Research Article

Bioinformatic Analysis Identifies Potential Key Genes in the Pathogenesis of Polycystic Ovary Syndrome

Qian-Qian Liang[1*], Dai-Jun Wang[2]

[1]Division of Health Management, Yuncheng central hospital, Eighth Clinical Medical College of ShanXi Medical University, Shanxi, China

[2]Department of Human Anatomy, Weifang Medical University, Shandong, China.

***Corresponding author:** Qian-Qian Liang, Division of Health Management, Yuncheng central hospital, Eighth Clinical Medical College of ShanXi Medical University, Shanxi, China.

Dai-Jun Wang, Department of Human Anatomy, Weifang Medical University, Shandong, China

Received: 01 April 2021; **Accepted:** 18 April 2022; **Published:** 22 April 2022

Citation: Qian-Qian Liang, Dai-Jun Wang. Bioinformatic Analysis Identifies Potential Key Genes in the Pathogenesis of Polycystic Ovary Syndrome. Journal of Bioinformatics and Systems Biology 5 (2022): 78-92.

Abstract

Polycystic ovary syndrome (PCOS) is one of the factors leading to infertility; however, the specific pathogenesis of PCOS is still unclear. The purpose of this study was to determine the key changes in gene expression during the formation of PCOS and provide a theoretical basis for the clinical diagnosis and treatment of PCOS.We analyzed differentially expressed genes (DEGs) in the GSE34526 dataset from the online bioinformatics array research tool (BART) (bart.salk.edu). Then, the Database for Annotation, Visualization and Integrated Discovery (DAVID) (https://david.ncifcrf.gov/) online analysis software for gene ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) rich path analysis, STRING (https://string-db.org/) online analysis tool for protein-protein interaction (PPI) network, Cytoscape software for Mcode module and HUB gene analysis were used. To verify the HUB genes, the GSE59456 dataset was analyzed, and it includes female Sprague-Dawley rats that were implanted daily with silicone capsules that continuously released 5α-dehydrotestosterone (DHT) for 12 weeks to mimic the hyperandrogen state of women with PCOS and a control (CTL) group that received empty capsules.A total of 91 DEGs (7 upregulated and 84 downregulated) were found. Seven central HUB genes were identified, i.e., integrin alpha-M (ITGAM), cytochrome BMUR 245 beta chain (CYBB), toll like receptor 1 (TLR1), platelet activating factor receptor (PTAFR), CD163 molecule, caspase 1 (CASP1), and matrix

metallopeptidase 9 (MMP9). The HUB genes were verified using GSE59456, and compared with the CTL group, the expression of the CYBB and CASP1 genes was reduced in the DHT group.The DEGs, HUB genes and signaling pathways identified in this study provide insights on the molecular mechanism underlying PCOS formation and reveal new targets for the diagnosis and treatment of PCOS.

Keywords: Polycystic ovary syndrome; Ovarian granulosa cells; genes; GSE34526; GEO59456

1. Background

Polycystic ovary syndrome (PCOS) is a common reproductive endocrine disease in women of childbearing age, and it has an incidence of 5.6-16% and is increasing annually. PCOS is considered a systemic multisystem disease that includes hyperandrogenemia, anovulation, irregular menstruation, infertility and metabolic abnormalities, including insulin resistance and hyperlipidemia [1, 2]. PCOS has many causal factors, including genetic and psychosocial factors, poor living habits and environmental factors (e.g., chemicals: pesticides, industrial pollutants; and personal care products: perfumes, deodorants, hair dyes, perfume compounds and bisphenol A). The specific etiology and pathogenesis of the disease are not currently clear [3-6]. Ovarian granulosa cells (GCs) are secreted and play an important role in the process of folliculogenesis [7], and understanding the gene expression of PCOS GCs is of great significance for effective diagnosis and treatment. In previous literature, potential differentially expressed genes in the GSE34526 gene expression profile were assessed by gene ontology (GO) enrichment, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway, PPI network and Mcode module analyses; however, HUB gene analyses have not been performed [8-12].

In this study, we used the online bioinformatics array research tool (BART) to analyze the original microarray dataset GSE34526 (healthy samples and PCOS female ovarian GCs) for differentially expressed genes (DEGs). GO enrichment, KEGG pathway, PPI network, Mcode module and Hub analyses and verification were performed to determine the genes, pathways and molecular mechanisms related to ovarian granulosa cells in women with PCOS to provide a theoretical basis for the clinical diagnosis, treatment and prevention of PCOS.

2. Methods

2.1 Microarray Data and DEG Identification

GEO (http://www.ncbi.nlm.nih.gov/GEO) [13] is a public functional genome database that contains high-throughput gene expression data, chips and microarrays. A gene expression dataset [GSE34526] was selected through GEO (GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array). The GSE3526 dataset includes 10 samples, including 3 normal female ovarian granulosa cell samples and 7 PCOS female ovarian granulosa cell samples (human granulosa cells were isolated from ovarian fluid aspiration of normal women and women with PCOS who received in vitro fertilization).

DEGs were downloaded from the BART platform (bart.salk.edu) [14], which can process raw microarray data from GEO or local data into a list of differential genes and related pathways. DEGs were determined by a logFC value greater than 1 or less than -1 and t-tests with adj. $P < 0.05$.

2.2 GO Enrichment and KEGG Pathway Analyses of DEGs

The Database for Annotation, Visualization and Integrated Discovery (DAVID) (https://david.ncifcrf.gov/) (Version 6.8) online analysis software was used for the GO enrichment and KEGG pathway analyses of differential genes. The GO analysis included three terms: biological process (BP), cellular component (CC), and molecular function (MF). A value of $P < 0.05$ was considered statistically significant when screening important GO terms and KEGG pathways.

