Mechanistic study of lipid metabolism disorders in diabetic kidney disease treated with GLQMP based on network pharmacology, molecular docking and in vitro experiments

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Author contributions
Xie YQ and Xiao M designed the study and offered funds. Liu SM, Yan ZI participated in the experiment. Liu SM wrote the paper. All the authors approved the final edited version of the manuscript.

Competing interests
The authors declare no conflicts of interest.

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Abbreviations
DKD, diabetic kidney disease; GLQMP, Gualou Qumai pill; PPI, protein-protein interaction; TG, triglycerides; TC, total cholesterol; TCM, traditional Chinese medicine; SD, Sprague Dawley; HG, high glucose; RT-qPCR, real-time polymerase chain reaction; KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene Ontology.

Citation

Abstract

Background: In this study, we used network pharmacology and molecular docking combined with vitro experiments to explore the potential mechanism of action of Gualou Qumai pill (GLQMP) against DKD. Methods: We screened effective compounds and drug targets using Chinese medicine systemic pharmacology database and analysis platform and Chinese medicine molecular mechanism bioinformatics analysis tools; and searched for DKD targets using human online Mendelian genetics and gene cards. The potential targets of GLQMP for DKD were obtained through the intersection of drug targets and disease targets. Cytoscape software was applied to build herbal medicine-active compound-target-disease networks and analyze them; protein-protein interaction networks were analyzed using the STRING database platform; gene ontology and Kyoto Encyclopedia of Genes and Genomes were used for gene ontology and gene and genome encyclopedia to enrich potential targets using the DAVID database; and the AutoDock Vina 1.1.2 software for molecular docking of key targets with corresponding key components. In vitro experiments were validated by CCK8, oil red O staining, TC, TG, RT-qPCR, and Western blot. Results: Through network pharmacology analysis, a total of 99 potential therapeutic targets of GLQMP for DKD and the corresponding 38 active compounds were obtained, and 5 core compounds were identified. By constructing the protein-protein interaction network and performing network topology analysis, we found that PPARA and PPARG were the key targets, and then we molecularly docked these two key targets with the 38 active compounds, especially the 5 core compounds, and found that PPARA and PPARG had good binding ability with a variety of compounds. In vitro experiments showed that GLQMP was able to ameliorate HK-2 cell injury under high glucose stress, improve cell viability, reduce TC and TG levels as well as decrease the accumulation of lipid droplets, and RT-qPCR and Western blot confirmed that GLQMP was able to promote the expression levels of PPARA and PPARG. Conclusion: Overall, this study revealed the active compounds, important targets and possible mechanisms of GLQMP treatment for DKD, and conducted preliminary verification experiments on its correctness, provided novel insights into the treatment of DKD by GLQMP.

Keywords: Gualou Qumai pill; diabetic kidney disease; disorder of lipid metabolism; network pharmacology; molecular docking
Highlights
In this study, we applied network pharmacological analysis and validation experiments to investigate the potential mechanisms of Gualou Qumai pill for the treatment of diabetic kidney disease.

Medical history of objective
Gualou Qumai pill is derived from the book "Synopsis of the Golden Chamber" (Written in the late Eastern Han dynasty) which was written by Zhang Jing Zhang. This prescription to reduce the treatment of thirst, edema and other diseases, achieved good results. Clinical studies have shown that Gualou Qumai pill can effectively reduce blood creatinine and urinary microalbumin in patients with diabetic kidney disease (DKD), improve the Chinese medical symptoms such as thirsty drinking, increased nocturia and coldness in hands and feet in DKD patients, delay the course of DKD.

