-α, IL-6, and CRP) were measured in FF supernatants. TNF-α, IL-6, and CRP were determined using an ELISA (R&D Systems, Minneapolis, MN, USA).

Western blot

Western blot was performed as described elsewhere (Zhang et al. 2014). Briefly, total protein samples were isolated and purified using Total Protein Extraction Kit from Merck (Burlington, MA, USA), as per the manufacturer's instructions. ~25 mg of protein samples were separated by on SDS/PAGE and transferred to Polyvinylidene difluoride (PVDF) membrane (Thermo Fisher Scientific), followed by incubation with the primary antibodies at 4℃ overnight. The primary antibodies employed in the current study were rabbit anti-TLR1, anti-TLR2 and anti-TLR8 polyclonal (Thermo Fisher Scientific) and goat

anti-CD14 polyclonal (Abcam, Shanghai, China). Final signals were finally detected using an ECL system (Amersham Biosciences) according to the manufacturer's instructions. Densitometric scanning of immunoblots was performed with the aid of Image J software.

**Statistical analysis.**

The statistical analyses were performed with SPSS13.0 software package (SPSS Inc., Chicago, IL, USA). Results were presented as the mean ± S.E.M. Continuous variables between two groups were compared using *Student's t*-test. The *P*-value < 0.05 was considered significantly different.

## Results

### Identification of DEGs

The GSE34526 dataset contained 10 granulosa cell samples, including 7 PCOS and 3 controls. The gene expression profiles were analyzed using GCBI and identified the DEGs. Based on the GCBI analysis, P value < 0.05 and |logFC| ≥ 2 were used as cut-off criteria. 426 DEGs, which included 418 up-regulated and 8 down-regulated genes, were identified. The details of DEGs expression heat map were shown in Figure 1.

### Function and pathways enrichment analysis

All DEGs was uploaded to the DAVID software to identify GO function. GO enrichment analysis results were presented in Table 1. For biological processes (BP), the top 5 GO terms of DEGs, namely inflammatory response, innate immune response, leukocyte migration, interferon-gamma-mediated signaling pathway, and adaptive immune response, were included. The top 5 GO terms of DEGs for molecular function (MF) were enriched in receptor activity, signaling pattern recognition receptor activity, low-density lipoprotein particle binding, transmembrane signaling receptor activity, and receptor binding. In addition, GO cell component (CC) analysis also showed that the top 5 GO terms of DEGs were significantly enriched in plasma membrane, extracellular exosome, integral

component of plasma membrane, cell surface, and cytosol.

In order to visualize the gene interactions among DEGs, a pathway relation network for the DEGs was constructed using the ClueGO plug-in of Cytoscape software (Figure 2). The pathway relation network for DEGs consisted of B cell receptor signaling pathway, Natural killer cell mediated cytotoxicity, TNF signaling pathway, cytokine-cytokine receptor interaction, chemokine signaling pathway, *etc.* These pathways were primarily associated with immune and inflammation.

**PPI network construction and module analysis**

Based on the information in the STRING database, PPI network was constructed using the MCODE plug-in of Cytoscape software. A total of 118 nodes and 421 edges were included in the total of DEGs with significant interaction relation. The top 3 significant modules were selected, and the KEGG pathway enrichment of the genes involved in the modules were analyzed (Figure 3). Enrichment analysis revealed that the genes in top 3 modules were mainly associated with Fc gamma R-mediated phagocytosis, B cell receptor signaling pathway, Toll-like receptor signaling pathway, Cell adhesion molecules (CAMs), Chemokine signaling pathways. These selected pathways were also associated with immune and inflammation. Furthermore, PPI network and module analysis showed that two modules were involved in the Toll-like receptor signaling pathway in the top 3 modules, and these candidate genes in this signaling pathway consisted of TLR1, TLR2, TLR8, and CD14.

**Patients characteristics**

A total of 78 patients were enrolled in this study, including 38 PCOS patients and 40 controls. Demographic and clinical characteristic parameters of the subjects were summarized in Table 2. Women in PCOS and control groups had a similar mean age, and duration of infertility except BMI. In basal hormonal levels, there was no significant difference in LH, E2 and P levels. However, the FSH, T and LH/FSH was significantly different between PCOS and Control groups ($p < 0.05$). In addition, concentration of cytokines (IL-6, TNF-a, and CRP) in FF as inflammatory markers was detected by ELISA (Table 2). The results showed that IL-6, TNF-a, and CRP levels of FF were significantly

197 increased in PCOS group compared with those in control group.