2.3 PPI network construction and Module Analysis

A differential protein -protein interaction (PPI) network analysis was performed using STRING (https://string-db.org/) (Version 11.0) online analysis software. PPI analyses can provide a better understanding of the pathogenesis of PCOS, and a minimum required interaction score of 0.400 indicates statistical significance. Cytoscape (www.Cytoscape.org) (version 3.7.2), which is an open source systems biology analysis software that can be used for data visualization, was downloaded and used to analyze the TSV files output by the STRING analysis. Mcode (version 1.6.1) is a plug-in of Cytoscape software. The functional module of the STRING protein gene network was constructed by clustering, and the network formed by the TSV files was analyzed again. The selection criteria were as follows: MCODE degree cutoff = 2, node score cutoff = 0.2, max depth = 100, and k-score = 2. Then, the DEGs in Mcode were analyzed using KEGG and GO software.

2.4 HUB gene selection and verification

The cytoHubba plug-in of Cytoscape software was used for HUB gene selection. The first 10 HUB genes were screened by Radility, MHC, Degree, Stress and Closeness, and an overlapping HUB gene network was constructed. The GEO59456 dataset was used to verify the selected HUB genes.

3. Results

3.1 Identification of DEGs

We used the BART online analysis software to analyze the DEGs in the GSE34526 dataset. BART software can automatically download data from GEO and analyze it using the LIMMA bioinformatics software package. The original fluorescence CEL file was used as input and divided into a PCOS group and a normal group. All samples were isolated from the ovarian fluid of normal and polycystic ovary syndrome patients undergoing in vitro fertilization. A total of 54675 genes were assessed. The Hclust R function was used to cluster the first 1000 expressed normalized genes (see Fig. 1a) based on logFC values greater than 1 or less than -1 and t-tests with adj. $P < 0.05$. The results showed that there were 91 DEGs, with 7 upregulated genes and 84 downregulated genes (see Fig. 1b).

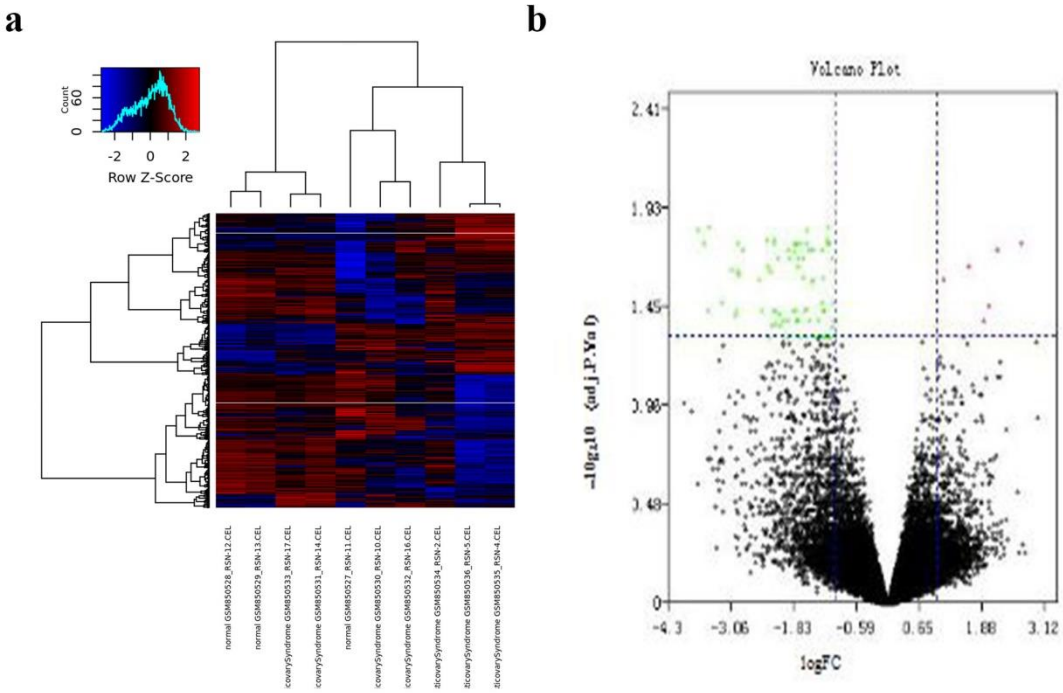

Figure 1: Cluster of the first 1000 expressed normalized genes and a volcano plot of all DEGs

a. Cluster of the first 1000 expressed normalized genes

b. Volcano plot of all DEGs. DEGs were determined by logFC values greater than 1 or less than -1 and t-tests with adj. $P < 0.05$.

