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Bioinformatics-based analysis of autophagy-related genes and prediction of potential Chinese medicines in diabetic kidney disease

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ABSTRACT

Objective To predict the autophagy-related pathogenesis and key diagnostic genes of diabetic kidney disease (DKD) through bioinformatics analysis, and to identify related Chinese medicines.

Methods Data from sequencing microarrays GSE30528, GSE30529, and GSE1009 in the Gene Expression Omnibus (GEO) were employed. Differentially expressed genes (DEGs) with adjusted $P < 0.05$ from GSE30528 and GSE30529 were identified. Combining these DEGs with the human autophagy gene database, Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses, and protein-protein interaction (PPI) network analysis were conducted on the obtained DKD autophagy-related genes. Subsequently, the least absolute shrinkage and selection operator (LASSO) regression and support vector machine-recursive feature elimination (SVM-RFE) algorithms were adopted to select autophagy-related genes. The diagnostic capability of these genes was assessed through analysis with the external validation set from microarray GSE1009, and relevant Chinese medicines were inversely predicted using the SymMap database.

Results A total of 2014 DEGs were selected from GSE30528 and GSE30529, leading to the identification of 37 DKD autophagy-related genes. GO analysis indicated 681 biological mechanisms, including autophagy regulation and plasma membrane microdomain activity. KEGG enrichment analysis identified 112 related signaling pathways. PPI network analysis showed a marked enrichment of autophagy-related genes in DKD. Through LASSO regression and SVM-RFE, four core diagnostic genes for autophagy in DKD were identified: protein phosphatase 1 regulatory subunit 15A (*PPP1R15A*), hypoxia inducible factor 1 alpha subunit (*HIF1α*), deleted in liver cancer 1 (*DLC1*), and ceroid lipofuscinosis neuronal 3 (*CLN3*). The external validation set demonstrated high diagnostic efficiency for these genes. Finally, 146 kinds of potential Chinese medicines were predicted using the SymMap database, with heat-clearing and detoxifying medicine and blood-activating and stasis-eliminating medicine accounting for the largest proportion (25/146 and 13/146, respectively).

Conclusion This study analyzed and validated bioinformatics sequencing databases to elucidate the potential molecular mechanisms of DKD autophagy and predicted key diagnostic genes, potential therapeutic targets, and related Chinese medicines, laying a solid foundation for clinical research and application.

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1 Introduction

Diabetes is a metabolic disorder syndrome caused by the imbalance of insulin secretion and the reduced sensitivity of target cells to insulin. With the improvement in living standards, the incidence of diabetes in China and globally is increasing annually, and individuals with diabetes is expected to 783 million in 2045 worldwide [1]. As one of the most dangerous and common complications of diabetes, diabetic kidney disease (DKD) also remains the main cause of chronic kidney disease and end-stage kidney disease globally. Approximately 35% of patients with diabetes will develop DKD, which can cause serious damage to kidney function and ultimately necessitate renal dialysis [2, 3]. The pathogenesis of DKD remains to be elucidated. Currently, angiotensin-converting enzyme inhibitors, metformin (kidney dose), and various Chinese medicines are mostly used in clinical treatment, but these treatment modalities have failed to reverse kidney injury [4]. Therefore, the early identification and diagnosis of DKD are of great importance.

A growing body of evidence shows that impaired autophagy can cause the accumulation of damaged organelles and proteins, thus disrupting cell homeostasis and continuously promoting the occurrence and development of DKD [5, 6]. Autophagy protects kidney cells and alleviates kidney injury through multiple mechanisms. It removes damaged organelles and misfolded proteins, regulates the morphology and function of podocytes, and modulates the metabolism of renal tubular epithelial cells. Additionally, autophagy regulates extracellular matrix deposition and alleviates inflammatory responses [6]. Specifically, the metabolic disorders of persistent hyperglycemia and hyperlipidemia can result in a state of excess nutrition and inhibit autophagy in diabetic kidney cells, while promoting autophagy can reduce kidney damage in patients with diabetes [7, 8]. All these clues indicate that activation of autophagy may be a novel therapeutic target for the prevention of DKD, demonstrating the significance of treating DKD based on autophagy balance.

The Gene Expression Omnibus (GEO) is a public library of gene expression data, containing data from a wide range of organisms and experimental conditions, including data from single-cell sequencing experiments [9]. In the context of precision medicine, gene sequencing technology has developed rapidly, and the use of bioinformatics to predict the core targets of diseases has received wide usage [10]. This study conducts differential gene expression analysis based on the GEO database to identify potential molecular mechanisms and related pathways involved in autophagy during the development of DKD. Additionally, it aims to predict related Chinese medicines, thereby providing a theoretical basis for the early diagnosis and treatment of DKD in clinical settings.