198 **Validation of candidate Genes **

199 To verify the candidate genes revealed by microarray, four above-mentioned genes (TLR1,
200 TLR2, TLR8, and CD14) were re-examined by qRT-qPCR and Western blot in the 78
201 samples (38 from PCOS patients and 40 from Controls). As shown in Figure 4A, the
202 mRNA levels of TLR2, TLR8, and CD14 were significantly increased in GCs from PCOS
203 when compared to those in GCs from the controls ($P < 0.01$). However, no significant
204 difference in the expression levels of TLR1 was observed between two experimental
205 groups. These results were further validated at the protein level by Western blot analysis
206 (Figure 4B).

207 **Discussion**

208 In the current study, we used published microarray data and bioinformatics analysis
209 method to explore the DEGs in ovarian granulosa cells of PCOS, and our systematic
210 analysis will help to understand the complicated pathogenesis of PCOS at the molecular
211 level.

212 The GO term analysis showed that the top 5 GO terms of DEGs were mainly
213 engaged in inflammatory response, innate immune response, leukocyte migration,
214 interferon-gamma-mediated signaling pathways, and adaptive immune response in
215 biological processes (BP). In order to visualize of gene interactions among DEGs, KEGG
216 pathway enrichment analysis was performed using ClueGO plug-in of Cytoscape software.
217 The results showed that these significant pathways with the DEGs, including B cell
218 receptor signaling pathway, Natural killer cell mediated cytotoxicity, TNF signaling pathway,
219 cytokine-cytokine receptor interaction, chemokine signaling pathway, etc. Hence, we
220 speculated that inflammation may play an essential role in the induction of PCOS.

221 PCOS is towel known to be correlated to low-grade chronic inflammation (El
222 Khoudary et al. 2011), and the alternation of ovarian environment is an fundamental
223 pathophysiological characteristic of PCOS, while GCs as the dominant cell community,
224 regulate the development of follicles and oocytes (Zuo et al. 2017). In 2001, Kelly *et al*.
225 firstly reported that women with PCOS have significantly higher CRP levels compared to

those in healthy women with normal menstrual rhythm and normal androgens (Kelly et al. 2001). In recent years, several studies have shown that the levels of inflammatory factors (TNF, CRP, Il-6, et al) in peripheral blood and FF from PCOS patients are significantly increased (Atabekoglu et al. 2011; Escobar-Morreale et al. 2011). These data suggest that low degree of chronic inflammation may be related to the pathogenesis of PCOS. In our study, the expressions of TNF-a, IL-6 and CRP were all upregulated in the FF of PCOS patients. At present, chronic low inflammation has not been applied in the clinical diagnosis and treatment of PCOS patients. It is necessary to further study the potential mechanisms of low degree inflammation in PCOS pathophysiological process.

To further understand the possible mechanisms underlying the low degree inflammation in PCOS, we constructed the PPI network with DEGs. Module analysis of the PPI network revealed that the development of PCOS was associated with Fc gamma R-mediated phagocytosis, B cell receptor signaling pathway, Toll-like receptor signaling pathway, Cell adhesion molecules (CAMs), Chemokine signaling pathway. Moreover, toll-like receptor (TLR) signaling pathway was involved in two of top three modules, so we speculate that TLR signaling pathway may play an important role in PCOS. TLR is one of pathogen pattern recognition receptors (Aderem and Ulevitch 2000; Akira and Sato 2003). It has been reported that TLRs are important in adipose tissue inflammation of chronic disease (Lucas and Maes 2013). Meanwhile, it is suggested that TLRs are associated with tissue damage and inflammation (Kawai and Akira 2010). Moreover, Liu et al. indicated that TLRs localized in mammalian granulosa cells, cumulus cells and theca cells, and TLRs expression are related with cumulus-oocyte complex expansion and fertilization (Liu et al. 2008). In addition, the activation of toll-like receptor signaling pathway leads to the stimulation of chemokine and cytokine expression including IL-6 and IL-8 (Zarember and Godowski 2002). The cytokines modulated local and systemic inflammatory and immune responses (Lotteau et al. 1990). The study found that TLR activation resulted in excessive expression of COX-2, which was associated with the inflammatory response (Sirois et al. 2004; Williams and DuBois 1996).Conversely, IL-10 as an anti-inflammatory cytokine controlled inflammation response via inhibiting TLR signaling pathways (Williams

et al. 2004). In addition, our results showed that toll-like receptor signaling pathway was related to TLR1, TLR2, TLR8, CD14. But, qRT-PCR results showed that TLR2, TLR8 and CD14 expression increased in PCOS ovary granulosa cells in the validation cohort. While, Western blot showed that the expression level of TLR8 had significantly difference between PCOS and control group.

