Original Article



# Exploring the molecular targets and mechanism of *S. miltiorrhiza-C. aromatica* in treating polycystic ovary syndrome based on network pharmacology

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**Background:** *S. miltiorrhiza-C. aromatica* (Danshen-Yujin; red sage and turmeric) is a frequently used Chinese herbal medicine pair in treating polycystic ovary syndrome (PCOS). This study aimed to classify the molecular targets and mechanisms participating in treating PCOS through network pharmacology.

**Methods:** The Traditional Chinese Medicine Systems Pharmacology (TCMSP) platform was employed for screening the active ingredients of *S. miltiorrhiza-C. aromatica*. The molecular targets from the UniProt database were identified and compared to the differentially expressed genes (DEGs) in the Gene Expression Omnibus (GEO) dataset GSE34526; the intersecting genes were obtained by constructing a Venn diagram. Protein-protein interaction (PPI) network construction and Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) enrichment analyses were made on the crossover genes. A key protein 3-dimensional (3D) structure was created using the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCDB PDB) database. Finally, the clinical data of 104 hospital-admitted PCOS patients from January 2018 to December 2020 were retrospectively analyzed to explore and analyze the clinical value of *S. miltiorrhiza-C. aromatica* in treating PCOS.

**Results:** In the TCMSP database, we found a total of 80 active ingredients in *S. miltiorrhiza-C.* A high clustering and three key proteins were obtained A high-scoring cluster and 3 key proteins, AOAH, HCK, and C1orf162, were obtained through the construction of protein mutual aid network and module analysis of differential genes. KEGG and GO enrichment analyses indicated that the *S. miltiorrhiza-C. aromatica* treatment mechanism in PCOS was mainly involved with inflammation-related pathways. The clinical data of PCOS patients were retrospectively analyzed. In the end, The long diameter of the ovary, the thickness of the endometrium, and the antral follicle count in the combined treatment group of *S. miltiorrhiza-C. aromatica* combined with clomiphene were higher after treatment than before treatment, and the clinical symptoms and hormone levels were also improved.

**Conclusions:** This study expounds the research value of *S. miltiorrhiza-C. aromatica* in treating PCOS from the perspectives of active ingredients, targets, signaling pathways, and clinical research. These findings also provide an important reference for treating PCOS with traditional Chinese medicine (TCM).

**Keywords:** Polycystic ovary syndrome (PCOS); network pharmacology; traditional Chinese medicine (TCM); bioinformatics; gene expression profile

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## Introduction

Polycystic ovary syndrome (PCOS) is a metabolic and endocrine disease with an incidence of 6–20% among reproductive-aged women (1), which is frequently found to cause infertility (2). It is usually manifested by obesity, hirsutism, oligoovulation or anovulation, and polycystic changes in the ovaries, often coexisting with insulin resistance and dyslipidemia, with significant risk of the development of cardiovascular disease or metabolic sequelae (e.g., diabetes mellitus, and metabolic syndrome) (3).

There is no special article of PCOS in traditional Chinese medicine. According to its symptoms, it can be seen in the description of "amenorrhea", "metrorrhagia", "irregular menstruation", "infertility" and other diseases. Traditional Chinese medicine believes that the female physiological rhythm is controlled by the kidney, which plays a leading role; At the same time, "women are born with the liver". The liver stores blood. Women's menstruation, pregnancy and childbirth all use blood. And the liver and kidney belong to the lower energizer, the kidney essence and liver blood complement each other, and the spring is inexhaustible. The liver is in charge of catharsis, the kidney is in charge of sealing up, and one is in charge of catharsis, so that the uterus has a period of catharsis, and menstruation comes on time. For PCOS patients, kidney deficiency is the basic pathogenesis, and liver depression is the important pathogenesis. Deficiency of kidney essence, lack of energy in days, stagnation of liver qi, and disorder of catharsis will result in obstruction of qi and blood circulation, deficiency of Chong and Ren cells, loss of nourishment, irregular menstruation and even amenorrhea. Despite the clinical prevalence of the disease, the etiology and pathophysiology of PCOS remain unclear. Strong epigenetic and environmental factors have been linked to PCOS, suggesting that it may be a complex polygenic condition (4). At present, the treatment mainly focuses on oral medication, surgery, and lifestyle adjustment to adjust the menstrual cycle, reducing androgen levels, ovulation induction, and preventing complications, which has achieved certain results (5). However, due to certain limitations and adverse reactions of western medicine treatment, traditional Chinese medicine (TCM) and integration of both medical practices have gradually become the choice of more and more PCOS patients. Therefore, exploring the target and mechanism of action related to the disease has important clinical significance for improving and treating PCOS.

Network pharmacology is based on multidisciplinary theories such as systems biology, proteomics, genomics, and pharmacology, and uses technologies such as network database salvage and high-throughput omics data analysis to construct the molecular biology network of disease drugs, predict the pathogenesis of diseases, and find drug targets and molecular mechanisms for treating diseases (6). Using network pharmacology to elucidate the TCM mechanism of action in PCOS treatment has been a hot topic in recent years.

TCM treatment of PCOS comprises tonifying the kidneys, relieving stagnation, and regulating energy, and this method has achieved good results (7). *S. miltiorrhiza-C. aromatica* (Danshen-Yujin; red sage-turmeric) is the core drug pair that is used to soothe the liver, relieve stagnation, remove blood stasis, and promote blood circulation (8). *S. miltiorrhiza* is slightly cold in nature, bitter in taste, and boosts blood circulation and promotes menstrual flow, relieving depression and restlessness (9). *C. aromatica* has a bitter and pungent smell, is cold in nature, and into the blood qi. It removes blood stasis, promotes blood circulation, relieves stagnation, and clears the heart. *S. miltiorrhiza-C. aromatica* has various active ingredients, and network pharmacology can be applied to screen out the core compounds of the drug, and then predict the target of the drug acting on the disease and the pathway involved. This study analyzed the core components of *S. miltiorrhiza-C. aromatica*, its targets in PCOS, and related pathways,

### Highlight box

**Key findings**

- The role of *S. miltiorrhiza-C. aromatica* in the treatment of polycystic ovary syndrome (PCOS) was discussed from the aspects of active ingredients, target, signaling pathway, and clinical studies, which provided an important reference for the treatment of PCOS by traditional Chinese medicine (TCM).

**What is known and what is new?**

- *S. miltiorrhiza-C. aromatica* is the core drug pair that plays the role of soothing the liver, relieving stagnation, removing blood stasis, and promoting blood circulation.
- This study aimed to explore the mechanism of its action on PCOS by analyzing the core components of Danshen-Yujin, the target of its action on PCOS, and related pathways.

**What is the implication, and what should change now?**

- The data of the study have shown the research value of *S. miltiorrhiza-C. aromatica* in treating PCOS from multiple levels and perspectives.

to explore its mechanism of intervention and treatment of PCOS, and to help guide future studies and clinical applications. We present the following article in accordance with the STREGA reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-23-29/rc).

## Methods

### *Active ingredients and target genes screening*

Using the Traditional Chinese Medicine Systems Pharmacology database (TCMSP; http://lsp.nwu.edu.cn/tcmsp.php) to screen the active ingredients of *S. miltiorrhiza-C. aromatica* pharmaceuticals, including *S. miltiorrhiza and C. aromatica*, which have similar drug properties with oral bioavailability (OB) ≥30%, and drug-like properties (DLP) ≥0.18. Predicted compounds targets were screened from the DrugBank (https//www.drugbank.ca/) database. Additionally, target information was compared from the UniProt database (https://www.uniprot.org/).

### *Acquisition of differentially expressed genes*

PCOS patients and healthy individuals' genetic samples were retrieved from the Gene Expression Omnibus (GEO) database dataset GSE34526. According to the conditions of P<0.005 and absolute value of log2FC >1, statistically significant differentially expressed genes (DEGS) were screened and a volcano map was constructed. Next, we selected the top 20 most significantly upregulated and downregulated genes to draw a heatmap.

### *Protein-protein interaction network analysis of common DEGs*

We used the online database Search Tool for the Retrieval of Interacting Genes/Proteins (STRING; https://string-db.org/) to analyze the protein-protein interaction (PPI) network of common DEGs, and imported the results into Cytoscape software (https://cytoscape.org/) for visualization and correlation analysis. The molecular complex detection (MCODE) plug-in was used to screen out key protein expression molecules.

