Exploring the medication pattern and mechanism of action of traditional Chinese medicine in treating polycystic ovary syndrome with kidney deficiency and blood stasis based on data mining and network pharmacology

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Author contributions
Liu Yiling collected and revised this article. Liu Jun wrote this article.

Competing interests
The authors declare no conflicts of interest.

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Abbreviations
PCOS, polycystic ovary syndrome; TCM, traditional Chinese medicine; BC, betweenness centrality; PPI, protein-protein interaction; KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene Ontology.

Abstract
Background: Using network pharmacology to explore the potential molecular mechanism of traditional Chinese medicine in treating polycystic ovary syndrome (PCOS) with kidney deficiency and blood stasis syndrome. Method: Collect the related literature materials of PCOS with kidney deficiency and blood stasis syndrome treated by traditional Chinese medicine in four databases in recent ten years, extract the information of prescriptions and complete the frequency analysis. Traditional Chinese Medicine Systems Pharmacology Database was used to screen out the effective components. Use Online Mendelian Inheritance in Man and other databases to screen PCOS disease targets. The intersection targets obtained by clustering prescription and PCOS disease targets were submitted to STRING database for protein-protein interaction network analysis, and Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes pathways were analysed by Metascape. Result: There are 155 kinds of traditional Chinese medicines used in the literature. The most commonly utilized ones are Cuscutae Semen, Angelicae Sinensis Radix, and Rehmanniae Radix Praeparata. The results of the cluster analysis indicated that the plants most commonly found throughout the prescription were Leonuri Herba, Lycopii Herba, Dipsaci Radix, etc. GO results show that biological processes include cell reaction to organic nitrogen compounds and cell reaction to nitrogen compounds. The functional display of GO molecule includes cytokine receptor binding, signal receptor regulator activity and so on. Kyoto Encyclopedia of Genes and Genomes results show that the possible mechanisms of action are cancer pathway, an endocrine resistance signal pathway. Conclusion: Through data mining, the cluster prescription for PCOS with kidney deficiency and blood stasis syndrome is Leonuri Herba, Lycopii Herba, Dipsaci Radix, etc. The network pharmacology research of cluster prescription shows that the main drug components for treating PCOS with kidney deficiency and blood stasis syndrome are quercetin, kaempferol, luteolin, tanshinone II, etc., which act on PTGS2, NCOA2, and other targets, and treat PCOS with kidney deficiency and blood stasis syndrome through cancer and endocrine resistance.

Keywords: polycystic ovary syndrome; data mining; syndrome of kidney deficiency and blood stasis; network pharmacology
Introduction

Polycystic ovary syndrome (PCOS) is a common reproductive endocrine disease in gynecology, which causes amenorrhea, obesity, and infertility and brings great trouble to women’s health and life [1]. In recent years, the incidence of this disease has increased significantly, and the pathogenesis of this disease is mainly explained by genetic factors [2], abnormalities in lipid metabolism [3], dysfunctional intestinal flora homeostasis [4], insulin resistance [5], and so on. At present, the treatment of PCOS in Western medicine is mainly based on drugs and surgery [6, 7], but the Western medical treatment has problems such as drug side effects and high postoperative recurrence rate, and the cost is high, which brings great pressure to the physiology and psychology of patients [8, 9]. There is no direct record of PCOS in ancient times, but from the clinical manifestations, it can be categorized as “amorenorrhoea,” “infertility”, “obstruction in the abdomen” and other diseases. Most modern medical doctors believe that the core pathogenesis of PCOS is kidney deficiency [10], with phlegm-dampness [11], blood stasis and other pathological products [12], and the kidney deficiency and blood stasis syndrome of PCOS is more common in clinical practice [13].

Traditional Chinese medicine (TCM) can often achieve significant therapeutic effects through targeted treatment based on the patient’s condition and evidence-based therapy [14]. Therefore, data mining and network pharmacology were applied to study the medication pattern and mechanism of action of TCM for the treatment of renal deficiency and blood stasis in PCOS. In this study, we mainly used data mining to analyze the published clinical literature on the treatment of PCOS renal deficiency and blood stasis by traditional Chinese medicine, to summarize the characteristics of the medication and prescription rules of traditional Chinese medicine for treating patients with PCOS renal deficiency and blood stasis, and to derive the clustered prescriptions by applying the association rules and clustering analysis. Network pharmacology can study the disease from a holistic, multi-faceted and molecular level, and more clearly illustrate the mechanism of action of clustered prescriptions in the treatment of renal deficiency and blood stasis in PCOS.

Materials and methods

Data mining

Sources of information. Four databases were searched: Chinese Biomedical Database, Wanfang Database, Chinese Periodicals Full Text Database, and Chinese Science and Technology Journals Full Text Database for literature from August 2012 to August 2022 on the treatment of renal deficiency and blood stasis of PCOS by traditional Chinese medicine.