TLR2 recognizes LPS ligands and mediates signal transductions. Accumulated evidences indicate that TLR2-mediated inflammation condition may favor sustained cytokine production. On the other hand, it may further favor androgen excess in women (Ojeda-Ojeda et al. 2016). Shimada, et al. proved that the expression of IL-6 can be regulated by TLR2 and TLR4 (Shimada et al. 2006). In 2008, Shimada rediscovered that TLR2/4-stimulated COCs secreted chemokines, which could induce sperm capacitation and enhance fertilization (Shimada et al. 2008). In an in vitro fertilization assay, Jiang *et al* proved that cumulus cells activated by TLR2/4 enhanced fertilization by releasing cytokines and chemokines (Jiang et al. 2005).

TLR8 is an inert receptor, but recent studies have shown that it can identify pathogens RNA and induce immune inflammation (Cervantes et al. 2013; Guiducci et al. 2013). Taghavi, S. A. investigated the expression of TLRs in follicular cells of infertile PCOS women, and found the expression levels of TLR1-6, TLR8 and TLR9 were higher in PCOS (Taghavi et al. 2013). Forsbach observed that the activation of TLR-8 led to the secretion of inflammatory cytokines such as IFN-γ, TNF-α and IL-12 (Ospelt and Gay 2010). Moreover, the accumulating evidence indicates that CPG-52364 as TLR antagonist, could block several inflammatory autoimmune diseases induced by activation of TLR7, TLR8 and TLR9 in clinical trials (Lai et al. 2017). It meant that TLR8 was associated with systemic inflammation, which was involved in pathophysiology of PCOS.

Clusters of differentiation 14 (CD14), as a pattern recognition receptor, is expressed on the surfaces of monocytes and macrophages, and contributes to TLR-induced cell activation (Aderem and Ulevitch 2000; Antal-Szalmas 2000). In 2012, Lei *et al* indicated that inhibition of CD14 by RNA interference could inhibit TNF-α secretion and NO production in RAW264.7 cells induced by LPS (Lei et al. 2012).Moreover, using in vitro

test Thorgersen EB, *et al*. found CD14 antibody binding sites on the surface, prevented their combination with LBP and made LPSCD14 TLR4-MyD2 receptor complexes formation blocked, which reduce the secretion of TNF-α and IL-1β (Thorgersen et al. 2009).They all suggested that the regulation of CD14 can inhibit the inflammatory response to a certain extent. Above all, the results indicate that these candidate gene (TLR2, TLR8 and CD14) and their related Toll-like receptor signaling pathway may play key roles in PCOS.

Additionally, there were eight DEGs including HAS2, THSD7A, LPHN3, DLX2, COCH, SPOCK3, CNTN4 and ACOT4, whose expression levels were found to be significantly down-regulated in GCs from PCOS patients when compared to those in GCs from Ctrl patients (data not shown). Some of these downregulated DEGs may also play potential roles during the pathogenesis of PCOS. For example, HAS2 and CNTN4 are both distinctly expressed in GCs (Liu et al. 2016; Wigglesworth et al. 2015). Overexpression of HAS2 promotes resistance to apoptosis in GCs (Liu et al. 2016). More importantly, HAS2 and CNTN4 are both regulated fundamentally by insulin signaling pathway (Grado-Ahuir et al. 2009; Kuroda et al. 2001). Given the close association between insulin-signaling pathway and gonadotrophin hormone action in PCOS (Aydos et al. 2016; Szczuko et al. 2016), we therefore propose that HAS2 and CNTN4 are both functionally involved in the pathogenesis of PCOS. This intriguing hypothesis is currently under investigation in our lab.

In conclusion, our data collectively provide a comprehensive bioinformatics analysis of DEGs in PCOS. Our results confirm that the inflammation and immune play important roles in the occurrence and development of PCOS. Meanwhile, toll-like receptor signaling pathway mediating inflammation and immune might be involved in the pathogenesis of PCOS, and TLR2, TLR8, CD14 may be core target genes. Therefore, the hub genes and pathways may be potential therapeutic targets of PCOS treatment.