### *Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) enrichment analysis*

In the Encyclopedia of Traditional Chines Medicine (ETCM) database (http://www.tcmip.cn/ETCM/index.php/Home/), the functional enrichment analysis (FEA) and signaling pathway prediction analysis of the candidate target genes of *S. miltiorrhiza and C. aromatica* were performed, respectively. Then, Metascape (https://metascape.org/gp/index.html#/main/step1) was used to conduct functional analysis online again, and the differential genes were added to Metascape for FEA common to the 2 TCMs.

### *Selection of clinical data*

The clinical data of 104 hospital-admitted PCOS patients at the Second People's Hospital of Wuhu from January 2018 to December 2020 were selected. The inclusion criteria were as follows: (I) Meet the PCOS-related criteria established in the 2003 Dutch PCOS Conference (10), including at least 2 of the following: oligomenorrhoea or amenorrhea, clinical or chemical testosterone >0.75 ng/mL, hyperandrogenism and/or polycystic ovary morphology (PCOM) by ultrasound examination; (II) The age range from postmenarche to within 40 years old; (III) The reproductive function of the spouse is good, and the semen quality is normal; (IV) Hysterosalpingography (HSG)/laparoscopy shows at least 1 fallopian tube (V) Did not receive other treatment 3 months before enrollment; (VI) Have complete clinical data and follow-up data, and at the same time give informed consent to the study. The exclusion criteria were as follows: (I) Those who do not meet the diagnostic criteria; (II) Gonadal hypoplasia, abnormal menstruation caused by reproductive tract abnormalities, organic lesions of the reproductive organs, and irregular menstrual cycles; (III) Those who have taken sex hormones within the 3 months prior to this study; (IV) Those with diabetes, liver and kidney disease, abnormal thyroid function, hyperprolactinemia, and other diseases. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by institutional of the Second People's Hospital of Wuhu (No. SZ2018001) and informed consent was taken from all the patients.

### *Treatment methods and observation indicators of enrolled patients*

Among the finally enrolled patients, 52 cases received the treatment of *S. miltiorrhiza-C. aromatica* combined with clomiphene, and 52 cases received clomiphene alone. *S. miltiorrhiza-C. aromatica* treatment involved 1 dose/d; participants started taking it on the 5th day of menstruation, took continuously for 3 menstrual cycles until menstruation commenced, and stopped the medicine during menstruation. For amenorrhea or progesterone withdrawal bleeding, patients resumed the TCM on the 5th day of menstruation. The type of clomiphene was H31021107, produced by Shanghai Hengshan Pharmaceutical Co., Ltd. (Shanghai, China), with a specification of 50 mg. Clomiphene was taken on the fifth day of progesterone withdrawal bleeding or menstruation, and the dose was maintained at 50 mg/d continuously for 3 menstrual cycles (by specialist physician guidance). The observation indicators included the following: (I) ovarian diameter, endometrial thickness, and antral follicle number observed by color Doppler ultrasound; (II) comparison of clinical symptoms including evaluation of hirsutism and acne by Ferriman Gallwey method and acne score; (III) comparison of sex hormone levels.

### *Statistical analysis*

Statistical analyses in the bioinformatics section were automatically calculated using the aforementioned online databases. The clinical data were subjected to $\chi^2$ test to compare and analyze the clinicopathological conditions, and the measurement data were analyzed with t test and multiple hypothesis testing. A P value <0.05 or a log rank P<0.05 was considered statistically significant.

## Results

### *Screening of active ingredients and target genes*

In the TCMSP database, with the aforementioned screening conditions, we found that there are 80 active ingredients in *S. miltiorrhiza-C. aromatica* medicine, of which 65 are in *S. miltiorrhiza*, and there are 15 species (*Table 1*) (11).

### *DEGs screening*

By comparing 3 normal and 7 disease samples in the GSE34526 dataset, a total of 21,655 DEGs were obtained (10,121 upregulated and 11,534 downregulated genes). The conditions for screening statistically significant DEGs were P<0.005 and absolute value of log2FC >1, which yielded 1,148 upregulated and 544 downregulated genes. The DEGs in the disease samples followed a normal distribution, with more substantially upregulated genes than downregulated genes, as indicated in the gene volcano plot (*Figure 1*). *Figure 2* and *Table 2* show the top 20 most upregulated and downregulated genes.

### *Protein mutual aid network construction and module analysis of common DEGs and core gene screening*

We intersected the DEGs in the GSE34526 dataset and all the protein-gene information in the UniProt database through a Venn diagram, and finally obtained 75 crossover genes (*Figure 3A*). Then we imported these 75 genes into Cytoscape software to build a PPI network diagram (*Figure 3B*). At the same time, we used the Network Analyzer tool in Cytoscape to perform non-directional score calculations on each node in the PPI network to obtain the value of degree of each node. The details of the top 20 genes sorted by Degree in Cytoscape software are shown in *Table 3*. Next, the degree's value was represented by the node's size. The node's color was from red to green to represent the neighborhood connectivity of each node from high to low. The edge thickness represented the combined_ score value of the edge, and the Attribute Circle Layout was used to arrange all the protein nodes. The MCODE plug-in were used to perform cluster association analysis on these important protein molecules with the default parameters of node score cutoff as 0.2, K-core as 2, and Max.Depth as 100, which yielded a cluster with a higher score (*Figure 3C*). Finally, 3 key proteins, AOAH, HCK, and C1orf162, were obtained. According to the size of the absolute value of logFC, the information of the top 20 genes of the cross-gene species is shown in *Table 4*.

### *GO functions and KEGG enrichment analysis of related targets*

We first performed FEA on the candidate target genes of *S. miltiorrhiza and C. aromatica* in the ETCM database, and we found that the top 20 gene functions involved in the GO analysis of *S. miltiorrhiza* include excitatory chemical synaptic transmission, intracellular receptor signaling, calcium ion transmembrane import into the cytosol, transcriptional initiation of RNA polymerase II promoter,

**Table 1** The total effective compounds and corresponding candidate target genes of S. miltiorrhiza-C. aromatica in the ETCM database

| Drug | ID | Compound | OB (%) | DL |
|---|---|---|---|---|
| Radix Salviae | MOL001601 | 1,2,5,6-tetrahydrotanshinone | 38.75 | 0.36 |
| Radix Salviae | MOL001659 | Poriferasterol | 43.83 | 0.76 |
| Radix Salviae | MOL001771 | poriferast-5-en-3beta-ol | 36.91 | 0.75 |
| Radix Salviae | MOL001942 | isoimperatorin | 45.46 | 0.23 |
| Radix Salviae | MOL002222 | sugiol | 36.11 | 0.28 |
| Radix Salviae | MOL002651 | Dehydrotanshinone II A | 43.76 | 0.4 |
| Radix Salviae | MOL002776 | Baicalin | 40.12 | 0.75 |
| Radix Salviae | MOL000569 | digallate | 61.85 | 0.26 |
| Radix Salviae | MOL000006 | luteolin | 36.16 | 0.25 |
| Radix Salviae | MOL006824 | α-amyrin | 39.51 | 0.76 |
| Radix Salviae | MOL007036 | 5,6-dihydroxy-7-isopropyl-1,1-dimethyl-2,3-dihydrophenanthren-4-one | 33.77 | 0.29 |
| Radix Salviae | MOL007041 | 2-isopropyl-8-methylphenanthrene-3,4-dione | 40.86 | 0.23 |
| Radix Salviae | MOL007045 | 3α-hydroxytanshinoneIIa | 44.93 | 0.44 |
| Radix Salviae | MOL007048 | (E)-3-(2-(3,4-dihydroxyphenyl)-7-hydroxy-benzofuran-4-yl)acrylic acid | 48.24 | 0.31 |
| Radix Salviae | MOL007049 | 4-methylenemiltirone | 34.35 | 0.23 |
| Radix Salviae | MOL007050 | 2-(4-hydroxy-3-methoxyphenyl)-5-(3-hydroxypropyl)-7-methoxy-3-benzofurancarboxaldehyde | 62.78 | 0.4 |
| Radix Salviae | MOL007051 | 6-o-syringyl-8-o-acetyl shanzhiside methyl ester | 46.69 | 0.71 |
| Radix Salviae | MOL007058 | formyltanshinone | 73.44 | 0.42 |
| Radix Salviae | MOL007059 | 3-beta-Hydroxymethyllenetanshiquinone | 32.16 | 0.41 |
| Radix Salviae | MOL007061 | Methylenetanshinquinone | 37.07 | 0.36 |
| Radix Salviae | MOL007063 | przewalskin a | 37.11 | 0.65 |
| Radix Salviae | MOL007064 | przewalskin b | 110.32 | 0.44 |
| Radix Salviae | MOL007068 | Przewaquinone B | 62.24 | 0.41 |
| Radix Salviae | MOL007069 | przewaquinone c | 55.74 | 0.4 |
| Radix Salviae | MOL007070 | (6S,7R)-6,7-dihydroxy-1,6-dimethyl-8,9-dihydro-7H-naphtho(8,7-g)benzofuran-10,11-dione | 41.31 | 0.45 |
| Radix Salviae | MOL007071 | przewaquinone f | 40.31 | 0.46 |
| Radix Salviae | MOL007077 | sclareol | 43.67 | 0.21 |
| Radix Salviae | MOL007079 | tanshinaldehyde | 52.47 | 0.45 |
| Radix Salviae | MOL007081 | S. miltiorrhizaol B | 57.95 | 0.56 |
| Radix Salviae | MOL007082 | S. miltiorrhizaol A | 56.97 | 0.52 |
| Radix Salviae | MOL007085 | Salvilenone | 30.38 | 0.38 |
| Radix Salviae | MOL007088 | cryptotanshinone | 52.34 | 0.4 |
| Radix Salviae | MOL007093 | dan-shexinkum d | 38.88 | 0.55 |
| Radix Salviae | MOL007094 | S. miltiorrhizaspiroketallactone | 50.43 | 0.31 |