Search method. Computerized search. To collect relevant literature on TCM treatment of renal deficiency and blood stasis in PCOS in the above four databases: enter subject = (“polycystic ovary syndrome” OR “PCOS” OR “late menopause”), (“polycystic ovary syndrome” OR “PCOS” OR “late menopause” OR “late menstruation” OR “late menstruation” OR “late menstruation”), OR “obstruction in the abdomen” OR “infertility”) AND (“tonifying kidney and promoting blood circulation” OR “kidney deficiency and blood stasis”) for fuzzy search”) was used for fuzzy search, and the starting and ending dates of the search were from 08/2012 to 08/2022.

Inclusion criteria. (1) Clinical observation and case-control trials published publicly from August 2012 to August 2022 on the treatment of kidney deficiency and blood stasis in PCOS by TCM or a combination of TCM and Western medicine; (2) the treatment modality in the literature is oral Chinese medicine decoction; (3) compliance with the diagnostic criteria of kidney deficiency and blood stasis syndrome in TCM [15] and PCOS diagnostic criteria in Western medicine [16]; (4) the efficacy was exact after treatment; (5) only the main formula was included; drugs with additions or subtractions for individual symptoms were not included; (6) the literature in the category of one manuscript with multiple submissions, the one with the most comprehensive record was selected.

Exclusion criteria. (1) Incomplete composition of the formula will not be accepted; (2) in the literature, Chinese medicines were combined with other therapies (e.g., acupuncture, moxibustion, acupressure, external treatment, etc.); (3) the use of Chinese minority medicine in the literature; (4) review, animal experiments, Meta-analysis, and conference literature will not be accepted; (5) the literature will not be accepted if it meets any one of the above criteria.

Research methods. Firstly, literature search was carried out sequentially in four databases according to the search strategy, and the literature on the treatment of kidney deficiency and blood stasis in PCOS by traditional Chinese medicine was screened according to the inclusion criteria. Literature information from the four databases that met the inclusion criteria was summarized and organized, and the herbal prescription information of PCOS renal deficiency and blood stasis evidence was extracted as the research data information, and the herbal prescription information was entered into Excel 2016. IBM SPSS modeler18.1 software and IBM SPSS Statistics25.0 software were applied to the association rule and clustering of the drugs for PCOS renal deficiency and blood stasis evidence. Herb of PCOS were analyzed by association rules and clustering.

Data entry and quality control. Firstly, literature search was carried out in four databases through computerized search methods, and all the retrieved literature was imported into NoteExpress 3.5.0 software to read the specific literature content, and literature screening was carried out according to the discharge standard to screen out the literature on the treatment of renal deficiency and blood stasis syndrome of PCOS by traditional Chinese medicine. This process uses a two-person, two-machine data entry method to ensure the accuracy and authenticity of the data.

Data information pre-processing. The names of drugs collated from the literature were normalized and standardized according to the 2020 edition of the Pharmacopoeia of the People’s Republic of China [17] and the Chinese Materia Medica (Editorial Board of Chinese Materia Medica, 1999) [18].

Research methodology

Screening of active ingredients of drugs. Ten drugs were searched separately through the Traditional Chinese Medicine Systems Pharmacology Database (https://old.tcmsp-e.com/tcmsp.php) [19] database, according to the oral utilization oral bioavailability ≥ 30% and drug likeness ≥ 0.18 [20], the results of the active ingredients and corresponding targets were organized and entered into an Excel table. The names of the relevant targets corresponding to the active ingredients were normalized using the Uniprot database (https://www.uniprot.org/) at the end of the query.

Screening of PCOS-related targets. PCOS was used as the keyword in Online Mendelian Inheritance in Man (https://www.omim.org/) [21], DrugBank (https://go.drugbank.com/), GeneCard (https://www.genecards.org/) [22] for disease targets of PCOS. After sorting out and merging all targets, the results obtained are PCOS disease target data.

Construction of single-flavored drug-active ingredient-action target network. The single drug, active ingredient and target were made into network and type files, and the two files were imported into Cytoscape 3.9.0 software [23], after which the data were used to construct the network diagram of the single traditional Chinese medicine-active ingredient-target. And the data are analyzed by using parameters such as Degree and Betweenness Centrality (BC) are used to analyze the data.

Construction of protein-protein interaction (PPI) network of cluster prescription-PCOS target. Using Venny (http://www.interactivevenn.net/) to draw the Wayne diagram will be clustered prescription action targets and PCOS disease targets to take the intersection. The common targets of clustered prescription action targets and PCOS disease targets were entered into the STRING v11.5 database (https://cn.string-db.org) to construct the PPI network [23]. The biological species was selected as Homo sapiens, and the minimum interaction threshold was set to “highest confidence > 0.9” to hide
targets with no protein interactions and obtain the targets with the closest protein interactions. The results were organized in Excel format and analyzed by Cytoscape 3.9.0 software for Degree, BC and other parameters to obtain the core target of the drug action in the disease.

**Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and Gene Ontology (GO) biofunction enrichment analysis.** Metascape database [25] (http://metascape.org/gp/index.html) includes various functions such as enrichment, conversion, annotation, etc. It integrates several authoritative databases and provides the most updated and accurate biological annotation and pathway enrichment. The metascape platform was utilized to perform GO biofunction enrichment and KEGG pathway analysis on the common targets of clustered prescription targets and PCOS disease targets. The GO biofunctions include biological processes, cellular components, and molecular functions. To obtain the final GO bioenrichment analysis and KEGG pathway analysis results.

**Results**

**Data mining result**

**Results of literature organization.** Searching through four databases, a total of 3912 pieces of literature were queried, and the retrieved literature was screened according to the discharge standard, and finally 144 pieces of literature and 154 Chinese medicine prescriptions were included.

**Statistical results of frequency of medication use.** The frequency statistics of 155 Chinese herbal medicines in 154 prescriptions were analyzed, and the results showed that 155 Chinese herbal medicines appeared a total of 1864 times. The top 10 Chinese herbal medicines included: Cuscutae Semen 129 times (83.77%), Angelicae Sinensis Radix 117 times (75.97%), Rehmanniae Radix Praeparata 91 times (59.09%), Salviae Miltiorrhizae Radix et Rhizoma 86 times (55.84%), Lycii Fructus 76 times (49.35%), Chaenxiong Rhzoma 69 times (44.81%), Paonaeae Rubra Radix 58 times (37.66%), Epimedi Foliun 53 times (34.42%), Dipsaci Radix 51 times (33.12%), Corni Fructa 47 times (30.52%).

**Statistical results of the frequency of attribution of menstruation.** The statistics of drug attribution to meridians showed a total of 4537 occurrences. It was mainly dominated by the liver meridian, which appeared 1523 times, accounting for 33.57% of the total number of times, followed by the kidney meridian (1007 times, 22.20%), spleen meridian (678 times, 14.94%), heart meridian (471 times, 10.38%), lung meridian (274 times, 6.04%), stomach meridian (150 times, 3.31%), pericardium meridian (140 times, 3.09%), bladder meridian (88 times, 1.94%), gallbladder meridian (81 times, 1.79%), large intestine meridian (68 times, 1.50%), Sanjiao meridian (51 times, 1.12%), and small intestine meridian (6 times, 0.13%), and the distribution is shown in Figure 1.

**Results of drug frequency statistics.** TCM has four Qi, namely, cold, cool, warm and hot. Besides the four natures, there is also a kind of neutral medicine, which refers to a kind of medicine with mild cold and hot bias. The statistics of the four Qi of drugs showed a total of 1780 occurrences. It was found that warm drugs were predominant 844 times, 47.42%, followed by neutral (524 times, 29.44%), cold (342 times, 19.21%), cool (51 times, 2.87%), and hot (19 times, 1.07%), the distribution of which is shown in Figure 2.

**Statistical results of drug flavor frequency.** The five flavors of TCM refer to sour, bitter, sweet, pungent and salty, but TCM is not limited to these five flavors, so astringency is attached to acid and tasteless to sweetness. The statistical results of medicinal flavors of drugs showed that most of the drugs used were sweet 1192 times, totaling 38.58%, followed by pungent (795 times, 25.73%), bitter (756 times, 24.47%), sour (175 times, 5.66%), salty (74 times, 2.39%), tasteless (64 times, 2.07%), and astringent (34 times, 1.10%). The distribution map is shown in Figure 3.

**Statistical results of drug efficacy attribution.** In this study, the Chinese medicines appearing in 154 prescriptions were categorized and counted according to the Traditional Chinese Pharmacology (Wu Qingguang, 2022) as well as the Chinese Materia Medica, and could be divided into 17 categories in total. The results showed that the most frequent occurrence of Chinese medicines was tonifying deficiency medicine, which was 961 times, accounting for 51.56%, followed by blood-activating and stasis-transforming medicines (460 times, 24.68%), interior heat-clearing medicines (102 times, 5.47%), astringing medicines (88 times, 4.72%), Qi (Qi refers to the basic substance that constitutes the human body and maintains life activities, and is the unity of substance and function) regulating medicines (72 times, 3.86%), wind-eliminating and dampness-resolving medicines (45 times, 2.41%), urination-promoting and dampness-draining medicines (40 times, 2.15%), Hemostatic medicines (27 times, 1.45%), interior-warming medicines (17 times, 0.91%), dampness-transforming medicines (14 times, 0.75%), phlegm-transforming, cough-stopping and panting-alleviating medicines (13 times, 0.7%), exterior-releasing medicines (12 times, 0.64%), tranquillizing medicines (5 times, 0.27%), orifice-opening medicines (3 times, 0.16%), digestion-promoting medicines (3 times, 0.16%), poisonous insecticidal and relieve itching medicines (1 time, 0.05%), and purgative medicines (1 time, 0.05%). The distribution of which is shown in Figure 4.

