THE LINK BETWEEN METABOLIC SYNDROME AND CHRONIC KIDNEY DISEASE: FOCUS ON DIAGNOSIS AND THERAPEUTICS

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THE LINK BETWEEN METABOLIC SYNDROME AND CHRONIC KIDNEY DISEASE: FOCUS ON DIAGNOSIS AND THERAPEUTICS

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Table of Contents

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Editorial: The link between metabolic syndrome and chronic kidney disease: Focus on diagnosis and therapeutics

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Editorial on the Research Topic

The link between metabolic syndrome and chronic kidney disease: Focus on diagnosis and therapeutics

Metabolic syndrome (MS), a summation of interrelated disorders (obesity, dyslipidemia, hyperglycemia and hyperuricemia, etc.), is related to proteinuria and incident chronic kidney disease (CKD). These metabolic disorders lead to various kidney dysfunction, including obesity-related kidney disease (ORKD), diabetic kidney disease (DKD), gouty nephropathy and even end-stage renal disease (ESRD). The increasing prevalence of MS attracts much attention to MS-related renal injury. However, the pathobiology and pathophysiology of kidney injury are different due to various metabolic risk factors; this increases the difficulty of diagnosis and therapy for MS-induced CKD. Thus, studies on interactions of MS-related diseases with CKD are essential for a better understanding of diagnosis and therapeutic strategies for metabolic-induced CKD. This Research Topic provides a platform for recent advances in diagnosis and therapeutic strategies for MS-related CKD. The special issue represents a collection of 6 original research articles and 5 review articles ranging from laboratory to patient-oriented studies.

Due to metabolic disorders and impaired renal function, the incidence of MS is significantly more prevalent in CKD patients than in the general population. Thus, MS has become an obvious risk factor for CKD. Lin et al. provide a comprehensive discussion on the diagnosis and treatment of MS and CKD. This review presents a comparison of several MS criteria and analyzes their differences. The authors summarize the epidemiology, pathogenesis, diagnosis, and treatment advances of MS and MetS-related renal injury. And interventions for MetS-related kidney damage are also the focus of this review.

Obesity, the hallmark characteristic of MS, has become a worldwide epidemic associated with several complications. Adipose accumulation and renal lipotoxicity lead to

inflammation and glomerular injury. Original research studies by Ye et al. showed that empagliflozin, sodium-glucose cotransporter 2 inhibitor, reduced obesity-renal injury by activating heme oxygenase-1(HO-1)–adiponectin axis. Transcriptome analysis indicated that empagliflozin influences key genes closely related to inflammation and NLRP3 inflammasome. The study provides new knowledge concerning potential targets for ORCD. Additionally, obesity is related to many adverse pregnancy outcomes and health status of offspring. The review by Wei et al. concluded the role of obesity in infertility development, fetus growth, the health of offspring and the occurrence of CKD. Meanwhile, they also outlined the therapeutic effect of weight loss on pregnancy and ORCD.

DKD, the most devastating microvascular comorbidity of diabetes, has been the leading severe cause of ESRD. Screening and identifying special biomarkers for diagnosis and therapeutic of DKD is essential. Using bioinformatics algorithms (WGCNA, LASSO, SVM-RFE and RF) and Venn diagrams, Han et al. finalized two powerful genes relevant to infiltrating immune cells (PRKAR2B and TGFBI) as diagnostic biomarkers for DKD, which were further validated in the test data. They developed a diagnostic model that combines these two genes to assess the risk of glomerular injury. Another research group, Wei et al., identified 176 up-regulated and 91 down-regulated genes by building a protein interaction network to select hub pathogenic genes. Four of these hub genes, FOS, EGR1, ATF3 and JUN, were closely linked to immune response or inflammatory genes in early DKD. These two studies demonstrate that immune response or inflammatory genes are associated with DKD.

Patients with ESRD are at risk for various complications and therapy difficulties. For example, uremic peripheral neuropathy is a serious neurological complication in CKD stage 5 dialysis (CKD5D). Li et al. studied the use of shear wave elastography (SWE) in diagnosing peripheral neuropathy in hemodialysis patients (CKD5D). The authors carried out Young's modulus measurements of the tibial nerve. It was found that the Young's modulus of the tibial nerve was 48.35 kPa, which is the best threshold for diagnosing uremic peripheral neuropathy in CKD5D.

Besides diagnosis, the optimal treatment for DKD also remains a major challenge. TangShenWeiNing formula (TSWN) is an expertly developed traditional Chinese herbal formula. And TSWN has been used clinically for over 20 years to treat DKD. The original article by Chang et al. revealed that TSWN reduced albuminuria and renal fibrosis and prevented renal cell apoptosis by modulating SIRT1/HIF-1a signaling in diabetic mice kidneys. This result indicates that TSWN has a significant protective effect on DKD therapy.

Bone, as a powerful organ, has a powerful endocrine function. Bone cells in the skeleton help regulate phosphorus balance, and the proteins produced by bone cells can influence insulin secretion and regulate glucose metabolism. Focusing on bone-derived hormones such as fibroblast growth factor 23, osteocalcin, sclerostin and lipocalin 2, Li et al. summarize their roles in regulating glucose metabolism and DKD. They concluded that bone-derived hormones are therapeutic targets for diabetes and its complications and are closely tied to insulin secretory, insulin resistance and glucose metabolism.

Peritoneal dialysis is one of the most commonly used alternative therapies for patients with ESRD. However, it has been suggested that urgent initiation of peritoneal dialysis (USPD) may carry the risk of catheter mobilization and dialysate outflow. Hu et al., discussed the safety and feasibility of a ≤24-hour break-in period for diabetic patients receiving USPD. This real-world study found that Break-in Period ≤24 h was not an independent risk factor for complications and technical failure compared to diabetic patients with a Break-in Period >24 h after catheter implantation to start peritoneal dialysis. Therefore, Break-in Period ≤ 24 hours for USPD initiation may be safe and feasible for patients with ESRD.

Primary cilia are a class of organelles that protrude from the surface of eukaryotic cells and have microtubule-based structures that can sense various pericellular signals. In a Mini-Review, Bai et al. focused on the relationship between cilia defects and kidney disease. Studies show that HDAC6, a key regulator of glomerular hyperfiltration-induced cilia breakdown, is downregulated, promoting cilia elongation and accelerating the progression of DKD. Remote control of ciliary motility by lipid nanoparticles targeting renal cilia would be a possible therapeutic target for DKD.

Hyperuricemia, caused by an increase in uric acid, usually leads to gouty nephropathy and increases in severity with the deterioration of kidney function. However, urates might have protection effects *via* their antioxidant properties. Mei et al. focus on the role of uric acid and gout in kidney disease and the problems encountered in the current treatment of gouty kidney disease. Hyperuricemia may participate in CKD development and progression, and uric acid-lowering therapy may slow CKD progression.

In summary, this Research Topic highlights the critical role of various metabolic risks in the progression, diagnosis and treatment of CKD. Metabolic risks have a significant negative impact on CKD. More in-depth research is required to explore the new diagnosis and therapy strategies for MS-related CKD.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Identification and Verification of Diagnostic Biomarkers for Glomerular Injury in Diabetic Nephropathy Based on Machine Learning Algorithms

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Diabetic nephropathy (DN) is regarded as the leading cause of end-stage renal disease worldwide and lacks novel therapeutic targets. To screen and verify special biomarkers for glomerular injury in patients with DN, fifteen datasets were retrieved from the Gene Expression Omnibus (GEO) database, correspondingly divided into training and testing cohorts and then merged. Using the limma package, 140 differentially expressed genes (DEGs) were screened out between 81 glomerular DN samples and 41 normal ones from the training cohort. With the help of the ConsensusClusterPlus and WGCNA packages, the 81 glomerular DN samples were distinctly divided into two subclusters, and two highly associated modules were identified. By using machine learning algorithms (LASSO, RF, and SVM-RFE) and the Venn diagram, two overlapping genes (PRKAR2B and TGFBI) were finally determined as potential biomarkers, which were further validated in external testing datasets and the HFD/STZ-induced mouse models. Based on the biomarkers, the diagnostic model was developed with reliable predictive ability for diabetic glomerular injury. Enrichment analyses indicated the apparent abnormal immune status in patients with DN, and the two biomarkers played an important role in the immune microenvironment. The identified biomarkers demonstrated a meaningful correlation between the immune cells' infiltration and renal function. In conclusion, two robust genes were identified as diagnostic biomarkers and may serve as potential targets for therapeutics of DN, which were closely associated with multiple immune cells.

Keywords: diabetic nephropathy, glomerular injury, biomarker, diagnostic model, machine learning algorithm

INTRODUCTION

Diabetic nephropathy (DN) is a serious cause of end-stage renal disease, resulting in heavy economic and medical burdens. Tubulointerstitial lesions, glomerular basement membrane thickening, mesangial matrix accumulation, and nodular glomerulosclerosis are the basic pathological features of DN (1). The current treatment strategy is either to strengthen glucose

control or to reduce glomerular intracapsular pressure to slow the progression of renal injury (2, 3). In fact, because of the individual heterogeneity of DN, not all patients can benefit from these drugs. Genome-wide expression profiles can be easily obtained from public databases, analyzed, and visualized on the R platform, thanks to the advancement and widespread application of bioinformatics analysis and high-throughput sequencing technology (4, 5). The changes in gene expression profiles involved in the initiation and progression of DN have been identified by high-throughput microarray technology (6).

According to the flow chart shown in **Supplementary Figure S1**, gene expression profiles of DN patients and normal samples were obtained and analyzed to identify the differentially expressed genes (DEGs). Highly associated modules were identified to determine the critical biomarkers, and a diagnostic model was developed based on the biomarkers. Moreover, enrichment analysis was performed to explore the potential mechanisms of the identified biomarkers in DN. It particularly illustrates the relationship between the biomarkers and immune cell infiltration.

MATERIALS AND METHODS

Data Collection and Preprocessing

A total of fifteen human microarray datasets, namely GSE96804, GSE47183-GPL11670, GSE47183-GPL14663, GSE99339-GPL19109, GSE99339-GPL19184, GSE104948-GPL22945, GSE104948-GPL24120, GSE30122, GSE1009, GSE30528, GSE30529, GSE47184-GPL11670, GSE47184-GPL14663, GSE104954-GPL22945, and GSE104954-GPL24120, were downloaded from the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/). More details of the collected datasets are presented in **Table 1**. After eliminating the batch effects by the Surrogate Variable Analysis (SVA) algorithm (7), seven glomerular DN (GDN) datasets (GSE96804, GSE47183-GPL11670, GSE47183-GPL14663, GSE99339-GPL19109, GSE99339-GPL19184, GSE104948-GPL22945, GSE104948-GPL24120), three GDN datasets (GSE30122, GSE1009, GSE30528), and five tubulointerstitial DN (TDN) datasets (GSE30529, GSE47184-GPL11670, GSE47184-GPL14663, GSE104954-GPL22945, GSE104954-GPL24120) were merged, normalized, and utilized as the GDN training cohort, GDN testing cohort, and TDN testing cohort, respectively. The distribution patterns between DN and normal samples were visualized by principal component analysis (PCA).

Identification of DEGs

DEGs between GDN and normal subjects in the GDN training cohort were detected by using the limma R package (8) with |log2 fold change (FC)|>1 and adjusted $p < 0.05$ as the cutoff threshold. Meanwhile, Gene Ontology (GO) enrichment analysis of DEGs was conducted using the clusterProfiler package. Gene Set Enrichment Analysis (GSEA) was also performed to investigate the significant differences in Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways between GDN and normal samples, with the Molecular Signature Database (MSigDB)-derived gene sets "c2.cp.kegg.v7.4.symbols.gmt" selected as a reference. Enriched pathways with $p < 0.05$ and false discovery rate (FDR) <0.25 were considered statistically significant.

Consensus Cluster Analysis

The ConsensusClusterPlus algorithm (9) was used to perform clustering analysis to identify potential subclusters of the GDN samples from the GDN training cohort. The maximum cumulative distribution function (CDF) index was selected as the optimal *k*-value. Meanwhile, principal component analysis (PCA) was employed to verify this classification based on gene expression patterns among different subgroups.

Weighted Gene Coexpression Network Analysis

The Weighted Gene Coexpression Network Analysis (WGCNA) method (10) was applied to build potential modules related to different subclusters of the 81 GDN samples. After filtering abnormal samples and calculating the Pearson correlation coefficient, the correlation adjacency matrix was constructed. Highly associated modules were selected for subsequent analysis. Functional enrichments of the genes within given modules were

TABLE 1 | The essential information of included microarray datasets in this study.

GEO series	Normal	DN	Tissue	Data type
GSE96804	20	41	Glomerulus	Training
GSE47183-GPL11670	0	7	Glomerulus	Training
GSE47183-GPL14663	0	7	Glomerulus	Training
GSE99339-GPL19109	0	7	Glomerulus	Training
GSE99339-GPL19184	0	7	Glomerulus	Training
GSE104948-GPL22945	18	7	Glomerulus	Training
GSE104948-GPL24120	3	5	Glomerulus	Training
GSE30122	13	9	Glomerulus	Testing
GSE1009	3	3	Glomerulus	Testing
GSE30528	13	9	Glomerulus	Testing
GSE30529	12	10	Tubulointerstitium	Testing
GSE47184-GPL11670	0	7	Tubulointerstitium	Testing
GSE47184-GPL14663	4	11	Tubulointerstitium	Testing
GSE104954-GPL22945	18	7	Tubulointerstitium	Testing
GSE104954-GPL24120	3	10	Tubulointerstitium	Testing

performed to interpret the diverse biological effects based on the KEGG, GO, and Disease Ontology (DO) analyses using the ClusterProfiler, DOSE, and ggplot2 packages.

Diagnostic Gene Screening and Diagnostic Model Construction

The Least Absolute Shrinkage and Selection Operator (LASSO) logistic regression (11), Support Vector Machine-Recursive Feature Elimination (SVM-RFE) (12), and Random Forest (RF) (13) algorithms were employed independently to screen the diagnostic genes from the selected modules. Ultimately, genes that overlapped among the three machine learning algorithms were regarded as diagnostic biomarkers. A receiver operating characteristic (ROC) curve was generated, and the area under the ROC curve (AUC) value was calculated to estimate the predictive utility of the identified biomarkers using the pROC package. The differential expression and predictive reliability of the biomarkers were further confirmed in the external testing cohorts. A diagnostic model was constructed using logistic regression analysis and visualized as a nomogram (14) to predict the glomerular injury in DN patients. The Concordance index (C-index), calibration curve, and decision curve analysis (DCA) were employed to visualize its discrimination performances. Besides, using the training datasets (**Table 1**), the expressions of the identified biomarkers were also explored in other chronic kidney diseases (CKD), including hypertensive nephropathy (HN) and systemic lupus erythematosus nephropathy (SLEN). Furthermore, based on the median expression level of each gene, 81 GDN samples from the GDN training dataset were divided into two groups (high- and low-expression group), and then Gene Set Variation Analysis (GSVA) was employed to clarify the enriched KEGG pathways with MSigDB gene sets "c2.cp.kegg.v7.4.symbols.gmt" used as a reference.

Verification and Clinical Correlation Analysis of the Identified Biomarkers

The expression patterns of identified biomarkers were reconfirmed by the Nephroseq v5 online database (http://v5.nephroseq.org) (15). A correlation analysis between the biomarkers and renal function was also carried out.

Evaluation of Immune Cell Infiltration

Based on the single-sample Gene-Set Enrichment Analysis (ssGSEA) method and the 29 gene sets of immune-related responses (16), the ssGSEA scores were quantified and designed to represent the activity and infiltrating fractions of immune cells and pathways in the GDN training cohort and the TDN testing cohort. The result of ssGSEA was shown as a heatmap. Furthermore, the cell-type identification by estimating relative subsets of RNA transcripts (CIBERSORT) algorithm (17) was performed to calculate the relative proportion of the infiltrating immune cells in each sample from the GDN training cohort and the TDN testing cohort. The abundances of infiltrating immune cells in DN patients and normal subjects were compared and visualized using the vioplot package. The differences in immune characteristics between the samples with low and high expression of the identified biomarkers were clarified. In the GDN training cohort, using the corrplot package, the correlations between the enrichment levels of infiltrating immune cells and the expressions of the diagnostic genes were also investigated.

Animal Experiments

A total of 15 male C57BL/6 mice (8 weeks old; ~25 g) were purchased from the Chongqing Medical University Animal Experiment Center (Chongqing, China). Mice were randomly divided into normal groups (n = 5) and high-glucose-induced renal injury models (n = 10) and given access to a normal chow diet (NCD) or a high-fat diet (HFD) for 4 weeks. A mouse model of hyperglycemia was induced by an intraperitoneal injection of streptozotocin (STZ; Sigma-Aldrich, USA). The random blood glucose levels ≥16.7 mmol/L 72 h after the injection were considered a successful establishment (18). At the end of 8 weeks, five NCD mice and six HFD/STZ-induced mice were fasted overnight, blood and 24-h urine samples were collected, and then mice were sacrificed. The kidney was harvested for subsequent study. All animal experiments were carried out following the Guide for the Care and Use of Laboratory Animals, and the procedures were approved by the Research Ethical Committee of Chongqing Medical University.

Blood glucose levels were measured using the Roche Dynamic Blood Glucose Monitoring System (Roche, Mannheim, Germany) by blood sampling from the tail vein. Urine albumin, blood urea nitrogen (BUN), and serum creatinine (Scr) were detected using an automatic biochemical analyzer (Hitachi, Tokyo, Japan). The obtained renal tissues were fixed, embedded, and cut into slices. Subsequently, hematoxylin and eosin (H&E), Masson, Periodic Acid-Silver (PAS), Oil Red O staining, and immunofluorescence (IF) staining for the selected biomarkers were performed. The stained slices were visualized and pictured with a light or fluorescence microscopy (Olympus, Tokyo, Japan). According to the manufacturer's instructions, the RT-qPCR was performed. The $2^{-\Delta\Delta Ct}$ method was used to quantify protein kinase cAMP-dependent regulatory type II beta (PRKAR2B) and transforming growth factor-beta-induced (TGFBI) expression with GAPDH as an internal control. The primer sequence is shown in **Supplementary Table S1**. A Western blot analysis was carried out. Primary antibodies against PRKAR2B (Santa Cruz, CA, USA) and antibodies against TGFBI (Abcam, Cambridge, UK) were used, respectively.

Statistical Analysis

All statistical analysis was performed using the R software (version 3.6.3) or GraphPad Prism 8.0 (GraphPad Software, CA, USA). A Wilcoxon test was performed to compare immune cell infiltration and the identified biomarker expressions between normal subjects and DN patients. The logistic regression algorithm was used to develop the predictive model. A ROC curve was used to judge the diagnostic accuracy of selected biomarkers. Correlation analysis was realized by Pearson's analysis. Moreover, an unpaired t-test was used to analyze the RT-qPCR, Western blot data, biochemical detection data, the differential expression levels of the two biomarkers from the Nephroseq v5 online database, and the differential expression levels of the two biomarkers in other CKD.

If not specially indicated, $p < 0.05$ was defined as statistical significance.

RESULTS

Identification of DEGs and Enrichment Analysis

There was a clearly pronounced discrimination between GDN and normal samples (**Figure 1A**). A total of 140 DEGs were identified including 75 upregulated and 65 downregulated genes, displayed in the Volcano plot and heatmap (**Figures 1B, C**). These DEGs were mainly involved in the biological processes associated with the extracellular structure organization and tumor necrosis factor production ($p < 0.05$, **Figure 1D**). The results of GSEA illustrated that metabolism-related pathways were enriched in the normal samples, while the immune-related signaling pathways were enriched in the GDN subjects (**Figure 1E**).

Unsupervised Cluster Construction and Key Module Identification

With the batch effects stripped, the consensus clustering was performed based on the gene expression profiles of the merged 81

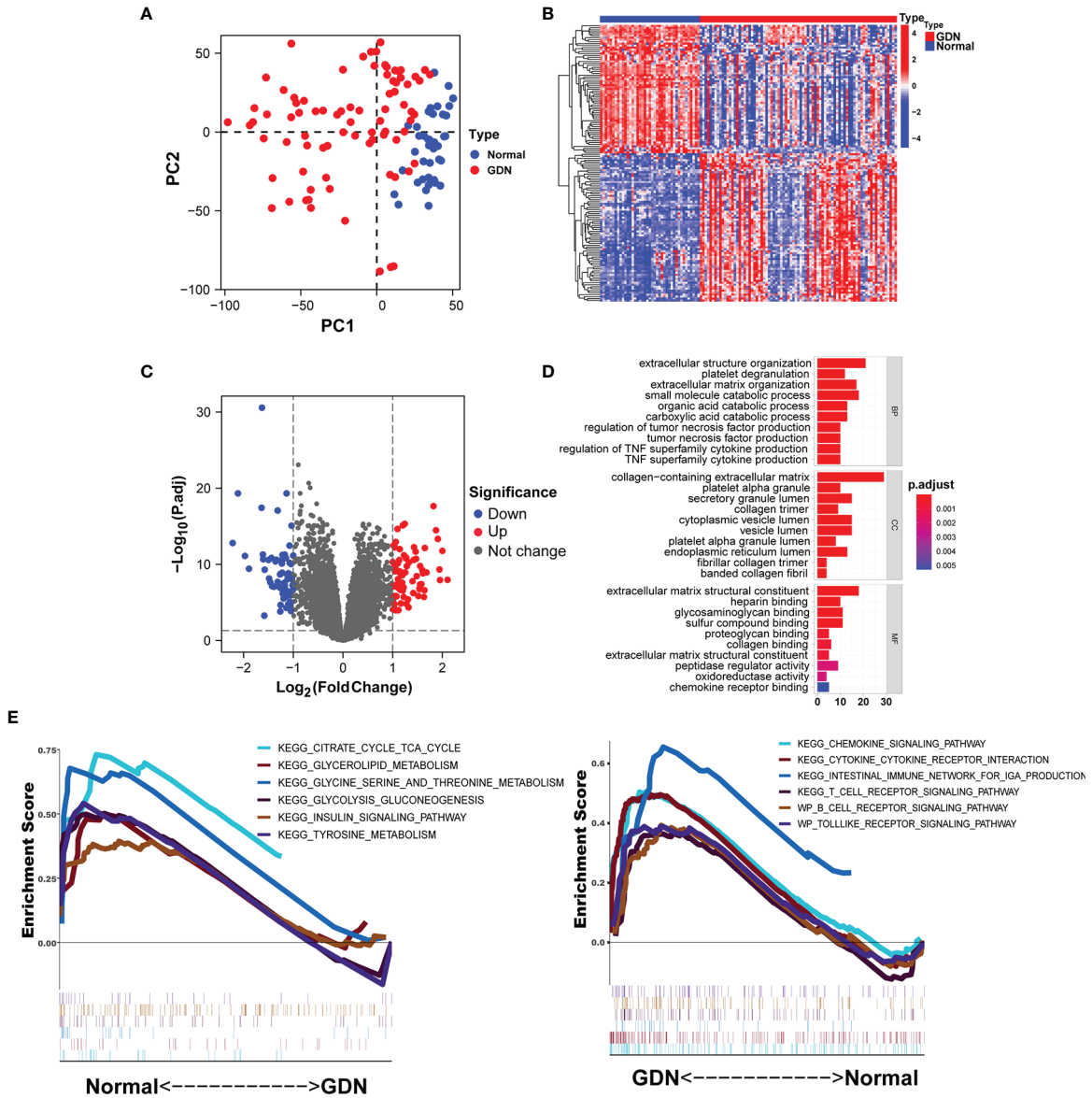

FIGURE 1 | Identification of DEGs in the GDN training cohort. **(A)** The principal component analysis (PCA) for the samples. **(B, C)** Heatmap and the Volcano plot of the DEGs. **(D, E)** Six enriched signaling pathways in normal or DN samples.