**Table 1** (*continued*)

**Table 1** (*continued*)

| Drug | ID | Compound | OB (%) | DL |
|---|---|---|---|---|
| Radix Salviae | MOL007098 | deoxyneocryptotanshinone | 49.4 | 0.29 |
| Radix Salviae | MOL007100 | dihydrotanshinlactone | 38.68 | 0.32 |
| Radix Salviae | MOL007101 | dihydrotanshinoneI | 45.04 | 0.36 |
| Radix Salviae | MOL007105 | epiS. miltiorrhizaspiroketallactone | 68.27 | 0.31 |
| Radix Salviae | MOL007107 | C09092 | 36.07 | 0.25 |
| Radix Salviae | MOL007108 | isocryptotanshi-none | 54.98 | 0.39 |
| Radix Salviae | MOL007111 | Isotanshinone II | 49.92 | 0.4 |
| Radix Salviae | MOL007115 | manool | 45.04 | 0.2 |
| Radix Salviae | MOL007118 | microstegiol | 39.61 | 0.28 |
| Radix Salviae | MOL007119 | miltionone II | 49.68 | 0.32 |
| Radix Salviae | MOL007120 | miltionone II | 71.03 | 0.44 |
| Radix Salviae | MOL007121 | miltipolone | 36.56 | 0.37 |
| Radix Salviae | MOL007122 | Miltirone | 38.76 | 0.25 |
| Radix Salviae | MOL007123 | miltirone II | 44.95 | 0.24 |
| Radix Salviae | MOL007124 | neocryptotanshinone ii | 39.46 | 0.23 |
| Radix Salviae | MOL007125 | neocryptotanshinone | 52.49 | 0.32 |
| Radix Salviae | MOL007127 | 1-methyl-8,9-dihydro-7H-naphtho(5,6-g)benzofuran-6,10,11-trione | 34.72 | 0.37 |
| Radix Salviae | MOL007130 | prolithospermic acid | 64.37 | 0.31 |
| Radix Salviae | MOL007132 | (2R)-3-(3,4-dihydroxyphenyl)-2-((Z)-3-(3,4-dihydroxyphenyl)acryloyl)oxy-propionic acid | 109.38 | 0.35 |
| Radix Salviae | MOL007140 | (Z)-3-(2-((E)-2-(3,4-dihydroxyphenyl)vinyl)-3,4-dihydroxy-phenyl)acrylic acid | 88.54 | 0.26 |
| Radix Salviae | MOL007141 | salvianolic acid g | 45.56 | 0.61 |
| Radix Salviae | MOL007142 | salvianolic acid j | 43.38 | 0.72 |
| Radix Salviae | MOL007143 | salvilenone I | 32.43 | 0.23 |
| Radix Salviae | MOL007145 | salviolone | 31.72 | 0.24 |
| Radix Salviae | MOL007149 | NSC 122421 | 34.49 | 0.28 |
| Radix Salviae | MOL007150 | (6S)-6-hydroxy-1-methyl-6-methylol-8,9-dihydro-7H-naphtho(8,7-g)benzofuran-10,11-quinone | 75.39 | 0.46 |
| Radix Salviae | MOL007151 | Tanshindiol B | 42.67 | 0.45 |
| Radix Salviae | MOL007152 | Przewaquinone E | 42.85 | 0.45 |
| Radix Salviae | MOL007154 | tanshinone iia | 49.89 | 0.4 |
| Radix Salviae | MOL007155 | (6S)-6-(hydroxymethyl)-1,6-dimethyl-8,9-dihydro-7H-naphtho(8,7-g)benzofuran-10,11-dione | 65.26 | 0.45 |
| Curcumae Radix | MOL000358 | beta-sitosterol | 36.91 | 0.75 |
| Curcumae Radix | MOL000359 | sitosterol | 36.91 | 0.75 |
| Curcumae Radix | MOL004241 | curcolactone | 51.51 | 0.2 |

**Table 1** (*continued*)

**Table 1** (*continued*)

| Drug | ID | Compound | OB (%) | DL |
|---|---|---|---|---|
| Curcumae Radix | MOL004244 | (4aR,5R,8R,8aR)-5,8-dihydroxy-3,5,8a-trimethyl-6,7,8,9-tetrahydro-4aH-benzo(f) benzofuran-4-one | 59.52 | 0.2 |
| Curcumae Radix | MOL004253 | Curcumenolactone C | 39.7 | 0.19 |
| Curcumae Radix | MOL004260 | (E)-1,7-Diphenyl-3-hydroxy-1-hepten-5-one | 64.66 | 0.18 |
| Curcumae Radix | MOL004263 | (E)-5-Hydroxy-7-(4-hydroxyphenyl)-1-phenyl-1-heptene | 46.9 | 0.19 |
| Curcumae Radix | MOL004291 | Oxycurcumenol | 67.06 | 0.18 |
| Curcumae Radix | MOL004305 | Zedoalactone A | 111.43 | 0.19 |
| Curcumae Radix | MOL004306 | Zedoalactone B | 103.59 | 0.22 |
| Curcumae Radix | MOL004309 | zedoalactone E | 85.16 | 0.19 |
| Curcumae Radix | MOL004311 | Zedoarolide A | 87.97 | 0.3 |
| Curcumae Radix | MOL004313 | Zedoarolide B | 135.56 | 0.21 |
| Curcumae Radix | MOL004316 | 1,7-Diphenyl-3-acetoxy-6(E)-hepten | 48.47 | 0.22 |
| Curcumae Radix | MOL004328 | naringenin | 59.29 | 0.21 |

ETCM, Encyclopedia of Traditional Chinese Medicine; OB, oral bioavailability; DL, drug-likeness.

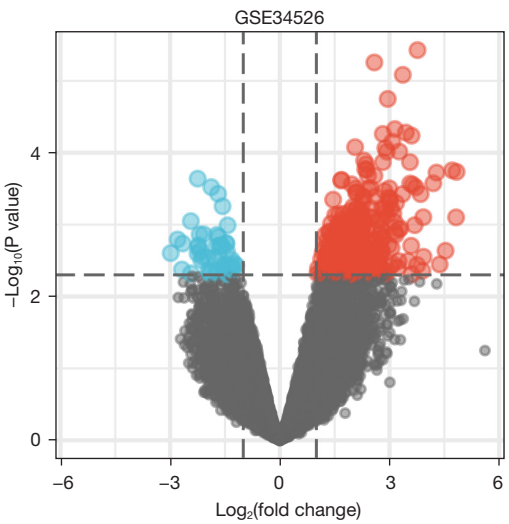


**Figure 1** Gene volcano map showing the distribution of genes in a disease sample. Red and blue represent up-regulated genes (logFC >1) and down-regulated genes (logFC <−1), respectively, while black represents no significant difference. FC, fold change.

steroid hormone-mediated signaling pathways, and so on (*Figure 4A*). In *C. aromatica's* GO analysis, the functions of the top 20 genes involved include regulation of lipid metabolism, positive regulation of transcriptional DNA templates, ion transmembrane transport, transcriptional initiation of RNA polymerase II promoters, steroid hormone-mediated signaling pathway, and intracellular receptor signaling pathway, and so on (*Figure 4B*). Next, we used Metascape to analyze and verify the functions of *S. miltiorrhiza* and *C. aromatica* online. The GO FEA explained gene functions from three levels: biological process (BP), cellular component (CC), and molecular function (MF). BP was mainly involved in the positive regulation of leukocyte migration, vasoconstriction, vasoconstriction regulation, and T cell-mediated cytotoxicity; CC was mainly related to the outer side of the plasma membrane, membrane domains, membrane microdomains and membrane rafts; MF was mainly related to actin binding, scaffold protein binding, lipopolysaccharide binding, and lipopeptide binding were related (*Figure 4C*), the details of which are shown in *Table 5*.