3.2 GO Term Enrichment and KEGG Pathway Analysis

The DAVID online tool was used to perform GO enrichment and KEGG pathway analysis on 91 DEGs. As shown in Fig. 2, each part of the GO analysis shows the top 10 enrichment analysis results. The results of the BP enrichment analysis showed that the predicted genes were primarily involved in the following categories: inflammatory response (GO:0006954), interferon-gamma-mediated signaling pathway (GO:0060333), leukocyte migration (GO:0050900), signal transduction (GO:0007165), adaptive immune response (GO:0002250), immune response (GO:0006955), innate immune response (GO:0045087), B cell receptor signaling pathway (GO:0050853), neutrophil chemotaxis (GO:0030593), and regulation of cell shape (GO:0008360) (Fig. 2a). The results of the cellular component (CC) enrichment analysis showed that the predictive genes were primarily involved in the following categories: plasma membrane (GO:0005886), integral component of plasma membrane (GO:0005887), integral component of membrane (GO:0016021), extracellular exosome (GO:0070062), endosome membrane (GO:0010008), clathrin-coated endocytic vesicle membrane (GO:0030669), IPAF inflammasome complex (GO:0072557), phagocytic vesicle membrane (GO:0030670), proteinaceous extracellular matrix (GO:0005578), and endocytic vesicle membrane (GO:0030666) (Fig. 2b). The results of the molecular function (MF) enrichment analysis showed that the predictive genes were primarily involved in the following categories: protein binding (GO:0005515), transmembrane signaling receptor activity (GO:0004888), receptor activity (GO:0004872), N-

formyl peptide receptor activity (GO:0004982), MHC protein binding (GO:0042287), complement receptor activity (GO:0004875), collagen binding (GO:0005518), GTPase activator activity (GO:0005096), RAGE receptor binding (GO:0050786), and IgG binding 2 (GO:0019864) (Fig. 2c).

The results of the KEGG pathway analysis showed that the predictive genes were primarily involved in Staphylococcus aureus infection (hsa05150), tuberculosis (hsa05152), phagosome (hsa04145), osteoclast differentiation (hsa04380), leukocyte transendothelial migration (hsa04670), cRap1 signaling pathway (hsa04015), leishmaniasis (hsa05140), Fc gamma R-mediated phagocytosis (hsa04666), influenza A (hsa05164), and amoebiasis (hsa05146) (Fig. 2d).

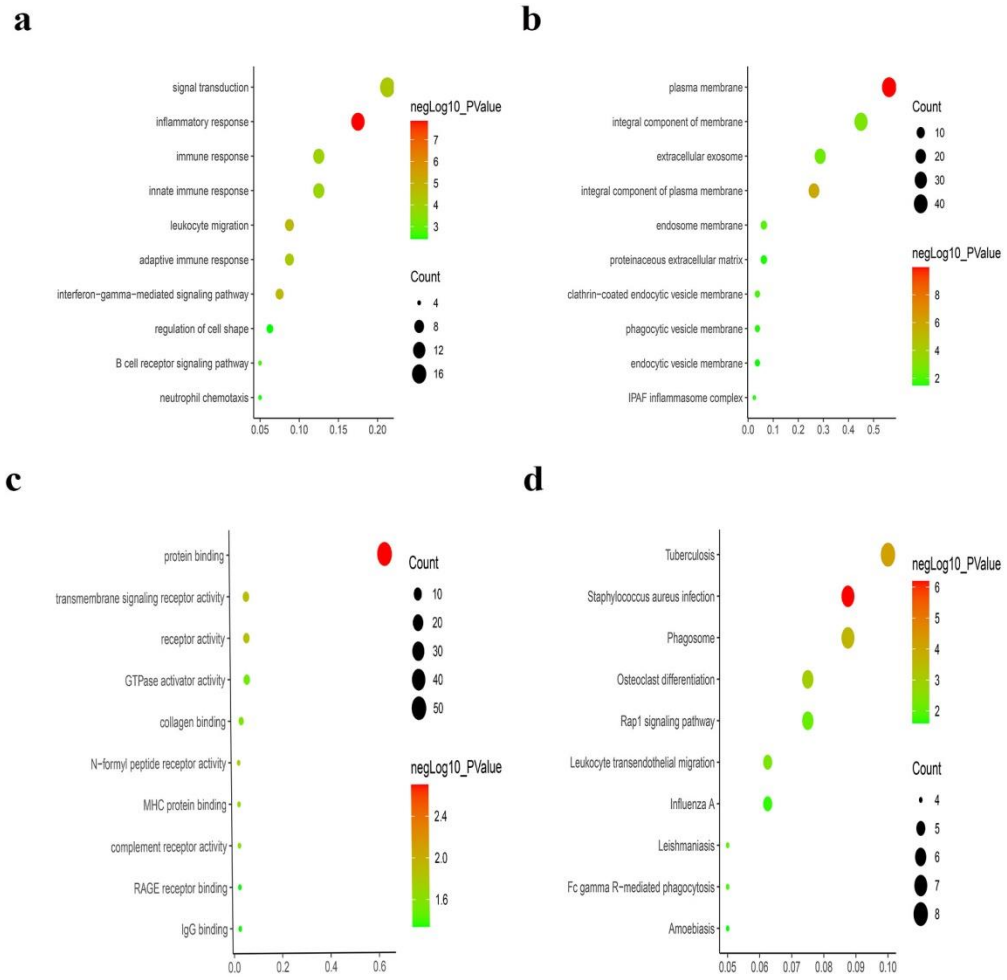

Figure 2: GO enrichment and KEGG pathway analysis of DEGs a. BP. b. CC. c. MF. d. KEGG. The picture shows the top ten GO enrichment and KEGG pathway analysis results.

3.3 PPI network construction and Mcode Analysis

A TSV file was downloaded and analyzed by STRING online analysis software, imported into Cytoscape, and inserted into the Mcode module, and it revealed 60 nodes and 193 edges. In the PPI network, the nodes represent the DEGs and the edges represent the interactions between DEGs. Based on this, two modules were obtained from the PPI network, as shown in Fig. 3. Next, the GO enrichment and KEGG pathway analyses of the DEGs of the two modules were performed again using the DAVID online tool. The results of the GO enrichment analysis of the DEGs in Mcode1 were as follows: the BP enrichment analysis results primarily included innate immune response (GO: 0045087) and inflammatory response (GO: 0006954); the CC enrichment analysis results included the IPAF inflammasome complex (GO: 0072557); and the MF enrichment analysis results included protein binding (GO: 0005515).