GDN samples in the GDN training cohort, and when k = 2, the classification was highly reliable and stable (**Figures 2A–C**). PCA confirmed that there was a distinct difference between the two subclusters (**Figure 2D**). GDN samples were divided into cluster 1 (C1, N = 48) and cluster 2 (C2, N = 33). With the soft-threshold power of β = 12 (scale-free R^2 = 0.906) set and the corresponding Pearson's correlation coefficient calculated (**Figure 2E**), four modules were identified (**Figure 2F**). Brown and blue modules had the highest correlation with the subclusters, and therefore were selected as the associated modules for further analysis. The genes from the two selected modules were mainly responsible for extracellular structure organization and cytokine chemotaxis

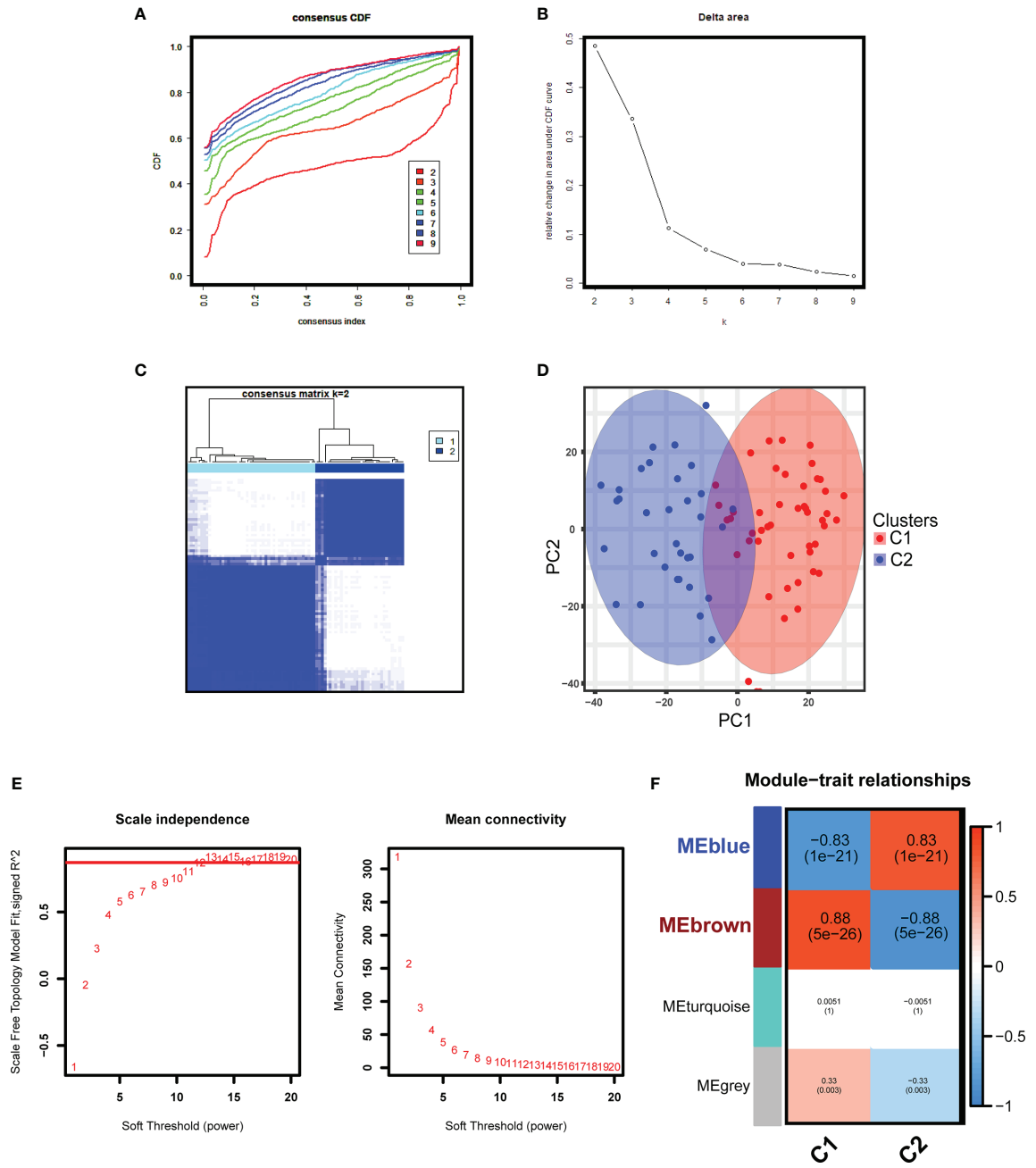

FIGURE 2 | Unsupervised consensus clustering and WGCNA analyses in the GDN training cohort. **(A)** Cumulative distributive function (CDF) for k = 2 to 9. **(B)** Delta diagram showing the variations of the area under the consensus clustering CDF curve for k = 2 to 9. **(C)** Heatmap exhibiting the two clusters of DN samples with k = 2. **(D)** The principal component analysis (PCA) based on the results of consensus clustering analysis. **(E)** Analysis of the network topology for various soft-threshold powers. **(F)** Heatmap displaying the module-trait correlations.

reactions (**Supplementary Figure S2A**). KEGG analysis indicated that they were significantly enriched in complement and coagulation cascades, PI3K-Akt signaling pathway and cytokine–cytokine receptor interaction (**Supplementary Figure S2B**). DO analysis revealed that the genes were mostly involved in urinary system disease, urinary system cancer, and lung disease (**Supplementary Figure S2C**).

Diagnostic Biomarker Identification and Verification

Using the LASSO regression algorithm, 22 genes from the selected modules were identified as potential diagnostic biomarkers (**Figures 3A, B**). By SVM-RFE algorithm, 13 genes were extracted from these modules as candidate biomarkers (**Figure 3C**). Two diagnostic genes were identified by the RF

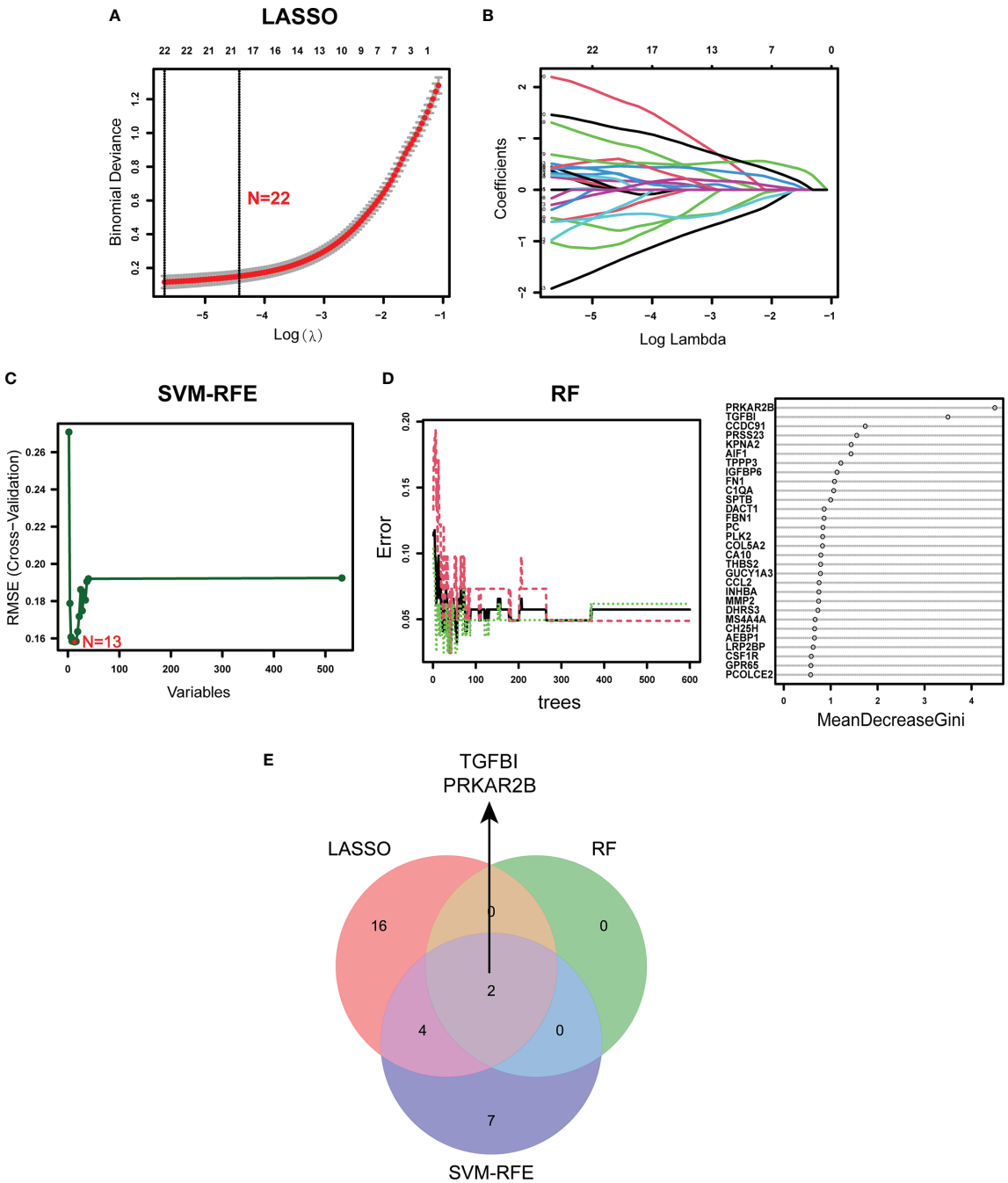

FIGURE 3 | Identification of the diagnostic biomarkers from the selected modules. **(A, B)** LASSO regression analysis. **(C)** SVM-RFE algorithm. **(D)** RF algorithm. **(E)** Venn plot exhibiting the reliable biomarkers among LASSO, SVM-RFE, and RF.

algorithm (**Figure 3D**). Two genes (PRKAR2B and TGFBI) were then overlapped *via* a Venn diagram, and served as robust diagnostic biomarkers (**Figure 3E**). Compared with normal control, decreased PRKAR2B expression ($p < 0.001$) and increased TGFBI expression ($p < 0.001$) were observed in the glomerular samples from the GDN training cohort (**Figure 4A**). The results were validated in the GDN testing cohort, and the consistent gene expression patterns were obtained (**Figure 4B**). Interestingly, the expression of TGFBI was still significantly upregulated in tubulointerstitial samples from the TDN testing

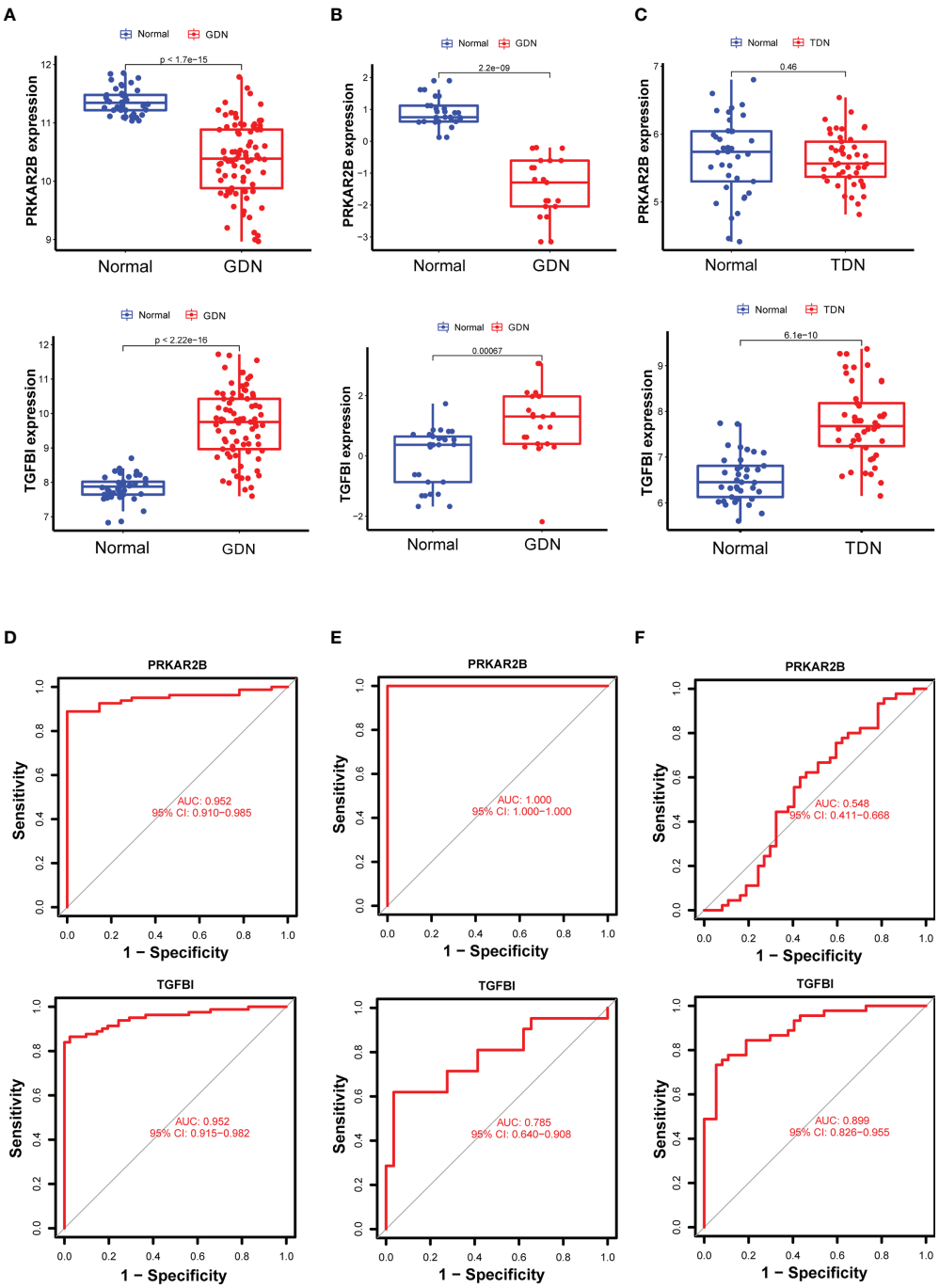

FIGURE 4 | Verification of the identified biomarkers. **(A–C)** Box plots for the differential expression analysis in the GDN training cohort, GDN testing cohort, and TDN testing cohort, respectively. **(D–F)** ROC curves for evaluating the diagnostic ability in the GDN training cohort, GDN testing cohort, and TDN testing cohort, respectively. $p < 0.05$ was considered statistically significant.

cohort ($p < 0.001$), while the expression of PRKAR2B had no significant change (**Figure 4C**). To estimate the predictive utility, the ROC curve was performed and found that the PRKAR2B and TGFBI illustrated a remarkably distinguishing efficiency with AUC values of 0.952 (95% CI: 0.910–0.985) and 0.952 (95% CI: 0.915–0.982) in the GDN training cohort, respectively (**Figure 4D**). Consistently, in the GDN testing cohort, the AUC value of PRKAR2B was 1.000 (95% CI: 1.000–1.000) and that of TGFBI was 0.785 (95% CI: 0.640–0.908) (**Figure 4E**). Unlike the low AUC value of PRKAR2B (0.548, 95% CI: 0.411–0.668), TGFBI still maintained a high AUC value of 0.899 (95% CI: 0.826–0.955) in the TDN testing cohort (**Figure 4F**). Furthermore, the similar expression patterns were also observed in HN and SLEN (**Supplementary Figure S3**).

Establishment of Nomogram

Based on the expressions of PRKAR2B and TGFBI from the GDN training cohort, a diagnostic model was constructed by logistic regression and visualized as a nomogram (**Figure 5A**). The C-index of the diagnostic model was 0.976 with an appropriate calibration plot. Also, the model showed a high AUC value (0.965), confirming the excellent prediction performance (**Figures 5B, C**). Additionally, DCA curves indicated the combined nomogram model showed the highest efficacy in predicting glomerular damage in DN patients compared with other single biomarker models (**Figure 5D**).

Expression Patterns and Clinical Correlation of the Biomarkers

Based on the Nephroseq v5 online tool, the expression patterns of both PRKAR2B and TGFBI in the glomerular and tubulointerstitial tissues of DN patients were further confirmed (**Figures 6A, B**). When compared with normal subjects, PRKAR2B expression was downregulated in DN glomerular tissue but not in DN tubulointerstitial tissue. The TGFBI expression was upregulated in both glomerular and tubulointerstitial tissue of DN patients. Correlation analysis revealed that PRKAR2B expression in DN glomerular tissue was positively correlated with glomerular filtration rate (GFR) ($r = 0.687$, $p = 0.013$) and negatively correlated with Scr ($r = -0.699$, $p = 0.011$) (**Figure 6C**). The TGFBI expression in DN tubulointerstitial tissue was found to be negatively correlated with GFR ($r = -0.749$, $p = 0.0005$) and positively correlated with Scr ($r = 0.664$, $p = 0.003$) (**Figure 6D**). Curiously, the expression of TGFBI in DN glomerular tissue was not associated with GFR and Scr. It suggested that the biomarkers were related to renal function in patients with DN, whereas their roles may be different.

Correlation Between the Two Biomarkers and Immune Cell Infiltration

The immune infiltration landscape in DN was obviously changed (**Supplementary Figure S4**). According to the GSVA results, the gene sets in the GDN samples with high PRKAR2B expression

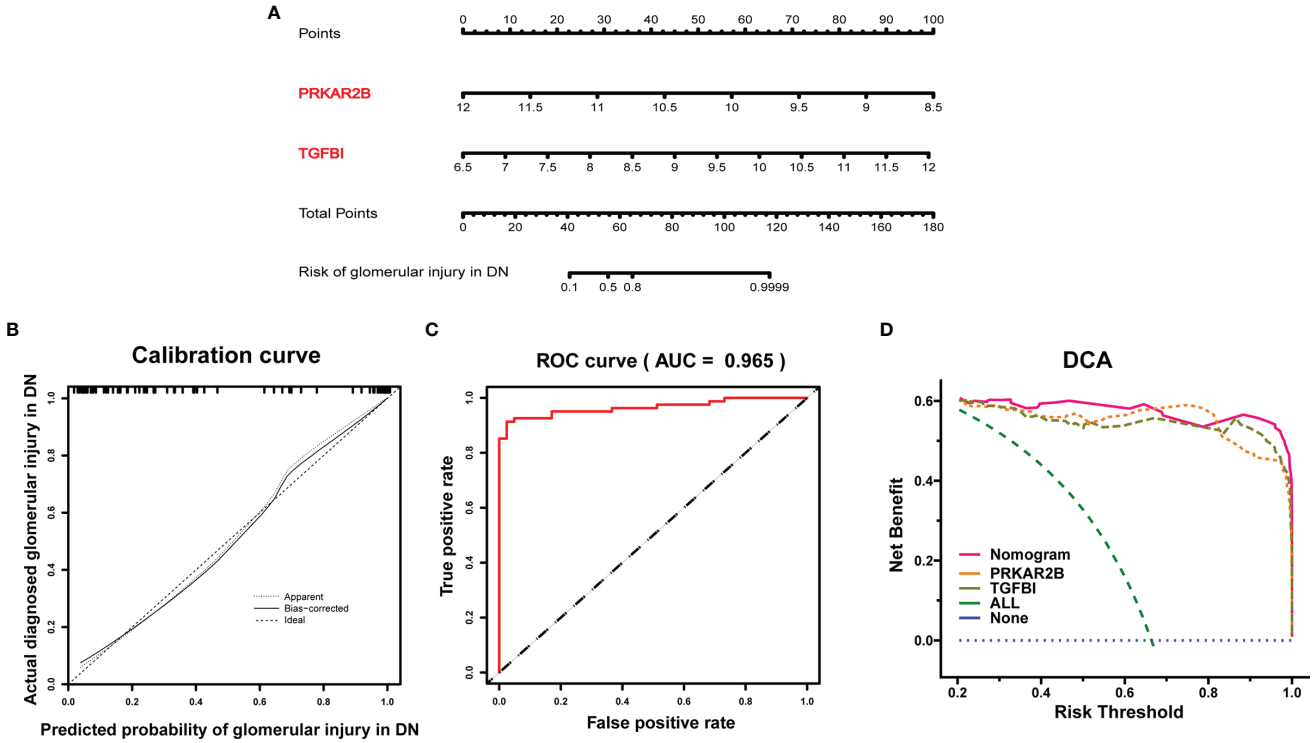

FIGURE 5 | Establishment of the diagnostic model in the GDN training cohort. **(A)** Nomogram for the diagnostic model of glomerular injury. **(B)** Calibration curve. **(C)** ROC curves to evaluate the discrimination ability. **(D)** DCA for the diagnostic model.