Background
Diabetic kidney disease (DKD) is the most serious microvascular complications of type 2 diabetes mellitus, which can lead end-stage renal disease [1]. About third of all of diabetic patients in developed countries have DKD, with a high morbidity and mortality. The pathogenesis of DKD is not fully understood and is mainly thought to be connected with a combination of multiple factors such as hemodynamic changes caused by long-term hyperglycemia, lipid metabolism disorders, inflammation and oxidative stress [2]. Among them, disturbances in lipid metabolism are typical features that lead to the progress of diseases such as diabetes, atherosclerosis and hyperlipidemia, and renal lipid accumulation is one of the important factors that promote the progression of DKD [3]. During the developmental process of DKD, when too much lipid is accumulated in excess of normal requirements, it leads to lipid deposition in the kidney, interfering with normal kidney function and causing kidney damage [4]. Disturbances in lipid metabolism can damage the kidney both by interfering with normal renal lipid metabolism and indirectly by systemic inflammatorome and oxidative stress, vascular damage, and changes in hormones and other signaling molecules that affect renal function [5, 6]. Therefore, it is important to improve the disturbance of renal lipid metabolism to slow down the progression of DKD. Gualou Qumai pill (GLQMP) is derived from the book “Synopsis of the Golden Chamber" and consists of five drugs, namely, Trichosanthis Radix, Poria, Dioscoreae Rhizoma, Aconiti Lateralis Radix Praeparata and Dionthus superbus L. Clinical studies have shown that GLQMP can effectively reduce blood creatinine and urinary microalbumin in patients with DKD, improve the Chinese medical symptoms such as thirsty drinking, increased nocturia and coldness in hands and feet in DKD patients, delay the course of DKD [7]. However, the biologically active ingredients of GLQMP and its pharmacological mechanism of action are unknown. As the bioinformatics grows by leaps and bounds, network pharmacology has become as a strong approach to explore traditional Chinese medicine, which assimilates a series of disciplines and technologies to regularly analyze the interactions of active compounds, diseases, targets and other elements [8-10]. Molecular docking is a very popular technology in recent years and has been involved in most aspects of new drug design and development, bringing great help to new drug discovery and development [11]. In this study, we used network pharmacology and molecular docking combined with vitro experiments to explore the potential mechanism of action of GLQMP against DKD.

Methods
Network pharmacology analysis
Effective active ingredients and potential targets of GLQMP.
GLQMP consisted of five herbal medicines, including Trichosanthis Radix, Poria, Dioscoreae Rhizoma, Aconiti Lateralis Radix Praeparata and Dionthus superbus L. The bioactive compounds (oral bioavailability ≥ 30% and drug-likeness ≥ 0.18) of the above five drugs were selected based on the drug system pharmacology database TCMSp. The 2D structures of the compounds were obtained from the PubChem Compound Database, and the structures were imported into the Swiss Target Prediction Database to obtain the targets of the relevant active ingredients, target of the relevant active ingredient. Targets with a probability > 0.1 were defined as relevant potential targets for GLQMP.

Acquisition of potential targets for lipid metabolism disorders and DKD. The keywords “diabetic kidney disease”, “diabetic nephropathy”, and “lipid metabolism disorders” were searched through the GeneCard database and the OMIM database to obtain targets related to DKD and lipid metabolism disorders.

TCM-active ingredient-therapeutic target network. The drug component-disease therapeutic target interaction maps were drawn by Cytoscape 3.9.1 software. The obtained drug, DKD and lipid metabolism disorder-related targets were intersected, and defined these three cross-targets as potential therapeutic targets for drug-disease. Venn diagrams of drug-disease cross-targets through bioinformatics and evolutionary genomics.

Gene Ontology and Kyoto Encyclopedia of Genes and Genomes enrichment analysis. Importing potential targets for drug-disease treatment into the DAVID database for Gene Ontology (GO) analysis (biological process, cellular composition and molecular function) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. Finally, visualization analysis were performed using the Microbial Information Online platform.

Constructing protein-protein interaction (PPI) networks. Interaction networks of therapeutic targets were constructed using the STRING database (http://string-db.org) and imported into Cytoscape 3.9.1 for network topology analysis and visualization.

Molecular docking
Download a mol2 format file of active compounds from the TCMSp database. Protein structures of core targets were downloaded from the Protein Data Bank and water molecules and other small-molecule ligands were removed using PyMol software. Molecular docking simulations were performed after converting the components and targets to PDBQT format files using AutoDock Vina 1.1.2. Finally, PyMol was used to create three-dimensional maps of the docking to visualize the receptor-ligand interactions.