![Figure 1 Radar map of Chinese medicine distribution by meridian](https://www.tmrjournals.com/mdm)
Figure 2 Distribution of the four Qi of Chinese medicine

Figure 3 Distribution of five flavors of Chinese medicine

Figure 4 Histogram of drug efficacy
Results of drug association rule analysis. The Apriori algorithm with minimum support set to 10%, minimum confidence set to 80% and maximum number of antecedent terms set to 2 was used to obtain the potential combinations between drugs, after which the drug association rule analysis was performed in the IBM SPSS Modeler 18.1 software. The results of the top 10 drug pair combinations in order of support were: Cuscutae Semen and Angelicae Sinensis Radix, Angelicae Sinensis Radix and Rehmanniae Radix Praeparata, Cuscutae Semen and Rehmanniae Radix Praeparata, Cuscutae Semen and Salviae Miltiorrhizae Radix et Rhiizoma, Angelicae Sinensis Radix with Rehmanniae Radix Praeparata and Cuscutae Semen, Angelicae Sinensis Radix and Lycii Fructus, Cuscutae Semen and Lycii Fructus, Cuscutae Semen with Rehmanniae Radix Praeparata and Angelicae Sinensis Radix, Angelicae Sinensis Radix and Chuanxiong Rhiizoma, Angelicae Sinensis Radix with Lycii Fructus and Cuscutae Semen. Association rules by confidence percentage (ranked results are: Lycopi Herba with Typhae Pollen and Spatholobi Caulis, Taxilli Herba with Typhae Pollen and Spatholobi Caulis, Dipisac Radix with Typhae Pollen and Spatholobi Caulis, Typhii Fructus with Typhae Pollen and Spatholobi Caulis, Saxieli Herba with Typhae Pollen and Lycopi Herba, Lycopi Herba with Typhae Pollen and Taxilli Herba, Lycopi Herba with Typhae Pollen and Lycistii Lucidi Fructus. The results of the association rules sorted by elevation were: Typhae Pollen with Spatholobi Caulis and Lycistii Lucidi Fructus, Typhae Pollen with Spatholobi Caulis and Achyranthis Bidentatae Radix, Typhae Pollen with Lycistii Lucidi Fructus and Dipisac Radix, Typhae Pollen with Lycistii Lucidi Fructus and Paoniae Rubrae Radix, Typhae Pollen with Taxilli Herba and Lycistii Lucidi Fructus, Typhae Pollen with Taxilli Herba and Lycistii Lucidi Fructus, Typhae Pollen with Lycistii Lucidi Fructus and Leonuri Herba, Typhae Pollen with Spatholobi Caulis and Lycopi Herba, Typhae Pollen with Lycopi Herba and Lycii Fructus. IBM SPSS Modeler 18.0 software was utilized to construct the network diagram of TCM association display as in Figure 5, while the number of occurrences of two TCM in the same prescription < 15 times was set as a weak link, and ≥ 35 times was set as a strong link.

Results of cluster analysis. High-frequency drugs with frequency ≥ 35 in the prescription were included and analyzed by clustering using IBM SPSS Statistics 25.0 software. A total of 19 Chinese medicines were included, and the clustering results divided the medicines into 2 groups with a distance of 23, which were (1) Leonuri Herba, Lycopi Herba, Dipisac Radix, Taxilli Herba, Achyranthis Bidentatae Radix, Lycistii Lucidi Fructus, Paoniae Rubrae Radix, Salviae Miltiorrhizae Radix et Rhiizoma, Lycii Fructus, and Cuscutae Semen. (2) Rehmanniae Radix Praeparata, Corni Fructus, Carthami Flos, Angelicae Sinensis Radix, Chuanxiong Rhiizoma, Paoniae Alba Radix, Gyperti Rhiizoma, Glycyrrhizae Radix, and Epiemdi Follum, and the spectra of systematic clustering analysis are shown in Figure 6.

Network pharmacology results
Results of active ingredient and target screening of drugs. After querying Traditional Chinese Medicine Systems Pharmacology Database, 119, 41, 202, 188, 119, 46, 29, 31, 51 and 16 active ingredients were obtained from Paoniae Rubrae Radix, Achyranthis Bidentatae Radix, Salviae Miltiorrhizae Radix et Rhiizoma, Lycii Fructus, Lycistii Lucidi Fructus, Taxilli Herba, Cuscutae Semen, Dipisac Radix, Leonuri Herba, and Lycopi Herba, in order of predominance of the active ingredients. After screening, the number of remaining active ingredients was 29, 4, 65, 45, 13, 2, 11, 8, 2 and 8 in that order. The number of targets obtained were 128, 191, 805, 318, 312, 143, 298, 49 and 47 in that order.

Results of PCOS disease target screening. In the disease databases, 4793, 3142 and 32 disease targets were obtained from Online Mendelian Inheritance in Man, DrugBank and Genecard databases, respectively, of which 787 disease targets were obtained by screening in Genecard database with Score median ≥ 13.73. Finally, after deleting the duplicate disease targets, we obtained PCOS-related target data, totaling 5228.