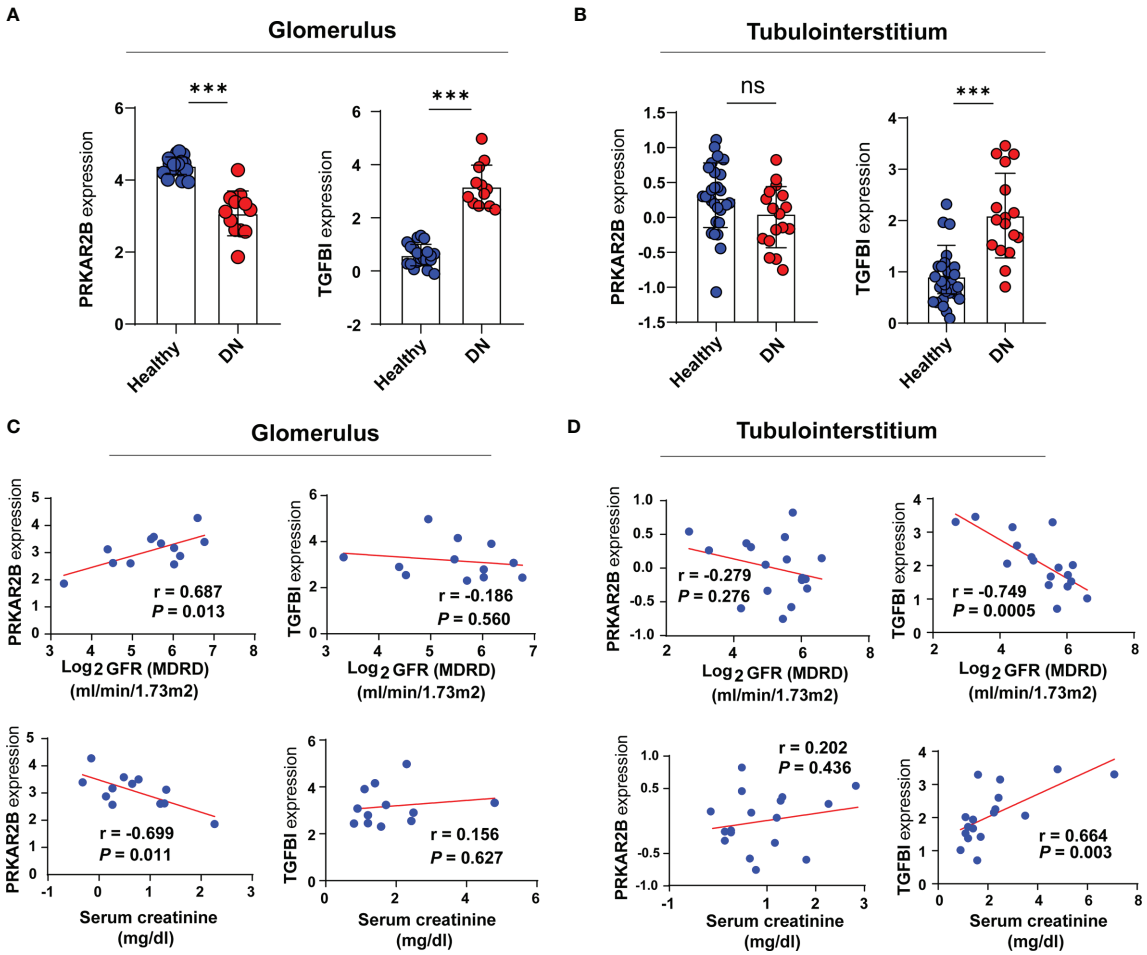

FIGURE 6 | Verification of the two identified biomarkers. **(A, B)** The expression patterns of the identified biomarkers. **(C, D)** Correlation analysis between the expression of the biomarkers and renal function indexes. ***$p < 0.001$ vs. healthy subjects. ns, not significant.

were markedly associated with multiple activated metabolism-related pathways and immune suppression biological functions, as well as the GDN samples with low expression of TGFBI (**Figure 7A**). Thus, given the roles of PRKAR2B and TGFBI in immune regulations, their effects on the immune cells' infiltration and biological processes were also explored. A proliferation of neutrophils, regulatory T cells (Tregs), macrophages, and plasmacytoid dendritic cells (pDCs) were observed. In addition, the activities of check-point, tumor-infiltrating lymphocytes (TIL), chemokine C-C-Motif receptor (CCR), T-cell coinhibition, and type II interferon (IFN) response were markedly enhanced in the GDN subjects with low PRKAR2B expression or subjects with high TGFBI expression (**Figure 7B**). Correlation analysis revealed that the infiltration of naive B cells was most positively correlated with PRKAR2B and was most negatively correlated with TGFBI. However, the infiltration of gamma-delta T cells was most negatively correlated with PRKAR2B and was most positively correlated with TGFBI ($p < 0.001$). More details were exhibited in **Figure 7C**.

Validation in Animal Models

According to the treatment schedule (**Figure 8A**), four mice in the HFD+STZ group did not meet the established protocols and were excluded. The levels of blood glucose, Scr, BUN, and 24 h urinary protein were significantly elevated in the HFD/STZ-induced mice compared with the NCD mice ($p < 0.01$, **Figure 8B**). As shown in **Figure 8C**, glomerular hypertrophy, proliferation of glomerular mesangial cells, dilation of the mesangial matrix, and irregular thickening of the glomerular and tubular basement membrane were observed in the renal tissue of HFD/STZ-induced mouse model. Masson staining revealed the formations of renal blue-stained extracellular collagen, mostly in the glomerular tissue. Oil Red O staining showed the number of lipid droplets increased, and the lipid accumulation in the glomerulus was more obvious than that in the tubuleinterstitium. Thus, the HFD combined with high-glucose-induced renal injury model was considered successfully established. The downregulated PRKAR2B expression in glomerular tissue (**Figure 8D**) and the upregulated TGFBI

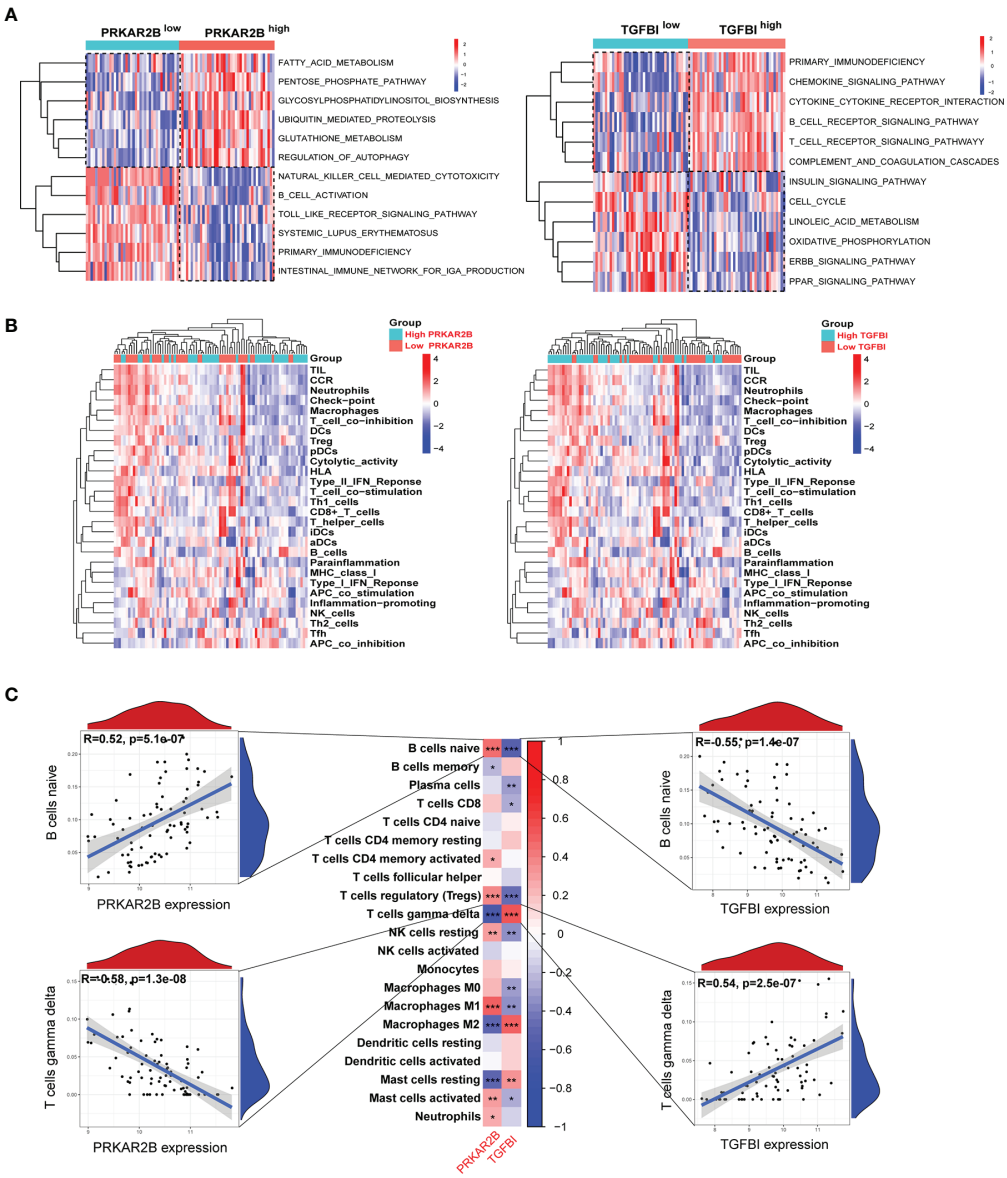

FIGURE 7 | The association between the biomarkers and immune infiltration in the GDN samples. **(A)** Heatmap of metabolism and immune-related gene sets by GSVA. **(B)** Heatmap of the immune landscape by ssGSEA. **(C)** Heatmap of the correlations between the biomarkers and infiltrating immune cells. ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$.

expression in both glomerular and tubulointerstitial tissues of the mouse model were observed (**Figure 8E, F**). Moreover, the reduced PRKAR2B expression and increased TGFBI expression were also confirmed in the renal tissues of the mouse model by RT-qPCR and Western blot ($p < 0.01$, **Figure 8G, H**).

DISCUSSION

Diabetic nephropathy results from the interactions of multiple genes. However, its potential mechanisms remain unclear. Recently, a large number of studies have focused on the screening of related biomarkers. Wang et al. analyzed five DN-associated gene datasets and identified fibronectin 1 (FN1) and complement component 3 (C3) as the immune infiltration-related biomarkers for DN (19). Wang et al. revealed the different pathological abnormalities between glomerulus and kidney tubules in DN and indicated that the changes of key regulated genes in methylation status might contribute to the pathogenesis of DN (20). However, although many efforts have been made to explore novel targets for DN, the present knowledge seems to be insufficient. Potential biomarkers with high specificity and sensitivity are still urgently required.

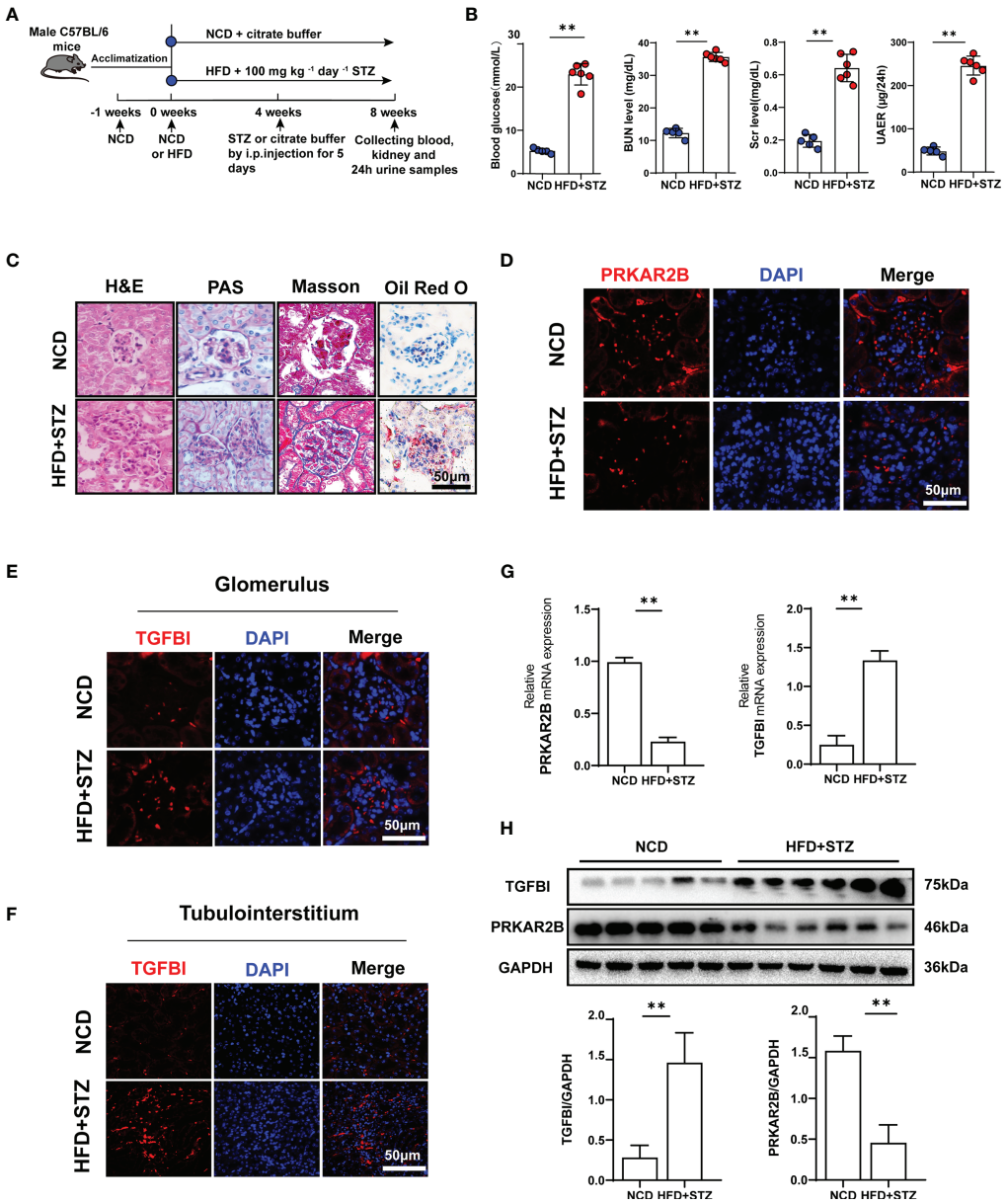

FIGURE 8 | Verification in animal experiments. **(A)** The treatment protocol for mouse model. **(B)** Blood glucose, Scr, BUN, and 24 h proteinuria levels. **(C)** Renal pathological sections stained with H&E, PAS, Masson, and Oil Red O. **(D)** Immunofluorescence staining of PRKAR2B in renal glomerular tissue. **(E, F)** Immunofluorescence staining of TGFBI in glomerular and tubulointerstitial tissues. **(G)** The mRNA expression of PRKAR2B and TGFBI in kidney tissue (RT-qPCR). **(H)** The protein levels of PRKAR2B and TGFBI in kidney tissue (Western blot). $^{**}p < 0.01$ vs. NCD mice. NCD, normal chow diet; HFD, high-fat diet; STZ, streptozotocin.

PRKAR2B is a cAMP-dependent protein kinase (PKA) (21) regulatory subunit that is abundantly expressed in various tumor tissues (22). However, there are few studies on the role of PRKAR2B in the progression of DN. Our study identified that PRKAR2B, with an excellent diagnostic value (AUC >0.95), was downregulated in the glomerulus but there was no significant change in the tubulointerstitium. TGFBI is a secretory protein induced by TGF-β in various cells and can be detected in serum and urine (23, 24). It was demonstrated that TGFBI was involved in the fibrotic processes of chronic cyclosporine-induced nephropathy by affecting the synthesis and degradation of the extracellular matrix (25). In the present study, the expression of TGFBI was upregulated in both glomerular and tubulointerstitial tissues, and it was proved to have a reliable diagnostic ability for DN. It was reported that the expression of TGFBI was prominently increased in the kidneys of diabetic patients, whereas the concentration of TGFBI in urine was also raised (26). Elevated urinary TGFBI concentration has been shown to

predict the prognosis of DN (27). This evidence enhanced the accessibility and feasibility of the clinical applications of TGFBI as a diagnostic marker. However, it is unclear why there was no significant correlation between TGFB expression in glomerular tissue and renal function indexes (such as GFR and Scr) in DN patients. Most notably, a novel diagnostic model combining the two biomarkers was developed with a high AUC value and favorable calibration, which exhibited excellent accuracy and reliability for estimating the glomerular damage in DN patients. Compared with any other single biomarker, the above model showed the highest efficacy for glomerular injury prediction in the GDN training cohort.

In this study, we found that the downregulated PRKAR2B expression in glomerular tissue may indicate the deterioration of kidney function in patients with DN, and so did the upregulated TGFBI in tubulointerstitial tissue. However, similar expression patterns of PRKAR2B and TGFBI were also found in patients with HN or SLEN, which suggested the differential expressions of PRKAR2B and TGFBI were not specific for DN but related to the renal injury.

It had been reported that extracellular matrix organization and extracellular matrix structural constituent lead to the accelerated deposition of extracellular matrix and renal fibrosis in DN (28). In this study, DEGs were demonstrated to be involved in this process in DN glomerular tissue. Multiple metabolism-related pathways were mainly enriched in normal samples, while immune inflammation pathways were mostly concentrated in GDN samples. It confirmed the notion that metabolic disorders and abnormal immune inflammation responses play a critical role in DN (29). Meanwhile, both PRKAR2B and TGFBI were disclosed to be involved in immune-related pathways and cell functions in the glomerular injury of DN. Moreover, both of them were associated with various immune cells such as naïve B cells, gamma delta T cells, Tregs, resting NK cells, resting mast cells, and macrophages. Previous studies reported that the deposition of macrophages, an important feature of DN, could be discovered in the kidney tissue of DN patients, indicating a decline in renal function (30). Mast cells were reported to participate in renal interstitial fibrosis, and the density of mast cells was related to serum creatinine levels in DN (31). It was reported that increased Tregs contributed to the improvement of DN and promoted the transplant tolerance to DN-induced renal allografts (32, 33). However, the roles of naïve B cells and gamma delta T cells in the pathological processes of DN have not been reported. Overall, the infiltrating immune cells are involved in the development and progression of DN. Improving abnormal immune status by targeting PRKAR2B and TGFBI may be a promising approach for the treatment of DN.

Some limitations need to be considered. First, different pathological stages of DN may affect the results of the study. Second, because of the potential heterogeneity from different annotation platforms and clinical covariates of samples, the batch effects cannot be completely eliminated among datasets. Third, the sample size may not be large enough. Finally, the present study was based on public data, so the biological functions of the two biomarkers need to be verified by further experiments.

In this study, using WGCNA, LASSO, SVM-RFE, and RF algorithms, PRKAR2B and TGFBI were identified as the potential biomarkers of DN. A diagnostic model combining PRKAR2B and TGFBI was established to evaluate the risk of diabetic glomerular injury with high sensitivity and accuracy. The potential association with infiltrating immune cells was also demonstrated, providing a fresh perspective on their roles in DN. Therefore, the findings may shed light on the management and treatment of patients with DN.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The animal study was reviewed and approved by the Research Ethical Committee of Chongqing Medical University.

AUTHOR CONTRIBUTIONS

HH, YC, and KL: conceptualization and methodology. HH, YC, HY, SZ, and WC: software and data curation. YL and QL: validation. DL and GY: reviewed and edited the manuscript. KL is the guarantor of this work and, as such, has full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.876960/full#supplementary-material

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ORIGINAL RESEARCH
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TangShenWeiNing Formula Prevents Diabetic Nephropathy by Protecting Podocytes Through the SIRT1/HIF-1α Pathway

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Background: Diabetic nephropathy (DN) represents a major complication of diabetes, and podocyte injury has a critical function in DN development. TangShenWeiNing formula (TSWN) has been demonstrated to efficiently decrease proteinuria and protect podocytes in DN. This work aimed to explore the mechanism by which TSWN alleviates DN and protects podocytes.

Methods: The major bioactive components of TSWN were detected by mass spectrometry (MS) and pharmacological databases. Eight-week-old male C57BLKS/J db/m and db/db mice were provided pure water, valsartan, low dose TSWN, middle dose TSWN and high dose TSWN by gavage for 12 weeks, respectively.

Results: MS and network pharmacology analyses suggested that TSWN might prevent DN through the sirtuin (SIRT)1/hypoxia-inducible factor (HIF)-1α pathway. Diabetic mice showed elevated urinary albumin in comparison with non-diabetic mice, and TSWN decreased urinary albumin in diabetic mice. Histological injury increased in the kidney in diabetic mice, which could be improved by TSWN. Fibrosis and collagen I expression were induced in the diabetic mouse kidney in comparison with the non-diabetic mouse kidney; TSWN alleviated these effects. Apoptosis and cleaved caspase-3 were induced in the diabetic mouse kidney in comparison with the non-diabetic mouse kidney, and TSWN blunted these effects. Podocytes were damaged in the diabetic mouse kidney, which was improved by TSWN. Podocin and nephrin amounts were decreased in the diabetic mouse kidney in comparison with the non-diabetic mouse kidney, and podocalyxin was increased in urine of diabetic animals in comparison with non-diabetic counterparts. After TSWN treatment, podocin and nephrin were raised in the diabetic mouse kidney, and urinary podocalyxin was depressed in diabetic animals. Diabetic mice had lower

SIRT1 and higher HIF-1α amounts in kidney specimens in comparison with non-diabetic mice, and TSWN promoted SIRT1 and inhibited HIF-1α in the diabetic mouse kidney. Moreover, co-staining of SIRT1 and podocin revealed that SIRT1 decreased in podocytes from diabetic mice in comparison with those from non-diabetic mice, and TSWN elevated SIRT1 in podocytes.

Conclusions: This study indicated that TSWN alleviates DN by improving podocyte injury through the SIRT1/HIF-1α pathway in diabetic mouse kidneys.

Keywords: TangShenWeiNing formula, diabetic nephropathy, podocytes (MeSH: D050199), SIRT1, HIF-1α

INTRODUCTION

Diabetic nephropathy (DN) or diabetic kidney disease (DKD) represents a common complication of diabetes mellitus and a major cause of end-stage renal disease (1). Podocyte injury is the major pathogenesis of DN. Podocytes are highly specialized, terminally differentiated cells. Hyperglycemia leads to abnormalities of podocyte-associated proteins and signaling pathways, and podocyte apoptosis accelerates disease progression (2). It is known that podocytes have a limited renewal ability. Podocyte injury has been identified as a major event resulting in proteinuric kidney diseases and renal failure (3). Therefore, a treatment that reduces podocyte injury can reduce urinary albumin, delay kidney function damage, and prevent or ameliorate DN progression.

So far, there is no ideal preventive and treatment methods to effectively delay diabetic kidney damage and prevent podocyte injury. Conventional therapeutic strategies, including glycemic control, weight control, and blockage of the renin-angiotensin-aldosterone system, may not achieve satisfactory therapeutic effects in many clinical practices. On the other hand, attention is being paid to traditional Chinese medicine, which can be used as the first or alternative therapy for the treatment of DN with good clinical efficacy. Increasing attention is paid to the identification and molecular mechanisms of bioactive compounds of traditional Chinese medicine on diabetic renal protection (4).

TangShenWeiNing formula (TSWN) represents a traditional Chinese herbal formula developed by experts of the Department of Traditional Chinese Medicine, Beijing Chao-yang Hospital, Capital Medical University. TSWN has been utilized clinically for treating DN for more than two decades, with favorable effects. Recently, TSWN was shown to reduce proteinuria and protect podocytes in patients with DN (data unpublished), suggesting that TSWN administration may result in pronounced therapeutic effects on DN. However, the mechanism by which TSWN alleviates DN remains undefined.

Network pharmacological analyses have demonstrated that hypoxia-inducible factor (HIF)-1 constitutes one of the main targets of TSWN. HIF represents a heterodimer comprising a constitutively expressed β-subunit and at least one of the oxygen-dependent α-subunits, i.e., HIF-1α and -2α. HIF activity is mostly modulated by oxygen-associated proteolysis of α-subunits (5). It was recently demonstrated that HIF-1α plays a dual role in DN. Studies have shown that HIF-1α elicits a protective effect in physiological or pathological hypoxia or ischemia, such as DN. However, the mainstream belief among scientists is that elevated HIF-1α is involved in the pathological process and proteinuria of glomerular diseases in DN. Podocyte damage may be susceptible to the accumulation of HIF-1α (6). In a previous work, increased HIF by knockout of prolyl hydroxylase domain protein-2 (PHD2), a factor degrading HIF, enhances renal fibrosis (7). HIF-1α upregulation is involved in kidney injury, and its inhibition results in DN prevention in diabetic mice (5, 8).