In vitro experiments
Drugs and reagents. minimum essential medium was purchased from Pricellia (Wuhan, China); Eastep™ super total RNA extraction kit was purchased from Promega Biological Products (Shanghai, China); Hifair® II 1st Strand cDNA Synthesis SuperMix for qPCR, Hieff® qPCR SYBR green master mix was purchased from Yeasen (Shanghai, China); BCA protein assay kit was purchased by Beyotime (Shanghai, China); anti-PPARA, anti-PPARG was purchased from Affinity (Jiangsu, China); anti-β-actin was purchased from Servicebio (Wuhan, China); 0.25% Trypsin EDTA, Ladders numbers of Western blot was purchased from Thermo Fisher Scientific (State of California, USA); Cell Counting Kit CCK8, D-anhydrous glucose dry powder was purchased from bioshop (Beijing, China); oil red O staining kit was purchased from Jiangsu KGI Biotechnology (Jiangsu, China).
Preparation of GLQMP-containing serum. GLQMP tablets were purchased from Beijing Tongrentang and identified by Professor Yi-Qiang Xie. First, The tablets of GLQMP were placed in a container, mixed with sterile water (eight times the weight of GLQMP by weight of water) and steeped for 1 h. The mixture is heated for 1 h and filtered to collect the filtrate. The liquid obtained after two repetitions was concentrated to obtain a combined decoction and to carry out the next experiments.
SPF grade Sprague Dawley (SD) rats were purchased and kept in Hainan Yunliang Biotechnology Company. The quality of experimental animals was tested by Beijing Vital River Laboratory
Animal Technology Company (quality certificate No. 110011230100954783). The SD rats were kept at an ambient temperature of (24 ± 2) °C, humidity of 50 ± 5%, light alternating between light and dark every 12 h. They were fed with free water and regular standard feed. This study was conducted by the National Institutes of Health Guide for the Care and Use of Laboratory Animals and was approved by the Ethics Committee of Hainan Medical College (ethics approval number: HYLL-2021-389).

SD rats were divided into a blank serum group and a GLQMP-containing serum group. The dose for the GLQMP group was obtained by converting the dose based on twice the body surface area of the adult rat, which was 5.94 g/kg/day. The blank serum group was given equal amount of sterile water. Gavage was performed once a day for 7 days. Blood was taken under anesthesia two hours after the last gavage (12 hours of fasting and water fasting). The blood of the same group was mixed, allowed to stand for 2 hours and centrifuged at 4 °C, 3,000 rpm/15 min. The upper serum layer obtained by centrifugation was dispensed into EP tubes, inactivated in a water bath at 56 °C for 30 minutes and finally stored at −80 °C for subsequent experiments.

Cell culture. Human proximal tubular epithelial cells (HK-2) were purchased from Pricella (Wuhan, China). Cells were cultured where in minimum essential medium containing 10% FBS and 1% penicillin-streptomycin solution. The temperature of the cell culture incubator was 37 °C and the CO₂ concentration was 5%.

CK8 cell viability assay. HK-2 cells were divided into five groups, which were: normal group; high glucose (HG) group (60 mM/L glucose); HG + low dose GLQMP group (8% Blank serum and 2% drug-containing serum); HG + medium dose GLQMP group (5% blank serum and 5% drug-containing serum); HG + high dose GLQMP group (10% drug-containing serum); HG + Canna group. HK-2 cell suspension (1,500 cells/well) was inoculated into 96-well plates. After modeling and drug treatment, the waste liquid was aspirated, add 10 μL CCK-8 solution and 100 μL culture medium to each well, and incubated at 37 °C in a cell culture incubator protected from light for a certain period of time. Finally, absorbance values of individual wells were measured at 450 nm using a zymograph.

Oil red O staining. HK-2 cells were inoculated onto the slides at a density of 2 × 10⁵ cells/well, and oil red O staining was performed according to the kit method after modeling as well as drug treatment. After staining, the slides were sealed and preserved with glycerol gelatin sealing solution, and then observed and photographed under an orthoscopic microscope.

Content determination of total cholesterol (TC) and triglycerides (TG). The model and drug-treated cells were digested with trypsin, the cell suspension was collected and centrifuged at 4 °C and 1,000 rpm for 5 min; the supernatant was discarded, washed again with PBS buffer, centrifuged, and finally broken by ultrasonication with the addition of PBS buffer under the condition of ice water bath. The prepared homogenates were measured directly according to the instructions.