Next, we analyzed the potential signaling pathways of the candidate target genes of *S. miltiorrhiza* and *C. aromatica* in the ETCM database, and found that the top 20 possible pathways of *S. miltiorrhiza* involved the unblocking of NMDA receptor-glutamate binding and activation, synthesis of bile acids and bile salts from 7α-hydroxycholesterol, reversible hydration of carbon dioxide, removal of amino-terminal propeptides from gamma-carboxylated proteins, gamma carboxylation of protein precursors, neurotransmitter receptors, and postsynaptic split transmission (*Figure 5A*). The top

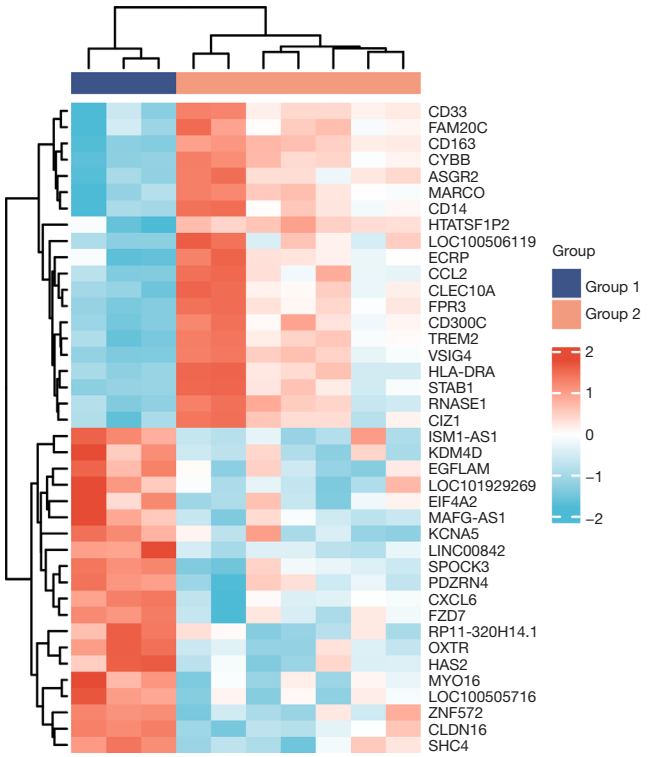


**Figure 2** Heat maps of the top 20 up-regulated and down-regulated differentially expressed genes. Red and blue represent up-regulated genes (logFC >1) and down-regulated genes (logFC <−1), respectively. FC, fold change.

**Table 2** The first 20 genes up-regulated and down-regulated

| Gene names | LogFC | P value | Regulation direction |
|---|---|---|---|
| MARCO | 4.834784125 | 0.000184655 | Up |
| CLEC10A | 4.821592459 | 0.000792618 | Up |
| TREM2 | 4.715156105 | 0.000175671 | Up |
| CCL2 | 4.532879131 | 0.002311245 | Up |
| RNASE1 | 4.371944656 | 0.003573333 | Up |
| VSIG4 | 4.28656872 | 0.000188864 | Up |
| CD33 | 4.198637982 | 0.000266157 | Up |
| CIZ1 | 3.930772716 | 0.002803201 | Up |
| CD14 | 3.924331622 | 0.000788682 | Up |

**Table 2** (*continued*)

**Table 2** (*continued*)

| Gene names | LogFC | P value | Regulation direction |
|---|---|---|---|
| ECRP | 3.899369465 | 0.004391934 | Up |
| HTATSF1P2 | 3.850684528 | 0.000375641 | Up |
| CD163 | 3.765991534 | 3.73671E-06 | Up |
| LOC100506119 | 3.757053362 | 0.003647718 | Up |
| CD300C | 3.720541152 | 0.000304087 | Up |
| FAM20C | 3.688800674 | 0.001017828 | Up |
| ASGR2 | 3.637079826 | 0.000279532 | Up |
| HLA-DRA | 3.60769455 | 0.004903838 | Up |
| CYBB | 3.602766861 | 5.7816E-05 | Up |
| STAB1 | 3.593157741 | 0.001968839 | Up |
| FPR3 | 3.566035274 | 0.000268063 | Up |
| EGFLAM | −2.984774836 | 0.002492964 | Down |
| CXCL6 | −2.796202653 | 0.001606384 | Down |
| KCNA5 | −2.677832819 | 0.004208824 | Down |
| ISM1-AS1 | −2.65578797 | 0.001830545 | Down |
| CLDN16 | −2.434348245 | 0.000894117 | Down |
| EIF4A2 | −2.431484168 | 0.004771586 | Down |
| SHC4 | −2.252093946 | 0.002517925 | Down |
| OXTR | −2.246951667 | 0.000229464 | Down |
| MAFG-AS1 | −2.2364217 | 0.002054656 | Down |
| MYO16 | −2.224922974 | 0.001936074 | Down |
| HAS2 | −2.194831415 | 0.001369181 | Down |
| RP11-320H14.1 | −2.184028108 | 0.002319949 | Down |
| KDM4D | −2.149884678 | 0.003108282 | Down |
| LOC100505716 | −2.105996852 | 0.002827683 | Down |
| SPOCK3 | −2.082157252 | 0.001362968 | Down |
| FZD7 | −1.972033027 | 0.00289799 | Down |
| ZNF572 | −1.94514354 | 0.004326402 | Down |
| PDZRN4 | −1.906035353 | 0.004712338 | Down |
| LOC101929269 | −1.894759116 | 0.004761204 | Down |
| LINC00842 | −1.875483606 | 0.000300999 | Down |

FC, fold change.

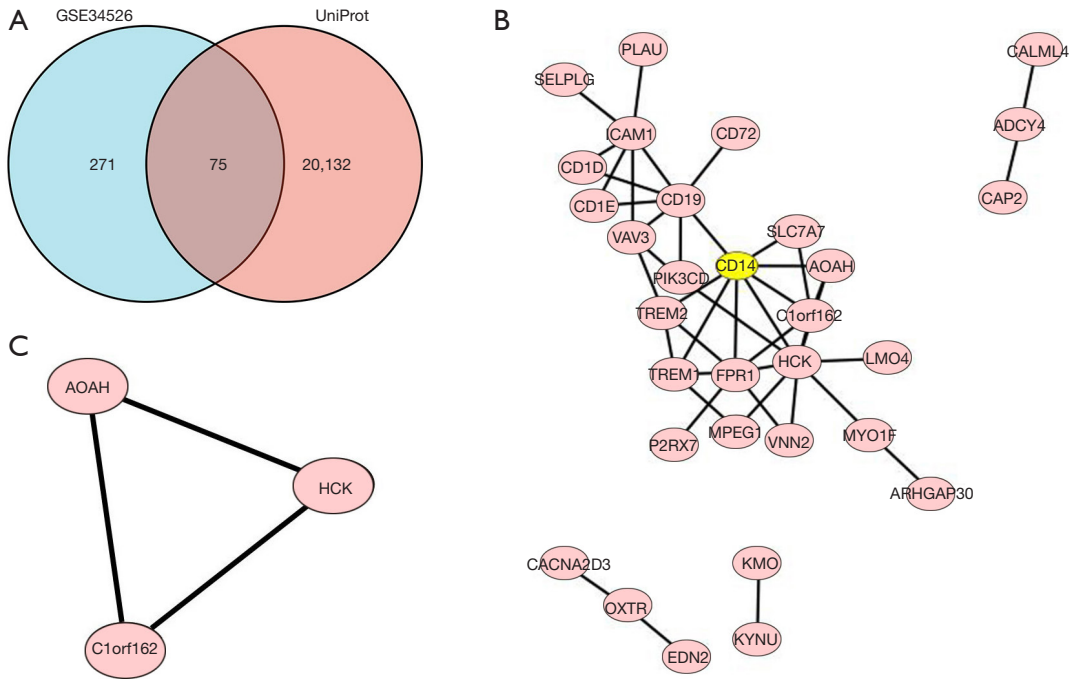


**Figure 3** PPI network and Venn diagram of candidate genes. (A) Candidate gene Venn diagram; (B) protein interaction network diagram; (C) Cluster with higher score. PPI, protein-protein interaction.