Recent studies have documented that HIF-1α is regulated by many factors, and sirtuin (SIRT) 1 is a major regulating factor of HIF-1α (9). The SIRT family consists of SIRT1-SIRT7 in mammals, and shares the same 275-amino acid catalytic core region. SIRT1, located in the nucleus, is a nicotinamide adenine dinucleotide dependent deacetylase (10). SIRT1 is closely related to aging-related diseases, diabetes, vascular diseases and kidney diseases, and widely involved in the regulation of various intracellular processes, including apoptosis, metabolism and autophagy (10, 11). Meanwhile, SIRT1 has been identified as a novel molecular target for the prevention and treatment of kidney diseases. Previous studies have revealed that SIRT deficiency sensitizes Ang-II-induced renal fibrosis (12). SIRT1 could delay the progression of various kidney diseases by inhibiting apoptosis and fibrosis (13, 14). It was indicated that overexpression of SIRT1 in podocytes attenuates proteinuria and kidney injury in an animal model of diabetes (15). On the contrary, the decrease of SIRT1 in podocytes was shown to increase urinary protein and exacerbate renal injury (16).

SIRT1 binds to and deacetylates HIF-1α at Lys674, which inactivates HIF-1α and suppresses HIF-1α targets (17). It was verified that upregulation of SIRT1 inhibits the development of diabetic microvascular diseases *via* downregulation of HIF-1α (18). Moreover, DN prevention could be achieved by regulating the SIRT1/HIF-1α pathway (19).

Based on network pharmacology analyses and previous studies, TSWN might regulate the SIRT1/HIF-1α pathway to prevent DN. However, whether TSWN alleviates DN by regulating the SIRT1/HIF-1α pathway in podocytes remains unclear. The current work aimed to assess how TSWN prevents DN.

MATERIALS AND METHODS

Assays involving animals had approval from the Animal Ethics Committee of Beijing Chao-Yang Hospital, Capital Medical University, and followed the animal care guidelines of Beijing Chao-Yang Hospital, Capital Medical University.

Medicines and Reagents

TSWN (Patent No. 202111331292.3 under review by the State Intellectual Property Office of China) contains 13 Chinese herbs, including Huangqi (ASTRAGALI RADIX) 20g (20/185), Taizishen (PSEUDOSTELLARIAE RADIX) 15g (15/185), Danggui (ANGELICAE SINENSIS RADIX) 10g (10/185), Dihuang (REHMANNIAE RADIX) 20g (20/185), Shanzhuyu (CORNI FRUCTUS) 10g (10/185), Shanyao (DIOSCOREAE RHIZOMA) 15g (15/185), Tianhuafen (TRICHOSANTHIS RADIX) 15g (15/185), Gouqizi (LYCII FRUCTUS) 15g (15/185), Danshen (SALVIAE MILTIORRHIZAE RADIX ET RHIZOMA) 15g (15/185), Fuling (PORIA) 15g (15/185), Zexie (ALISMATIS RHIZOMA) 10g (10/185), Taoren (PERSICAESE MEN) 15g (15/185), and Gancao (GLYCYRRHIZAE RADIX ET RHIZOMA) 10g (10/185). TSWN was purchased from Pharmacy of Chao-Yang Hospital, Capital Medical University.

Ultra-High Performance Liquid Chromatography-Tandem Mass Spectrometry Analysis

TSWN extraction and storage were carried out as follows. TSWN underwent centrifugation (3000 rpm, 5 min). The resulting supernatant underwent filtration through a 0.45 μm PTFE membrane and storage at 4°C until assessment.

A UPLC-MS comprising a HESI-II probe was utilized for mass spectrometry analysis. The operating parameters were as follows: positive and negative HESI spray voltages, 3.7 and 3.5 kV, respectively; oven temperature, 300°C; sheath and auxiliary gas, nitrogen; collision gas, nitrogen; pressure, 1.5 mTorr; flow rate, 0.3 mL/min; column temperature, 45°C. Data collection and processing utilized Masslynx 4.1 and the Scientific Information System. The main components were quantitated with the UPLC system equipped with an Acquity UPLC column (2.1 mm × 100 mm, 1.8 μm).

Detection of Pharmaceutical Components

In this study, to determine the effective targets of TSWN, the traditional Chinese medicine systems pharmacology (TCMSP) database (https://www.tcmsp-e.com/) (20) was utilized for assessing all herbs in TSWN, and the components with oral bioavailability (OB) ≥ 0.3 and drug-likeness (DL) ≥ 0.18 (21, 22) were selected. Then, the intersection of the selected components by network pharmacology and mass spectrometry results was considered.

In order to retrieve the disease targets, the online mendelian inheritance in man (OMIM) database (https://omim.org/) (23), drug bank database (https://go.drugbank.com/) (24), discover genes internet (DisGeNET) data (25) (https://www.disgenet.org/) and Gene Cards database (https://www.genecards.org/) (26) were used, and "Diabetic Nephropathy" was used as a search term in these databases.

Venn diagrams were performed for TSWN's active component targets as well as disease targets. The intersection of drug and disease targets was selected, and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis was carried out in the Metascape database (https://metascape.org/) (27). All figures were generated with the R 4.1.1 statistical software.

Animal Experiments

Male C57BLKS/J db/m and db/db mice (7 weeks old) were provided by Nanjing Biomedical Research Institute of Nanjing University, Nanjing, China. The animals were assigned to six groups, including the db/m, db/db, db/db+V, db/db+TSWN-L, db/db+TSWN-M and db/db+TSWN-H groups (n = 6 per group).

Totally 185 g crude drugs of TSWN underwent soaking in 400 ml pure water for decoction to yield a concentration of 2 g/mL. TSWN in the present research was utilized at low, medium and high doses of 6.01, 12.02 and 24.05g/kg, respectively, twice per day in the db/db+TSWN-L, db/db+TSWN-M and db/db+TSWN-H groups, respectively (the medium dose was based on the dosage commonly administered to adult humans). The db/db+V group was administered 10.29 mg/kg of valsartan (Beijing Novartis Pharmaceutical, Beijing, China; dose based on the dosage commonly administered to adult humans) dissolved in pure water once per day and pure water alone once per day. The db/m and db/db groups were administered pure water alone twice per day. The volumes of intragastric administration of different groups were consistent with db/db+TSWN-H group by supplementing pure water. All the above gavage treatments were carried out for 12 weeks from 8 weeks of age.

The conditions were as follows: n = 3/cage; light cycle, 12-h light/dark cycle (lights on 08:00 - 20:00 h); temperature, 22 ± 1°C; humidity, 40%; freely available water and food; litter replacement, once a day.

Following a 12-week administration, the animals were housed in individual metabolic cages for taking urine samples, and anesthesia was performed by intraperitoneally injecting Rompun 10 mg/kg (Bayer Korea, Ansan, Gyeonggi-Do, Korea) and Zoletil 30 mg/kg (Virbac, Carros, France) in combination at week 20. Blood samples were collected from the left ventricle and kept at -80°C for subsequent analysis. Euthanasia was followed by kidney removal.

Blood and Urine Tests

Blood and urine parameters were measured as follows. Blood glucose (GLU) level was detected with a HemoCue B-Glucose kit (HemoCue AB, Angelholm, Sweden). Insulin (INS) levels were detected with a radioimmunoassay kit (Linco Research, St Charles, MO, USA). Total cholesterol (TC) and triglycerides (TG) levels were detected by an auto-analyzer (Wako, Osaka, Japan). Blood urea nitrogen (BUN) was measured with a iStat-Kit (HESKA, Fort Collins, MO, USA). Serum and urine creatinine concentrations were detected by HPLC (Beckman Instruments, Fullerton, CA, USA). Urine albumin

concentration was detected by an immunoassay (Bayer, Elkhart, IN, USA). Urine albumin-to-creatinine ratio (UACR) was derived as urine albumin/urine creatinine (μg/mg). Serum creatinine and alanine aminotransferase (ALT) amounts were examined with an automatic biochemical analyzer (Olympus AU480, Japan). Urinary podocalyxin levels in mice were measured by enzyme linked immunosorbent assay (ELISA) (Exocell, Philadelphia, PA, USA). The above assays followed the directions of the respective manufacturers.

Light Microscopy

Kidney tissue specimens underwent fixation with 10% formalin (SF93-20; Fisher Scientific, Pittsburgh, PA, USA). Histological features were assessed by hematoxylin and eosin (H&E; Servicebio, Wuhan, China) and Periodic Acid Schiff (PAS; Servicebio) staining. Fibrosis was assessed by the ratio of fibrotic area to total area detected by Masson's trichrome (Servicebio) and Sirius red (Servicebio) staining. Kidney apoptosis was examined by TUNEL (Servicebio). Kidney specimens underwent embedding in frozen optimal cutting temperature compound (Fisher HealthCare, Houston, TX, USA) and sectioning at 8 μm for immunostaining. The specimens underwent incubation with primary antibodies targeting SIRT1 (1:100; Abcam, Cambridge, MA, USA) and podocin (1:100; Sigma, Shanghai, China). This was followed by incubation with second antibodies conjugated with fluorescein isothiocyanate (1:500). For quantitation, 10 random high-power fields in mouse kidney samples were assessed with Image J (NIH, Bethesda, MD, USA).

Transmission Electron Microscopy

Three kidney specimens per group were sliced through the hilum in a longitudinal fashion. Then, kidney specimens underwent mincing into rectangular pieces of approximately 1 mm, fixation with 2.5% glutaraldehyde (4 h at 4°C) and four rinsing steps with 0.1 mol/L phosphate buffer saline (PBS; 15 min each). After fixation with 1% citrate (2 h) and two rinsing steps with 0.1 mol/L PBS (5 min each), dehydration was carried out with acetone at 50, 70, 90 and 100%, successively (15 min each). Specimens underwent infiltration with acetone and plant fats at ratios of 1:1 and 2:1 for 2 h, respectively, followed by overnight infiltration with pure resin. After Epon 812 resin embedding, ultrathin sections at 60-70 nm were obtained with an Ultracut R microtome. Uranium acetate and lead nitrate were utilized for staining before observation.

Western Blot

Samples were randomly selected from the six groups. Assays were performed thrice. Kidney cortex tissue specimens underwent homogenization, followed by a 10-minute centrifugation (16000×g at 4°C). A bicinchoninic acid protein assay kit (Pierce Co, Rockford, IL, USA) was utilized for protein quantitation. Totally 20 μg of protein per sample underwent separation by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis gel and transfer onto polyvinylidene difluoride (PVDF) membranes. After blocking (5% skimmed milk in Tris-buffered saline), overnight incubation was carried out with primary antibodies targeting collagen I (1:1000; Abcam, Cambridge, MA, USA), cleaved caspase-3 (1:1000; Abcam), podocin (1:1000; Abcam), nephrin (1:1000; Abcam), HIF-1α (1:1000; Novus Bio, Littleton, CO, USA), SIRT1 (1:1000; Abcam) and β-actin (1:1000; Cell Signaling, Danvers, MA, USA). Next, a 2-hour incubation with secondary antibodies linked to horseradish peroxidase (1:5000; Santa Cruz, CA, USA) was carried out. Quantitation was performed by densitometry with the image acquisition and analysis software (Bio-Rad).

Ribonucleic Acid Extraction and Quantitative Reverse Transcriptase Polymerase Chain Reaction

RNA extraction from kidney tissue specimens utilized TRIzol (Invitrogen, Carlsbad, CA, USA) using standard protocols as directed by the manufacturer. qRT-PCR was carried out with QuantiTect SYBR Green PCR Kit (Qiagen, Valencia, CA). Primers were: SIRT1, 5'-GCTGACGACTTCGACGACG-3' (sense) and 5'-TCGGTCAACAGGAGGTTGTCT-3' (antisense); HIF-1α, 5'-CTCGGCGAAGCAAAGAGT-3' (sense) and 5'-GCCATCTAGGGCTTTCAG-3' (antisense); β-actin, 5'-CATCCGTAAAGACCTCTATGCCAAC-3' (sense) and 5'-ATGGAGCCACCGATCCACA-3' (antisense).

Statistical Analyses

Data are mean ± SEM. Multiple groups were compared by one-way ANOVA. SPSS 22.0 (SPSS, Inc., Chicago, IL, USA) was utilized for data analysis, and two-sided $P<0.05$ was deemed statistically significant.

RESULTS

Potential Targets of TSWN Effects on DN

Totally 15 bioactive components (**Table 1**) and 223 effective targets of TSWN as well as 1145 targets of DN were screened out by mass spectrometry (**Supplementary 1**) and network pharmacology analysis (**Supplementary 2A–D**). Venn diagrams were generated for TSWN's effective targets and DN targets (**Figure 1A**). The intersection of TSWN and DN targets was selected, and KEGG enrichment analysis was performed by using the Metascape database (https://metascape.org/) (27). The results are shown in **Figure 1B**, and HIF-1 was one of the main targets of TSWN. Since HIF-1α is regulated by SIRT1 (16, 17), systems pharmacology revealed that TSWN might prevent DN by regulating the SIRT1/HIF-1α pathway.

Mouse Biophysical Features

In this study, body and kidney weights, food intakes, blood glucose amounts, insulin levels and triglycerides were

TABLE 1 | Active components of TSWN formula.

Mol ID	Name	OB (%)	DL
MOL000422	Kaempferol	44.88	0.24
MOL000098	Quercetin	46.43	0.28
MOL001689	Acacetin	34.97	0.24
MOL000006	Luteolin	36.16	0.25
MOL008400	Glycitein	50.48	0.24
MOL001942	Isoimperatorin	45.46	0.23
MOL002776	Baicalin	40.12	0.75
MOL007088	Cryptotanshinone	52.34	0.4
MOL007154	Tanshinone IIa	49.89	0.4
MOL000291	Poricoic acid B	30.52	0.75
MOL000289	Pachymic acid	30.62	0.75
MOL002565	Medicarpin	49.22	0.34
MOL004328	Naringenin	59.29	0.21
MOL004908	Glabridin	53.25	0.47
MOL004949	Isolicoflavonol	45.17	0.84

significantly higher in the db/db, db/db+V, db/db+TSWN-L, db/db+TSWN-M and db/db+TSWN-H groups compared with the db/m group. These parameters were comparable in the db/db, db/db+V, db/db+TSWN-L, db/db+TSWN-M and db/db+TSWN-H groups (**Figures 2A–E, G**). It was found that blood urea nitrogen (BUN) level was significantly higher in the db/db, db/db+V, db/db+TSWN-L and db/db+TSWN-M groups compared with the db/m group, and there was no significant difference in BUN among db/db, db/db+V, db/db+TSWN-L, db/db+TSWN-M and db/db+TSWN-H groups (**Figures 2I**). All groups had comparable total cholesterol, ALT and serum creatinine (SCR) levels (**Figures 2F, H, J**).

Renal Phenotype of Mice

As depicted in **Figure 3**, the db/db group had elevated urinary albumin excretion (UAE) and UACR in comparison with the db/m group. In addition, the db/db+V, db/db+TSWN-L, db/db+TSWN-M and db/db+TSWN-H groups had markedly reduced UAE and UACR values in comparison with the db/db group (**Figures 3A, B**). H&E staining showed mesangial basement membrane thickening and KW nodule formation in the db/db group. PAS staining showed that the mesangial glomerular basement membrane was remarkably increased in db/db group. However, valsartan or TSWN treatment prevented renal pathological changes in mice with experimental diabetes (**Figures 3C, D**).

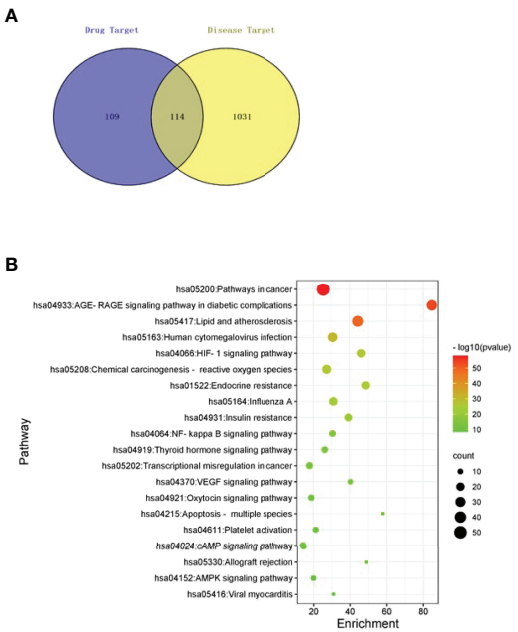

FIGURE 1 | Network pharmacological analyses. **(A)** Venn diagram of targets of TSWN formula and targets of diabetic nephropathy. **(B)** Pathway enrichment analysis of differential genes by TSWN formula treatment on diabetic nephropathy. The vertical axis represents the names of the 20 selected pathways, the color of the dot represents the -log10(P) value, the size represents the number of genes, and the horizontal axis is the enrichment of pathways.

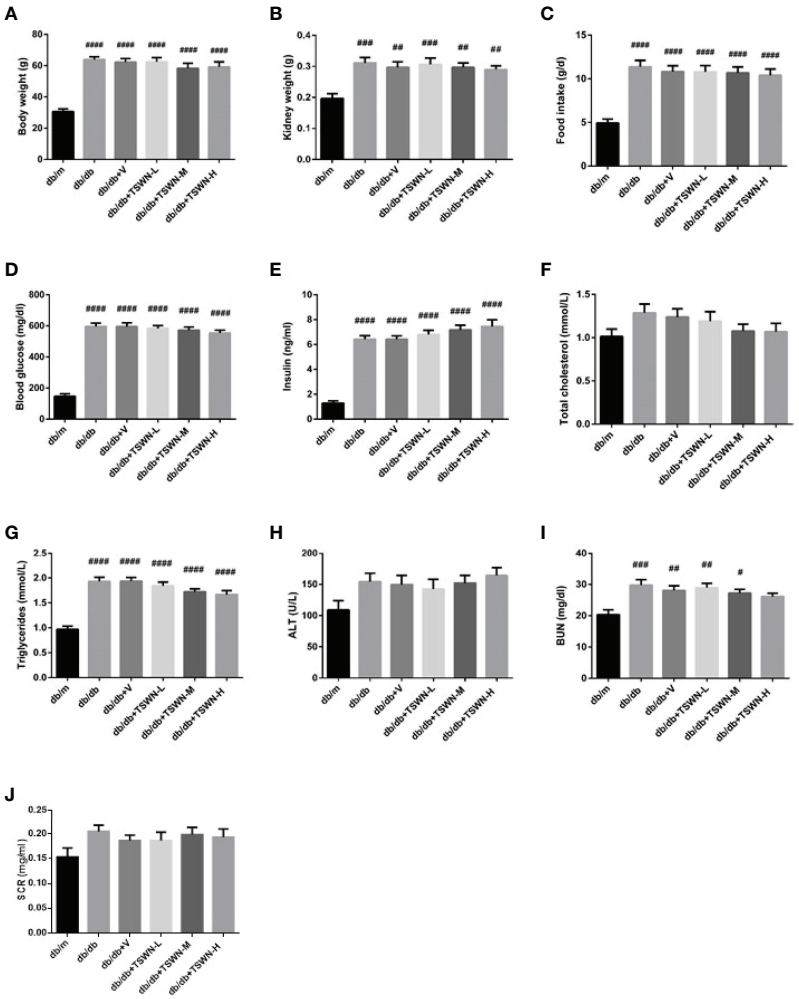

FIGURE 2 | Physical and biochemical characteristics of mice. **(A)** Body weight. **(B)** Kidney weight. **(C)** Food intake. **(D)** Fasting blood glucose level. **(E)** Fasting Insulin level. **(F)** Fasting total cholesterol. **(G)** Fasting triglycerides. **(H)** Alanine aminotransferase (ALT). **(I)** Blood urea nitrogen (BUN). **(J)** Serum creatinine (SCr). n = 6 mice/group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$, $^{\#\#\#\#}P < 0.0001$ vs db/m group. Db/m, db/m mice; db/db, db/db mice; db/db+V, db/db mice with valsartan treatment; db/db+TSWN-L, db/db mice with low dose TSWN treatment; db/db+TSWN-M, db/db mice with middle dose TSWN treatment; db/db+TSWN-H, db/db mice with high dose TSWN treatment. Data are means ± S.E.M.

Renal Fibrosis in Mice

Renal fibrosis was assessed by Masson's staining and Sirius red staining (**Figures 4A–C**). Both assays demonstrated that diabetes markedly induced renal fibrosis in mice, which was suppressed by valsartan or TSWN. Immunoblot further revealed that diabetes upregulated fibrosis associated protein-collagen I in the mouse kidney, which was alleviated by valsartan or TSWN (**Figure 4D**).

Renal Apoptosis in Mice

Subsequently, we measured apoptosis in the mouse kidney by TUNEL, and cleaved caspase-3 by Western blot. The TUNEL assay demonstrated that diabetes markedly induced mouse renal apoptosis, which was prevented by valsartan or TSWN (**Figures 5A, B**). Meanwhile, immunoblot demonstrated that diabetes upregulated apoptosis-associated cleaved caspase-3 in the mouse kidney, while valsartan or TSWN treatment suppressed renal cleaved caspase-3 expression in diabetic animals (**Figure 5C**).