Reverse transcription-quantitative real-time polymerase chain reaction (RT-qPCR). Total cellular RNA was extracted using the RNA extraction kit and cDNA was prepared using the reverse transcription kit. RT-qPCR analysis was performed under the q225 system according to the instructions of the real-time fluorescence quantitative polymerase chain reaction, and finally, the relative expression of the genes was calculated using the 2^(-ΔΔCt) method. The primer sequences used are shown in Table 1.

Western blot. Protein lysis samples were obtained using RIPA Lysis Buffer and Phenylmethanesulfonyl fluoride. roten concentration was quantified using BCA kit. Denaturation was performed at 100 °C for 10 min using loading buffer. The samples were separated electrophoretically in a gel and then transferred to a Polyvinylidene fluoride membrane. After incubation with 5% skimmed milk for 2 hours at room temperature, the primary antibody was incubated overnight at 4 °C, the membrane was washed three times with Tris Buffered Saline with Tween for 10 minutes each time, and the membrane was washed and incubated with the secondary antibody for 1 hour at room temperature. Finally, the membrane was developed with Enhanced Chemiluminescence luminescent solution and photographed. The expression levels of PPARA and PPARG were analyzed by image J using β-actin as internal reference.

Statistical analysis
Three biological replicates of the experiment were performed and the resulting data were statistically analyzed and plotted using Graphpad Prism (version 9.5). Statistical analyses of multiple groups of samples were performed using a one-way analysis of variance. The Tukey method was used to compare any two groups of data. All data were expressed as mean ± standard deviation. If P < 0.05, it was statistically significant.

Result
Network pharmacology analysis
Collection of active ingredients and corresponding targets of GLQMP. A total of 54 active ingredients were screened through the TCMSp website, including 2 from Trichosanthes Radix, 15 from Poria, 15 from Dioscorea Rhzomos, 21 from Aconitum Lateralis Radix Preparata and 1 from Dianthus superbus L. See Supplementary Table S1. The target information of the above active ingredients was obtained through the Swisstargetprediction website, and 677 predicted targets of GLQMP were identified and 41 corresponding active compounds. The correspondence between active ingredients and targets is shown in Supplementary Table S2.

Potential targets of GLQMP for the treatment of DKD. “diabetic kidney disease”, “diabetic nephropathy”, “lipid metabolism disorders” were used as keywords and were searched in GeneCards (https://www.genecards.org/) and OMIM (https://omim.org/) databases to collect DKD and lipid metabolism disorders-related genes. Detailed information is provided in Supplementary Table S3. Further, the intersection targets of GLQMP, DKD and lipid metabolism disorders were combined to create a venn diagram to obtain 99 potential therapeutic targets of raronoin for the treatment of lipid metabolism disorders in DKD, which as shown in Figure 1.

Construction of TCM-active ingredient-therapeutic target networks. We used Cytoscape 3.9.1 software to construct a TCM-active ingredient-target network. The network was mainly constructed by 38 active ingredients and the corresponding 99 potential therapeutic targets, as shown in Figure 2. The topological parameter data of the active compounds are shown in Supplementary Table S4. Among all active ingredients, the top five degree values were 11,4-ecidosadienoic acid (MOL002211), Deoxyandrographolide (MOL002395),(2R)-2-((5R,10S,13R,14R,16R,17R)-16-hydroxy-3-keto-4,10,13,14-pentamethyl-1,2,5,6,12,15,16,17-octahydrocyclopenta[a]phenanther-17-yl)-5-isopropyl-5-enoic acid (MOL000285),

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neokaduranic acid I (MOL002401), 3beta-Hydroxy-24-methylene-8-lanostene-21-oic acid (MOL000287). We consider these five substances to be the core compounds of GLQMP.