Construction of single Chinese medicine-active ingredient-target network. Through the analysis of Analyze Network in Cytoscape 3.9.0 software, there are 126 active ingredients and 824 targets in the clustered prescription, and there are 404 cut-off points and 1622 edges in the network diagram. The darker color and larger area of the nodes represent the higher Degree value. The network diagram is shown in Figure 7. The results showed that qunecetin had the largest Degree value, with a Degree value of 146 and a BC of 0.39, followed by kaempferol, with a Degree value of 60 and a BC of 0.08, lignanserin, with a Degree value of 57 and a BC of 0.09, and tanshinone, with a Degree value of 309, BC of 0.04; arachidonic acid, Degree value of 37, BC of 0.61; β-sitosterol, Degree value of 36, BC of 0.19; baicaline, Degree value of 33, BC of 0.06; vitexin hexetin, Degree value of 31, BC of 0.01; dihydropseuquiperene, Degree value of 31, BC of 0.01; isostanshinone, Degree value of 0.09; isostanshinones, Degree BC was 0.01; isorhamnetin, Degree value was 31, BC was 0.01. The main target of the clustered prescription was PFGS2, Degree value was 77, BC was 0.15, followed by NCOA2, Degree value was 47, BC was 0.07; PGR, Degree value was 42, BC was 0.05; PGS1, Degree value was 42, BC was 0.05; PGS1, Degree value was 0.05; Degree value of 42, BC of 0.05; SCN5A, Degree value of 37, BC of 0.03; ADRB2, Degree value of 37, BC of 0.02; NCOA1, Degree value of 36, BC of 0.01; CHRM1, Degree value of 36, BC of 0.01; RXRA, Degree value of 33, BC of 0.03; OPN1, Degree value of 31, BC of 0.00.
Clustering prescription-PCOS action target PPI network construction. The intersection of clustered prescription targets and PCOS disease targets was taken to obtain common targets as in Figure 8, in which there were 269 drug targets, 5228 disease targets and 106 common intersection targets. The obtained common targets were entered into the STRING database to obtain the PPI network of clustered prescription for PCOS-related targets, as shown in Figure 9, in which the PPI network contained 106 nodes, 428 interactions, with an average node degree of 8.26. The gene targets that clustered the heaviest prescription for PCOS were screened by the PPI network. The top 10 selected core genes are STAT3, JUN, AKT1, TP53, MAPK1, TNF, MAPK14, EGFR, ESR1, FOS.

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GO biofunction enrichment and KEGG pathway analysis. The 106 common targets obtained by clustering prescription-PCOS intersection were submitted to Metascape platform for GO biofunction and KEGG pathway enrichment analysis. Among them, biological processes was mainly cellular response to organic nitrogen compounds, cellular response to nitrogen compounds, and response to hormones, etc. Cellular components was mainly involved in membrane microregions, plasma membrane rafts, RNA polymerase II transcriptional regulatory complex, receptor complex, and cytosolic bases, etc. Molecular functions was mainly included in cytokine receptor binding, signaling receptor regulatory activity, cytokine activity, signaling receptor activator activity, and receptor ligand activity, etc., which were detailed in Figure 10. KEGG results suggest that the pathways for the treatment of PCOS are the pathways of cancer, Kaposi’s sarcoma-associated herpesvirus infection, human cytomegalovirus infection, hepatitis B, endocrine resistance, bladder cancer, hepatitis C, human papillomavirus infection, prostate cancer, etc., and the pathways were enriched as Figure 11.
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Figure 10 Predicted GO biofunctional enrichment analysis. GO, Gene Ontology; BP, biological process; CC, cell component; MF, molecular function.

Figure 11 Predictive KEGG pathway analysis. KEGG, Kyoto Encyclopedia of Genes and Genomes.