Podocyte Injury in Mouse Kidneys

Podocyte injury has an important function in DN. Therefore, we investigated TSWN's effects on podocytes in mouse kidneys. As depicted in **Figure 6**, the protein levels of the podocyte markers podocin and nephrin were lower in the kidneys of the db/db group in comparison with the db/m group (immunoblot), and podocalyxin, another marker of podocytes, had elevated urine amounts in the db/db group compared with the db/m group, as detected by ELISA. Meanwhile, podocin and nephrin were raised in the diabetic mouse kidney, and urine podocalyxin was decreased in mice

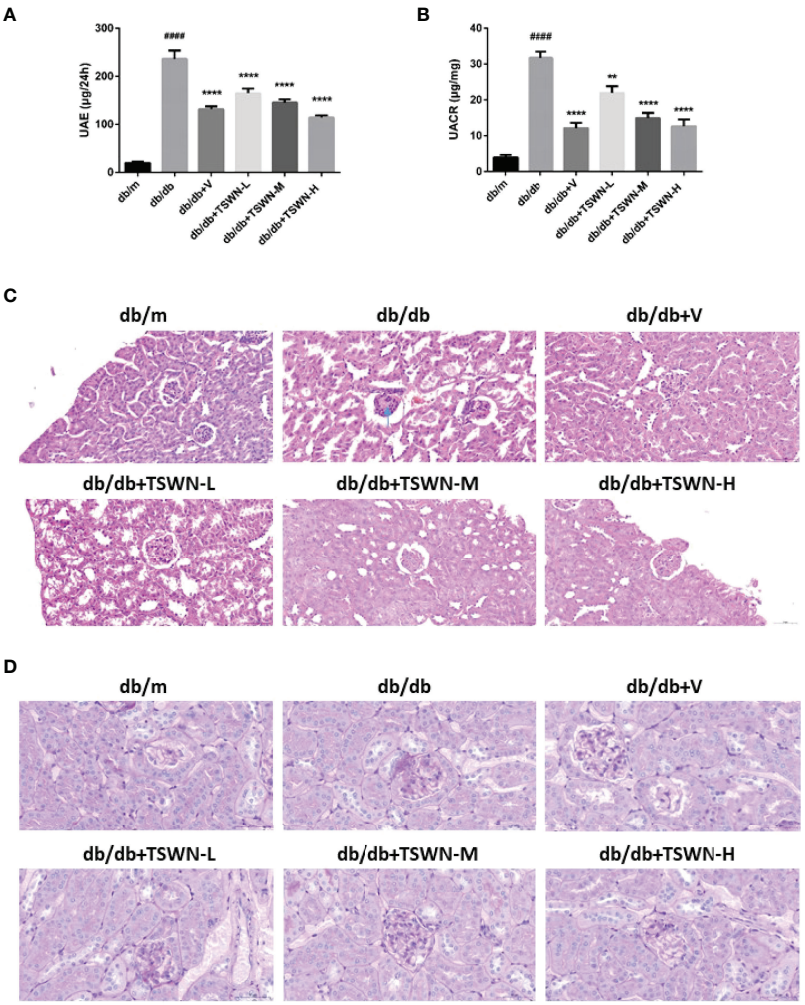

FIGURE 3 | Renal phenotype of mice. **(A)** Urinary albumin excretion (UAE). **(B)** Urinary albumin-to-creatinine ratio (UACR). **(C)** Representative photographs of mouse kidneys by Hematoxylin & Eosin (H&E) staining. **(D)** Representative photographs of mouse kidneys by Periodic Acid Schiff (PAS) staining. n = 6 mice/group. $^{\#\#\#\#}P < 0.0001$ vs db/m group; $^{**}P < 0.01$ vs db/db group. Db/m, db/m mice; db/db, db/db mice; db/db+V, db/db mice with valsartan treatment; db/db+TSWN-L, db/db mice with low dose TSWN treatment; db/db+TSWN-M, db/db mice with middle dose TSWN treatment; db/db+TSWN-H, db/db mice with high dose TSWN treatment. Data are means ± S.E.M.

with experimental diabetes after valsartan or TSWN treatment (**Figures 6A–C**). Next, podocyte morphology was examined by transmission electron microscopy. Damaged podocytes with perforation were found in the kidneys of db/db mice, while podocyte injury was improved in the kidneys of diabetic mice after valsartan or TSWN treatment (**Figures 6D**).

Assessment of SIRT1/HIF-1α Pathway in Mouse Kidneys

Then, renal SIRT1 and HIF-1α amounts were assessed in mice. As shown in **Figure 7**, the db/db group showed lower SIRT1 and elevated HIF-1α in kidneys measured by both qRT-PCR and immunoblot compared with the db/m group. After valsartan or TSWN treatment, SIRT1 was upregulated and HIF-1α was suppressed in diabetic mouse kidneys (**Figures 7A–D**). Moreover, the immunostaining fractions of renal SIRT1 and podocin were decreased in the db/db group in comparison with the db/m group, and valsartan or TSWN treatment raised SIRT1 and podocin in diabetic mouse kidneys. Furthermore, co-staining of SIRT1 and podocin showed that SIRT1 amounts were decreased in podocytes of the db/db group versus db/m animals, whereas valsartan or TSWN treatment increased SIRT1 levels in the podocytes of diabetic mouse kidneys (**Figures 7E–G**).

DISCUSSION

This work demonstrated that UAE and UACR were increased in diabetic mice, and diabetes accelerated pathological changes, promoted fibrosis and apoptosis, and induced podocyte injury

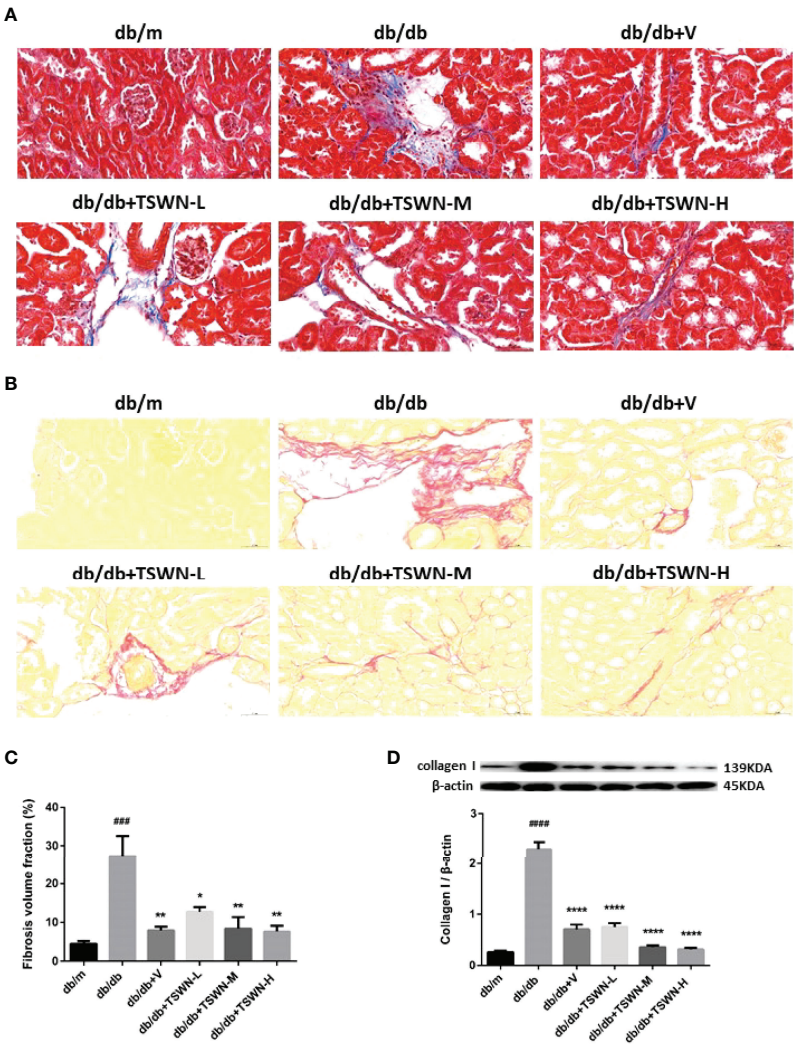

FIGURE 4 | Fibrosis in mouse kidneys. **(A)** Representative photographs of renal fibrosis measured by Masson's staining. **(B)** Representative photographs of renal fibrosis measured by Sirius red staining. **(C)** Quantification of renal fibrosis. **(D)** Representative photographs and quantification of collagen I in mouse kidneys detected by western blot. n = 6 mice/group. $^{###}P < 0.001$, $^{####}P < 0.0001$ vs db/mgroup; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{****}P < 0.0001$ vs db/db group. Db/m, db/m mice; db/db, db/db mice; db/db+V, db/db mice with valsartan treatment; db/db+TSWN-L, db/db mice with low dose TSWN treatment; db/db +TSWN-M, db/db mice with middle dose TSWN treatment; db/db+TSWN-H, db/db mice with high dose TSWN treatment. Data are means ± S.E.M.

in mouse kidneys. Furthermore, SIRT1 was decreased and HIF-1α was increased in diabetic mouse kidneys compared with non-diabetic mouse kidneys. TSWN reduced UAE and UACR, improved renal pathological changes, inhibited renal fibrosis, decreased renal apoptosis and prevented podocyte injury in diabetic mice. Importantly, TSWN regulated the SIRT1/HIF-1α pathway in the podocytes of diabetic mouse kidneys.

DN represents a commonly detected microvascular complication of diabetes, accounting for adverse clinical outcome (28). Treatment options for DN mainly reduce albuminuria to improve the prognosis of clinical adverse events (29). At present, drugs commonly used to prevent DN and reduce albuminuria in clinic, including angiotensin receptor blockers (ARB), reduce renal fibrosis and apoptosis, and protect podocytes (30, 31). In addition, ARB can delay DN through the SIRT1/HIF-1α pathway (32, 33). TSWN, a traditional Chinese herbal formula, contains 13 Chinese herbs. TSWN provides an effective outcome of therapeutic effects in patients with DN by preventing podocyte injury. Based on mass spectrometry and network pharmacology, TSWN might prevent DN and decrease urinary albumin *via* the HIF-1 pathway.

The pathological characteristics of DN include a variety of structural and functional changes in the kidney, leading to albuminuria (34). In the present study, hyperglycemia damaged the kidneys of diabetic mice, leading to increased albuminuria and aggravated kidney histology in mice with experimental diabetes. However, TSWN treatment could reduce albuminuria and improve kidney histology in animals

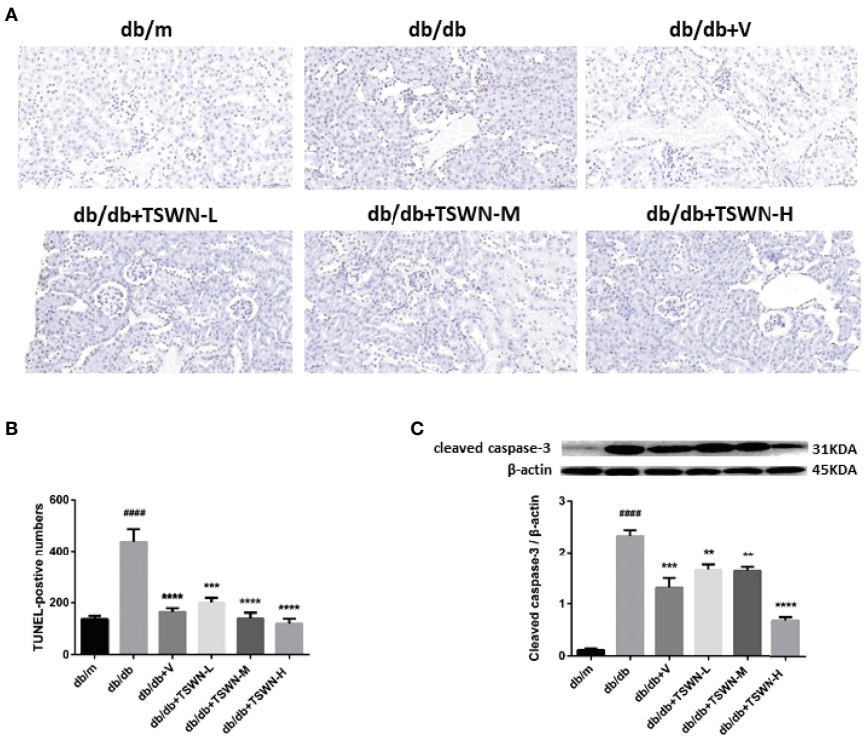

FIGURE 5 | Apoptosis in mouse kidneys. **(A, B)** Representative photographs and quantification of renal apoptosis measured by TUNEL assay. **(C)** Representative photographs and quantification of cleaved caspase-3 in mouse kidneys detected by western blot. n = 6 mice/group. $^{\#\#\#\#}P < 0.0001$ vs db/m group; $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$ vs db/db group. Db/m, db/m mice; db/db, db/db mice; db/db+V, db/db mice with valsartan treatment; db/db+TSWN-L, db/db mice with low dose TSWN treatment; db/db+TSWN-M, db/db mice with middle dose TSWN treatment; db/db+TSWN-H, db/db mice with high dose TSWN treatment. Data are means ± S.E.M.

with experimental diabetes, suggesting TSWN's curative effect on DN.

Fibrosis represents a hallmark of progressive chronic renal disorders, potentially leading to renal failure. High blood glucose upregulates the expression fibrotic factors, further resulting in damaged glomerular filtration barrier and causing DN (29). In the current study, diabetes caused fibrosis and increased collagen I amounts in the mouse kidney, and these effects were alleviated by TSWN, suggesting that TSWN reduces renal fibrosis in diabetic mice.

Hyperglycemia induces apoptosis in podocytes (35). Accumulating evidence demonstrates that apoptosis accelerates the pathogenesis of DN (36). In the present study, diabetes enhanced apoptosis and upregulated cleaved caspase-3 in the mouse kidney. However, TSWN treatment was confirmed to prevent renal apoptosis, downregulating cleaved caspase-3 in the diabetic mouse kidney. These findings confirmed that TSWN could reduce renal apoptosis in diabetic mice.

Podocytes are cells with high level of differentiation, found outside the glomerular basement membrane. They form the last line of defense for the glomerular filtration barrier (37). Since podocytes show limited capabilities of repair and regeneration, the degree of podocyte injury is considered the main prognostic determinant of DN (29). Podocytes are critical for renal function, and constitute the primary focus in multiple renal disorders, especially DN. Damage of podocytes contributes to the accumulation of podocyte-derived cell debris and podocyte-specific molecular targets in urine, which could be detected by specific tests (38). In this study, diabetic mice had lower levels of podocin and nephrin in renal tissues and higher levels of podocalyxin in urine compared with non-diabetic mice. After TSWN treatment, the levels of podocin and nephrin in renal tissues were increased and the levels of podocalyxin in urine were decreased in diabetic mice. In addition, podocyte damage was observed by transmission electron microscopy in diabetic mouse kidneys, which was improved by TSWN treatment. These findings indicated TSWN reduces podocyte injury in diabetic animals.

According to mass spectrometry and network pharmacology, TSWN might prevent DN and decrease urinary albumin *via* the HIF-1α pathway. HIF-1α has a known association with DN, and could play a protective role in DN (6). Accumulating evidence reveals that elevated HIF-1α leads to DN and podocyte injury. Under hyperglycemic conditions, elevated expression of HIF-1α, a transcriptional factor mediating hypoxia adaptation, could stimulate renal fibrosis and proteinuria (39, 40). HIF-1α expression is accompanied by renal fibrosis in diabetes, and HIF-1α upregulation can cause renal injury (41). Conversely, experimental knockout or inhibition of HIF-1α attenuates renal fibrosis (42, 43). Recent reports

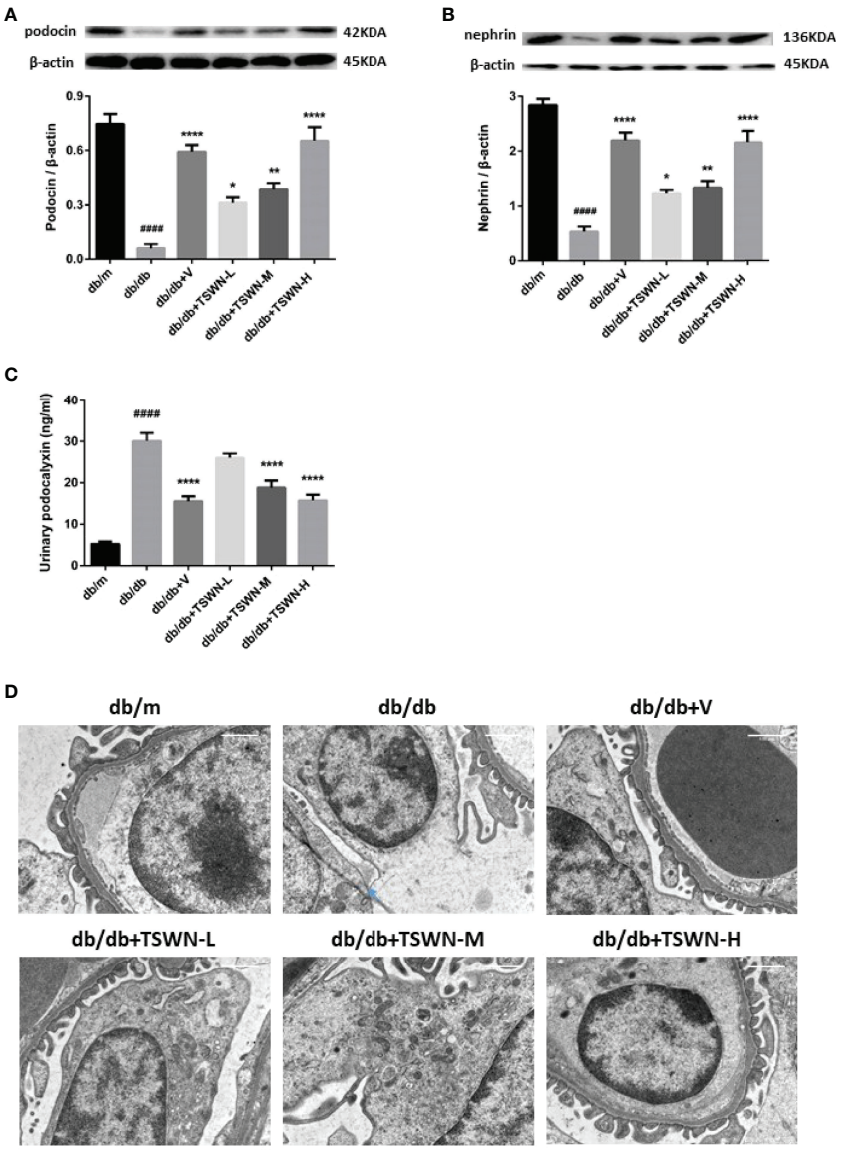

FIGURE 6 | Podocytes in mouse kidneys. **(A)** Representative photographs and quantification of podocin in mouse kidneys measured by western blot. **(B)** Representative photographs and quantification of nephrin in mouse kidneys measured by western blot. **(C)** Quantification of urinary podocalyxin measured by enzyme linked immunosorbent assay (ELISA). **(D)** Representative photographs of podocytes in mouse kidneys detected by transmission electron microscopy. n = 6 mice/group. $^{\#\#\#\#}P < 0.0001$ vs db/m group; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{****}P < 0.0001$ vs db/db group. Db/m, db/m mice; db/db, db/db mice; db/db+V, db/db mice with valsartan treatment; db/db+TSWN-L, db/db mice with low dose TSWN treatment; db/db+TSWN-M, db/db mice with middle dose TSWN treatment; db/db+TSWN-H, db/db mice with high dose TSWN treatment. Data are means ± S.E.M.

have documented that HIF-1α is regulated by SIRT1, a nicotinamide adenine dinucleotide dependent deacetylase that deacetylates HIF-1α at Lys674 and inactivates HIF-1α, leading to the suppression of HIF-1α target genes (17). SIRT1 was recently identified as a novel molecular target for the prevention and treatment of several renal diseases, including DN. Overexpression of SIRT1 in podocytes attenuates proteinuria and kidney injury in an animal model of diabetes (15). Furthermore, SIRT1 regulates renal apoptosis and fibrosis. Hyperglycemic conditions caused SIRT1 in the kidney to decline, inducing apoptosis and fibrosis (10, 11, 13–15). Then, all these effects promoted the development of DN. In the present study, diabetic mice had lower SIRT1 and higher HIF-1α in the kidney in comparison with non-diabetic mice, and TSWN promoted SIRT1 and inhibited HIF-1α in diabetic mouse kidneys. Moreover, co-staining of SIRT1 and podocin revealed that SIRT1 was decreased in the podocytes of diabetic mouse kidneys in comparison with non-diabetic mouse kidneys, and SIRT1 was elevated in the podocytes of diabetic mouse kidneys upon TSWN treatment. These results suggested that TSWN

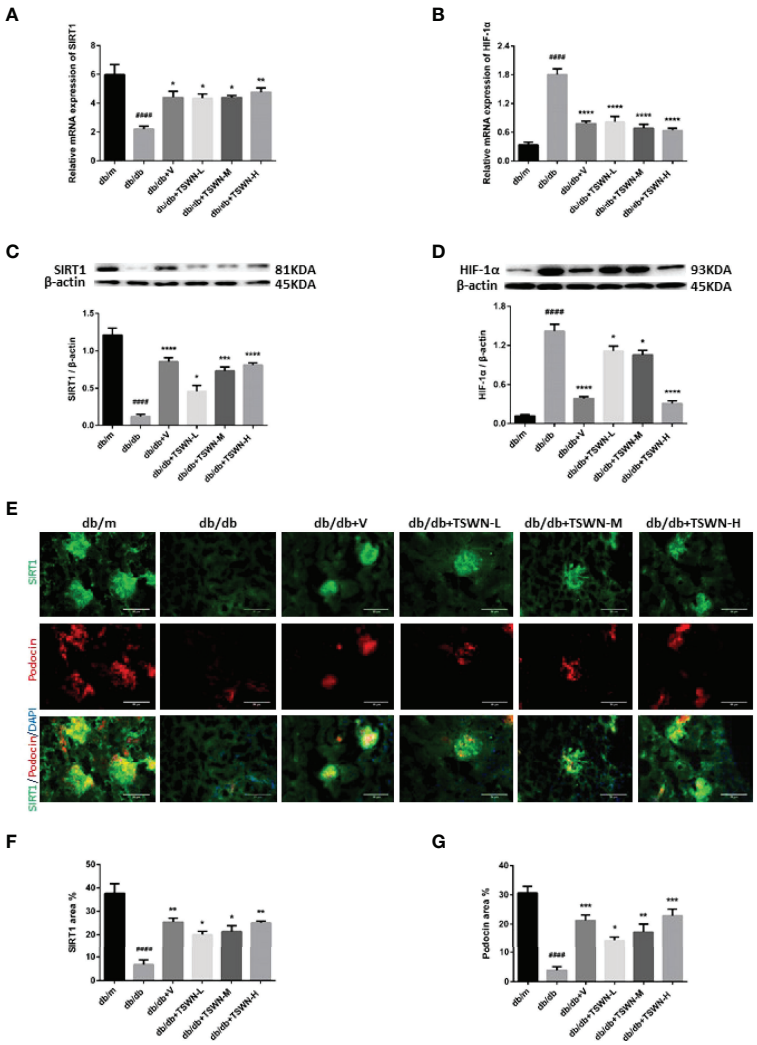

FIGURE 7 | SIRT1 and HIF-1a in mouse kidneys. **(A)** Messenger ribonucleic acid (mRNA) expression of SIRT1 in mouse kidneys by quantitative reverse transcriptase polymerase chain reaction (RT-PCR). **(B)** MRNA expression of HIF-1a in mouse kidneys by RT-PCR. **(C)** Representative photographs and quantification of SIRT1 in mouse kidneys measured by western blot. **(D)** Representative photographs and quantification of HIF-1a in mouse kidneys measured by western blot. **(E–G)** Representative photographs and quantification of co-staining of SIRT1 and podocin in mouse kidneys. n = 6 mice/group. $^{\#\#\#\#}P < 0.0001$ vs db/m group; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$ vs db/db group. Db/m, db/m mice; db/db, db/db mice; db/db+V, db/db mice with valsartan treatment; db/db+TSWN-L, db/db mice with low dose TSWN treatment; db/db+TSWN-M, db/db mice with middle dose TSWN treatment; db/db+TSWN-H, db/db mice with high dose TSWN treatment. Data are means ± S.E.M.

prevents DN by modulating the SIRT1/HIF-1α pathway in the podocytes of diabetic mouse kidneys.