2 Data and methods

2.1 Screening of differentially expressed genes in DKD

Using the keywords “diabetic kidney disease” and “diabetic nephropathy”, we conducted a search in the GEO database (http://www.ncbi.nlm.nih.gov/geo/). After selecting “expression profiling by array” and “homo sapiens”, we obtained three sets of gene chip data from glomerular biopsy tissues related to DKD: GSE30528, GSE30529, and GSE1009. Subsequently, GSE30528 and GSE30529 were designated as the training set, while GSE1009 as the external validation set. Using the limma and sva packages in R software (v4.3.0), we carried out batch correction and combined analysis on the gene expression matrices of two datasets from the training group. The Benjamini-Hochberg method for multiple testing correction was employed. To control the overall false discovery rate, an adjusted P value (adj. P) < 0.05 was set as the criterion for identifying differentially expressed genes (DEGs). The pheatmap package was used to construct a heatmap of the DEG expressions.

2.2 DKD-related autophagy genes screening and protein-protein interaction (PPI) network analysis

The autophagy-related gene set was derived from the human autophagy database (HADb) (http://www.autophagy.lu/). The intersection of DEGs and autophagy genes was used to obtain the DKD autophagy genes. Then, the pheatmap package in R software (v4.3.0) was used to draw the expression heatmap. Subsequently, a PPI network analysis was performed for the core autophagy genes implicated in DKD using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database (https://cn.string-db.org/), then we obtained the core protein interaction network.

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

Using the Bioconductor in the R software package (v4.3.0), autophagy genes in DKD were analyzed with a significance threshold of adj. P < 0.05 for GO and KEGG enrichment analyses. For the GO enrichment analysis, categories including biological process (BP), cellular component (CC), and molecular function (MF) were examined. In addition, the KEGG enrichment analysis was performed to explore the involvement of these genes in specific signaling pathways.

2.4 Screening of DKD autophagy-related genes

Machine learning methods, specifically least absolute shrinkage and selection operator (LASSO) regression and support vector machine-recursive feature elimination (SVM-RFE) algorithms, were used to screen autophagy feature genes in DKD. Initially, a preliminary screening of DEGs was conducted. Subsequently, LASSO regression and SVM-RFE were utilized to identify candidate hub genes. LASSO regression was performed using the glmnet package with a binomial response type and α set to 1. SVM-RFE was conducted using R software (v4.3.0), and variables with the minimum classification error were determined through 10-fold cross-validation to select the penalty parameter.

2.5 Diagnostic value of autophagy-related genes in the validation set

The external GSE1009 dataset was standardized to construct a disease-control model, which was used to evaluate the degree of association between DKD autophagy-related genes and diseases. Using the pROC package, the receiver operating characteristic (ROC) curve was plotted, and the diagnostic efficacy of autophagy-related genes was evaluated using the area under the curve (AUC). The AUC ranges from 0 to 1.000, serving as an indicator to assess the diagnostic efficacy of the core genes: an AUC value closer to 1 indicates better diagnostic efficacy. To evaluate the robustness of the predictive model in this study, a logistic regression model was built using the DKD-specific autophagy genes as predictors. The model's performance was then assessed through ROC curve analysis with 10-fold cross-validation, and the resulting AUC along with its 95% confidence interval (CI) was reported.

2.6 Prediction of Chinese medicines

The SymMap database (http://www.symmap.org/) is a syndrome association database of Chinese medicines. By inputting the identified DKD-specific autophagy genes into this database, we searched for various Chinese medicines that can act on these genes and classified the drugs according to their efficacy regarding the *Pharmacopoeia of the People's Republic of China 2020* [11].

2.7 Statistical analysis

The Benjamini-Hochberg method for multiple testing correction was employed, with adj. $P < 0.05$ set to screen for DEGs. LASSO regression was performed using the glmnet package, adopting a binomial response type and setting α to 1. SVM-RFE was conducted using R software (v4.3.0).

3 Results

3.1 Expression of DEGs in DKD

The comparison before and after batch correction of the data distribution from GSE30528 and GSE30529 is shown in Figure 1A and 1B. After correction, the data dispersion from GSE30528 and GSE30529 were less than before which meant background errors caused by different samples were avoided. Further analysis showed 2 014 DEGs in total, of which 1 082 were up-regulated and 932 down-regulated (Figure 1C). The heatmaps of the top 50 significantly up-regulated and down-regulated genes was drawn based on the adj. P (< 0.05) of DEGs (Figure 1D).

3.2 Autophagy genes of DKD and PPI network analysis

Overall, 222 autophagy genes were obtained from the HADb. By intersecting DEGs with these autophagy genes, we identified 37 DKD autophagy genes (Figure 2A). Among these, 25 were up-regulated autophagy genes and 12 down-regulated autophagy genes (Figure 2B). After performing a PPI network analysis on the autophagy genes implicated in DKD, we observed a significant enrichment of PPIs, characterized by a higher-than-expected number of edges (197) and an average node degree of 10.6. Additionally, the average local clustering coefficient was 0.657, while the expected number of edges was only 62. This enrichment was further confirmed by a PPI enrichment P value < 1.0×10^{-16}, indicating its statistical significance (Figure 2C).