Discussion

According to the statistics of drug frequency, the top 6 drugs in Chinese medicine were Cuscutae Semen, Angelicae Sinensis Radix, Rehmanniae Radix Praeparata, Salviae Miltiorrhizae Radix et Rhizoma, Lycii Fructus, Chuanxiong Rhizoma can invigorate Qi, activate blood circulation and remove blood stasis, while Cuscutae Semen, Rehmanniae Radix Praeparata and Lycii Fructus benefit the kidney and regulate menstruation. The frequency of Cuscutae Semen is in the first place, which shows the importance of Cuscutae Semen in the treatment of kidney deficiency and blood stasis in PCOS. Cuscutae Semen has the function of tonifying Yang (in Chinese philosophy, the masculine, active and positive principle, characterized by light, warmth, dryness, activity, etc.) and benefiting Yin (in Chinese philosophy, the female, latent, passive principle, characterized by dark, cold, wetness, passivity, disintegration, etc.), and filling the marrow. According to Yu-qiu’s Explanation of Medicinals (Huang Yuanyu, 1754 C.E.), Cuscutae Semen has a sour flavor and a relatively calm Qi, and enters the liver and kidney meridians, with the function of tonifying the liver and kidney. Moreover, modern pharmacological research has shown [26] that Cuscutae Semen can significantly improve ovarian endocrine function in female reproductive system damage caused by drugs, endocrine disorders, stress environment, etc., and thus can treat a variety of gynecological diseases. Angelicae Sinensis Radix is commonly used in gynecological clinics, not only has the effect of generating blood and activating blood circulation, but also can promote the circulation of Qi, complementary to the line, the line in the complementary, so that the complementary without stagnation. PCOS renal deficiency and blood stasis is a disease with the underlying deficiency and the underlying reality, the use of Angelicae Sinensis Radix can be used to complement the Qi and blood together, complementary to the deficiency and eliminate the stasis, and treat the symptoms and the root cause, which is the key drug for treating the PCOS renal deficiency and blood stasis. Because of the above effects of Angelicae Sinensis Radix, Zhang Jingyue (1563–1640 C.E.)
[27] believes that Angelicae Sinensis Radix is the holy medicine in blood, and the Medicine Properties in Verse (Lin Weijie, Jin and Yuan dynasties) believes that Angelicae Sinensis Radix can make up for all the deficiencies of women, so Angelicae Sinensis Radix has always been "the holy medicine of gynecology". Chen Shiduo (1627–1707) believes that Rehmanniae Radix Praeparata, for the Yin in the Yang, into the liver and kidney meridian, with nourishing blood and Yin, replace essence to fill the liver and kidney beneﬁt essence and blood function. Salviae Miltiorrhizae Radix et Rhizoma is bitter and cold in nature, and enters the heart meridian. It has the effect of calming the mind, removing blood stasis and replenishing new blood, and can treat hema-tochezia in women. 

Salviae Miltiorrhizae Radix et Rhizoma is good at nourishing blood and promoting menstruation, and it can also activate blood circulation, eliminate blood stasis and relieve pain, support the positive and eliminate the evil, and eliminate blood stasis without hurting the positive, so there is a “Salvia Miltiorrhizae Radix et Rhizoma and Siwu decoction are similar in function” said PCOS kidney deﬁciency and blood stasis in the body of the patient, stasis of the blood can be a long time into the heat, Salviae Miltiorrhizae Radix et Rhizoma not only activate blood circulation and dissipate blood stasis, but also clear the heat and cool the blood. Zhang Xichun (1860–1933 C.E.)[29] believes that Lycii Fructus is a good medicine to nourish the liver and kidney. The explanation of the Explanations of the Classic of Materia Medica (Ye Gu, 1644–1911 C.E.) that the Lycii Fructus can beneﬁt the heart and kidneys, the heart and kidneys together, then the king of fire and kidney water phase peace and harmony, can make the muscles and bones strong, strong bones and muscles make body light, blood and Qi is sufﬁcient to the face of the rosy cheeks, to prevent the body from aging. Chuanxiong Rhizoma has the effect of promoting Qi and blood circulation and relieving pain, and is known as the Qi medicine of blood, because PCOS patients with kidney deﬁciency and blood stasis can further lead to Qi stagnation, so the application of Chuanxiong Rhizoma can promote the Qi stagnation of blood, so as to make Qi and blood ﬂow smoothly.