CONCLUSIONS

In summary, this study indicated that TSWN plays an important role in DN treatment, and reduces albuminuria through the regulation of SIRT1/HIF-1α signaling in the podocytes of diabetic mouse kidneys. Further study of the therapeutic mechanism of TSWN in DN should be explored in the future.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The animal study was reviewed and approved by Animal Ethics Committee of Beijing Chao-Yang Hospital, Capital Medical University.

AUTHOR CONTRIBUTIONS

JC: Design, Experimentation, Statistics, Article revision. JZ: Statistics, Article revision. XG: Experimentation. HD: Experimentation. HY: Experimentation. MH: Experimentation. ZS: Experimentation. XF: Design, Experimentation, Statistics, Article revision. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.888611/full#supplementary-material

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ORIGINAL RESEARCH
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Screening and Identification of Hub Genes in the Development of Early Diabetic Kidney Disease Based on Weighted Gene Co-Expression Network Analysis

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Objective: The study aimed to screen key genes in early diabetic kidney disease (DKD) and predict their biological functions and signaling pathways using bioinformatics analysis of gene chips interrelated to early DKD in the Gene Expression Omnibus database.

Methods: Gene chip data for early DKD was obtained from the Gene Expression Omnibus expression profile database. We analyzed differentially expressed genes (DEGs) between patients with early DKD and healthy controls using the R language. For the screened DEGs, we predicted the biological functions and relevant signaling pathways by enrichment analysis of Gene Ontology (GO) biological functions and Kyoto Encyclopedia of Genes and Genomes (KEGG) signaling pathways. Using the STRING database and Cytoscape software, we constructed a protein interaction network to screen hub pathogenic genes. Finally, we performed immunohistochemistry on kidney specimens from the Beijing Hospital to verify the above findings.

Results: A total of 267 differential genes were obtained using GSE142025, namely, 176 upregulated and 91 downregulated genes. GO functional annotation enrichment analysis indicated that the DEGs were mainly involved in immune inflammatory response and cytokine effects. KEGG pathway analysis indicated that C-C receptor interactions and the IL-17 signaling pathway are essential for early DKD. We identified FOS, EGR1, ATF3, and JUN as hub sites of protein interactions using a protein–protein interaction network and module analysis. We performed immunohistochemistry (IHC) on five samples of early DKD and three normal samples from the Beijing Hospital to label the proteins. This demonstrated that FOS, EGR1, ATF3, and JUN in the early DKD group were significantly downregulated.

Conclusion: The four hub genes FOS, EGR1, ATF3, and JUN were strongly associated with the infiltration of monocytes, M2 macrophages, and T regulatory cells in early DKD samples. We revealed that the expression of immune response or inflammatory genes was suppressed in early DKD. Meanwhile, the FOS group of low-expression genes

showed that the activated biological functions included mRNA methylation, insulin receptor binding, and protein kinase A binding. These genes and pathways may serve as potential targets for treating early DKD.

Keywords: bioinformatics, WGCNA, GSEA analysis, early diabetic kidney disease, hub gene

INTRODUCTION

Diabetic kidney disease (DKD) is a formidable health challenge that we are faced with. It occurs in up to 50% of patients with diabetes and is the dominant cause of end-stage renal disease (1, 2). However, microalbuminuria is the most common early clinical symptom of DKD and is usually undetected and easily ignored by patients. Following the onset of early DKD, chronic renal failure eventually develops into uremia as the disease progresses. As a result, when patients exhibit obvious symptoms, such as massive proteinuria and renal failure, most patients have progressed to advanced DKD (3–5). Screening key genes in early DKD and clarifying their biological functions is expected to predict the development of DKD as early as possible.

Glomerular endothelial dysfunction plays a crucial role in the pathogenesis of early DKD (6). Hyperglycemia induces oxidative stress, endoplasmic reticulum stress, and apoptosis in the early stages of DKD (7, 8). Inflammation and immune regulation are the fundamental mechanisms underlying the development and progression of DKD. Epigenetic contributions to inflammation and fibrogenesis occur at different regulatory levels, namely, DNA methylation and non-coding RNA modulation (9). The cytokine–cytokine (C–C) receptor interaction pathway and activated biological functions are also essential parts of the above network.

In recent years, thanks to the rapid development of bioinformatics and gene chip technology, the establishment and improvement of many disease databases have provided the theoretical basis for revealing pathogenesis and new therapeutic targets. Differentially expressed genes (DEGs) and hub genes could help us to better understand the molecular mechanisms underlying DKD progression and provide candidate targets for the diagnosis and treatment of DKD (10–14). In this study, we aimed to screen DEGs in early DKD patients compared with those in normal kidney tissue and to explore the biological functions and possible mechanisms of the DEG signaling pathway. This finding may provide a promising direction for clarifying the diagnosis and pathogenesis of early DKD.

MATERIALS AND METHODS

Data Collection

The National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/) was used to obtain early DKD relevant gene expression profile data. We obtained the original data from human kidney tissue gene chip GSE142025, which contained the data of nine normal kidney tissues and six early DKD samples, using the keyword "early diabetic nephropathy" or "early diabetic kidney disease" in the GEO database.

Data Processing and Differential Expression Analysis

We ran the R language script to read the dataset downloaded from the GEO database and normalize the data. Our research analyzed the standardization of chip expression spectrum differences *via* R language functions and limma packages, tested correction through the Bayes method multiple, and filtered DEGs with a standard of $|\log2FC| > 2$ and $P < 0.05$. We performed cluster analysis of DEGs and created a heatmap using gplots in the R language.

GO Enrichment Analysis of Differential Genes and KEGG Pathway Analysis

We analyzed the DEGs of selected data using the DAVID database (https://david.ncifcrf.gov/) (15), according to the GO analysis of gene function annotation enrichment. Molecular functions, cellular components, and biological processes were part of the data that was analyzed (16). KEGG was used to annotate DEGs, which mainly covered gene function, biological pathways, cell localization, and signaling pathways. The KEGG signaling pathway was analyzed using the KOBAS database (17), with a screening condition of $P < 0.05$.

Differential Gene Protein Interaction Network Analysis

Protein–protein interaction (PPI) analysis and modular analysis of differential genes were performed using the STRING database (http://string-db.org/) (18) and Cytoscape software (19). We imported DEGs into the STRING database and analyzed the PPI of the differential genes. Then, we used the degree plug-in of Cytoscape software to analyze the module of results and mined the most closely connected modules in PPI to predict the interaction between proteins encoded by DEGs. Finally, we screened the most critical genes.

GSEA Enrichment Analysis of Hub Genes Pathway and Biological Function

Gene set enrichment analysis (GSEA; Broad Institute, Inc., Massachusetts Institute of Technology, and Regents of the University of California) is a widely used computational method that determines whether there is a statistically significant difference between two gene groups (20). In this

Abbreviations: DKD, Diabetic Kidney Disease; IHC, immunohistochemistry; DEGs, Differentially Expressed Genes; NCBI, National Center for Biotechnology Information; GEO, Gene Expression Omnibus; GO, Gene Ontology; MF, Molecular function; CC, Cellular components; BP, Biological Process; KEGG, Kyoto Encyclopedia of Genes and Genomes; PPI, Protein–Protein Interaction; GSEA, Gene Set Enrichment Analysis; Tregs, T cells regulatory.

study, we used the GSEA software (version 3.0) to analyze the GO and KEGG pathways of the hub genes.

Immune State Was Evaluated Based on CIBERSORT and ssGSEA Algorithms

Our study used the CIBERSORT (21) and ssGSEA algorithms (20) to evaluate the immune status of early DKD tissues. With "CIBERSORT" (R packet), we used the CIBERSORT algorithm to analyze gene expression data. Using the standard P value <0.05, we screened samples and calculated the percentage of 22 immune cells. Our study compared standardized data with gene sets by using "GSVA" (R package). The ssGSEA algorithm classified genes with common biological functions, chromosomal localization, and physiological regulation. Ultimately, we identified 29 immune-related genes.

Immunohistochemistry

This study was approved by the Beijing Hospital Institutional Review Board. The ethics approval letter number is 2022BJYYEC-025-01. As described under the approved protocols, DKD kidney biopsy samples from patients were collected by ultrasound-guided renal biopsy. Early DKD patients were defined as having a urine albumin–creatine ratio of between 30 and 300 mg/g and an estimated glomerular filtration rate of >90 ml/min/1.73 m^2. Biopsy samples included five cases of early DKD and three normal tissues adjacent to the tumor nephrectomy samples. Histological analysis of all patients was performed by investigators who were blinded to the experimental design. Using specific primary antibodies and biotinylated secondary antibodies, immunostaining was performed on five samples of early DKD and three normal samples from nephrectomies. The sections were incubated with rabbit anti-c-Jun (ab40766; antibody diluted 1:100; Abcam, USA), anti-c-EGR1 (ab194357; antibody diluted 1:100; Abcam, USA), anti-c-Fos (ab222699; antibody diluted 1:100; Abcam, USA), and anti-c-ATF3 (ab254268; antibody diluted 1:100; Abcam, USA), followed by secondary antibodies (Cell Signaling Technology, USA). The slides were photographed under a microscope with a digital camera (Nikon Eclipse ci). The imaging system was a Nikon Digital Sight DS-FI2. We used ImageJ software to assess 5–10 horizons (200× magnification) for semiquantitative analysis.

Statistical Analysis

The analysis in this study was performed using R software version 3.6.2, in which the "limma" package was used for differential gene acquisition, the "cluster Profiler" and "org.Hs.eg.db" packages were used for functional enrichment analysis, and the "survival" package was used to perform Kaplan–Meier survival analysis. The study conducted a statistical analysis between two independent samples based on SPSS Statistics 22 using the t-test, and P <0.05 was considered statistically significant.

RESULTS

Screening of DEGs

We analyzed the differences in gene types and visualized genetic variations. Volcano plots were used for figure analysis of identified DEGs. Compared with the normal control, red dots represent the upregulation of genes in the tissues of early DKD patients, while green dots represent the downregulation of genes, as shown in **Figure 1A**. In the heatmap, the limma package of the R language screened 267 DEGs. Compared with the normal control, the red area in early DKD patient tissues represents adequately expressed genes, while the green area represents poorly expressed genes. As shown in **Figure 1B**, 91 genes were adequately expressed and 176 were poorly expressed (see the attached table DEGs 1).

Weighted Gene Co-Expression Network Analysis (WGCNA)

Using WGCNA, we identified the key modules relevant to early DKD formation in the GEO dataset. In **Figures 2A, B**, we analyzed the scale-free fitting index (left) and average connectivity (right) of various soft threshold powers based on

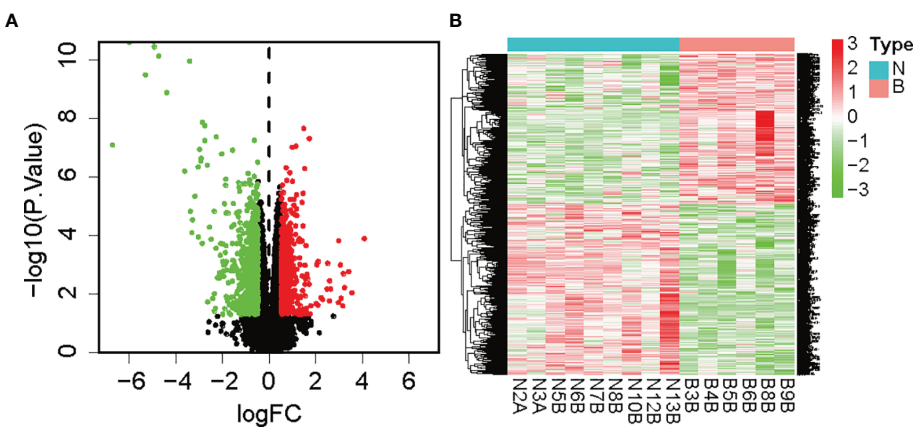

FIGURE 1 | **(A)** Volcano map of DEGs (green was the downregulated gene, red was the upregulated gene, and black was the undifferentiated gene); **(B)** Heatmap of DEGs (note: green was downregulated and red was upregulated).

the scale-free R2. In **Figure 2C**, the tree graph of all genes is based on different metric (1 – Tom) clustering. Each branch in the tree represents a gene, and the color of each module represents a co-expression module. The heatmap representing the correlation between the epigenome and early DKD formation traits, with each group containing a correlation coefficient and a *P*-value, is shown in **Figure 2D**. Numbers in parentheses on the left represent the number of genes in the corresponding epigenetic module. As shown in **Figure 2**, the most positively correlated gene was magenta, whereas the most negatively correlated gene was blue (see the attached table).

Venn Diagram

Using the Venn Diagram R package (22), we conducted Venn diagram analysis of DEGs and epigenetics filtered from the data set to screen for genes associated with early DKD. These diagrams show overlapping genes and biological complementarities. **Figure 3A** shows the intersection of upregulated and positively relevant genes in early DKD (see the attached table). **Figure 3B** shows the intersection of downregulated and negatively relevant genes in early DKD (see the attached table).

GO Enrichment Analysis and KEGG Pathway Analysis of Differential Genes

Using the DAVID database, we conducted a GO biological function enrichment analysis for 103 significant differences. In terms of biological processes, differential genes were mainly involved in biological processes, which included leukocyte migration, cell chemotaxis, leukocyte chemotaxis, adipocyte differentiation, and organophosphorus response. In terms of cell composition, the different genes were most abundant in the areas of secretory granules and on the external side of the plasma membrane. In terms of molecular functions, the differential genes were mainly enriched in DNA-binding transcription activator activity, RNA polymerase II-specific, C–C chemokine binding, and chemokine binding, as shown in **Figure 3C**. As shown in **Figure 3D**, KEGG signal pathway enrichment analysis suggested that DEGs were involved in C–C receptor interaction, the IL-17 signaling pathway, and viral protein interaction with cytokines and

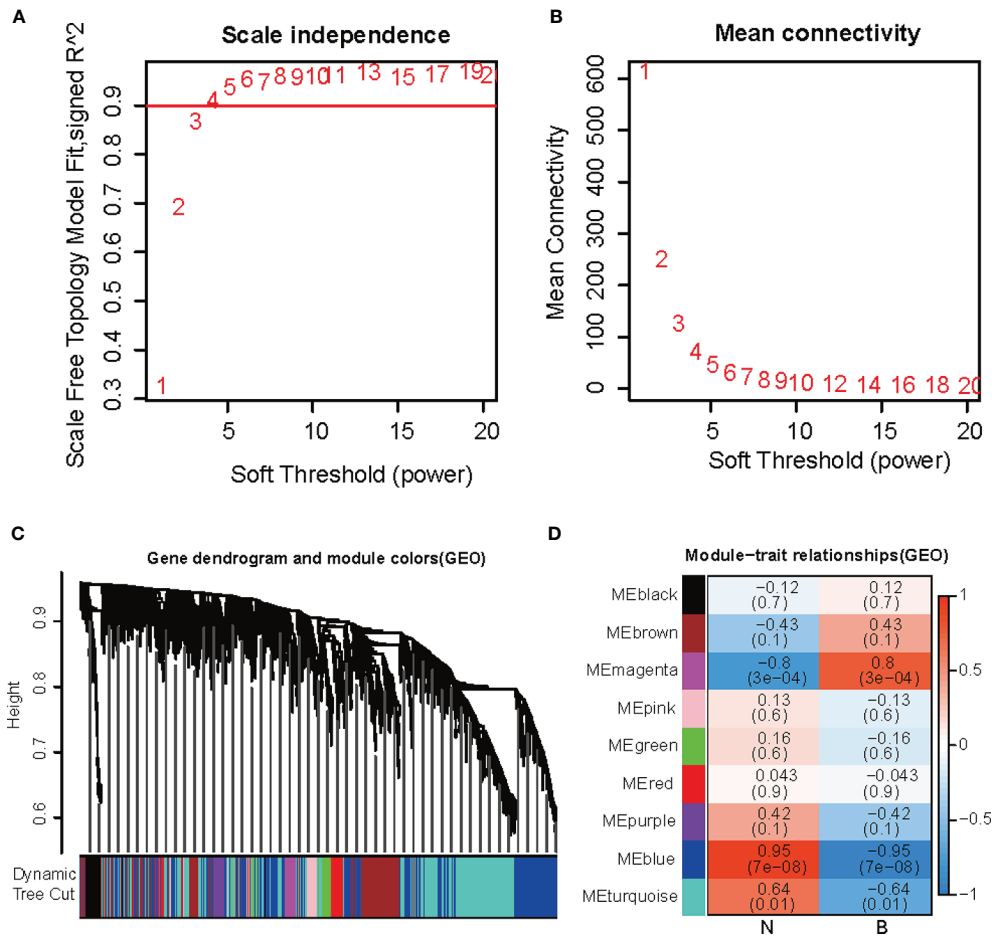

FIGURE 2 | WGCNA co-expression network analysis **(A)** Escale-free fitting index. **(B)** Mean connectivity. **(C)** Each branch in the tree represented a gene, and the color of each module was on behalf of a co-expression module. **(D)** The heatmap for the correlation between the epigenome and early DKD formation traits.

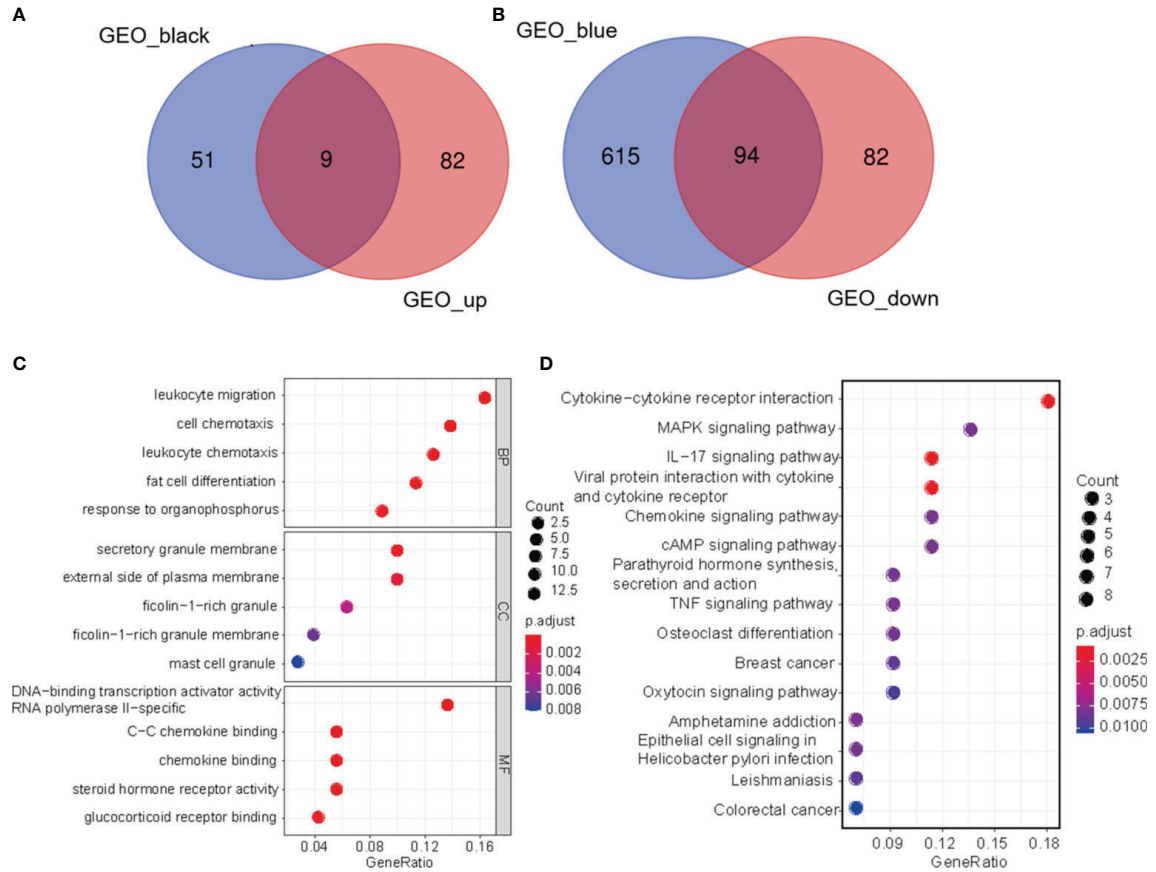

FIGURE 3 | Venn diagram. **(A)** The intersection of up-regulated genes and positively relevant genes in early DKD. **(B)** the intersection of downregulated genes and negatively relevant genes in early DKD. **(C)** Enrichment analysis of GO biological function of DEGs. **(D)** KEGG signaling pathway analysis of DEGs.

cytokine receptors. The results of GO functional biological function enrichment analysis and KEGG signaling pathway enrichment analysis indicated that the biological functions relevant to early DKD were immune inflammatory responses and cytokine effects.

Screening of Hub Genes of Protein Interaction Network

To screen the differential genes that were strongly linked to early DKD, we performed protein interaction network analysis on the basis of 103 differential genes using the STRING database and Cytoscape software, as shown in **Figure 4A**. As a basis, the top 10 positioning hub genes were further screened, and included FOS, JUN, EGR1, ATF3, FOSB, ZFP36, DUSP1, PTGS2, BTG2, and NR4A1, shown in **Figure 4B**. JUN, EGR1, FOS, and ATF3 proteins are closely correlated with other proteins, with darker colors indicating higher grades.

GSEA Enrichment Analysis of Biological Functions and Pathways of Hub Genes

We found that FOS was a hub gene. We conducted a GSEA enrichment analysis of genes with low expression in the FOS group. As shown in **Figure 5**, four activated biological functions, namely, mRNA methylation, sulfation, insulin receptor binding, and protein kinase A binding, were identified. Also shown in **Figure 5**, are four activated pathways, namely, adherens junction, ABC transporters, butanoate metabolism, and steroid hormone biosynthesis, that were identified.

Immune Status of DKD Tissues Were Evaluated Based on CIBERSORT and ssGSEA Algorithms

The heatmap represents the levels of 29 immune genes in normal tissues and early DKD tissues based on the ssGSEA algorithm, as shown in **Figure 6A**. The expression levels of 22 immune cells in normal and early DKD tissues were determined using the CIBERSORT algorithm, as shown in **Figure 6B**. The four hub genes, FOS, EGR1, ATF3, and JUN, were positively correlated with immune cell infiltration in early DKD tissues. ATF3 was positively correlated with monocyte infiltration (P <0.05) and memory resting CD4 T cells (P <0.05). ATF3 was negatively correlated with M2 macrophages (P <0.01) and regulatory T cells (Tregs) (P <0.01). The FOS group was positively correlated with the infiltration of monocytes (P <0.05) and resting natural killer cells (P <0.05). As shown in **Figure 6C**, JUN was positively correlated with monocyte infiltration (P <0.05).