**Table 3** Detailed information of the top 20 genes sorted by Degree in Cytoscape software

| Gene | Betweenness | BottleNeck | Closeness | Clustering coefficient | Degree | DMNC | MCC | MNC | Radiality |
|---|---|---|---|---|---|---|---|---|---|
| *HCK* | 171.23333 | 12 | 14.33333 | 0.16667 | 9 | 0.38896 | 18 | 5 | 3.97947 |
| *CD14* | 151.6 | 9 | 14.5 | 0.32143 | 8 | 0.32929 | 21 | 7 | 4.11437 |
| *CD19* | 168.1 | 8 | 13.41667 | 0.19048 | 7 | 0.25931 | 10 | 5 | 3.94575 |
| *FPR1* | 64.63333 | 4 | 13.16667 | 0.33333 | 7 | 0.33284 | 15 | 6 | 3.8783 |
| *ICAM1* | 86.33333 | 3 | 11.45 | 0.2 | 6 | 0.2842 | 8 | 4 | 3.50733 |
| *C1orf162* | 10.66667 | 2 | 12 | 0.6 | 5 | 0.38896 | 14 | 5 | 3.77713 |
| *VAV3* | 37.86667 | 5 | 11.41667 | 0.33333 | 4 | 0.30898 | 5 | 3 | 3.7434 |
| *TREM2* | 23.46667 | 1 | 11.58333 | 0.5 | 4 | 0.46346 | 7 | 3 | 3.77713 |
| *TREM1* | 11.73333 | 2 | 11.08333 | 0.5 | 4 | 0.46346 | 7 | 3 | 3.64223 |
| *PIK3CD* | 53.3 | 13 | 11.5 | 0.33333 | 3 | 0.30779 | 3 | 2 | 3.84457 |
| *AOAH* | 0 | 1 | 10.83333 | 1 | 3 | 0.46346 | 6 | 3 | 3.67595 |
| *MPEG1* | 3.06667 | 1 | 9.56667 | 0 | 2 | 0 | 2 | 1 | 3.37243 |
| *VNN2* | 0 | 1 | 9.73333 | 1 | 2 | 0.30779 | 2 | 2 | 3.40616 |
| *CD1D* | 0 | 1 | 9.28333 | 1 | 2 | 0.30779 | 2 | 2 | 3.33871 |
| *CD1E* | 0 | 1 | 9.28333 | 1 | 2 | 0.30779 | 2 | 2 | 3.33871 |
| *OXTR* | 2 | 3 | 2 | 0 | 2 | 0 | 2 | 1 | 0.33871 |
| *SLC7A7* | 0 | 1 | 9.41667 | 1 | 2 | 0.30779 | 2 | 2 | 3.43988 |
| *MYO1F* | 42 | 2 | 9.4 | 0 | 2 | 0 | 2 | 1 | 3.33871 |
| *ADCY4* | 2 | 3 | 2 | 0 | 2 | 0 | 2 | 1 | 0.33871 |
| *KYNU* | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0.19355 |

MCC, maximal clique centrality; DMNC, density of maximum neighborhood component; MNC, maximum neighborhood component.

**Table 4** Information of the top 20 crossover genes sorted by logFC absolute value from high to low

| Gene | LogFC | P value |
|---|---|---|
| *TREM2* | 4.715156105 | 0.000175671 |
| *CD14* | 3.924331622 | 0.000788682 |
| *COL24A1* | 3.092087182 | 0.003520422 |
| *PLAU* | 2.998535901 | 0.000313225 |
| *FTHL17* | 2.765544598 | 0.001130595 |
| *ADCY4* | 2.717574461 | 0.003875534 |
| *CAPNS2* | 2.709504574 | 0.003545901 |
| *KCNA5* | –2.677832819 | 0.004208824 |
| *C1orf162* | 2.592848998 | 0.003126336 |
| *HCK* | 2.585015946 | 0.002710348 |
| *KYNU* | 2.5761862 | 0.003572205 |
| *FPR1* | 2.506625056 | 0.000799221 |
| *CD72* | 2.496959501 | 0.000594997 |
| *ICAM1* | 2.365725873 | 0.001181154 |
| *CD1E* | 2.341110442 | 0.004016336 |
| *CCDC15* | 2.324436483 | 0.003188285 |
| *SLC7A7* | 2.318271305 | 0.002886925 |
| *FCGBP* | 2.271693711 | 0.000405885 |
| *TMEM106A* | 2.27007665 | 0.001106818 |
| *OXTR* | –2.246951667 | 0.000229464 |

FC, fold change.

20 possible action pathways of *C. aromatica* involve RORA-activated gene expression, PPARA-activated gene expression, transcriptional regulation of white adipocyte differentiation, synthesis of bile acid and bile salt, transient receptor potential channel, intracellular receptor sumoylation, and activation of nuclear receptor transcriptional pathways (*Figure 5B*). Then, we used Metascape to conduct KEGG analysis of *S. miltiorrhiza-C. aromatica* online again, and we found that the mechanism of *S. miltiorrhiza-C. aromatica* in treating PCOS mainly focuses on the cAMP signaling pathway, amoebiasis, hematopoietic cell lineage, B cell receptor signaling pathways, and tryptophan metabolism (*Figure 5C*), the details of which are shown in *Table 6*.

### *The 3D structure of the key proteins in the DEGs network in the RCDB PDB database*

In the above study, we obtained 3 key proteins in the DEGs network, namely AOAH, HCK, and C1orf162. Then, we further explored the structure of these key proteins on the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCDB PDB) website (https://www.rcsb.org/), and finally found that only AOAH and HCK have 3-dimensional (3D) structure information, as shown in *Figure 6*.

### *General clinical data of two groups of patients*

Among the finally enrolled patients, 52 cases received the treatment of *S. miltiorrhiza-C. aromatica* combined with clomiphene, and 52 cases received clomiphene alone. We found no substantial differences in terms of age, disease duration, and body mass index (BMI) (*Table 7*).

### *Comparison of color Doppler ultrasound examination results before and after treatment in the two groups*

After 3 menstrual cycles, we re-conducted color Doppler ultrasound examinations on the 2 groups of patients, and the findings demonstrated that the long diameter of the ovaries, endometrial thickness, and the number of antral follicles in the combined treatment group of *S. miltiorrhiza-C. aromatica* combined with clomiphene were all higher than before treatment, and were substantially higher than those in the clomiphene alone treatment group. The difference was substantial, which also reflected the clinical value of our research direction from the side (*Table 8*).

### *Comparison of clinical symptoms between the two groups before and after treatment*

We used the acne score and the Ferriman Gallwey method to compare the changes of clinical symptoms before and after treatment in the 2 groups (*Table 9*). We found that the clinical symptoms of the patients in the *S. miltiorrhiza-C. aromatica* combined with clomiphene treatment group improved after treatment, and the improvement rate was higher than that of the patients treated with clomiphene alone.