Cluster analysis of high-frequency drugs for the treatment of kidney deﬁciency and blood stasis in PCOS yielded Leonuri Herba, Lycopii Herba, Dipsaci Radix, Taxilli Herba, Achyranthis Bidentatae Radix, Ligustri Lucidi Fructus, Paeoniae Rubrae Radix, Salviae Miltiorrhizae Radix et Rhizoma, Lycii Fructus, and Cassiae Semen as the ﬁrst group, which had the effects of beneﬁting the kidney and ﬁlling in the vital essence, activating blood circulation and removing blood stasis. The kidney is the foundation of the innate nature and has the function of sealing and storing. Suppose kidney Qi deﬁciency, loss of sealing and storing, Qi imbalance, the normal operation of the blood in the human body is affected. In that case, the bloodline is stagnant, the formation of stagnant blood stasis and blockage of Chong and Ren meridians (the Chong meridian is called the “sea of the twelve channels”, Ren meridian is particularly associated with premenopause and childbirth. They both related to the menstruation, leukorrhea, pregnancy, childbirth and lactation of women), and the serious and prolonged illness can injure the kidneys, and the degree of stagnation of the blood is deepened. Therefore, due to the deﬁciency of the kidneys, the stagnation of the blood, and the addition of blood stasis, blood stasis activates and eliminates the products. The second group consists of Rehmanniae Radix Praeparata, Cori Fructi, Carthami Flos, Angelicae Sinensis Radix, Chuanxiong Rhizoma, Paeoniae Albae Radix, Coryi Rhizoma, Glycyrrhizae Radix, and Epimedi Flos and so on, to strengthen the effect of tonifying the kidneys and nourishing the blood and strengthening the efﬁcacy of the kidneys and nourishing the blood and for the etiology of the blood stasis, using Carthami Flos to strengthen the activation and elimination of blood stasis in the formula. Coryi Rhizoma is a “Qi medicine in blood,” which not only has the function of promoting Qi but also has the effect of promoting blood circulation. Combined with Chuanxiong Rhizoma, the formula is tonifying without stagnation, and the Qi and blood circulation together, Quercetin, kaempferol, lignans and tanshinone IIA were the main active ingredients in the clustered prescription. Yang Z et al. [30] found that PCOS rats induced with dehydroepiandrosterone were highly similar to PCOS patients in terms of the degree of ovarian pathology and hormone levels and that intervention with quercetin improved the abnormal oxidative stress state in PCOS rats. The study of Jiang XJ et al. [31] showed that quercetin was able to inhibit the activity of the AGES/RAGE pathway, which led to the proliferation of ovarian granulosa cells in PCOS rats thereby improving the PCOS status. Studies have shown [32] that quercetin not only reduces the level of insulin resistance, IL-6, tumor necrosis factor and other Inflammatory factors in PCOS rats but also restores the cycle of PCOS insulin-resistant rats. Kaempferol [33] belongs to the natural ﬂavonoids, which can affect T-cell signaling and have a series of pharmacological effects such as anti-inﬂammatory, antioxidant, and immunomodulatory. Quercetin and kaempferol modulate the activity and expression of MRp2, thereby enhancing antioxidant, anti-inﬂammatory, and anticancer effects [34]. Studies [35] found that kaempferol has anticancer effects on various cancers, and kaempferol may inhibit prostate cancer growth and metastasis by regulating the Wnt/β-catenin pathway. Studies have shown [36, 37] that ovarian development and follicular growth are closely related to the Wnt pathway, especially the Wnt/β-catenin signaling pathway, and the occurrence of PCOS is related to the Wnt signaling pathway. β-catenin is also related to ovarian steroid hormones and luteinization, and if the pathway is abnormal, it will cause premature ovarian aging, ovarian dysfunction, luteal insufﬁciency and other diseases. Lignans [36] have been found to beneﬁt the heart and kidneys, and can protect against cardiovascular disease, antioxidant and anti-inﬂammatory effects and can inhibit tumor cell proliferation. Hui XL [39] and other studies found that lignans can inhibit the AKT/STAT3 pathway, inhibit the proliferation of cancer cells, and thus inhibit tumor growth. It was found [40] that lignans can reduce the production of PGE2, signiﬁcantly reducing the inﬂammatory response and the expression of inﬂammatory factors such as IL-6, IL-1β, and TNF-α, thus alleviating the inﬂammatory response. Tanshinone IIA has anti-cardiac hypertrophy, anti-tumor and anti-inﬂammatory effects [41]. Study [42] found that tanshinone IIA can regulate ovarian reproductive endocrine function, most PCOS patients have abnormal P450 aromatase function, and tanshinone IIA can inhibit the activity of the cytochrome C450 enzyme system. Zhang RX et al. [43] found that the combination of tanshinone and GnRH-a could reduce androgen and luteinizing hormone levels and improve glucose metabolism disorders in PCOS patients, as well as improve endometrial tolerance and pregnancy rate in patients. Feng L [44] found that for patients with PCOS combined with insulin resistance, tanshinone has the effect of reducing the levels of androgens, dehydroepiandrosterone sulfate and androstenedione, and increasing the level of sex hormone-binding globulin and the level of AKT3 mRNA in the endometrial tissue of the patient was significantly reduced after treatment. 

PPI network construction yielded clustered prescriptions acting on the core targets of PCOS as STAT3, JUN, EGR1, TP53, MAPK1, TNF, MAPK14, EGFR, ERβ, FOS and so on. Among them, IL-6/STAT3 pathway is associated with inﬂammatory response, insulin resistance, etc. Study [45] conﬁrmed that activated STAT3 can lead to the development of inﬂammatory response. Experimental studies by Wang MK et al. [46] found that insulin resistance and signiﬁcant improvement in ovarian morphology in PCOS rats may be associated with metformin inhibition of the IL-6/STAT3 pathway. JUN is an important branch in the MAPK signaling pathway. Studies have shown [47] that dehydroepiandrosterone has a therapeutic effect of PCOS infertility by regulating the JNK signaling pathway and improving ovarian function. AKT1 can affect androgen levels. Studies have shown that metformin can alleviate insulin resistance by regulating the AKT1 signaling pathway [48]. AKT1 is involved in the regulation of primordial follicle quiescence and activation, and has an important role in processes such as human granulosa lutein cell survival and differentiation [49]. EGFR, EGR2, FOXO1, MAPK1, IGF1, and TP53 may be closely related to autophagy activation and PCOS [50]. Some experiments have shown that TP53 can upregulate p53 involved in PCOS, which suggests that TP53 is closely related to the pathological process of PCOS [51]. MAPK is a key signaling pathway.
related to androgen biosynthesis and insulin resistance in PCOS, which can lead to insulin resistance in PCOS [52]. From the above we can see that clustering prescription can act on the targets of PTGS2, NCOA2, PGR, STAT3, JUN, AKT1, TP53 and MAPK1 to regulate the pathogenesis of PCOS and thus treat PCOS.