3.3 GO and KEGG enrichment analysis of DKD autophagy genes

The GO analysis of 37 DKD autophagy genes showed 681 biological mechanisms, mainly through 610 BPs, including regulation of autophagy, cellular response to chemical stress, and cellular response to external stimulus. These genes also participated in 41 MFs, such as cysteine-type endopeptidase activity, cysteine-type peptidase activity, and cysteine-type endopeptidase activity in the apoptotic process. Furthermore, these genes were mainly localized in 30 CCs, including membrane rafts, membrane microdomains, and autophagosomes (Figure 3A and 3B).

Overall, 112 related signaling pathways were obtained by KEGG enrichment analysis (Figure 3C and 3D). Among these pathways, apoptosis, Kaposi sarcoma-associated herpesvirus infection, and autophagy (animal) were identified as being associated with DKD autophagy.

3.4 Autophagy-related genes of DKD

Overall, 12 autophagy-related genes for DKD were identified using LASSO regression (Figure 4A and 4B). Additionally, 8 signature genes with the smallest expression errors were recognized using SVM-RFE (Figure 4C and

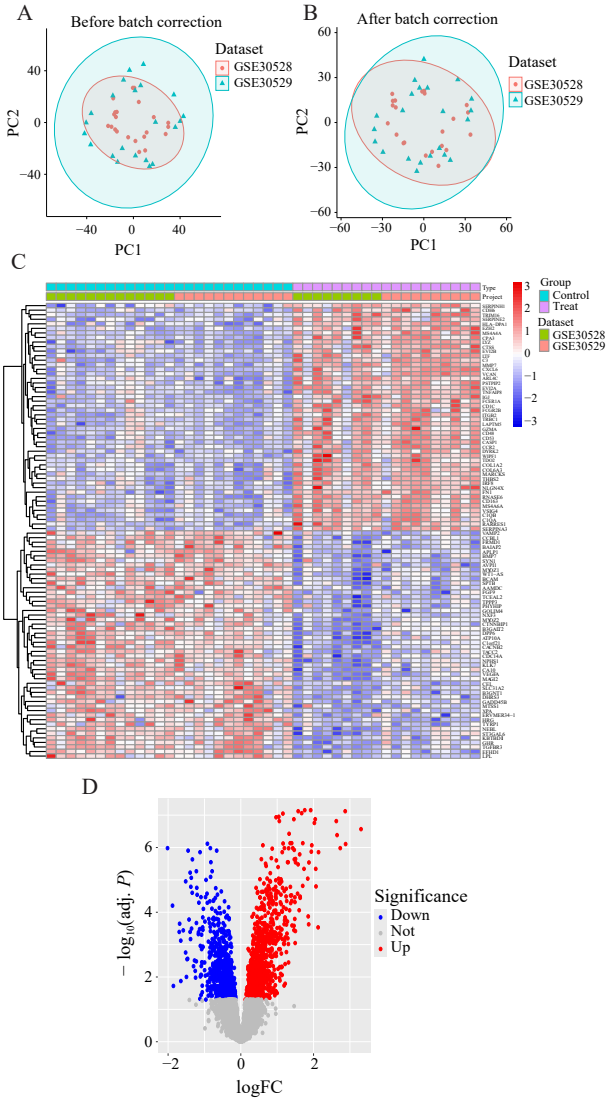

Figure 1 Differentially expressed genes of DKD

A, the principal component analysis of before batch correction from gene sets GSE30528 and GSE30529. B, the principal component analysis of after batch correction from gene sets GSE30528 and GSE30529. C, the expression of DEGs in the batch corrected dataset analysis. D, the heatmap of the top 50 up-regulated (red) and down-regulated (blue) genes when adj. $P < 0.05$. Treat, the external validation set. Control, the training set. FC, fold change.