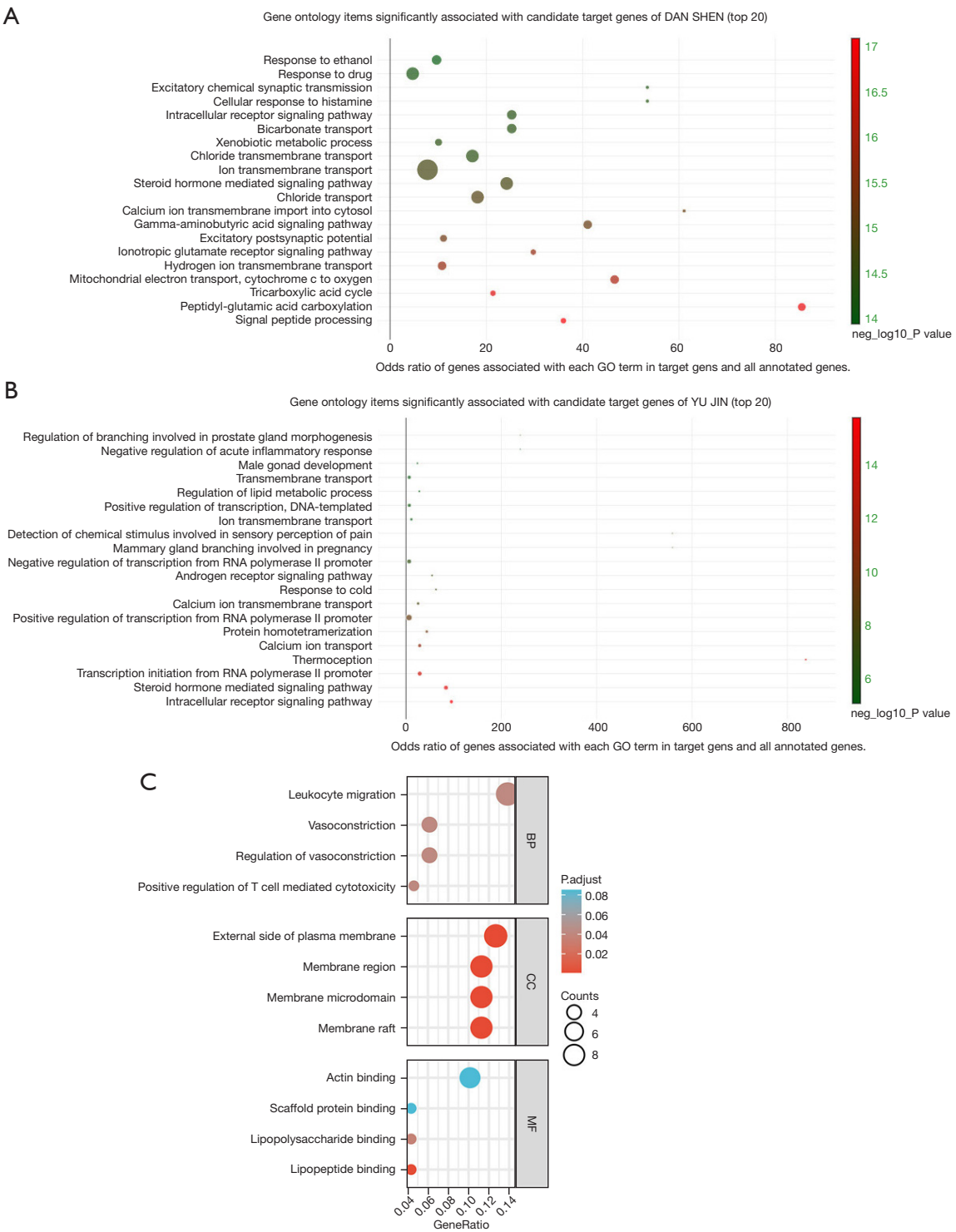


**Figure 4** GO enrichment analysis of *S. miltiorrhiza-C. aromatica* targets in treating PCOS. (A) FEA of candidate target genes of *S. miltiorrhiza* in ETCM database; (B) FEA of candidate target genes of *C. aromatica* in ETCM database; (C) function of *S. miltiorrhiza-C. aromatica* targets in Metascape online database enrichment analysis. P<0.05 is considered statistically significant. GO, Gene Ontology; PCOS, polycystic ovary syndrome; ETCM, Encyclopedia of Traditional Chinese Medicine; FEA, functional enrichment analysis; BP, biological process; CC, cell component; MF, molecular function.

**Table 5** Details of the GO analysis of the *S. miltiorrhiza-C. aromatica* targets in the Metascape online database

| Ontology | ID | Description | GeneRatio | BgRatio | P value | P.adjust | qvalue |
|---|---|---|---|---|---|---|---|
| BP | GO:0019229 | Regulation of vasoconstriction | 4/65 | 58/18670 | 4.93e-05 | 0.042 | 0.033 |
| BP | GO:0050900 | Leukocyte migration | 9/65 | 499/18670 | 5.48e-05 | 0.042 | 0.033 |
| BP | GO:0001916 | Positive regulation of T cell mediated cytotoxicity | 3/65 | 26/18670 | 9.89e-05 | 0.042 | 0.033 |
| BP | GO:0042310 | Vasoconstriction | 4/65 | 76/18670 | 1.42e-04 | 0.042 | 0.033 |
| CC | GO:0009897 | External side of plasma membrane | 9/71 | 393/19717 | 1.13e-05 | 7.14e-04 | 6.55e-04 |
| CC | GO:0045121 | Membrane raft | 8/71 | 315/19717 | 1.72e-05 | 7.14e-04 | 6.55e-04 |
| CC | GO:0098857 | Membrane microdomain | 8/71 | 316/19717 | 1.76e-05 | 7.14e-04 | 6.55e-04 |
| CC | GO:0098589 | Membrane region | 8/71 | 328/19717 | 2.30e-05 | 7.14e-04 | 6.55e-04 |
| MF | GO:0071723 | Lipopeptide binding | 3/69 | 10/17697 | 6.68e-06 | 0.001 | 0.001 |
| MF | GO:0001530 | Lipopolysaccharide binding | 3/69 | 35/17697 | 3.40e-04 | 0.037 | 0.034 |
| MF | GO:0003779 | Actin binding | 7/69 | 431/17697 | 0.001 | 0.085 | 0.079 |
| MF | GO:0097110 | Scaffold protein binding | 3/69 | 59/17697 | 0.002 | 0.085 | 0.079 |

P<0.05 is considered statistically significant. BP, biological process; MF, molecular function; CC, cellular component.

### *Comparison of sex hormone levels in two groups of patients*

After the treatment cycle ended, we took blood samples from the 2 groups of patients for laboratory comparison. We found that the reproductive hormone levels of patients in the *S. miltiorrhiza-C. aromatica* combined with clomiphene group were significantly improved, and the improvement was better than that of clomiphene alone group (*Table 10*).

## Discussion

PCOS is relatively common in women of reproductive age and is one of the main causes of infertility. Its main pathological feature is the disorder of sex hormone secretion (12,13). Studies have shown that the main pathological mechanisms of PCOS are hyperandrogenism and insulin resistance, but abnormal immune function, activation of inflammatory factors, and oxidative stress also affect the progression of PCOS (14). The adverse prognosis such as infertility caused by PCOS seriously affects female reproductive function, and at the same time brings heavy physical and psychological burdens to patients and their families (15). Due to the heterogeneity of disease treatment, TCM and integrated TCM and Western medicine have gradually become the choice of more and more PCOS patients. Our in-depth understanding of the possible PCOS mechanism from the perspective of TCM treatment plays a vital role in formulating targeted treatment measures.

We conducted a network pharmacology analysis of *S. miltiorrhiza-C. aromatica* in treating PCOS. Previous studies have shown that for patients with pregnancy-induced hypertension syndrome, compound *S. miltiorrhiza* injection mixed with magnesium sulfate as a therapeutic plan can improve infant and maternal health, reduce 24-hour urine protein and serum C-reactive protein (CRP), homocysteine (Hcy) and endothelin 1 (ET-1) levels, control blood pressure, and improve blood coagulation function (16); *S. miltiorrhiza* induces the expression of aquaporin 3 in isolated human primary amniotic epithelial cells through the ERK1/2 signaling pathway (17). Turmeric, also known as Wenyujin, nano-curcumin relieves insulin resistance and pancreatic defects in rats with PCOS through PI3K/AkT/mTOR and tumor necrosis factor-α (TNF-α) regulation (18). Curcumin attenuates pro-angiogenesis and pro-inflammatory factors in human endometrial stromal cells through the NF-κB signaling pathway (19). These studies reflect that *S. miltiorrhiza-C. aromatica* also plays an important role in some gynecological diseases, which also reflects the value of our research.