The KEGG pathway analysis of these DEGs primarily included phagosome (hsa04145) and tuberculosis (hsa0515) (Table 1). The results of the GO enrichment analysis of the DEGs in Mcode 2 were as follows: the BP enrichment analysis results included the interferon-gamma-mediated signaling pathway (GO: 0060333) and immune response (GO: 0006955); the CC enrichment analysis results included clathrin-coated endocytic vesicle membrane (GO: 003066) and plasma membrane (GO: 0005886); and the MF enrichment analysis results included MHC class II receptor activity (GO: 0032395). The KEGG pathway analysis of these DEGs primarily included Staphylococcus aureus infection (hsa05150) (Table 2).

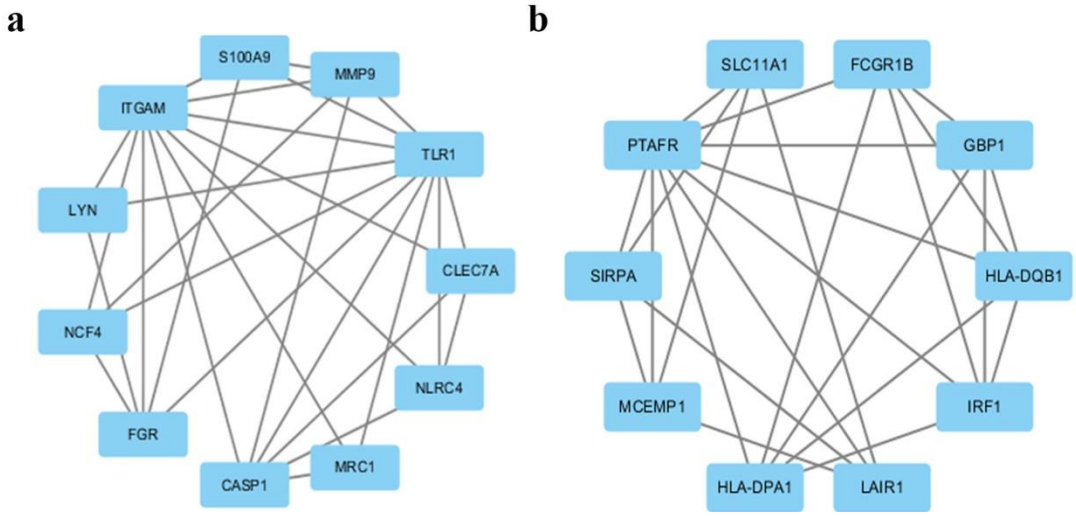

Figure 3: Two modules from the PPI network a. Module 1. b. Module 2.

Table 1: Top ten GO enrichment and KEGG pathways analyzed by DAVID for the DEGs in Module 1

Description	P Value	Count	Gene symbol
GO:0045087~	2.44E-06	6	LYN,TLR1,FGR,CLEC7A, NLRC4, S100A9

Innate immuner esponse			
GO:0006954~ inflammatory response	4.81641E-05	5	LYN, TLR1, CLEC7A, NLRC4, S100A9
hsa04145:Phagosome	0.000775204	4	ITGAM,CLEC7A, NCF4, MRC1
GO:0006919~ activation of cysteine-typeendopeptidase activity involved in apoptotic process	0.001058638	3	CASP1,NLRC4, S100A9
hsa05152:Tuberculosis	0.001254976	4	TLR1, ITGAM, CLEC7A, MRC1
GO:0005515~protein binding	0.001453344	11	LYN, TLR1, FGR, ITGAM, CLEC7A,NCF4,MRC1,CASP1, NLRC4, S100A9, MMP9
hsa05134:Legionellosis	0.002102061	3	ITGAM, CASP1, NLRC4
GO:0050900~leukocyte migration	0.002267864	3	LYN, ITGAM, MMP9
GO:0002768~immune response-regulating cell surface receptor signaling pathway	0.002380172	2	LYN, FGR
GO:0072557~IPAF inflammasome complex	0.002740926	2	CASP1, NLRC4

Table 2: Top ten GO enrichment and KEGG pathways analyzed by DAVID for the DEGs in Module 2

Description	*P* Value	Count	Gene symbol
GO:0060333~interferon-gamma- mediated signaling pathway	6.49E-11	6	IRF1, PTAFR, FCGR1B, GBP1,HLA-DPA1, HLA-DQB1
GO:0006955~immune response	2.5173E-05	5	SLC11A1, PTAFR, FCGR1B, HLA-DPA1, HLA-DQB1

GO:0030669~clathrin-coated endocytic vesicle membrane	0.00017601	3	FCGR1B, HLA-DPA1, HLA-DQB1
hsa05150:Staphylococcus aureus infection	0.0005958	3	PTAFR, HLA-DPA1, HLA-DQB1
GO:0005886~plasma membrane	0.0006987	8	SLC11A1, PTAFR, SIRPA, FCGR1B, GBP1, LAIR1, HLA-DPA1, HLA-DQB1
GO:0016021~integral component of membrane	0.0029814	8	SLC11A1, PTAFR, SIRPA, MCEMP1,FCGR1B, LAIR1,HLA-DPA1, HLA-DQB1
GO:0010008~endosome membrane	0.00352092	3	SLC11A1, HLA-DPA1, HLA-DQB1
GO:0032395~MHC class II receptor activity	0.00708798	2	HLA-DPA1, HLA-DQB1
GO:0002504~antigen processing and presentation of peptide or polysaccharide antigen via MHC class II	0.00807213	2	HLA-DPA1, HLA-DQB1
GO:0042613~MHC class II protein complex	0.01081484	2	HLA-DPA1, HLA-DQB1