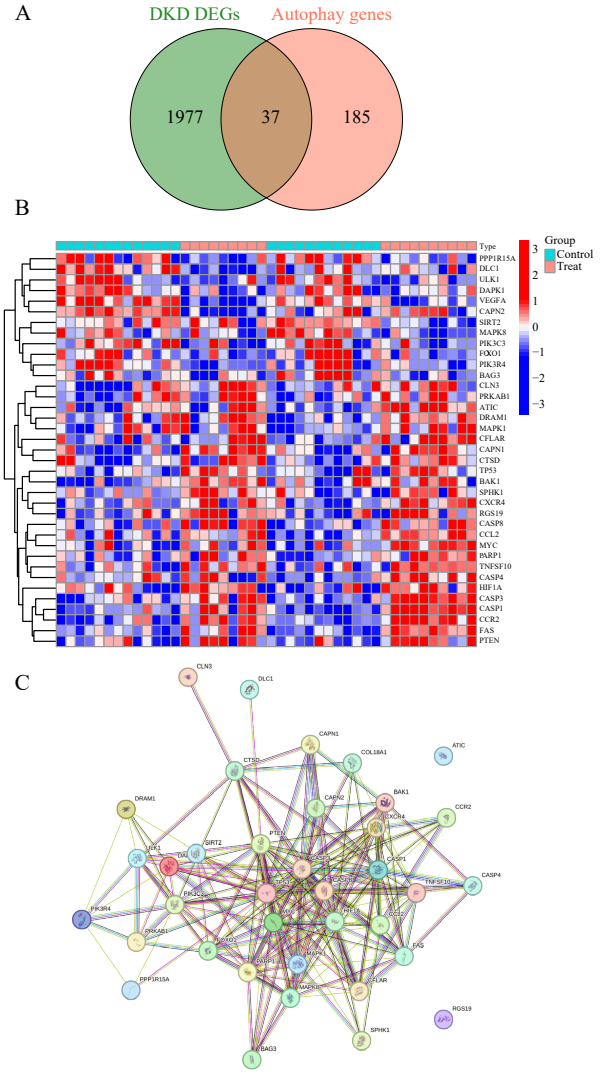

Figure 2 Autophagy genes of DKD and PPI network analysis

A, the intersection diagram of DKD DEGs and autophagy genes. B, the heatmap display of 37 DKD autophagy genes. Red represents up-regulation, and blue represents down-regulation. C, the PPI network analysis of DKD autophagy genes. The lines between genes represent PPIs, with different colors indicating various types of evidence: dark green for genes neighborhood, red for gene fusions, dark blue for gene co-occurrence, light green for text mining, black for co-expression, and light blue for protein homology.

4D). The intersection (Figure 4E) of genes identified by LASSO regression and SVM-RFE was selected, and 4 common genes were obtained: protein phosphatase 1 regulatory subunit 15A (*PPP1R15A*), hypoxia inducible factor 1 alpha subunit (*HIF1α*), deleted in liver cancer 1 (*DLC1*), and ceroid lipofuscinosis neuronal 3 (*CLN3*).

In the external validation set for DKD, the AUC values for *PPP1R15A, HIF1α, DLC1*, and *CLN3* were 0.667, 0.889, 0.667, and 1.000, respectively (Figure 4F), all exceeding the threshold of 0.60, indicating moderate to excellent diagnostic accuracy. Notably, the logistic regression model for DKD achieved an AUC of 1.000, with a 95% CI ranging from 1.000 to 1.000 (Figure 4G), showing the significant diagnostic potential of these feature genes.

3.5 Prediction of Chinese medicines targeting DKD autophagy-related core genes

The four core genes related to DKD autophagy (*PPP1R15A, HIF1α, DLC1*, and *CLN3)* were imported into the SymMap database. Using a screening criterion of $P < 0.01$, a total of 146 kinds of Chinese medicines were identified. Among them, the categories of heat-clearing and detoxifying medicine as well as blood-activating and stasis-eliminating medicine, represented the highest percentage (25/146 and 13/146, respectively), as shown in Table 1.