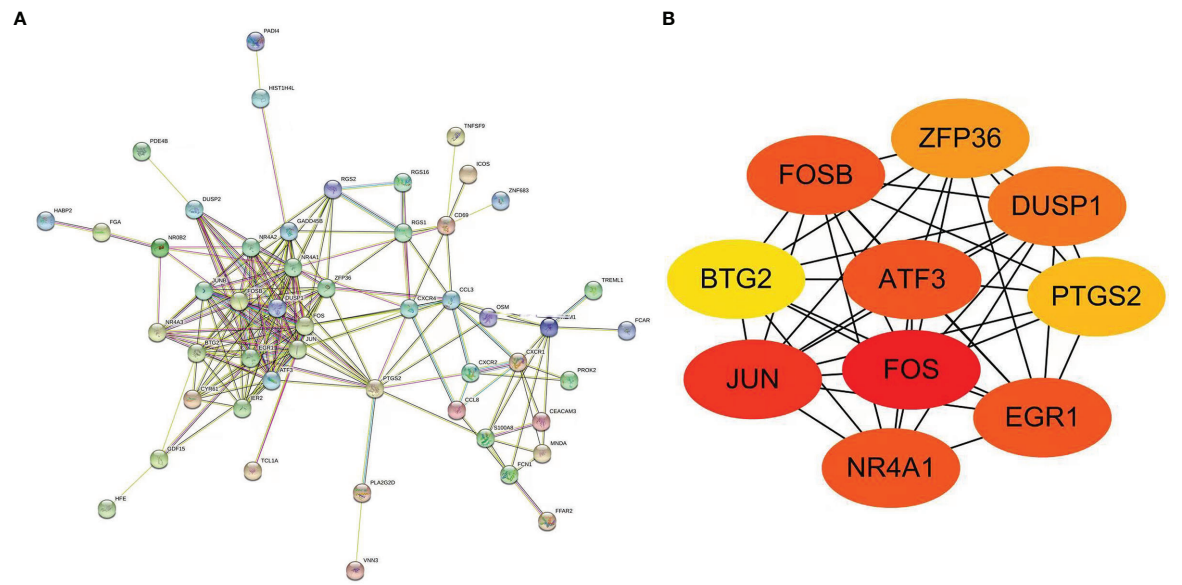

FIGURE 4 | **(A)** Protein–protein interaction network of DEGs. **(B)** Protein–protein interaction network of differentially expressed hub genes (darker color indicated higher grades).

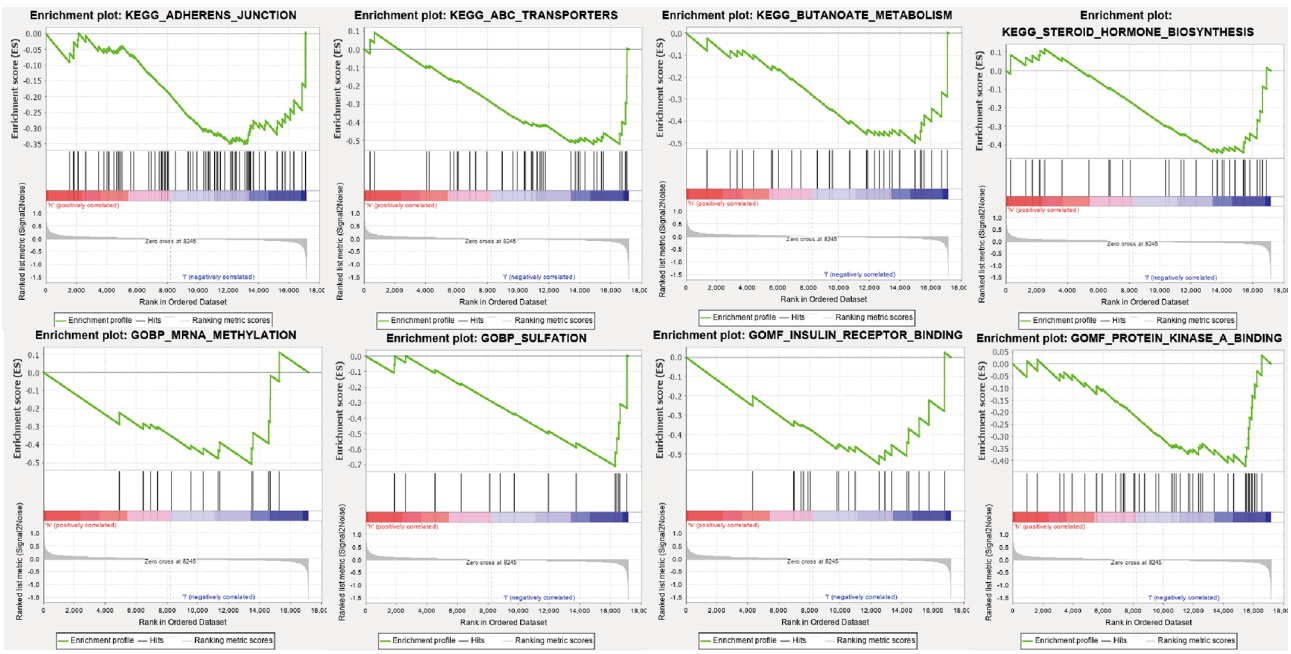

FIGURE 5 | Activated pathways and activated biological functions of GSEA enrichment analysis in the FOS group with low expression.

Immunostaining Results

As shown in **Figure 7**, we performed immunostaining on five early DKD samples and three normal samples from the Beijing Hospital to label proteins such as FOS, EGR1, ATF3, and JUN. The results demonstrated that the relative expression levels of FOS, EGR1, ATF3, and JUN proteins were significantly downregulated in the early DKD group compared with the normal group.

DISCUSSION

DKD is a common complication of type 2 diabetes, with a high prevalence of 20–50% in patients with diabetes (1). DKD accounts for 44.5% of end-stage renal disease cases in developed countries (23). In line with the global diabetes pandemic, the absolute number of DKD patients is increasing

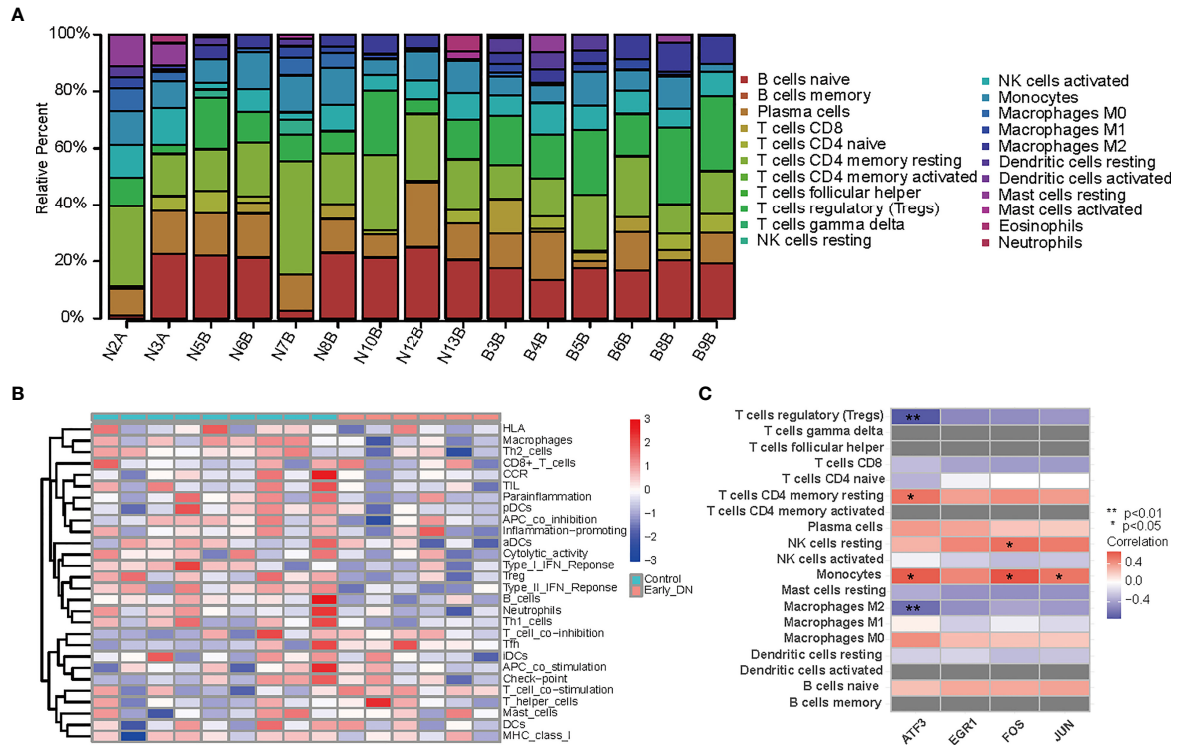

FIGURE 6 | **(A)** Heatmap of 29 immune genes in normal and early DKD tissues based on ssGSEA algorithm. **(B)** Heatmap of the expression levels of 22 kinds of immune cells in normal and early DKD tissues based on the CIBERSORT algorithm. **(C)** Heatmap of hub genes with relevant immune cells infiltration in early DKD tissue.

(24). The cost of diabetes management was estimated to be approximately $760 billion in 2019, and it is expected to rise to $845 billion by 2045, with the majority of the costs used to treat related complications (25). Despite the enormous economic health pressure, we urgently require promising clinical biomarkers of early DKD to effectively slow down and, ideally, halt the progression of DKD.

With the rapid development of high-throughput sequencing technology and gene chip technology, deep mining of sequencing data or gene chips in bioinformatics enables extensive and in-depth analysis of the mRNA expression profile of the whole genome. In this study, we explored the relevant target genes and gene interactions that influence the progression of early DKD. As far as we are aware, this is the first study to screen and identify hub genes in early DKD patients and control normal kidney tissue using WGCNA. We validated our conclusions using kidney specimens from the Beijing Hospital. Our study provides a theoretical basis and promising research proposals for the underlying molecular mechanisms, treatment, and prognosis of early DKD.

In this study, we downloaded the mRNA dataset GSE142025 from the GEO database. A total of 267 DEGs were found in the kidney tissue of patients with early DKD and in normal kidney tissue. We conducted GO biological function enrichment, KEGG signaling pathway enrichment, and protein interaction network analyses. Enrichment analysis suggested that C–C receptor interaction and the IL-17 signaling pathway were essential in early DKD. Using a PPI network and module analysis, we identified FOS, EGR1, ATF3, and JUN as hub sites of protein interaction. Meanwhile, the IHC results revealed that the relative expression levels of FOS, EGR1, ATF3, and JUN were significantly downregulated in the early DKD group compared with normal kidney tissue control.

KEGG signal pathway enrichment analysis suggested that DEGs were mainly enriched in the cytokine receptor interaction pathway, namely, the C–C receptor interaction, the IL-17 signaling pathway, and viral protein interaction with cytokines and cytokine receptors. Kim et al. and Mohamed et al. (26, 27) found that the IL-17 signaling pathway is essential for early DKD and that the application of IL-17A could prevent, treat, and reverse DKD effectively. Cytokine biology tends to be complex. They play various roles by interacting with expressed receptors, triggering signaling pathways and releasing cytokines. In early DKD-based network-centric analyses, the C–C receptor interaction pathway was critical. GSEA enrichment analysis of the FOS group of low-expression genes revealed that the activated biological functions included mRNA methylation, insulin receptor binding, and protein kinase A binding.

Fundamental theories and animal experiments were brought into correspondence with the conclusions of this study. 1) Animal experiments (28, 29) have confirmed that epigenetic regulation of gene expression is important for developing early

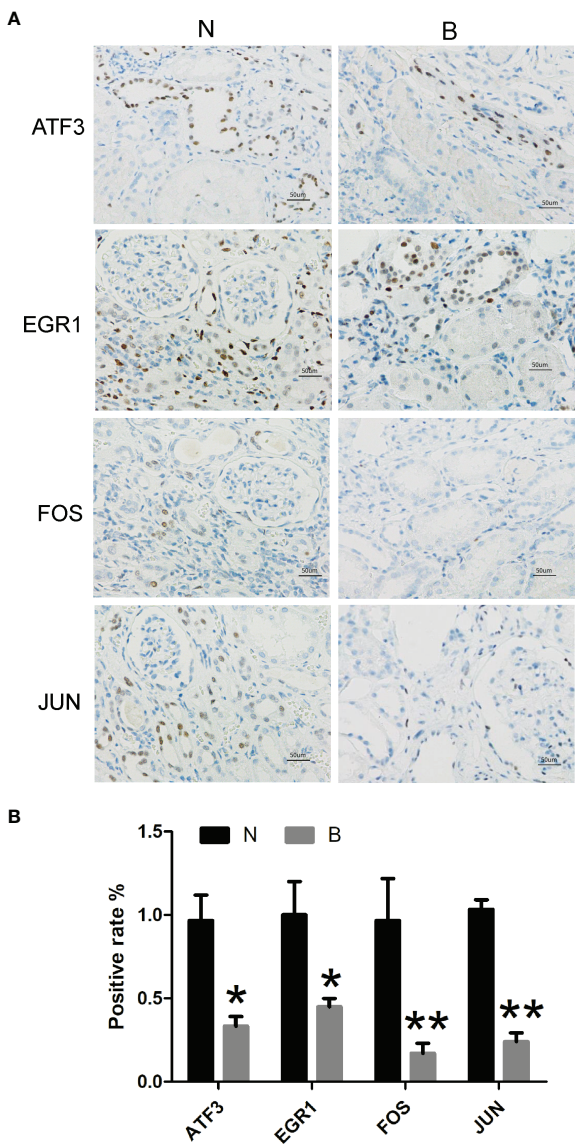

FIGURE 7 | Immunostaining of early DKD and normal samples from the Beijing Hospital to label proteins such as FOS, EGR1, ATF3, and JUN (N, normal kidney tissue; B, early diabetic kidney disease; *P <0.05, **P <0.01).

DKD. In the future, methylation changes promise to predict renal function changes in patients with early DKD (30, 31). 2) Early cellular insulin resistance may directly associate to the onset of early DKD (32). Park et al. and Garner et al. implied that increased insulin receptor signaling protected podocytes from high glucose, insulin, and inflammatory cytokines in the environment (33, 34). 3) Glucose transporter 1-mediated glucose influx, which drove glucose metabolism and ATP production, significantly increased cAMP production and protein kinase A activity in hyperglycemia. Extensive studies (35–37) have demonstrated that the cAMP-protein kinase A pathway plays a crucial role in the epigenetic regulation of profibrotic factors in diabetes. In general, multiple biological functions that are interrelated with low FOS expression are fundamental to the pathogenesis of early DKD.

Several studies have indicated that inflammation is the fundamental pathogenesis of DKD (38, 39). Immune regulation is also associated with disease development and progression (40, 41). Surprisingly, we found that the expression of immune response or inflammatory genes was suppressed in early DKD compared with that in normal kidney tissue. 1) As DKD progressed, the levels of pro-inflammatory monocytes and circulating inflammation increased. Monocytes were recruited to the kidneys and differentiated into macrophages. The recruitment of single cells/macrophages is closely related to the progression of DKD (42, 43). The hub genes, ATF3, FOS, and JUN, were positively correlated with monocytes. However, in our study of early DKD, ATF3, FOS, and JUN expression decreased, indicating fewer monocytes. 2) The imbalance of M1/M2 macrophages leads to persistent inflammation and fibrosis, which ultimately results in renal sclerosis with the release of growth factors (44–48). M2 macrophages can protect cells from damage in the DKD microenvironment (49–51). The hub gene, ATF3, was negatively correlated with M2 macrophage infiltration. However, ATF3 expression was decreased, indicating an increase in macrophage M2 in early DKD in our study. 3) Experimental results suggest that Tregs have potential therapeutic value in improving insulin resistance and slowing organ damage by limiting the pro-inflammatory environment (52–54). ATF3 expression is negatively correlated with Treg infiltration. In contrast, ATF3 expression decreased, whereas Tregs increased in early DKD in our study. This is similar to the finding of Fan et al. (55), who found that the expression of immune response or inflammatory genes was suppressed in early DKD but highly upregulated in advanced DKD.

Expression of c-FOS and c-JUN increased glomerular mesangial cell proliferation, which led to the excess production and accumulation of excreted extracellular matrix. This is an important characteristic of DKD (56). The transcription factor EGR1 is involved in the high glucose-induced proliferation and fibrosis of rat glomerular mesangial cells (57). The stress factor ATF3 is induced in the podocytes of patients with DKD, which increases podocyte apoptosis and injury (58). The four genes that were downregulated at an early stage but are likely to be upregulated with the progression of DKD mostly consist of genes associated with kidney disease pathogenesis, such as those related to immune response and fibrosis. In practice, more data must provide more accurate and reliable conclusions.

Our study has the following limitations. First, we need further validation in cell experiments. Cells were prepared from rats with streptozotocin-induced DKD. Construct plasmids that inhibit the expression of target genes and plasmids that promote the high expression of target genes and then transfer them into early diabetic kidney disease cells. We determined whether the protective effects of the cells were attributed to the upregulation of the expression levels of FOS, EGR1, ATF3, and JUN. This will further confirm our findings, and we are in the process of reversing early DKD by genetic modification. However, further research is required. Second, expression of

immune response or inflammatory genes was suppressed in early DKD in our study. This appears to contradict our previous research hypothesis. However, it is a further immune supplement to the mechanism of early DKD. Inflammation is lower in early DKD than in normal kidney tissues, leading to an immune imbalance. Our findings are critical, as they provide a new perspective on the pathogenesis of early DKD. Finally, there are a few early DKD gene chips in the GEO database. We look forward to more genetic data on the differences between early DKD and normal kidney tissue to provide more precise and reliable results.

SUMMARY

From a bioinformatics perspective, this study revealed early interrelated pathogenic genes. Compared to the normal patient group, the relative expression of FOS, EGR1, ATF3, and JUN proteins in the early DKD group was significantly downregulated. The four hub genes, FOS, EGR1, ATF3, and JUN, were strongly associated with the infiltration of monocytes, macrophage M2, and Tregs. We verified that the expression of immune response or inflammatory genes was suppressed in early DKD. Meanwhile, the FOS group of low-expression genes showed that the activated biological functions included mRNA methylation, insulin receptor binding, and protein kinase A binding. DEGs were mainly enriched in C–C receptor interaction and the IL-17 signaling pathway. Thus, these genes and pathways may be promising therapeutic targets for early DKD.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary Material**.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Beijing Hospital Ethics Committee. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

RW consulted literatures and wrote the manuscript. JQ assisted with writing and revising the manuscript. DC performed immunostaining of kidney issue. QP and LG designed the study. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.883658/full#supplementary-material

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Empagliflozin Attenuates Obesity-Related Kidney Dysfunction and NLRP3 Inflammasome Activity Through the HO-1–Adiponectin Axis

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Empagliflozin (EMPA) is a novel sodium-glucose cotransporter 2 inhibitor (SGLT2i) that produces protective cardiovascular-renal outcomes in patients with diabetes. However, the effects of EMPA on obesity-related kidney disease have not been determined. The heme oxygenase-1 (HO-1)–adiponectin axis is an essential antioxidant system with anti-apoptotic and anti-inflammatory properties. This study explored whether EMPA improves obesity-related kidney disease through regulation of the renal HO-1-mediated adiponectin axis. C57BL/6J mice were assigned to control, high-fat diet (HFD) groups, and EMPA (10 mg/kg) groups. HFD mice showed metabolic abnormality and renal injury, including increased urinary albumin excretion, morphologic changes, and lipid accumulation. EMPA treatment improved metabolic disorders and attenuated lipotoxicity-induced renal injury. Furthermore, EMPA treatment ameliorated renal NLRP3 inflammasome activity and upregulated the HO-1–adiponectin axis. Our findings indicate that EMPA improves obesity-related kidney disease through reduction of NLRP3 inflammasome activity and upregulation of the HO-1–adiponectin axis, suggesting a novel mechanism for SGLT2i-mediated renal protection in obesity.

Keywords: Empagliflozin, obesity, kidney disease, NLRP3, HO-1

INTRODUCTION

The prevalence of obesity, an important public health problem, has substantially increased over the past 30 years (1). This increased prevalence has implications for various complications, including renal damage known as obesity-related kidney disease (OKD) (2). Generally, the onset of OKD is unnoticed; most patients initially have no obvious clinical symptoms, with the exception of microalbuminuria identified during physical examination. In patients with obesity, hyperfiltration often occurs as a compensatory mechanism for the increased metabolic demands. This causes damage to renal structure and function, leading to OKD and the potential for end-stage kidney disease (3, 4). The pathogenesis of OKD usually involves high metabolic demand, insulin

resistance, chronic inflammation, and disordered lipid metabolism (5). However, the mechanisms by which obesity contributes to the induction or progression of OKD have remained unclear.

Heme oxygenase-1 (HO-1) is an inducible enzyme/protein that catalyzes the oxidative degradation of heme to bilirubin (6). HO-1 can sense and respond to various metabolic alterations, including oxidative and inflammatory stress. Increased HO-1 expression is considered a promising therapeutic method for metabolic disease alleviation through the regulation of cellular function and pathophysiology (7). Notably, HO-1 may mediate beneficial effects by enhancing adiponectin secretion; this pathway is known as the HO-1–adiponectin axis (8–10). The activation of this axis in obese animal models may suppress inflammatory cytokine activity and protect against OKD (8, 11). Adiponectin is mainly secreted from adipose tissue that has potent anti-inflammatory, antiatherogenic, and vasoprotective properties (12). Circulating adiponectin levels are usually decreased in obesity and metabolic disease. Adiponectin therapy has glucose-lowering effects and can ameliorate insulin resistance (13). Several studies have reported favorable results of adiponectin treatment in metabolic disease (14, 15). Notably, adiponectin is also produced in non-adipose tissue, particularly in the kidney (e.g., in glomerular endothelial cells and tubular cells) (16). However, there is a need to identify the mechanism by which the renal HO-1–adiponectin axis affects OKD.

Empagliflozin (EMPA), a new oral glucose-lowering drug, selectively acts on sodium-glucose cotransporter-2 inhibitor (SGLT2i) receptors in proximal kidney tubule epithelial cells; it inhibits sodium-glucose cotransporters to reduce blood glucose (17). The most direct effects of SGLT2i therapy include the restoration of tubule feedback and reduction of both oxidative stress and inflammation; these effects have renoprotective and cardioprotective outcomes (18). Although SGLT2i therapy improves diabetic nephropathy outcomes, no study has investigated whether OKD can be alleviated by SGLT2i therapy in patients or animals with obesity. This study examined whether EMPA could improve OKD through the HO-1–adiponectin axis in high-fat diet (HFD)-induced obese mice.

MATERIALS AND METHODS

Experimental Animals

Four-week-old male C57BL/6J mice were purchased from Jinan Pengyue Laboratory (China). All mice were randomly assigned to normal control (NC), HFD, and HFD-EMPA (HFD-E) groups. Mice in the NC group were fed a regular diet, while mice in the other groups were fed an HFD (45% fat, 530 kcal/100 g; Fanbo Biotechnology, Wuxi, China). After they had received an HFD for 24 weeks, mice in the HFD-E group were administered EMPA (10 mg/kg/day) (Boehringer Ingelheim) by oral gavage for another 8 weeks. Body weight and body composition analysis were measured weekly (Bruker Minispec LF50, Germany). After 32 weeks of feeding, glucose tolerance assessment, insulin resistance assessment, and 12-h urine collection were performed; mice were sacrificed 1 week later. The study protocol was approved by the Animal Ethics Committee of Weifang Medical University.