After screening DEGs, we obtained 1,148 upregulated and 544 downregulated genes, and further screened core genes through PPI network construction and module analysis, and finally obtained 3 key proteins, AOAH, HCK, and C1orf162. Based on this result, we speculate that these

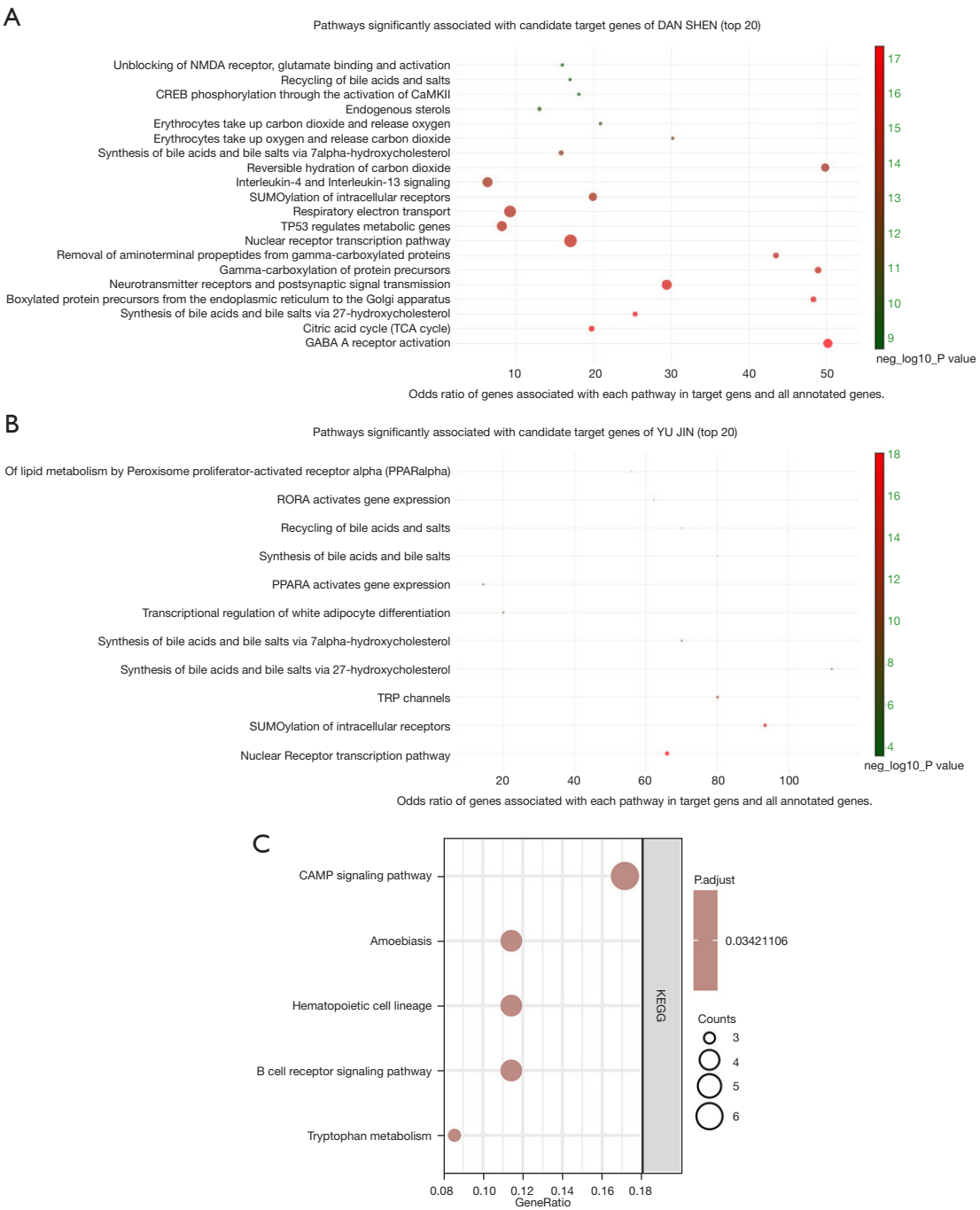


**Figure 5** Signaling pathway analysis of the potential role of *S. miltiorrhiza-C. aromatica* candidate target genes in treating PCOS. (A) Potential signaling pathway analysis of candidate target genes of S. miltiorrhiza in ETCM database; (B) Potential signaling pathway analysis of candidate target genes of *C. aromatica* in ETCM database; (C) *S. miltiorrhiza-C. aromatica* target in Metascape online database potential role signaling pathway analysis. P<0.05 is considered statistically significant. PCOS, polycystic ovary syndrome; ETCM, Encyclopedia of Traditional Chinese Medicine; KEGG, Kyoto Encyclopedia of Genes and Genomes.

**Table 6** Details of KEGG analysis of S. miltiorrhiza-C. aromatica targets in Metascape online database

| Ontology | ID | Description | GeneRatio | BgRatio | P value | P.adjust | qvalue |
|---|---|---|---|---|---|---|---|
| KEGG | hsa04024 | cAMP signaling pathway | 6/35 | 216/8076 | 2.90e-04 | 0.034 | 0.026 |
| KEGG | hsa04662 | B cell receptor signaling pathway | 4/35 | 82/8076 | 4.07e-04 | 0.034 | 0.026 |
| KEGG | hsa00380 | Tryptophan metabolism | 3/35 | 42/8076 | 7.63e-04 | 0.034 | 0.026 |
| KEGG | hsa04640 | Hematopoietic cell lineage | 4/35 | 99/8076 | 8.31e-04 | 0.034 | 0.026 |
| KEGG | hsa05146 | Amoebiasis | 4/35 | 102/8076 | 9.30e-04 | 0.034 | 0.026 |

P<0.05 is considered statistically significant. KEGG, Kyoto Encyclopedia of Genes and Genomes.

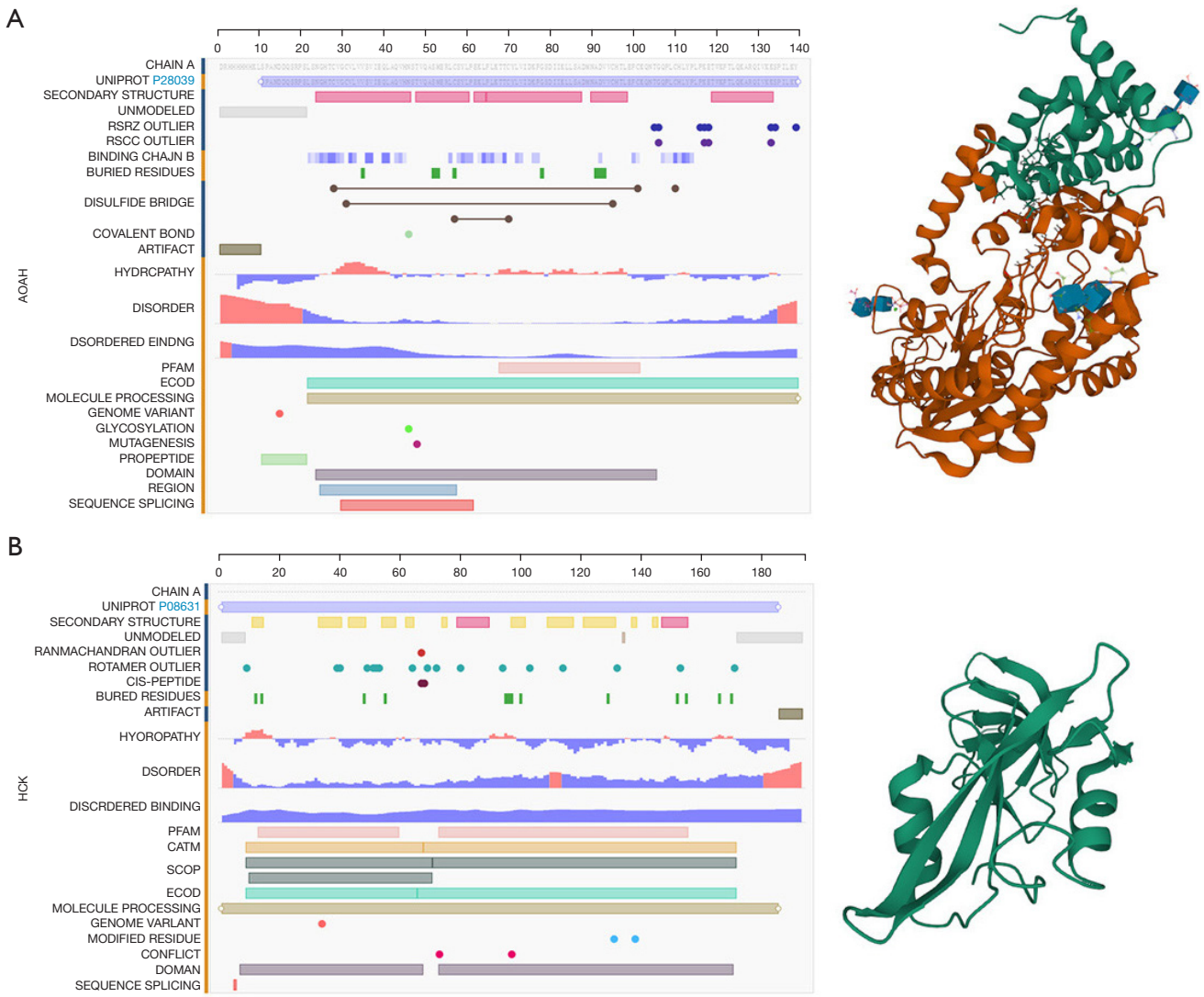


**Figure 6** The 3D structure information of key proteins in the DEGs network in the RCDB PDB database. (A) 3D structure information of AOAH protein; (B) 3D structure information of HCK protein. 3D, 3-dimensional; DEGs, differentially expressed genes; RCDB PDB, Research Collaboratory for Structural Bioinformatics Protein Data Bank.