**GO and KEGG enrichment analysis.** To explain the processes involved in the treatment of DKD by GLQMP, we performed KEGG pathway and GO enrichment analyses of 99 potential therapeutic targets using the DAVID database and selected entries with *P* < 0.05. Detailed information is in the uploaded zip file Supplementary Table S5. GO analysis identified 537 significantly enriched GO terms, mainly including 389 biological processes, 55 cellular components, and 93 molecular functions. We therefore filtered the top 10 GO terms as shown in Figure 3A. In the biological process category, these terms are mainly related to the response to xenobiotic stimuli, positive regulation of gene expression, and glucose metabolic processes. In the cellular component category, the main terms include receptor complexes, cell surfaces, depressions, etc. In the molecular function category, key terms include RNA polymerase II transcription factor activity, ligand-activated sequence-specific DNA binding, steroid binding, heme binding, and others. To identify potential signaling pathways, we analyzed 119 KEGG pathways. The top 20 significantly enriched pathways included AGE-RAGE signaling pathway in diabetic complications, epidermal growth factor receptor tyrosine kinase inhibitor resistance, HIF-1 signaling pathway, PI3K-Akt signaling pathway, and so on. The results are shown in Figure 3B.

**PPI network construction.** The 99 targets of GLQMP for the treatment of disorders of lipid metabolism in DKD were imported into the STRING database, resulting in protein interaction network data. The parameter restriction was “homo sapiens” and the minimum required interaction score was “medium confidence (0.400)”. The PPI network was visualized and protein network relationships were mapped using Cytoscape 3.9.1 software, see Figure 4. The network topology analysis is shown in Supplementary Table S6. Based on the degree values, the top 5 key targets for GLQMP treatment of DKD included TNF, PPARG, ESR1, PPARA, and MTOR.

Figure 1 Target map of drugs and diseases. DKD, diabetic kidney disease; GLQMP, Gualou Qumai pill.

Figure 2 Cytoscape construction of a GLQMP component-targeting pathway network. The nodes with a larger area and darker color have a larger degree value. Where triangles represent the party, squares represent the active compounds, and circles represent the targets. GLQMP, Gualou Qumai pill.
Figure 3 Enrichment analysis plot. (A) Gene Ontology enrichment analysis. (B) Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis.

Figure 4 Protein target PPI networks were obtained from the STRING database via Cytoscape. (Which the nodes with a larger area and darker color have a larger degree value). PPI, protein-protein interaction.

Molecular docking simulation validation
Due to the important role of the peroxisome proliferator-activated receptors family in lipid metabolism, as well as a large number of active ingredients with good docking ability with PPARα and PPARγ. Therefore, PPARα and PPARγ were taken as the key targets, and the relevant components were searched for molecular docking according to the TCM component-therapeutic target network in reverse. Our molecular docking results showed that there was a good binding capacity between the active compounds and the core target proteins when the binding energy of the active chemical components to the key target proteins was less than $-5$ kcal/mol, and the results are shown in Table 2. In addition, the visualization of the docking results of PPARα and PPARγ with the five core compounds which is shown in Figure 5, and the docking results of PPARα and PPARγ with other active compounds are shown in Supplementary Figure S1. Therefore, we hypothesize that GLQMP can intervene in lipid metabolism disorders through the co-regulation of the expression levels of PPARα and PPARγ by multiple components, thus treating DKD.

In vitro experimental validation
GLQMP ameliorates the reduction of HK-2 cells viability under high glucose stress. To determine the protective effect of GLQMP on

Table 2 Results of molecular docking between ligands and core target receptors

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<th>PPARG Binding energy (kcal/mol)</th>
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Figure 5 Molecular docking results between core components and pivotal targets

using the CCK-8 assay, as shown in Figure 6. The viability of HK-2 cells exposed to 60 mmol/L glucose was significantly reduced compared with normal cells. The survival of GLQMP-treated HK-2 cells was increased compared with the HG group, and the protective effect on HK-2 cells under high glucose stress was more pronounced at higher GLQMP dose concentrations. Therefore, high dose concentrations of GLQMP were used in subsequent experiments. GLQMP ameliorates lipid metabolism disorders in HK-2 cells under high glucose stress. To observe the influence of GLQMP on HG-induced lipid droplet generation in HK-2 cells, we used oil red O staining to detect the level of lipid droplet accumulation in HK-2 cells, which was shown in Figure 7A. Treatment of HK-2 cells with 60 mmol/L glucose significantly increased the area and number of intracellular lipid droplets compared with cells in the normal group, however, the aggregation of intracellular lipid droplets was significantly reduced after treatment with GLQMP. Cholesterol and triglycerides were detected in HK-2 cells using the corresponding kits, as shown in Figure 7B, 7C. Our experiments confirmed that TC and TG levels were significantly elevated in the model group and were reversed by GLQMP treatment. The above experimental results indicate that GLQMP has an inhibitory effect on lipid accumulation in HK-2 cells under 60 mmol/L glucose.