Nevertheless, the potential caveats and other alternative explanations would be very insightful for future research, such as the limited control numbers in the database, and future directions of single-RNA profiling.

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We are grateful to the researchers who have deposited their data in the public database.

**Conflict of interest**

The authors declare that there are no conflicts of interest.

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## Figure legends

**Figure 1** Heat map of the differentially expressed genes of GSE34526. Red: up-regulation; green: down-regulation.

**Figure 2** Pathway relation network for the total differentially expressed genes (DEGs). Each GO term is represented by a circle, and each group is represented by a different color. The larger the size of the circle is, the more the number of genes is contributing to the GO term.

**Figure 3** Top 3 modules from the protein-protein interaction network. (A) module 1, (B) the enriched pathways of module 1, (C) module 2, (D) the enriched pathways of module 2, (E) module 3, (F) the enriched pathways of module 3.

**Figure 4** (A) qRT-PCR analysis of the expression of TLR1, TLR2, TLR8, and CD14 gene in human ovarian luteinized granulosa cells (GCs) from PCOS and Controls. *$P < 0.05$, **$P < 0.01$. (B) Western blot analysis of the expression levels of TLR1, TLR2, TLR8, and CD14 in human ovarian luteinized GCs from PCOS and Controls. Densitometric scanning of immunoblots was performed in which the level of a target protein was normalized against the protein level in Ctrl #1, which was arbitrarily set at 1 (lower panel). Each bar represents the mean±S.E.M. of results from three experiments using different batches of GCs. Each experiment had replicate cultures. *$P < 0.05$, **$P < 0.01$.

**Supplementary Figure 1** Representative RT-PCR analysis of TLR2 expression in human ovarian luteinized GCs from PCOS and Controls. Parallel amplification of *GAPDH* mRNA served as internal control.

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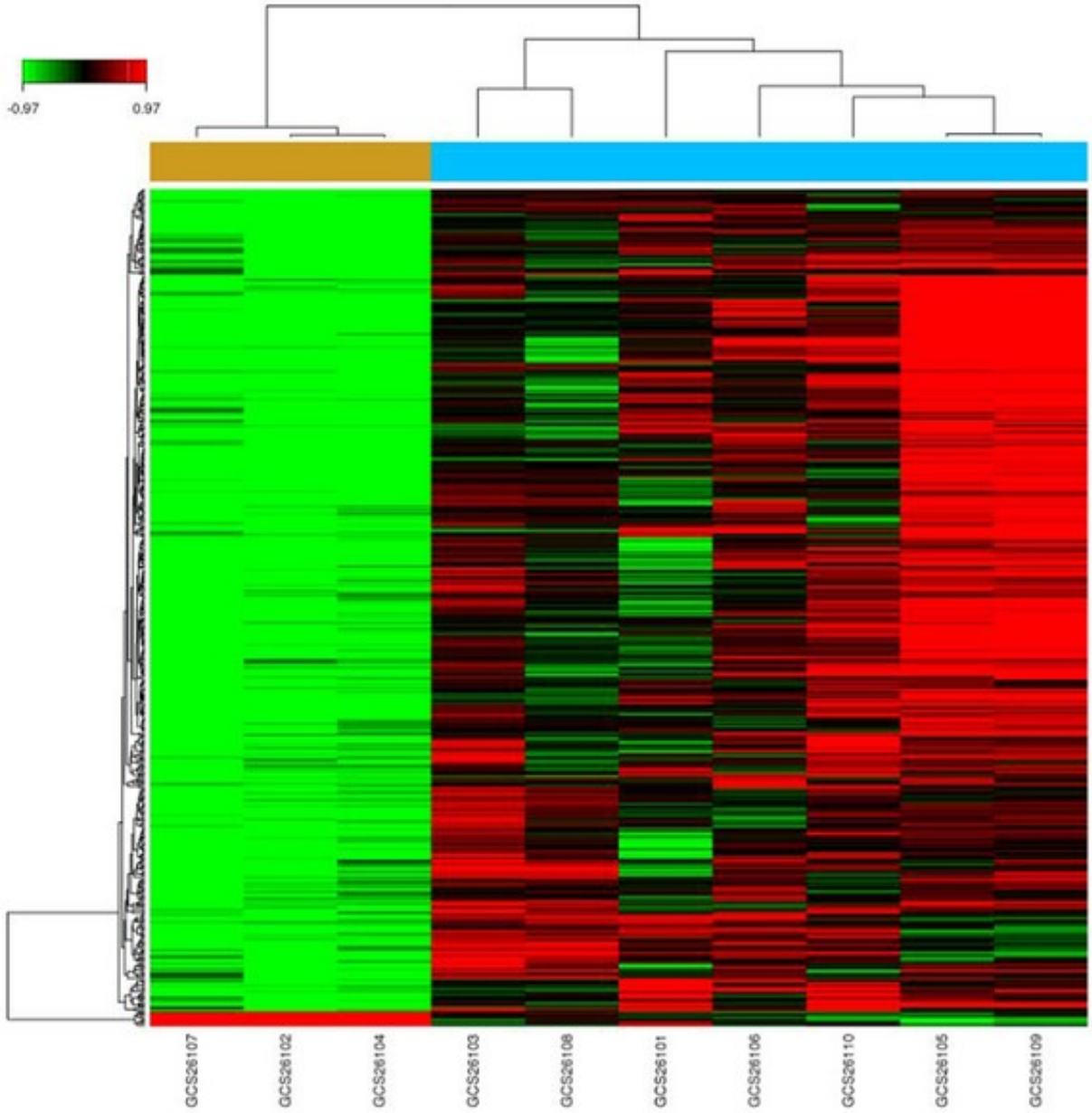