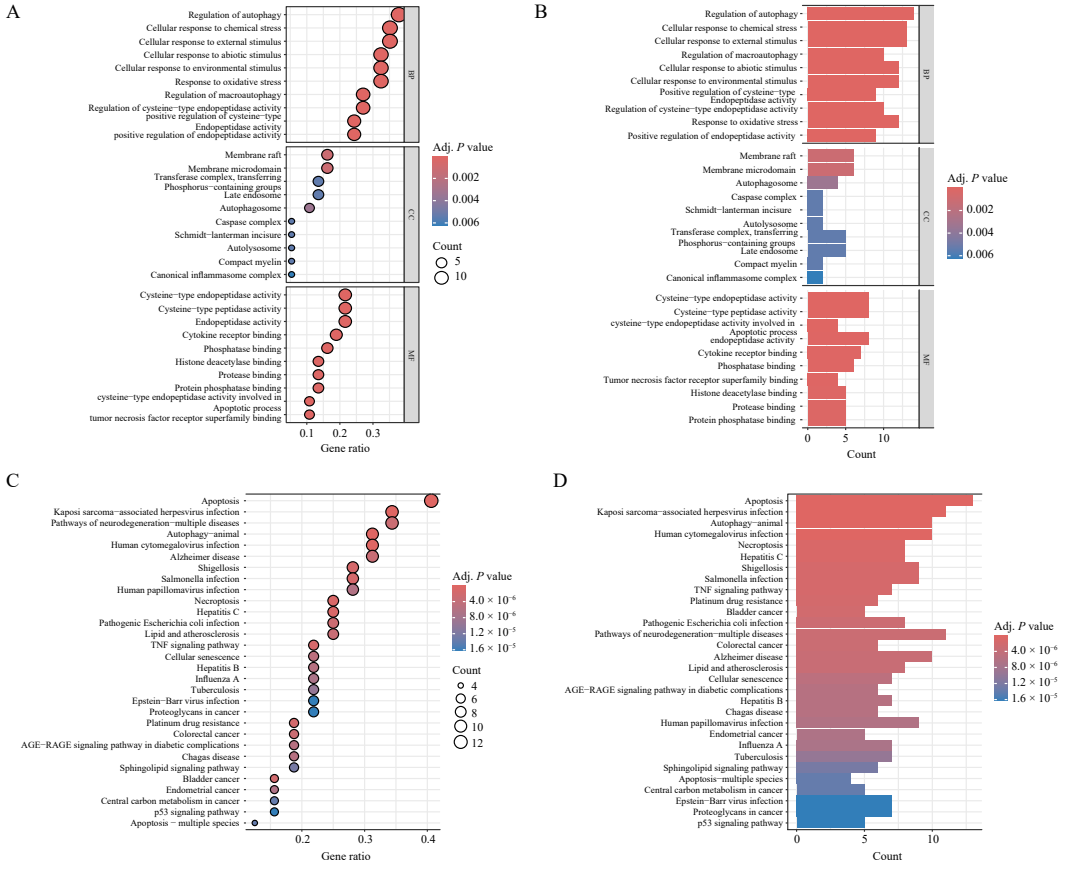

Figure 3 GO and KEGG analysis of DKD autophagy genes

A, the top 30 enriched terms in GO analysis. B, network visualization of GO enrichment analysis. C, the top 30 enriched KEGG signaling pathways. D, enrichment scatter plot of the significance and gene ratio of the top KEGG pathways.

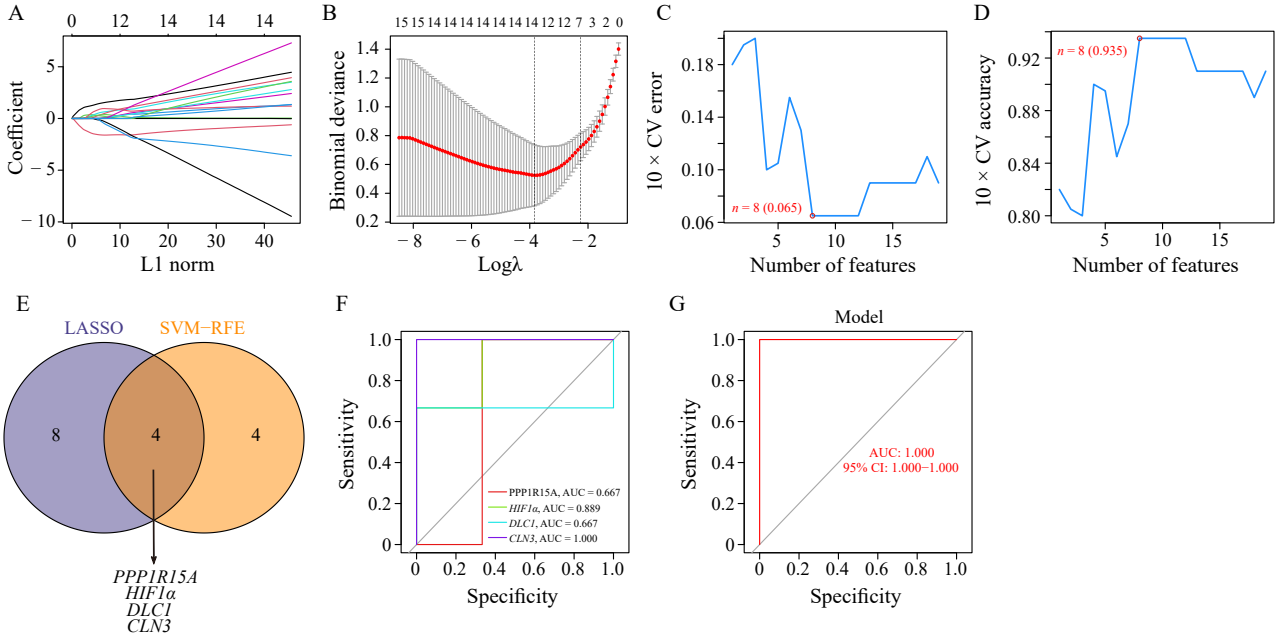

Figure 4 Characteristic genes of DKD autophagy

A, the coefficient path plot of DKD autophagy characteristic genes obtained by LASSO regression method L1 norm. B, the parameter map of DKD autophagy characteristic gene obtained by LASSO regression method. C, the minimum error obtained by 10-fold cross-validation (10 × CV) of the SVM-RFE method (n = 8). D, the highest accuracy otained by 10-fold cross-validation (10 × CV) of the SVM-RFE method achieves (n = 8). E, genes intersection identified by LASSO regression and SVM-RFE. F, the ROC curve of *PPP1R15A*, *HIF1α*, *DLC1*, and *CLN3* in the GSE1009 dataset. G, the ROC curve of feature genes under the logistic regression model.