Table 3: Top 10 HUB genes ranked using cytoHubba

Degree	Stress	MNC	Radiality	Closeness
ITGAM	ITGAM	ITGAM	ITGAM	ITGAM
CYBB	CYBB	CYBB	CYBB	CYBB
TLR1	TLR1	TLR1	TLR1	TLR1
CD163	CD163	CD163	CASP1	CD163
CASP1	MMP9	CASP1	CD163	CASP1
PTAFR	CASP1	FPR1	SLC11A1	MMP9
MMP9	PTAFR	PTAFR	FPR1	PTAFR
FPR1	MCEMP1	MMP9	PTAFR	SLC11A1

FCGR2B	IRF1	SLC11A1	FCGR2B	FPR1
SLC11A1	NCF4	FCGR2B	MMP9	FCGR2B

3.4 Gene selection and verification

In this study, we used the cytoHubba plug-in of Cytoscape software to select HUB genes and screened the first ten genes according to the Radility, MHC, Degree, Stress and Closeness methods (see Table 3). Finally, the following seven central genes were identified as HUB genes by overlapping the first 10 genes (see Figure 4): integrin alpha-M (ITGAM), cytochrome BMUR 245 beta chain (CYBB), toll like receptor 1 (TLR1), platelet activating factor receptor (PTAFR), CD163 molecule, caspase 1 (CASP1), and matrix metallopeptidase 9 (MMP9). The GSE59456 dataset was used to verified these Hub genes, and the analysis results showed that the expression of the CYBB and CASP1 genes in the DHT group was significantly reduced compared with that in the CTL group (P = 0.0286, P = 0.0286) (see Fig. 5b, Fig. 5F).

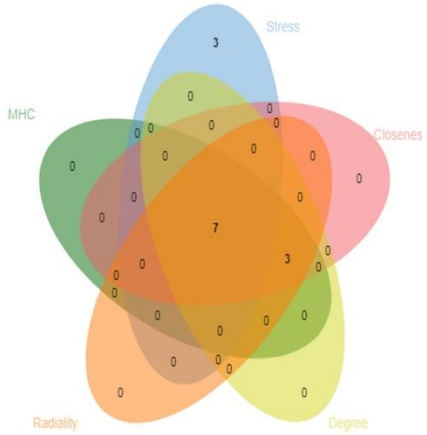

Figure 4: Seven overlapping central genes of the top 10 genes were screened by five methods using the cytoHubba plug-in. The selection criteria were as follows: MCODE degree cutoff = 2, node score cutoff = 0.2, max depth = 100, and k-score = 2.