Fig. 2 Download full resolution image

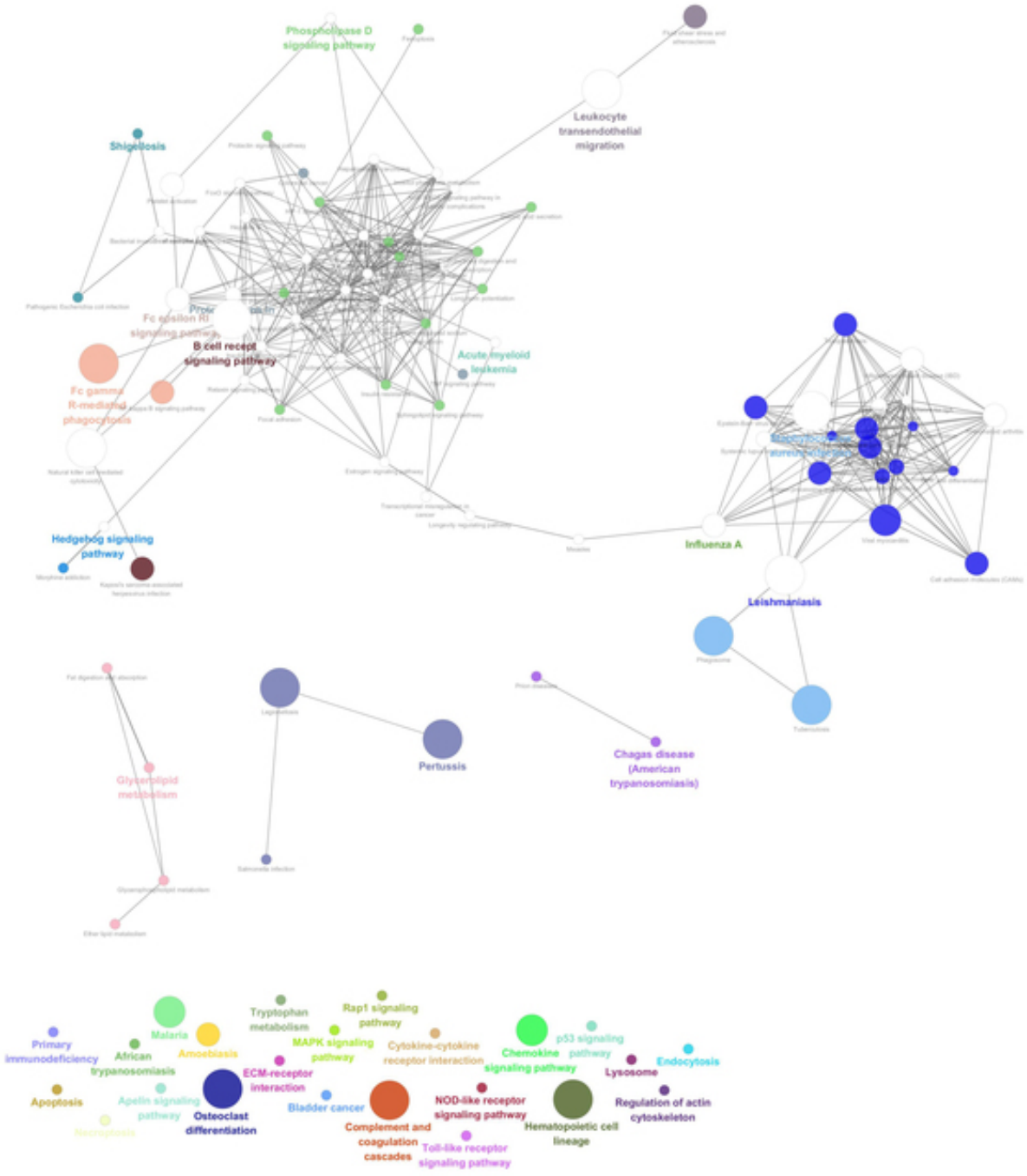
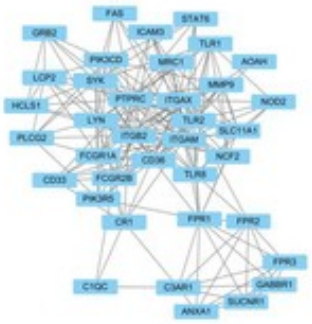