Table 1 Predicted Chinese medicines targeting the four core autophagy-related genes in DKD

Efficacy	Chinese medicine	Ratio
Heat-clearing and detoxifying medicine	Shidagonglaogen (Mahoniae Radix), Gonglaomu (Mahoniae Caulis), Jiubiying (Sargentodoxae Caulis), Shidagonglaoye (Mahoniae Folium), Muhudie (Oroxyli Semen), Weilingcai (Potentillae Herba), Baiyaozi (Radix Stephaniae Cepharanthae), Tufuling (Smilacis Glabrae Rhizoma), Sijiqing (Ilicis Folium),Huanglian (Coptidis Rhizoma), Jixuecao (Centellae Herba), Shandougen (Sophorae Tonkinensis Radix et Rhizoma), Longkui (Solani Nigrum Herba), Guizhencao (Bidens Pilosae Herba), Machixian (Portulacae Herba), Shuifeiji (Silybi Mariani Fructus), Baijiangcao (Patriniae Herba), Fengweicao (Herba Pteridis Multifidae), Bailian (Ampelopsis Radix), Banbianlian (Lobeliae Chinensis Herba), Banzhilian (Scutellariae Barbatae Herba), Heye (Nelumbinis Folium), Shegan (Belamcandae Rhizoma), Banlangen (Isatidis Radix), Chuanxinlian (Andrographitis Herba)	25/146
Blood-activating and stasis-eliminating medicine	Chuanniuxi (Cyathulae Radix), Honghua (Carthami Flos), Guijianyu (Euonymi Ramulus), Yujin (Curcumae Radix), Niuxi (Achyranthis Bidentatae Radix), Duyiwei (Herba Lamiophlomis), Yuejihua (Rosae Chinensis Flos), Shanzha (Crataegi Folium), Yimucao (Leonuri Herba), Mabiancao (Verbenae Herba), Jianghuang (Curcumae Longae Rhizoma), Guangzao (Choerospondiatis Fructus), Wangbuliuxing (Vaccariae Semen)	13/146

4 Discussion

4.1 Enrichment analysis of autophagy genes in DKD

Autophagy is one of the major pathological mechanisms involved in the progression of DKD, particularly basal autophagy in renal cells, which is essential for maintaining renal homeostasis, structure, and function [12]. Under stress conditions, autophagy undergoes changes as part of the adaptive response of renal cells and is strictly regulated by signaling pathways that can modulate cellular autophagic flux. Furthermore, dysregulated autophagic contributes to the pathogenesis of acute kidney injury, incomplete renal repair after acute kidney injury, and chronic kidney diseases of various etiologies, DKD in particular. Currently, clinical screening for DKD primarily relies on microalbuminuria as an important indicator, but 20% to 60% of patients still demonstrate normal urine protein levels when renal function is impaired [13]. In addition, renal biopsy is considered the gold standard for diagnosing kidney diseases but is rarely used in the early diagnosis of DKD due to its considerable invasive nature and potential harm to the kidney. Therefore, there is an urgent need to identify the core autophagy-related genes in DKD and analyze their pathogenesis. Based on this, the present study employs bioinformatics techniques and machine learning algorithms to identify these genes in DKD and elucidate their potential regulatory mechanisms, aiming to provide new insights into the clinical prevention and treatment of DKD.

First, we conducted a preliminary enrichment analysis of the 37 eligible autophagy-related genes in DKD. In the GO analysis of these genes, we found that they are involved in as many as 681 biological mechanisms, primarily including 610 BPs such as regulation of autophagy, cellular response to chemical stress, and cellular response to external stimuli. This indicates that autophagy genes play a central role in regulating autophagic activity during the onset and progression of DKD, suggesting that autophagy may be a key response mechanism of the body to intracellular environmental changes and external stimuli. A total of 41 MFs were identified, including cysteine-type endopeptidase activity, cysteine-type peptidase activity, and cysteine-type endopeptidase activity involved in the apoptotic process. These MFs are closely related to protein hydrolysis and processing, particularly in the involvement of cysteine-type endopeptidase activity in the apoptotic process, further implying a tight connection between autophagy and apoptosis. They are primarily localized in 30 CCs, such as membrane raft, membrane microdomain, and autophagosome. The autophagosome, as a hallmark structure in the autophagy process, signifies the crucial role of these autophagy genes in autophagosome formation and function. Membrane structures, such as membrane raft and membrane microdomain, may serve as aggregation and action sites for autophagy-related proteins or signaling molecules, providing a specific spatial environment and material basis for the autophagy process. The localization of autophagy genes in these specific CCs plays a beneficial part in a deeper understanding of the precise cellular sites and molecular interaction networks involved in autophagy initiation. It also offers a theoretical foundation for subsequent research on intervention strategies targeting specific membrane structures or organelles.

The KEGG enrichment analysis identified 112 relevant signaling pathways. Among these, the pathways related to apoptosis, Kaposi sarcoma-associated herpesvirus infection, and autophagy (animal) in the context of DKD autophagy are particularly noteworthy. The enrichment of the apoptosis pathway further confirms the close association of autophagy with apoptosis in DKD, suggesting that they may mutually influence and regulate each other, jointly determining the outcome of renal cells [14]. The emergence of the Kaposi sarcoma-associated

herpesvirus infection pathway indicates a potential interaction between virus infection-related signaling pathways and the autophagy mechanism in DKD [15]. Although its specific mechanism of action in DKD remains unclear, it is closely associated with the onset and progression of end-stage renal disease, providing a new research direction for exploring novel pathogenic factors or therapeutic targets for DKD. The enrichment of the autophagy (animal) pathway strongly supports the involvement of autophagy genes in the classic autophagy pathway in DKD. Moreover, we initially revealed the interaction between core autophagy genes and their potential links with other genes in key pathways such as apoptosis and inflammation through PPI analysis. By delving into the changes in and interactions of various molecules within these pathways, we can more comprehensively elucidate the molecular mechanisms of DKD autophagy, providing key insights for developing therapeutic strategies based on autophagy regulation [16].