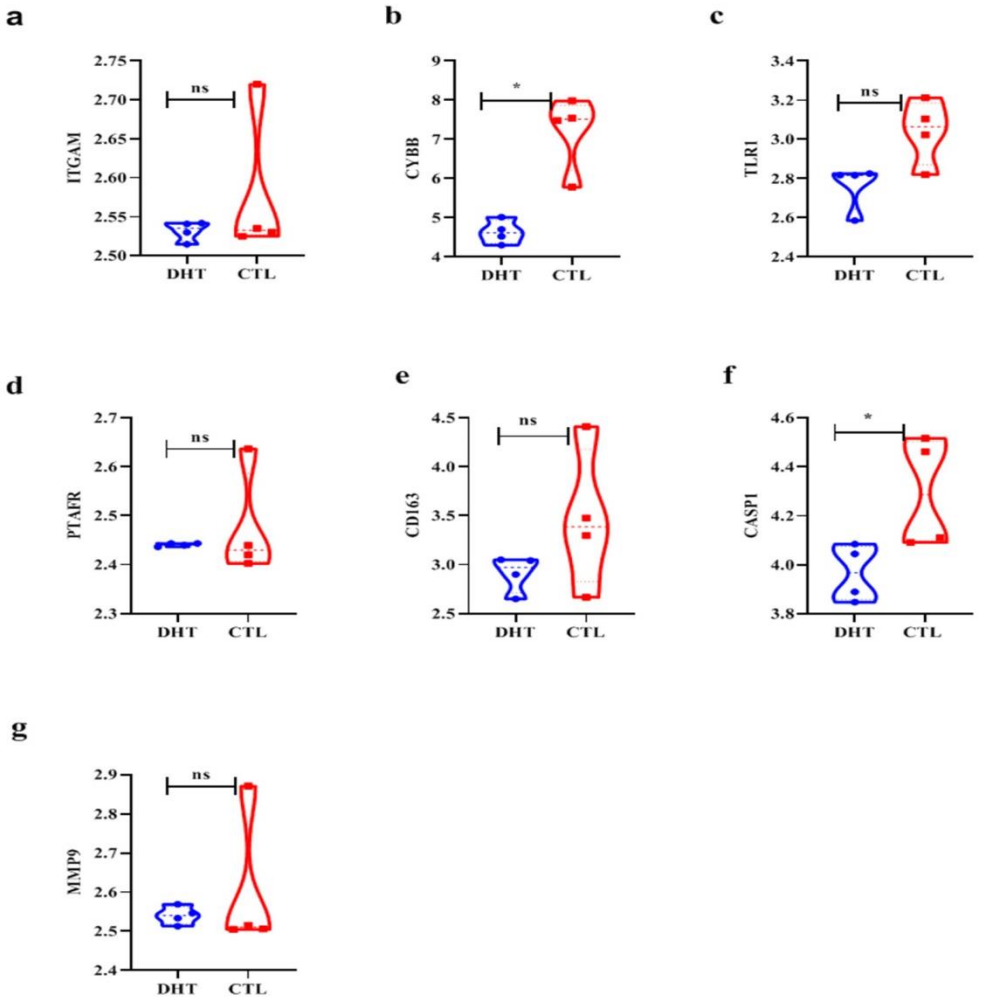

Figure 5: HUB genes were validated using the GSE59456 dataset. a. ITGAM; b. CYBB; c. TLR1; d. PTAFR; e. CD163; f. CASP1; g. MMP9. DHT group: n = 4; CTL group: n = 4 The data did not follow a normal distribution; therefore, we used the Mann-Whitney U test. ns=no significance, *=P<0.05

4. Discussion

GCs play an important role in the formation of follicles and cumulus-oocyte complexes around the egg [15]. Recently, many scholars have found that cumulus GC genes can predict oocyte development [16, 17].

Is the pathogenesis of PCOS related to GCs? Many scholars have found that changes occur in oocyte growth and embryonic potential in patients with PCOS. Abnormal GC function is one of the primary causes of follicular dysplasia in PCOS [18, 19]. Victor Blasco and colleagues investigated the decreased expression of TAC3, TACR3 and KISS1 mRNA in the mural granulosa and cumulus cells of patients with PCOS, and these changes may be related to abnormal follicular development and ovulation disturbance in PCOS patients [20]. These data show that abnormal gene expression in ovarian GCs is closely related to the pathogenesis of PCOS. However, many genes are involved in GCs, and whether additional gene abnormalities and gene interactions lead to the pathogenesis of PCOS needs to be further explored.

Many scholars have performed DEG analyses, PPI network analyses, GO enrichment analyses and KEGG pathway analyses of GSE34526 datasets [8-12]. DEGs were identified using different research methods, thus providing important clues for the diagnosis and treatment of PCOS. However, previous studies have not performed HUB gene screening of the GSE34526 dataset. In this dataset analysis, the DEGs in the GSE34526 dataset (3 normal GCs and 7 PCOS female GCs) was analyzed using BART online analysis software. This analysis tool has six modules, and users can test differential expression of the original microarray data from GEO or local data using the LIMMA bioinformatics software package [14]. The exclusion criteria included genes with logFC > 1 or < -1 and t-tests with adj. $P < 0.05$. A total of 91 DEGs (7 upregulated and 84 downregulated) were found. The GO enrichment analysis of DEGs using DAVID software showed that these genes were primarily involved in inflammatory reactions, plasma membrane and protein binding. Reports have indicated that abnormalities in inflammatory cytokines and GC cell membrane receptors are related to the pathogenesis of PCOS [21-23]. In addition, recent studies have reported that SRAGE plays a protective role in the development of PCOS by inhibiting inflammation [24]. The KEGG pathway analysis also showed that the DEGs were primarily associated with infection and bacteriophages, which is consistent with GO enrichment analysis.

The PPI network of DEGs was analyzed by STRING, and the TSV file was downloaded and imported into Cytoscape software, in which two modules were identified by the Mcode plug-in. The GO enrichment and KEGG pathway analyses of the DEGs in the module were performed using the DAVID online tool. Our research showed that module 1 significantly participates in the innate immune response, inflammatory response, phagosome and IPAF inflammasome complex while module 2 significantly participates in the interferon-gamma-mediated signaling pathway, clathrin-coated endocytic vesicle membrane, MHC class II receptor activity and Staphylococcus aureus infection. Previous research has confirmed that serum levels of interferon-γ in patients with PCOS are lower than those in healthy women; thus, interferon-γ may be a new biomarker for the diagnosis and treatment of PCOS [22]. Androgens can induce GC apoptosis, a process related to macrophages. Therefore, infection and immunity play an important role in the occurrence and development of PCOS [25].

In addition, we also analyzed the PPI network of DEGs and used five methods to identify seven HUB genes: ITGAM, CYBB, tTLR1, PTAFR, CD163, CASP1, and MMP9. ITGAM is the most prominent HUB gene and has been reported to be associated with the pathogenesis of PCOS [26, 27]; however, its expression in ovarian GCs or the specific pathogenesis of PCOS has not been previously studied. The relationship between polycystic ovary syndrome and CYBB or CASP1 also has not been studied. Saturated fat intake has been reported to promote an increase in circulating endotoxin levels and TLR-4 gene expression in obese women of childbearing age, especially in the presence of PCOS [28]. However, the role of TLR1 in the pathogenesis of PCOS is still unclear. PTAFR, a member of the G protein coupled receptor family, is detected in the luminal epithelial cells of embryonic diapause and strongly expressed in all stages of resuscitation [29]. The relationship between PTAFR and PCOS has not been previously reported. CD163 has been identified

a marker of macrophages, and Asa Lindholm and other scholars found that the expression of CD163 in peripheral blood is decreased in overweight women with PCOS [30]. Nine members of the matrix metalloproteinase family, which are the main proteases involved in extracellular matrix remodeling, were identified. The levels of MMP2 and MMP9 were higher in the circulation, follicular fluid and granulosa cells of patients with PCOS, while the levels of TIMP1 were constant or low. Increased MMP activity may disrupt the process of tissue remodeling as well as the availability of growth factors and gap junctional communication, thus leading to the development of abnormal ovarian phenotypes in women with PCOS [31]. The verification of these HUB genes using the GSE59456 dataset showed that CYBB and CASP1 were reduced in the DHT group. To determine whether CYBB plays an important role in the pathogenesis of PCOS, human and animal specimens must be used for further verification.