Fig. 3 [Download full resolution image]

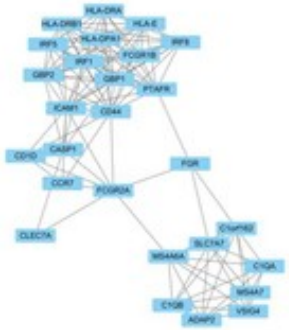
A



B

| Term | P value | FDR | Gene |
|---|---|---|---|
| Fc gamma R-mediated phagocytosis | 1.46E-06 | 0.00149 | PTPRC, LYN, FCGR2B, FCGR1A, PIK3CD, PLCG2, PIK3R5, SYK |
| B cell receptor signaling pathway | 5.62E-06 | 0.00573 | LYN, FCGR2B, GRB2, PIK3CD, PLCG2, PIK3R5, SYK |
| Leukocyte transendothelial migration | 7.61E-05 | 0.077493 | NCF2, MMP9, PIK3CD, PLCG2, PIK3R5, ITGB2, ITGAM |
| Hematopoietic cell lineage | 0.001911 | 1.930742 | CR1, CD36, CD33, FCGR1A, ITGAM |
| Toll-like receptor signaling pathway | 0.003439 | 3.450576 | PIK3CD, TLR1, TLR2, PIK3R5, TLR8 |

C



D

| Term | P value | FDR | Gene |
|---|---|---|---|
| Systemic lupus erythematosus | 4.40E-06 | 0.003611 | C1QA, C1QB, HLA-DRB1, HLA-DPA1, FCGR2A, HLA-DRA |
| Viral myocarditis | 3.14E-05 | 0.025781 | ICAM1, HLA-DRB1, HLA-DPA1, HLA-E, HLA-DRA |
| Autoimmune thyroid disease | 3.20E-04 | 0.262222 | HLA-DRB1, HLA-DPA1, HLA-E, HLA-DRA |
| Cell adhesion molecules (CAMs) | 3.55E-04 | 0.29062 | ICAM1, HLA-DRB1, HLA-DPA1, HLA-E, HLA-DRA |
| Hematopoietic cell lineage | 0.001486 | 1.212095 | CD44, HLA-DRB1, HLA-DRA, CD1D |

E

F

| Term | P value | FDR | Gene |
|---|---|---|---|
| B cell receptor signaling pathway | 0.001503 | 1.46896 | PIK3CG, PIK3AP1, BLNK, BTK |
| Chemokine signaling pathway | 0.002277 | 2.217082 | PIK3CG, DOCK2, NCF1, HCK, ELMO1 |
| Fc gamma R-mediated phagocytosis | 0.002965 | 2.878819 | PIK3CG, DOCK2, NCF1, HCK |
| Leukocyte transendothelial migration | 0.005471 | 5.252746 | PIK3CG, ACTB, CYBB, NCF1 |
| Toll-like receptor signaling pathway | 0.039114 | 32.45961 | PIK3CG, CD14, SPP1 |

Fig. 4 Download full resolution image