4.2 Analysis of the four autophagy-related genes

The protein encoded by the *PPP1R15A* gene involved in intracellular signal transduction and metabolic regulation [17]. In renal diseases, *PPP1R15A* may affect the autophagy pathway by regulating cell apoptosis, inflammatory response, oxidative stress, and pyroptosis, thereby influencing glomerular filtration and renal tubular reabsorption functions and intervening in renal injury [18]. The protein encoded by the *HIF1α* is activated under hypoxic conditions and participates in the cellular adaptive response to hypoxia [19]. In DKD, *HIF1α* may promote cellular adaptation to hypoxia by regulating the autophagy pathway, thus mitigating renal tissue damage. Study has shown that hyperglycemia and hypoxia upregulate *HIF1α* expression, while downregulation of *HIF1α* can reduce inflammation and oxidative stress levels in HUVECs. The HIF1α/Jumonji domain-containing protein 1A (JMJD1A) signaling pathway plays a role in the inflammation and oxidative stress induced by hyperglycemia and hypoxia in human umbilical vein endothelial cells (HUVECs) [20]. The study found that as renal function declines, serum HIF1α levels in patients gradually increase, indicating that HIF1α levels may be related to the progression of DKD [21]. Animal experiments showed that the improvement in urinary protein levels in diabetic nephropathy mice by the drug was associated with the regulation of autophagy and the reduction of HIF1α expression [22].

The protein encoded by the *DLC1* gene is a Rho GTPase-activating protein engaged in the regulation of the cytoskeleton and cell motility [23]. In DKD, *DLC1* may affect the structure and function of the glomerular basement membrane by regulating the autophagy pathway. However, some research has shown that no histological or clinical differences were observed between *DLC1* knockout mice and wild-type mice, and regulating DLC1 expression alone does not impair renal and hepatic function in mice, suggesting that further research on this gene is warranted [24]. The protein encoded by the *CLN3* gene is a lysosomal membrane protein that regulates lysosomal function. In DKD, *CLN3* may participate in the onset and progression of the disease by regulating the autophagy pathway and thereby affecting lysosomal degradation function [25].

Characteristic genes play an important role in benefiting the clinical diagnosis and treatment of DKD. In the external validation set for DKD in this study, the AUC values for *PPP1R15A, HIF1α, DLC1*, and *CLN3* all exceeded the threshold of 0.60, indicating good diagnostic accuracy for these four autophagy-related genes. This further demonstrates that these genes are of considerable importance in assisting clinical diagnosis and treatment of DKD.

4.3 Progress of Chinese medicines intervention in autophagy in DKD

Traditional Chinese medicine (TCM) does not have a corresponding term for DKD, but it is often categorized under conditions such as lower consumption and consumptive disease, with the accumulation of heat-toxicity and blood stasis exacerbating the progression of DKD. TCM treatment for DKD demonstrates marked efficacy and high safety. The types and components of Chinese medicines used in treating DKD are numerous, and their mechanisms of action are also complex and diverse. This study focuses on autophagy genes in DKD as targets and reversely explores Chinese medicines that participate in regulating these autophagy-related genes. This provides a new direction for drug screening in exploring the potential role of Chinese medicines in regulating autophagy in DKD and in its treatment. Among the 146 kinds of Chinese medicines screened, heat-clearing and detoxifying medicine and blood-activating and stasis-resolving medicine make up a large proportion. Antipyretic detoxicate drugs, such as Shidagonglaogen (Mahoniae Radix), Gonglaomu (Mahoniae Caulis), and Jiubiying (Sargentodoxae Caulis), are commonly used in TCM theory to eliminate heat and toxins from the body, which may have a positive effect on strengthening the inflammatory state of patients with DKD [26]. Meanwhile, medicines that promote blood circulation and remove blood stasis, such as Chuanniuxi (Cyathulae Radix), Honghua (Carthami Flos), and Guijianyu (Euonymi Ramulus), are mainly used to promote blood circulation and dissipate blood stasis [27]. These Chinese medicines may have a reparative effect on renal damage owing to microcirculatory disturbances in DKD patients. Further observation revealed that certain Chinese medicines, such as Tufuling (Smilacis Glabrae Rhizoma) and Muhudie (Oroxyli Semen), are associated with multiple autophagy-related genes,