Based on our analysis, we screened seven HUB genes and confirmed through the dataset that CYBB and CASP1 may play important roles in the formation of PCOS. However, our study is limited by a lack of analyses on the dataset related to the peripheral blood of women with PCOS. In our next study, we will further analyze whether genes related to the peripheral blood of women with PCOS are consistent with the expression of GCs. In addition, we will perform animal experiments to confirm the potential mechanism of Hub genes in the formation of PCOS to provide a theoretical basis for the clinical diagnosis and treatment of PCOS.

5. Conclusion

In conclusion, 91 DEGs, 2 network modules and 7 HUB genes were identified. Among the seven Hub genes ITGAM, CYBB, TLR1, PTAFR, CD163, CASP1 and MMP9 and their associated signaling pathways, the GSE59456 dataset verification further confirmed that the CASP1 and CYBB genes may play an important role in the occurrence and development of PCOS. In future studies, human peripheral blood samples and animal experiments should be performed to confirm the role of these genes in the formation of PCOS to provide a basis for clinical diagnosis and treatment.

Abbreviations

PCOS: Polycystic Ovary Syndrome;

DEGs: Differentially Expressed Genes;

BART: Bioinformatics Array Research Tool;

DAVID: Database for Annotation, Visualization and Integrated Discovery;

GO: Gene Ontology;

KEGG: Kyoto Encyclopedia Of Genes And Genomes;

PPI: Protein-Protein Interaction;

ITGAM: Integrin Alpha-M;

CYBB: Cytochrome BMUR 245 Beta Chain;

TLR1: Toll Like Receptor 1;

PTAFR: Platelet Activating Factor Receptor;

CASP1: Caspase 1;

MMP9: Matrix Metallopeptidase 9;

GCs: Granulosa Cells;

BP: Biological Process;

CC: Cellular Component;

MF: Molecular Function.

Acknowledgments:

None

Authors' contributions

Qian-Qian Liang and Dai-Jun Wan contributed equally to the work.

Funding

This work was supported by the Scientific and Technological Innovation Programs of Higher Education Institutions in Shanxi (NO.2020L0229).The funding bodies played no role in the design of the study and collection, analysis and interpretation of data and in writing the manuscript.

Availability of data and materials

Microarray datasets (GSE34526 and GSE59456) for this study are openly available in Gene Expression Omnibus database respectively (last accessed on 26 May 2021).

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that there are no conflicts of interest.

References:

1. Patel S: Polycystic ovary syndrome (PCOS), an inflammatory, systemic, lifestyle endocrinopathy. The Journal of Steroid Biochemistry and Molecular Biology 182 (2018): 27-36.
2. Basirat Z, Faramarzi M, Chehrazi M, Amiri M, Ghofrani F, Tajalli Z. Differences between infertile women with and without PCOS in terms of anxiety, coping styles, personality traits, and social adjustment: a case–control study. Archives of Gynecology and Obstetrics 301 (2019): 619-626.
3. Yang O, Kim HL, Weon J-I, Seo YR. Endocrine-disrupting Chemicals: Review of Toxicological Mechanisms Using Molecular Pathway Analysis. Journal of Cancer Prevention 20 (2015): 12-24.
4. Boberg J, Taxvig C, Christiansen S, Hass U. Possible endocrine disrupting effects of parabens and their metabolites. Reproductive Toxicology 30 (2010): 301-312.
5. Noonan GO, Ackerman LK, Begley TH. Concentration of Bisphenol A in Highly Consumed Canned Foods on the U.S. Market. Journal of Agricultural and Food Chemistry 59 (2011): 7178-7185.
6. Akash MSH, Sabir S, Rehman K. Bisphenol A-induced metabolic disorders: From exposure to mechanism of action. Environmental Toxicology and Pharmacology 77 (2020): 103373.
7. Chen Y, Zhang X, An Y, Liu B, Lu M. LncRNA HCP5 promotes cell proliferation and inhibits apoptosis via miR-27a-3p/IGF-1 axis in human granulosa-like tumor cell line KGN. Molecular and Cellular Endocrinology 503 (2020): 110697.
8. Yang D, Li N, Ma A, Dai F, Zheng Y, Hu X, Wang Y, Xian S, Zhang L, Yuan M et al. Identification of Potential Biomarkers of Polycystic Ovary Syndrome via Integrated Bioinformatics Analysis. Reproductive Sciences (2020).
9. Liu H-Y, Huang Y-L, Liu J-Q, Huang Q. Transcription factor-microRNA synergistic regulatory network revealing the mechanism

of polycystic ovary syndrome. Molecular Medicine Reports 13 (2016): 3920-3928.