In vitro experimental validation of the predicted targets PPARA and PPARG. RT-qPCR and Western blot were used to detect the mRNA and protein levels of PPARA and PPARG, as shown in Figure 8. We found that the mRNA and protein levels of PPARA and PPARG were decreased under HG conditions compared with normal cells; the mRNA and protein expression levels of PPARA and PPARG were increased after GLQMP intervention.

Discussion

DKD belongs to the categories of traditional Chinese medicine such as “edema” (A generalized term for diseases characterized by excessive drinking, eating, urination, emaciation, or urine with a sweet odor), “deficiency and fatigue” (Chronic weakness syndrome), and “Guan Ge” (Clinical features include inability to pass urine and vomiting) [12]. Most of the ancient Chinese medical texts are descriptions of its symptoms and variants, and there is no exact name of the disease. Based on the theoretical system of Chinese medicine and the understanding of modern medicine, contemporary medical practitioners have standardized the Chinese medical name of DKD as “thirsty kidney disease” (Increased urination caused by chronic nephritis, renal insufficiency, uremia, etc.) [13]. The disease is caused by the deficiency of kidney yang (Symptoms of fear of cold) over a long period of time, resulting in the retention of water-dampness (Excessive accumulation of localized water and fluid in the patient’s body, blocking the normal function of the local organs and meridians) and stagnation in the kidney channels, and its pathology is characterized by a combination of deficiency markers, deficiency and reality (complicated illness), and stagnation of blood (Poor gas flow in the body leads to impaired blood flow and the pathological condition of blood stasis) and phlegm throughout the disease (High phlegm congestion of the airways leading to shortness of breath, wheezing and dyspnea), which are important pathological products and pathogenic factors [14, 15]. Therefore, warming the yang (Ability to keep out the cold) and transforming the Qi (Evaporation of water into gases for elimination from the body), dipping the water and lowering the turbidity (Drain the body of dampness, phlegm, water and drink, and other pathological products of human metabolism) is the key to
treating DKD. In this formula, Aconitii Lateralis Radix Praeparata warms the kidneys and strengthens the yang (The source of all life activities in the human body), with Poria, which is used to relieve phlegm, Dioscoreae Rhizoma, which tonifies the middle and strengthens the spleen (Nurture the spleen and stomach) and regulates the waterway (relieve urination), Trichosanthis Radix, which clears heat and fire (Clearance of fire and heat effects), generates fluid and quenches thirst (Moisturizes the throat and quenches thirst), and Dianthus superbus L, which is used to eliminate evil heat (Antibacterial and antiviral), and to patent water (diuretic effect). The combination of the five herbs is effective and powerful. Zhang Qi, a master of national medicine, treated end-stage diabetic nephropathy with the addition of Juniperus communis pill, which was able to effectively relieve edema, reduce proteinuria, and improve renal function [16]. Shiji Luo used this formula in combination with cloxacillin to significantly reduce early urinary microalbumin excretion in patients with DKD and to better regulate lipid metabolism disorders in patients [17].