showing that they may have a multi-target mechanism of action and can regulate multiple biological processes related to DKD autophagy simultaneously [28]. Additionally, blood-activating stasis-removing drugs such as Honghua (Carthami Flos), although primarily associated with the *HIF1α* gene, may also indirectly promote renal repair and regeneration by influencing the autophagy process [29]. The study found that a Chinese medicines formula primarily containing Chuanniuxi (Cyathulae Radix) can reduce podocyte apoptosis in the kidneys and inhibit the mRNA expression of *HIF1α* [30]. Furthermore, it found that Honghua (Carthami Flos) intervention could upregulate the expression of p62 protein through the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) pathway, while reducing the levels of Beclin1 and microtubule-associated protein 1 light chain 3 (LC3) II/LC3 I proteins, thereby inhibiting autophagic activity, enhancing neuronal homeostasis, and exerting neuroprotective effects [31]. Taohong Siwu Decoction could upregulate the expression of Bcl-2 and the ratio of LC3 II/LC3 I in hypoxia-induced H9C2 rat cardiomyocytes, while inhibiting the expression of Bax and p62, thus promoting cellular autophagy, inhibiting cardiomyocyte apoptosis, and exerting a protective effect on cardiomyocytes [32]. Additionally, the active component of Honghua (Carthami Flos), safflor yellow, can boost autophagy in rat brain vascular endothelial cells, reduce the apoptosis levels of these cells, and alleviate endothelial injury, providing a protective effect [33]. These results further suggest that Chinese medicines can improve the progression of DKD by regulating key signaling pathways related to autophagy, offering better treatment options for DKD. However, their actual efficacy and safety still need to be validated through rigorous clinical trials despite the theoretical potential of these medicines in treating DKD.

Meanwhile, this study has some limitations. First, gene set enrichment analysis was not employed to elucidate the dynamic changes of characteristic genes during the progression of DKD; second, other machine learning methods (such as random forests or neural networks) were not introduced for cross-validation. The current research results may have certain biases and uncertainties due to several limitations. These include the limited amount of data, the lack of molecular docking analysis to validate the predicted herbal medicine targets, and the absence of clinical data verification. Additionally, the mechanisms of action of Chinese medicines are often complex and diverse. Future research needs to further explore how these Chinese medicines interact with autophagy-related genes and how they promote the pathological state of DKD by regulating the autophagy process. Therefore, we hope to further expand the sample size or use an independent cohort for further validation in future studies, and to verify the predicted effects of Chinese medicine targets and active ingredients through animal experiments, in order to more comprehensively assess the diagnostic efficacy of the characteristic genes. In addition, the effectiveness and safety of these Chinese medicines should be further verified in order to provide better treatment options for patients with DKD.

5 Conclusion

In summary, this study has preliminarily unveiled the autophagy-related BPs, MFs, CCs, and signaling pathways associated with DKD by utilizing bioinformatic analysis of the gene sets GSE30528, GSE30529, and GSE1009. It also identify diagnostic feature genes for DKD and predicted potential Chinese medicines for DKD treatment based on these feature genes, offering new insights and directions for drug screening and therapeutic strategies for DKD.

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Competing interests

The authors declare no conflict of interest.

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基于生物信息学分析糖尿病肾病自噬特征基因及潜在中药预测

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【摘要】目的 基于生物信息学分析预测糖尿病肾脏疾病（DKD）自噬相关的发病机制和关键诊断基因，并预测相关中药。**方法** 采用基因表达综合库（GEO）中测序芯片 GSE30528、GSE30529 和 GSE1009 的数据，从 GSE30528 和 GSE30529 芯片中鉴定出校正后 *P* 值 < 0.05 的差异表达基因（DEGs），结合人类自噬基因库，对获得的 DKD 自噬特征基因进行基因本体（GO）、京都基因与基因组百科全书（KEGG）通路富集分析及蛋白质相互作用（PPI）网络分析，再通过最小绝对收缩和选择算子（LASSO）回归和支持向量机-递归特征消除（SVM-RFE）算法获取自噬特征基因。通过使用微阵列 GSE1009 的外部验证集进行分析来评估这些基因的诊断能力，并使用 SymMap 数据库反向预测相关中药。**结果** GSE30528 和 GSE30529 芯片数据共筛选出 2014 个 DEGs，鉴定出 37 个 DKD 自噬相关基因，GO 分析显示 681 个包括自噬调节和细胞膜微区域活性在内的生物学机制，KEGG 富集分析获得 112 条相关信号通路，PPI 网络分析表明 DKD 自噬相关基因显著富集。通过 LASSO 回归和 SVM-RFE 算法确定了 4 个 DKD 自噬的核心诊断基因：蛋白磷酸酶 1 调控亚基 15A（*PPP1R15A*）、缺氧可诱导因子 1α 亚基（*HIF1α*）、肝癌缺失 1（*DLC1*）和蜡样色素脂褐质沉积神经元型 3（*CLN3*），且外部验证集表明这些基因的诊断效率较高。最终通过 SymMap 数据库预测出 146 味潜在中药，其中清热解毒药和活血祛瘀药类占比最大，分别为 25/146 和 13/146。**结论** 本研究从生物信息学测序数据库分析和验证，明确 DKD 自噬的潜在分子机制，预测关键诊断基因、潜在治疗靶点以及相关中药，为临床研究及应用奠定了坚实基础。

【关键词】生物信息学；差异表达基因；糖尿病肾脏疾病；自噬基因；中药预测