**Table 7** General clinical data of the two groups of patients (mean ± SD)

| Group | Age (years) | Disease course (years) | BMI (kg/m$^2$) |
|---|---|---|---|
| Salvia-turmeric combined with clomiphene | 28.98±4.25 | 6.73±4.43 | 24.92±1.56 |
| Clomiphene | 27.12±5.32 | 5.82±4.28 | 24.86±1.62 |
| t | 1.970 | 1.065 | 0.192 |
| P value | 0.052 | 0.289 | 0.848 |

P<0.05 is considered statistically significant. BMI, body mass index.

**Table 8** Comparison of color ultrasound examination results before and after treatment in the two groups (mean ± SD)

| Group | Long diameter of ovary (mm) | | Endometrial thickness (mm) | | Number of antral follicles | |
|---|---|---|---|---|---|---|
| | Before | After | Before | After | Before | After |
| Salvia-turmeric combined with clomiphene | 17.05±2.41 | 21.65±2.50 | 3.70±0.22 | 5.25±0.36 | 3.01±0.48 | 5.56±1.15 |
| Clomiphene | 17.21±2.43 | 18.01±2.46 | 3.72±0.32 | 3.75±0.32 | 3.05±0.47 | 3.01±0.49 |
| t | 0.337 | 7.484 | 0.371 | 22.46 | 0.429 | 14.71 |
| P value | 0.737 | <0.0001 | 0.711 | <0.0001 | 0.669 | <0.0001 |

**Table 9** Comparison of clinical symptoms between the two groups before and after treatment (mean ± SD)

| Group | Acne score | | Ferriman Gallwey score | | Hair loss | | Menstrual cycle adjusted to normal number | |
|---|---|---|---|---|---|---|---|---|
| | Before | After | Before | After | Before | After | Before | After |
| Salvia-turmeric combined with clomiphene | 1.36±0.2 | 1.06±0.2 | 20.08±1.2 | 18.02±1.2 | 41 | 16 | 11 | 39 |
| Clomiphene | 1.32±0.18 | 1.10±0.16 | 18.84±1.3 | 18.21±1.3 | 31 | 35 | 12 | 25 |
| t | | 1.126 | | 0.774 | | 13.89 | | 7.96 |
| P value | | 0.26 | | 0.44 | | <0.001 | | 0.005 |

P<0.05 is considered statistically significant.

**Table 10** Comparison of sex hormone levels (mean ± SD)

| Group | LH (IU/L) | | FSH (IU/L) | | T (nmol/L) | | E2 (pg/mL) | |
|---|---|---|---|---|---|---|---|---|
| | Before | After | Before | After | Before | After | Before | After |
| Salvia-turmeric combined with clomiphene | 17.08±2.53 | 9.64±1.85 | 5.64±0.78 | 5.56±0.81 | 2.88±0.76 | 1.26±0.64 | 130.05±18.99 | 104.80±10.46 |
| Clomiphene | 17.02±2.44 | 12.16±2.35 | 5.61±0.80 | 5.54±0.81 | 2.83±0.73 | 2.03±0.72 | 131.89±18.91 | 123.04±13.60 |
| t | 0.123 | 6.076 | 0.194 | 0.126 | 1.101 | 5.764 | 0.495 | 7.666 |
| P value | 0.902 | <0.0001 | 0.847 | 0.900 | 0.274 | <0.0001 | 0.622 | <0.0001 |

P<0.05 is considered statistically significant. LH, luteinizing hormone; FSH, follicle stimulating hormone; T, testosterone; E2, estradiol.

3 key proteins can be used as predictive targets for PCOS. At the same time, it provides reference for further research and development of targeted drugs in the future. In addition to the screening of key protein genes, we also performed KEGG enrichment of GO functions and related targets. We found that the mechanism of *S. miltiorrhiza-C. aromatica* in treating PCOS mainly focuses on inflammation-related pathways, cAMP signaling pathways, B cell receptor signaling pathways, tryptophan metabolism, and so on. At present, relevant studies have shown that these mechanism pathways are vital in treating PCOS. For example, the total flavonoids extracted from bergamot play a role in PCOS through the JAK2/STAT3 signaling pathway mediated by interleukin-6 (IL-6) (20). The activation of the TGF-β1/Smad3 signaling pathway inhibits ovarian follicle development in PCOS by promoting the granulosa cells' apoptosis (21). To selectively activate follicles through the mTOR signaling pathway, it is necessary to restrict nerve growth factor (NGF) induction in the ovarian stroma (22). Decanoic acid is a medium-chain fatty acid that may lower HSD3B2 transcription and protein production by inhibiting the orphan nuclear receptor Nur77 recruitment to the HSD3B2 promoter in response to cAMP. Decanoate, the conjugate base of decanoic acid, effectively manages hyperandrogenism and insulin resistance in a letrozole-induced rat model of PCOS by decreasing serum-free testosterone, decreasing fasting insulin, and restoring the estrous cycle (23). By controlling oxidative stress, mitochondrial membrane potential, inflammation, and apoptosis, cryptotanshinone protects ovarian tissue from PCOS-related injury (24). Positive correlations between testosterone, the free androgen index, and the ratio of luteinizing hormone to follicle-stimulating hormone (LH:FSH) have been found in patients with PCOS, suggesting that tryptophan-kynurenine metabolites may be useful biomarkers for diagnosing and treating PCOS (25). These studies also reflect the value and feasibility of our exploration of *S. miltiorrhiza-C. aromatica* treatment of PCOS.

We retrospectively analyzed the clinical data of PCOS patients in our hospital to further explore the effectiveness of *S. miltiorrhiza-C. aromatica* in treating PCOS. We selected the clinical data of 104 hospital-admitted PCOS patients from January 2018 to December 2020. Among them, 52 cases were treated with *S. miltiorrhiza-C. aromatica* combined with clomiphene, and 52 cases were treated with clomiphene alone. After the treatment of the 2 groups of patients for 3 menstrual cycles, it was revealed that the long diameter of the ovary, the endometrial thickness, and the antral follicle count in the combined treatment group of *S. miltiorrhiza-C. aromatica* combined with clomiphene were all increased compared with those before treatment, and were significantly higher than those of patients in the group treated with clomiphene alone. At the same time, the clinical symptoms and hormone levels of the patients in the former group were also improved. Therefore, these findings again reflect the clinical and scientific significance of *S. miltiorrhiza-C. aromatica* in treating PCOS, laying a certain foundation for the later basic experiments.

There are many ways to treat PCOS by traditional Chinese medicine or western medicine. Western medicine has strong and powerful efficacy, but because the efficacy is too strong, it will cause damage to other parts of the body, which is often said to be a side effect, but it is suitable for the period of rapid development of the disease. The traditional Chinese medicine is just and soft, and the dosage and time are well matched, and the effect is fast and good. It is mainly used in some cases of continuous development. Compared with western medicine, traditional Chinese medicine has another advantage. Western medicine can temporarily control the disease, while traditional Chinese medicine can cure the root cause. However, no matter which method can effectively improve the condition of patients, individualized treatment is the most suitable treatment. Therefore, we need to carry out further *in vitro* and *in vivo* experiments and relevant clinical trials to verify in the future.

## Conclusions

Although there are some limitations in this study, the data of the study have shown the research value of *S. miltiorrhiza-C. aromatica* in treating PCOS from multiple levels and perspectives. In the future, we can conduct relevant basic cytology experiments based on the predicted main active ingredients, signaling pathways, and targets to provide a more accurate basis for treating PCOS with TCM.

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## Footnote

*Reporting Checklist:* The authors have completed the

 

STREGA reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-23-29/rc

*Data Sharing Statement:* Available at https://atm.amegroups.com/article/view/10.21037/atm-23-29/dss

*Conflicts of Interest:* All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-23-29/coif). The authors have no conflicts of interest to declare.

*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by institutional of the Second People's Hospital of Wuhu (No. SZ2018001) and informed consent was taken from all the patients.



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