Modern pathology suggests that lipid deposition in the kidney has become an important factor leading to renal injury in DKD, and high levels of lipids in the body can cause fatty deposits in renal tissue, glomerulosclerosis and thylakoid expansion, aggravate the progression of proteinuria and tubulointerstitial fibrosis, and can directly damage cells and cause irreversible damage to renal function [18–20]. Although a great deal of research has been done on DKD, there is still a lot of undiscovered information about DKD. With the booming development of Chinese medicine, the research of Chinese medicine draw more and more attention. However, the complexity of TCM has resulted in many mechanisms of action that are still unclear. Studies have shown that GLQMP has significant efficacy in DKD, but the exact mechanism of action is still unclear. In our study, we first
Identified PPAR and PPARG, which are genes enriched in the lipid metabolism disorder pathway, as possible key targets in which to exert therapeutic effects based on network pharmacological predictions. Then the efficacy of GLQMP on DKD was determined by experimental validation, and it was found that GLQMP could increase the expression of PPARA and PPARG in HK2 cells. Peroxisome proliferator-activated receptors are lipid-activated transcription factors that are members of the nuclear receptor superfamily, which includes PPARA, PPARB, PPARG and other isofoms [21]. PPARs harness strengths in the self-balancing of lipid metabolism by regulating a variety of genes. Among them, PPARA in both glomerular and tubular cells is expressed, and its activation would upregulate the expression of genes which involved in fatty acid transport, binding and activation, and also regulates fatty acid catabolism and mitochondrial β-oxidation [22-24]. PPARG plays a key role in the regulation of adipogenesis, lipid storage, and glucose metabolism, and it has been reported that PPARA activation is closely related to the remission of DKD [25, 26]. Mishra et al. demonstrated that PPARA is a diabetes-inducible transcription factor that contributes to the control of the renal response to lipid response [27]. PPARA deficiency exacerbates the severity of diabetic nephropathy by increasing extracellular matrix formation, inflammation and circulating concentrations of free fatty acids and triglycerides, while increased PPARG levels accelerate the renal fatty acid metabolic process and reduce lipid deposition [28]. It was shown that the novel selective PPARA modulator, K-877, reduced renal lipid deposition in db/db mice and had a protective effect on the kidney [29]. PPARA deficiency exacerbates foot cell injury, and increased PPARG expression effectively alleviates foot cell injury and protects its integrity in diabetic patients, while renal tubular epithelial cell PPARA expression downregulation also affects podocyte function [30, 31]. Pioglitazone, a PPARG agonist, significantly improved plasma triglyceride concentrations in type 2 diabetes mellitus patients [32, 33]. While PPARA promotes lipid utilization, PPARG promotes lipid storage, and PPARA and PPARG and target genes play a dominant role in regulating lipid metabolism [34].

In this experiment, we firstly, predicted the pharmacological mechanism and possible active components of GLQMP on DKD by network pharmacology, and found that PPARA as well as PPARG might be the key targets to exert therapeutic effects among them. Therefore, we molecularly docked PPARA and PPARG with the active compounds, which led to the finding that these two targets bind well to a wide range of compounds in the GLQMP. Through in vitro experiments, we found that GLQMP increased the survival of HG-induced HK-2 cells and decreased TC and TG content, in addition, oil red O staining showed that GLQMP reduced HG-stimulated lipid accumulation. To further investigate whether GLQMP could affect the expression of PPARA and PPARG, we found that GLQMP could significantly increase the expression levels of PPARA and PPARG mRNA and protein by RT-qPCR and Western blot.

Combining the experimental results of this study and the findings of other scholars, we believe that GLQMP can regulate PPARA and PPARG to resist DKD, which provides a theoretical basis for the clinical application of GLQMP and also provides a basis for further research on GLQMP. However, due to the reasons of time, technology and funding, this study still has many shortcomings, firstly, animal experiments have not been added for further validation; secondly, there is no further knockdown validation of the two genes, PPARA and PPARG, in terms of cellular experiments; and lastly, the active ingredients of the drug-containing serum of GLQMP have not yet been analyzed in detail. In summary, we will make further improvement in the subsequent related experiments.

**Conclusion**

In our study, we first screened the active compounds of GLQMP, identified the potential targets of GLQMP for the treatment of DKD through network pharmacology, and revealed the possible mechanism of action of GLQMP on DKD. Then, through in vitro experiments, we found that GLQMP increased the survival of HK-2 cells in a high glucose environment and decreased TC, TG levels and lipid deposition, in addition, GLQMP increased the expression levels of PPARA, PPARG mRNA and protein. The above experiments illustrate the feasibility of the results of this study and provide research ideas for further study of GLQMP in the treatment of DKD.

**References**