10. Liu H-Y, Liu J-Q, Mai Z-X, Zeng Y-T. A Subpathway-Based Method of Drug Reposition for Polycystic Ovary Syndrome. Reproductive Sciences 22 (2014): 423-430.
11. Bi X, Zhai Z, Wang S. Identification of the key pathways and genes related to polycystic ovary syndrome using bioinformatics analysis. General physiology and biophysics 38 (2019): 205-2014.
12. Wang W, Ji J, Li J, Ren Q, Gu J, Zhao Y, Hong D, Guo Q, Tan Y. Several critical genes and microRNAs associated with the development of polycystic ovary syndrome. Annales d'Endocrinologie 81 (2020): 18-27.
13. R. Edgar MD, and A. E. Lash. Gene expression omnibus: NCBI gene expression and hybridization array data repository. Nucleic Acids Research 30 (2002): 207-210.
14. Amaral ML, Erikson GA, Shokhirev MN. BART: bioinformatics array research tool. BMC Bioinformatics 19 (2018): 296.
15. Dong J-p, Dai Z-h, Jiang Z-x, He Y, Wang L, Liao Q-y, Sun N-x, et al. CD24: a marker of granulosa cell subpopulation and a mediator of ovulation. Cell Death & Disease 10 (2019).
16. Bencomo E, Pérez R, Arteaga M-F, Acosta E, Peña O, Lopez L. Apoptosis of cultured granulosa-lutein cells is reduced by insulin-like growth factor I and may correlate with embryo fragmentation and pregnancy rate. Fertility and Sterility 85 (2006): 474-480.
17. Gebhardt KM, Feil DK, Dunning KR, Lane M, Russell DL: Human cumulus cell gene expression as a biomarker of pregnancy outcome after single embryo transfer. Fertility and Sterility 96 (2011): 47-52, e42.
18. Dumesic D, Abbott D. Implications of Polycystic Ovary Syndrome on Oocyte Development. Seminars in Reproductive Medicine 26 (2008): 053-061.
19. Dewailly D, Robin G, Peigne M, Decanter C, Pigny P, Catteau-Jonard S. Interactions between androgens, FSH, anti-Müllerian hormone and estradiol during folliculogenesis in the human normal and polycystic ovary. Human Reproduction Update 22 (2016): 709-724.
20. Blasco V, Pinto FM, Fernández-Atucha A, Prados N, Tena-Sempere M. Altered expression of the kisspeptin/KISS1R and neurokinin B/NK3R systems in mural granulosa and cumulus cells of patients with polycystic ovarian syndrome. Journal of Assisted Reproduction and Genetics 36 (2018): 113-120.
21. N A Cataldo 1 DAD, P C Goldsmith, R B Jaffe. Immunolocalization of Fas and Fas ligand in the ovaries of women with polycystic ovary syndrome: relationship to apoptosis. Human Reproduction 15 (2000): 1889-1897.
22. Amir Tirosh, Ediz S. Calay , Gurol Tuncman , Kathryn C. Claiborn, Karen E. Inouye, et al. The short-chain fatty acid propionate increases glucagon and FABP4 production, impairing insulin action in mice and humans. SCIENCE TRANSLATIONAL MEDICINE 11 (2019).
23. Brenjian S, Moini A, Yamini N, Kashani L, Faridmojtahedi M, Bahramrezaie M, Khodarahmian M, Amidi F. Resveratrol treatment in patients with polycystic ovary

syndrome decreased pro-inflammatory and endoplasmic reticulum stress markers. American Journal of Reproductive Immunology 83 (2019).

24. Wang BJ, Qian L, Li J, Wang F, Yang QL, Li G, Liang YL, Guo YH. sRAGE plays a role as a protective factor in the development of PCOS by inhibiting inflammation. Gynecological Endocrinology 36 (2019): 148-151.
25. Patricia DA Lima A-LN, Qi Wang, Yi-An Chen, Arthur Leader, Annie Cheung, et al. Polycystic ovary syndrome: possible involvement of androgen-induced, chemerin-mediated ovarian recruitment of monocytes/macrophages. Biology of reproduction 99 (2018): 838-852.
26. Nerurkar PV, Jang M, Lee MJ, Lee JM, Bae C-S, Kim S-H, Ryu JH, Cho I-H. Oriental Medicine Kyung-Ok-Ko Prevents and Alleviates Dehydroepiandrosterone-Induced Polycystic Ovarian Syndrome in Rats. PLoS ONE 9 (2014): e87623.
27. Willis GR, Connolly K, Ladell K, Davies TS, Guschina IA, Ramji D, et al. Young women with polycystic ovary syndrome have raised levels of circulating annexin V-positive platelet microparticles. Human Reproduction 29 (2014): 2756-2763.
28. González F, Considine RV, Abdelhadi OA, Acton AJ. Saturated Fat Ingestion Promotes Lipopolysaccharide-Mediated Inflammation and Insulin Resistance in Polycystic Ovary Syndrome. The Journal of Clinical Endocrinology & Metabolism 104 (2019): 934-946.
29. Fenelon JC, Shaw G, O'Neill C, Frankenberg S, Renfree MB. Paf receptor expression in the marsupial embryo and endometrium during embryonic diapause. Reproduction 147 (2014): 21-31.
30. Lindholm A, Blomquist C, Bixo M, Dahlbom I, Hansson T, Sundstrom Poromaa I, Buren J. No difference in markers of adipose tissue inflammation between overweight women with polycystic ovary syndrome and weight-matched controls. Human Reproduction 26 (2011): 1478-1485.
31. STAVROULA BAKA KZ, EVANGELIA KOUSKOUNI1, EVANGELOS MAKRAKIS, STELLA DEMERIDOU DT, DIMITRIS HASSIAKOS and GEORGE CREATSAS. Matrix Metalloproteinases 2 and 9 and their Tissue Inhibitors in the Follicular Fluid of Patients with Polycystic Ovaries Undergoing In Vitro Fertilisation. IIN Vivo 24 (2010): 293-296.