Clustering prescriptions may be involved in the treatment of PCOS by regulating biological processes such as cell proliferation, differentiation and apoptosis, modulating molecular activity, regulating receptor complexes, and modulating the binding of growth factors and cytokines.

KEGG enrichment out of the results showed that the main signaling pathways of clustering prescription for the treatment of kidney deficiency and blood stasis in PCOS include the pathway of cancer, Kapoor’s sarcoma, related herpesvirus infections, human cytomegalovirus infections, hepatitis B, endocrine resistance, bladder cancer, hepatitis C, human papillomavirus infections, and p53 signaling pathway, etc. The occurrence of PCOS is associated with the pathway of cancer. Endometrial cancer (EC) is one of the complications of PCOS. Most PCOS patients exhibit anovulation or sporadic ovulation, and estrogen can stimulate the endometrium for a long period of time without the effect of progesterone, which will lead to an increased chance of endometrial hyperplasia and even EC in PCOS patients in the long run. According to research statistics, the incidence of EC in PCOS patients is more than three times higher than the incidence in normal healthy women [53]. Zhang X et al. [54] found that CD36-005 was highly expressed and the proliferative activity of primary endometrial mesenchymal stromal cells was enhanced in the rat endometrium through the study of lncRNA CD36-005 in the endometrium of the rat model of PCOS, suggesting that differential expression of mRNA is associated with the regulation of this suggests that differential mRNA expression is related to the regulation of mesenchymal cell proliferation and metastasis, and that abnormal mRNA expression can lead to endometrial abnormalities. Cai LJ et al. [55] retrospectively studied 211 PCOS patients and found that increased intra-abdominal adiposity index could increase the risk of EC in PCOS patients, and intra-abdominal adiposity index could have a certain predictive value for the occurrence of EC in PCOS patients. Increased visceral fat can trigger hyperlipidemia. Some studies have shown that hyperlipidemia is closely related to the development of EC [56]. The function of normal cell membranes and enzymes can be affected by hyperlipidemia, causing changes that result in cell damage and accelerating the process of apoptosis; high triglycerides can induce tumorigenesis and increase the risk of EC [57].

Among them, endocrine resistance is mainly manifested as insulin resistance, which plays an important role in the pathogenesis of PCOS. PI3K/AKT pathway and MAPK/ERK pathway play an important role in insulin signaling, which is closely related to cell metabolism, survival, and apoptosis. PI3K/AKT signaling pathway plays an important role in the regulation of follicle and oogenesis, which might be associated with follicular differentiation, growth and survival in non-gonadotrophic follicles [58]. Study [59] found that the PI3K/AKT pathway is closely related to cell proliferation and apoptosis, and abnormal regulation of PI3K/AKT is seen in PCOS patients. It was found [60] that abnormalities in the PI3K/AKT pathway in ovarian tissues in a rat model of PCOS interfered with the normal metabolism of insulin. Gong et al. found that the PI3K/AKT pathway was importantly linked to the growth and apoptosis of ovarian granulosa cells, and that activation of the PI3K/AKT pathway protected the ovarian granulosa cells and improved the quality of the oocytes, which in turn ameliorated the developmental process of PCOS [61]. The study of Zhang N et al. [62] showed that PI3K/AKT signaling pathway could be activated by berberine and could improve the symptoms of insulin resistance in PCOS. Yang HY [63] demonstrated that ligand could activate the PI3K/AKT/FOXO1 signaling pathway in the ovary, which could restore the motility cycle in some PCOS rats, reduce the serum luteinizing hormone level and improve the symptoms of insulin resistance, which provided a theoretical basis for the clinical treatment of PCOS. Zhang Y et al. [64] showed that modulation of PI3K/AKT signaling improved hormone levels and insulin resistance in PCOS-IR model rats. Ovarian insulin resistance may be related to the abnormal activation of MAPK/ERK pathway, which may lead to the impairment of PI3K/AKT pathway conduction and affect the development and maturation of eggs [65]. Jiang XL et al. [66] combined warm acupuncture with spleen-strengthening and expectorant formula to treat normal-weight PCOS patients with significant therapeutic effects, and the mechanism may be related to blocking the activation of MAPK/ERK signaling pathway. Therefore, through analysis, it was found that the treatment of PCOS with kidney deficiency and blood stasis by clustering prescription may be mainly realized through the mechanisms of inhibiting the cancer pathway, improving endocrine resistance, and improving cellular senescence.

According to the results discussed above, it is known that the clustered prescriptions mainly achieve the treatment of renal deficiency and blood stasis in PCOS through the cancer pathway and improvement of endocrine resistance, and at the same time, it is concluded that the analysis of active ingredients, targets and pathways of the clustered prescription has the role of anti-tumor and anti-endocrine resistance and regulation of cell proliferation, apoptosis, etc., which reveals the potential mechanism of action of traditional Chinese medicine in the treatment of renal deficiency and blood stasis in PCOS.
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