Oral Glucose Tolerance Test and Insulin Tolerance Test

After they had been fasted for 6 h, the mice were administered 2 mg/g glucose by oral gavage (oral glucose tolerance test) or 0.75 U/kg regular insulin (diluted in saline) by intraperitoneal injection (insulin tolerance test). Tail venous blood were collected for assessment with a blood glucometer (On Call EzIII, China) at various time points.

Biochemical Assays

Plasma triglycerides (TG) and free fatty acid (FFA) concentrations were measured using commercial test kits (BC0625 and BC0596, Solarbio, China). Urinary albumin was measured using an enzyme-linked immunosorbent assay (CEB028Mu, Cloud-Clone Corp, China). Urinary creatinine was measured using a test kit from Jiancheng (Nanjing, China).

Immunofluorescence

For the detection of NLR family, pyrin domain containing 3 (NLRP3) expression patterns, frozen tissue blocks were cut into 5-μm sections. After they had been washed with phosphate-buffered saline, tissues were fixed with 4% paraformaldehyde solution for 10 min, permeabilized with 0.3% Triton-X 100 (TB8200, Solarbio, China) for 20 min, blocked with 1% bovine serum albumin, then followed by incubated with NLRP3 antibody (#15101, Cell Signaling Technology) (1:200) overnight. Subsequently, the tissues were incubated with goat secondary antibody (1:600; ab150077, Abcam) at room temperature, then incubated with 4′,6-diamidino-2-phenylindole (DAPI, 1:200) for 10 min. Finally, NLRP3 expression patterns were photographed using a microscope (Zeiss, Germany).

Histopathological Analysis

Kidney tissues were separated in cooled saline, then immediately fixed in 4% paraformaldehyde and embedded in paraffin. Five-millimeter sections were cut from paraffin blocks, then stained with hematoxylin and eosin for histopathology analysis. Kidney tubular interstitial fibrosis was evaluated by Masson's trichrome and Picrosirius Red staining, while lipid accumulation was assessed by Oil Red O staining. Photographs were acquired using Motic Digital Pathology Solution (Easyscan, Motic, China).

Transmission Electron Microscopy

Mouse kidney tissues were fixed in sodium cacodylate buffer. Fixed tissues were trimmed to 1-mm^3 cubes for embedding. Sixty-nanometer-thick sections were cut using an ultramicrotome (Leica UC7, Leica, Germany); the sections were placed in cuprum

grids. The cuprum grids were then imaged *via* transmission electron microscopy (HT7800/HT7700, Hitachi, Japan).

Western Blotting

Kidney tissue was ground with a manual homogenizer and homogenized in cold protease inhibitor lysis buffer for 30 min. It was then centrifuged at 12000 rpm for 10 min at 4°C; the supernatant was subjected to protein quantification *via* bicinchoninic acid assay. The samples were separated *via* 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis, transferred to polyvinylidene fluoride membranes, and incubated with antibodies to the following proteins: HO-1 (#43966, Cell Signaling Technology); Adiponectin (ab181281, Abcam) and β-actin (66009-1-Ig, Proteintech).

Reverse Transcription Polymerase Chain Reaction Analysis

Total RNA from the left kidney was isolated and extracted using TRIzol Reagent (Invitrogen). Then, the extracted RNA was reverse-transcribed using a PrimeScript™ RT Reagent Kit with gDNA Eraser (#RR047A, TaKaRa). TB Green® Premix Ex Taq™ II (#RR820A, TaKaRa) was used for quantitative polymerase chain reaction analysis. Relative changes in expression levels of amplified genes were determined using the comparative cycle threshold (Ct) method (i.e., $2^{-\Delta\Delta Ct}$). Relative expression levels of the interleukin (IL)-1β, IL-6, IL-18, and NLRP3 genes were normalized to the expression of β-actin. The primers used in this study are shown in **Table 1**.

RNA-Seq and Data Analysis

The RNA sequence and data analysis were prepared as previously described by our group (19). Differentially expressed genes (DEGs) were identified using limma packages in R 4.0.3 with the default parameters at (logFC) > 1, P.Value < 0.05 for the groups. And Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses of DEGs were conducted using the clusterProfiler package in R based on the criteria: Adjusted.P.Value<0.01.

Statistical Analysis

All statistical analyses were carried out using GraphPad Prism 8. The results are shown as means ± standard errors of the mean. One-way or two-way ANOVA and Turkey's test were utilized as appropriate. Differences were considered statistically significant based on the following setting: P<0.05.

RESULTS

EMPA Treatment Decreased Weight Gain, Fat Mass, and Fat%, While Normalizing Glucose Intolerance, in HFD Mice

After 32 weeks of HFD administration, HFD mice exhibited significant morphological changes compared with NC mice (**Figures 1A, B**). Specifically, HFD mice had significantly increased body weight (49.67 ± 1.48 g vs. 30.82 ± 1.08 g, P<0.05), fat mass (14.56 ± 0.43 g vs. 2.05 ± 0.18 g, P<0.05), and fat/weight% (29.37 ± 0.75% vs. 7.74 ± 0.27%, P<0.05), compared with NC mice. However, EMPA treatment significantly decreased the final body weight (44.87 ± 1.42 g vs. 49.67 ± 1.48 g, P<0.05), fat mass (11.75 ± 0.78 g vs. 14.56 ± 0.43 g, P<0.05), and fat/weight% (26.08 ± 1.09% vs. 29.37 ± 0.75%, P<0.05), compared with HFD alone (**Figures 1C–E**). To further explore the lipid metabolism profiles, we measured levels of serum TG and FFA. As expected, these indicators were remarkedly increased in the HFD mice, compared with NC mice (TG: 32.87 ± 1.69 mg/dL vs. 18.51 ± 2.49 mg/dL, P<0.05; FFA: 1303.00 ± 81.14 μmol/L vs. 618.60 ± 52.12 μmol/L, P<0.05); EMPA significantly decreased FFA (746.30 ± 56.59 μmol/L vs. 1303.00 ± 81.14 μmol/L, P<0.05) but had no beneficial effects on TG (29.31 ± 2.71 mg/dL vs. 32.87± 1.69 mg/dL, P>0.05), compared with HFD alone (**Figures 1F, G**). Furthermore, compared with NC mice, HFD mice showed higher fasting glucose levels (10.32 ± 0.66 mmol/L vs. 6.73 ± 0.37 mmol/L, P<0.05) and impairments of both glucose tolerance and insulin tolerance; these alterations were mitigated by EMPA treatment (P<0.05) (**Figures 1H–J**), suggesting that EMPA could alleviate HFD-induced metabolic disorders.

EMPA Treatment Decreased Kidney Injury in HFD Mice

Urinary albumin assessment and histopathology techniques were used to observe renal function. The ratio of urinary albumin to creatinine was higher in HFD mice than in NC mice (45.24 ± 4.71 μg/μmol vs. 14.26 ± 2.28 μg/μmol, P<0.05); EMPA treatment decreased this ratio compared with HFD alone (21.01 ± 1.99 μg/μmol vs. 45.24 ± 4.71 μg/μmol, P<0.05) (**Figure 2B**). HFD mice showed substantial glomerular hypertrophy and renal tubular lumen enlargement in hematoxylin and eosin staining analyses; they also exhibited considerable lipid deposition in Oil Red O staining analyses of renal tubules. Furthermore, HFD treatment induced significant renal fibrosis, compared with NC treatment, in Masson's trichrome

TABLE 1 | The primers used in the study.

Gene	Primer sequence (5'→3')	
β-actin	F: GGCTGTATTCCCCTCCATCG	R: CCAGTTGGTAACAATGCCATGT
IL-1β	F: GCAACTGTTCCTGAACTCAACT	R: ATCTTTTGGGGTCCGTCAACT
IL-18	F: GACTCTTGCGTCAACTTCAAGG	R: CAGGCTGTCTTTTGTCAACGA
IL-6	F: CTGCAAGAGACTTCCATCCAG	R: AGTGGTATAGACAGGTCTGTTGG
NLRP3	F: ATTACCCGCCCGAGAAAGG	R: TCGCAGCAAAGATCCACACAG

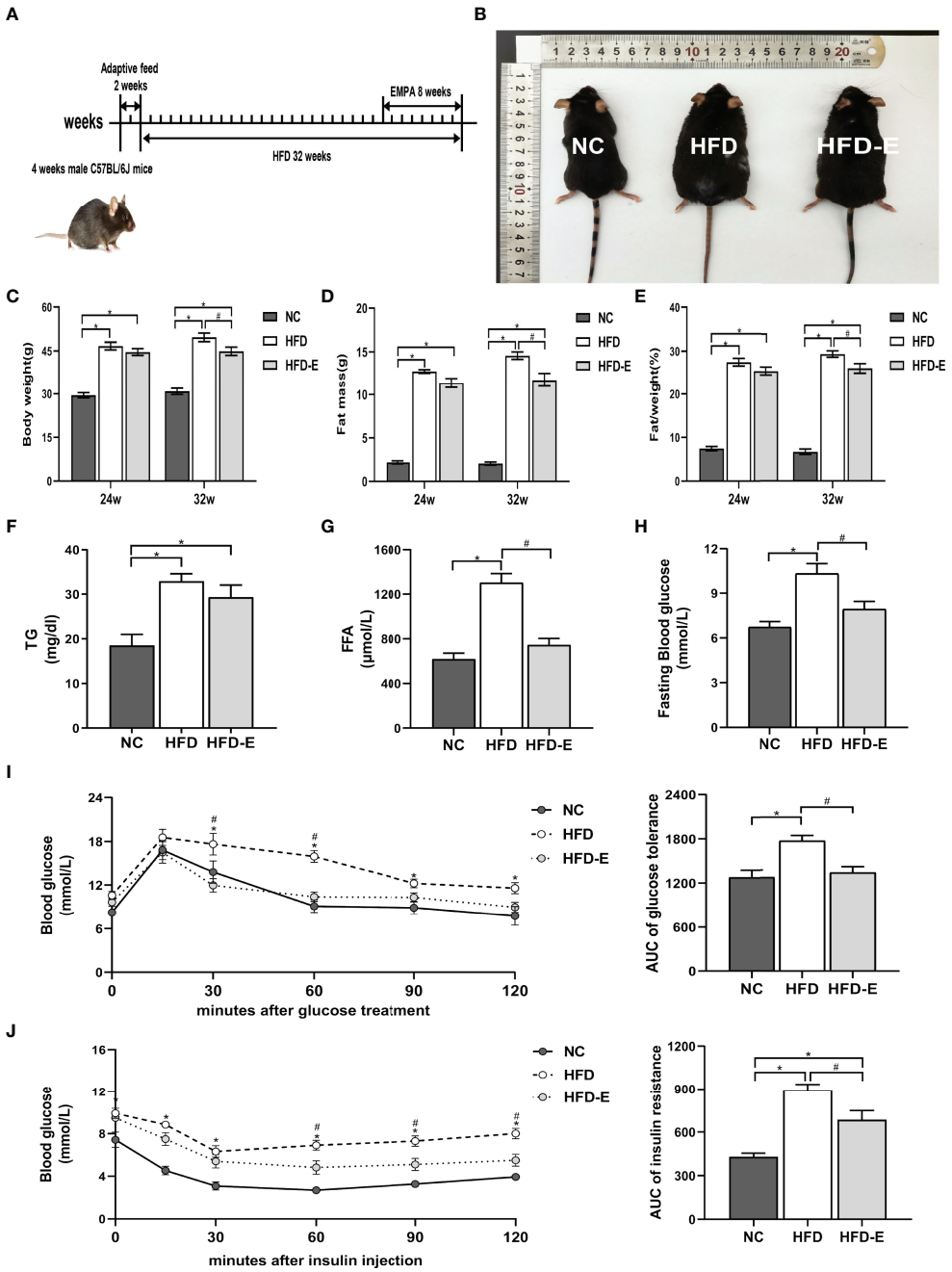

FIGURE 1 | EMPA reduced body weight and glycolipid metabolism. **(A)** 4-week-old male mice were fed HFD for 24 weeks and then treated with EMPA for another 8 weeks. **(B)** Morphology of mice. **(C)** Body weight. **(D)** Body fat mass. **(E)** Body fat mass%. **(F)** TG levels. **(G)** FFA levels. **(H)** Fasting blood glucose levels. **(I)** Oral glucose tolerance test and area under curve (AUC) of glucose tolerance. **(J)** Insulin tolerance test and AUC of insulin tolerance. Data are means ± SEM, n = 6/group, *P < 0.05 vs. NC; #P < 0.05 vs. HFD.

and Picrosirius Red staining. Contemporaneously with these changes, the mitochondria swell and rupture, crest disorder, and increased lipid droplets accumulation in the transmission electron microscope in HFD mice. These morphological alterations were partially reversed by EMPA treatment, indicating that EMPA exerts renoprotective effects in HFD mice (**Figure 2**).

Kidney Transcriptomic Analyses Revealed Novel EMPA-Induced Pathways in HFD Mice

To identify potential mechanisms by which EMPA alleviates OKD, three groups of kidneys were subjected to transcriptome profiling. These samples were divided into three groups (NC,

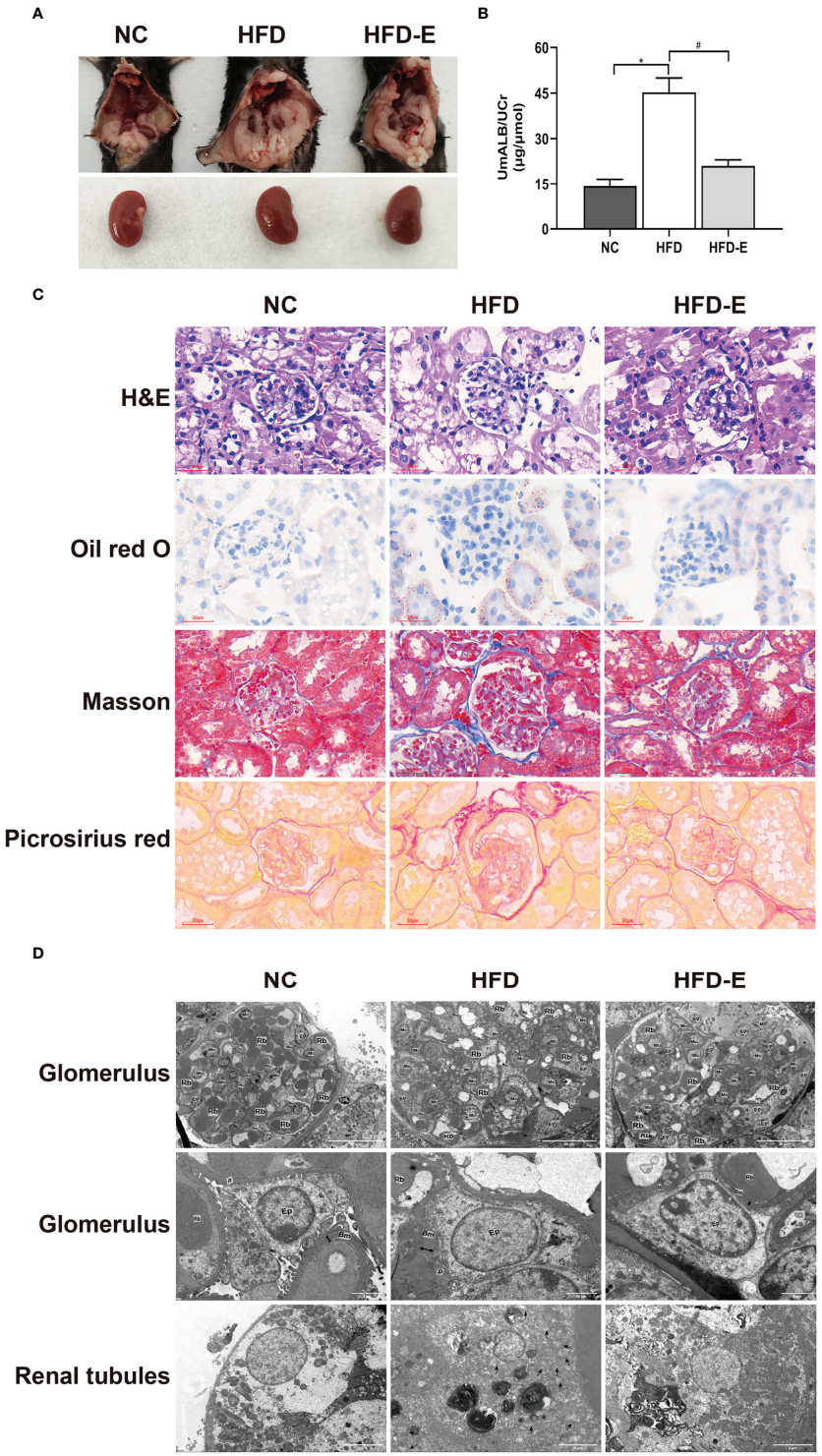

FIGURE 2 | EMPA improved kidney dysfunction and morphologic change. **(A)** Morphology of mice kidney. **(B)** The ratio of urinary albumin to creatinine (n= 6/group). Data are means ± SEM. *P < 0.05 vs. NC; #P < 0.05 vs. HFD. **(C)** H&E, Oil red O, Masson trichrome and picrosirius red staining. Scale bar = 30 μm. **(D)** TEM images of glomerular and tubular structures. Scale bar = 20 μm, 2 μm and 5 μm. BM, basement membrane; Ep, epithelial cells; Rb, red blood cell; Mc, mesangial cells; P, podocyte; Double arrow, basement membrane thickness; * lipid drops; ↑ Damaged mitochondria.

HFD, HFD-E), and limma package in R 4.0.3 were used to screening DEGs. DEGs were identified using two comparisons: HFD/NC, and HFD-E/HFD based on (logFC) > 1, P.Value < 0.05. We identified 1029 DEGs union in three groups (**Figure 3A**), 852 DEGs in comparing HFD vs. NC, 279 DEGs in the HFD-E vs. HFD groups, both of which 102 DEGs shared in HFD vs. NC and HFD-E vs. HFD (**Figure 3B**). Additionally, we identified DEGs for which expression levels were altered or reversed by EMPA. In total, 852 DEGs were detected in the HFD vs. NC comparison, of which 524 were upregulated and 328 were downregulated; 279 DEGs were detected in the HFD-E vs. HFD comparison, of which 127 were upregulated and 152 were downregulated. All DEGs were depicted using bar plots and volcano diagrams (**Figures 3C, D**). These DEGs shared in HFD-E vs. HFD and HFD vs. NC were enriched in GO and KEGG categories associated with cytokines and chemokines based on the criteria: Adjusted.P.Value<0.01 (**Figure 3E**).

EMPA Treatment Decreased NLRP3 Inflammasome Activity in HFD Mice

Immunofluorescence staining to quantify the protein expression of NLRP3 in mouse kidney tissue revealed significantly elevated expression in HFD mice, compared with NC mice; EMPA treatment reversed this expression pattern (**Figure 4A**). Additionally, we analyzed the transcription levels of NLRP3 and its related genes. Consistent with the immunofluorescence staining results, HFD induced increased transcription of NLRP3, IL-6, IL-1β, and IL-18; these alterations were reversed by EMPA treatment ($P<0.05$; **Figures 4B–E**).

EMPA Treatment Induced HO-1–Adiponectin Axis Activity in HFD Mice

To verify whether the renal HO-1–adiponectin axis is involved in OKD, we detected the levels of these proteins in kidney tissue; both were significantly decreased in HFD mice. Importantly, EMPA reversed this expression pattern through significant upregulation of HO-1–adiponectin levels (**Figure 5**).

DISCUSSION

This study investigated the effect of EMPA treatment on OKD in obese mice. We found that HFD induced clear metabolic abnormality and renal injury, accompanied by downregulation of the HO-1–adiponectin axis and enhancement of NLRP3 inflammasome activity. However, EMPA treatment reduced renal injury and NLRP3 inflammasome through activation of the HO-1–adiponectin axis. This study reveals a novel protective role for EMPA in OKD, with a potential underlying mechanism.

Obesity is an increasing public health problem that leads to metabolic syndrome and increased vascular complications, including OKD. Our HFD treatment induced a metabolic syndrome-like phenotype in mice, which included increased body weight and fat. We also observed hyperglycemia, hyperlipidemia, and impaired glucose tolerance in HFD mice. These pathological abnormalities alter metabolic homeostasis and exacerbate kidney damage, as indicated by increased levels of urinary albumin. Furthermore, HFD induced substantial pathological changes, including glomerular hypertrophy, tubule lumen enlargement, renal fibrosis, and mitochondrial injury. Oil Red O staining and transmission electron microscopy demonstrated that lipid droplet accumulation increased in HFD mice. These results indicated that HFD-induced lipotoxicity and associated metabolic abnormalities lead to renal injury, consistent with our previous findings (2, 20).

EMPA is an SGLT2i with cardioprotective, renoprotective, and glucose-lowering effects in diabetic patients. Lu et al. (21) found that EMPA can modulate myocardial contractility; it can also attenuate ischemia and reperfusion injury. Furthermore, EMPA alleviated cardiac inflammation and energy depletion *via* AMPK activation; it exhibited a renoprotective effect by enhancing endogenous ketone body-induced inhibition of mTORC1 (22, 23). Notably, Li et al. reported that EMPA could inhibit epithelial-mesenchymal transition and aberrant glycolysis in proximal tubules, thus protecting renal function (24). Furthermore, EMPA reduces metabolic derangements and restores altered tubule-glomerular feedback, protecting against diabetes-induced cardiorenal injury (25). Our findings demonstrated that EMPA had robust mitigating effects on metabolic and pathophysiological abnormalities in HFD-induced renal injury.

To further elucidate the mechanism by which EMPA protects OKD, we analyzed the mouse kidney transcriptome by RNA-Seq and found 102 DEGs shared in three groups. GO and KEGG enrichment showed that these DEGs were mainly enriched in cytokines, chemokines, and tumor necrosis factor signaling pathways, all of which were association with inflammation process. We discovered that HFD affects inflammatory processes; EMPA can attenuate these processes.

The NLRP3 inflammasome is an important component of pathological inflammation that can trigger local and systemic inflammation (26); it has crucial roles in various diseases (e.g., autoimmunity, diabetes, and cardiovascular disease). The NLRP3 inflammasome is also activated in both acute and chronic kidney disease in mice and humans (27–29). Activation of the NLRP3 inflammasome and subsequent excess production of IL1β, IL-6, and IL18 lead to exacerbation of kidney injury (30). The NLRP3 inflammasome participates in host–pathogen interactions; it recruits and activates pro-inflammatory proteases. Therefore, treatments targeting the NLRP3 inflammasome, the center of inflammatory response, may be useful for the management of various inflammation-related diseases. In the present study, immunofluorescence and RNA-Seq analyses indicated that EMPA treatment attenuated NLRP3 inflammasome activity and inflammation-related biological processes, but the detailed mechanism requires further exploration.

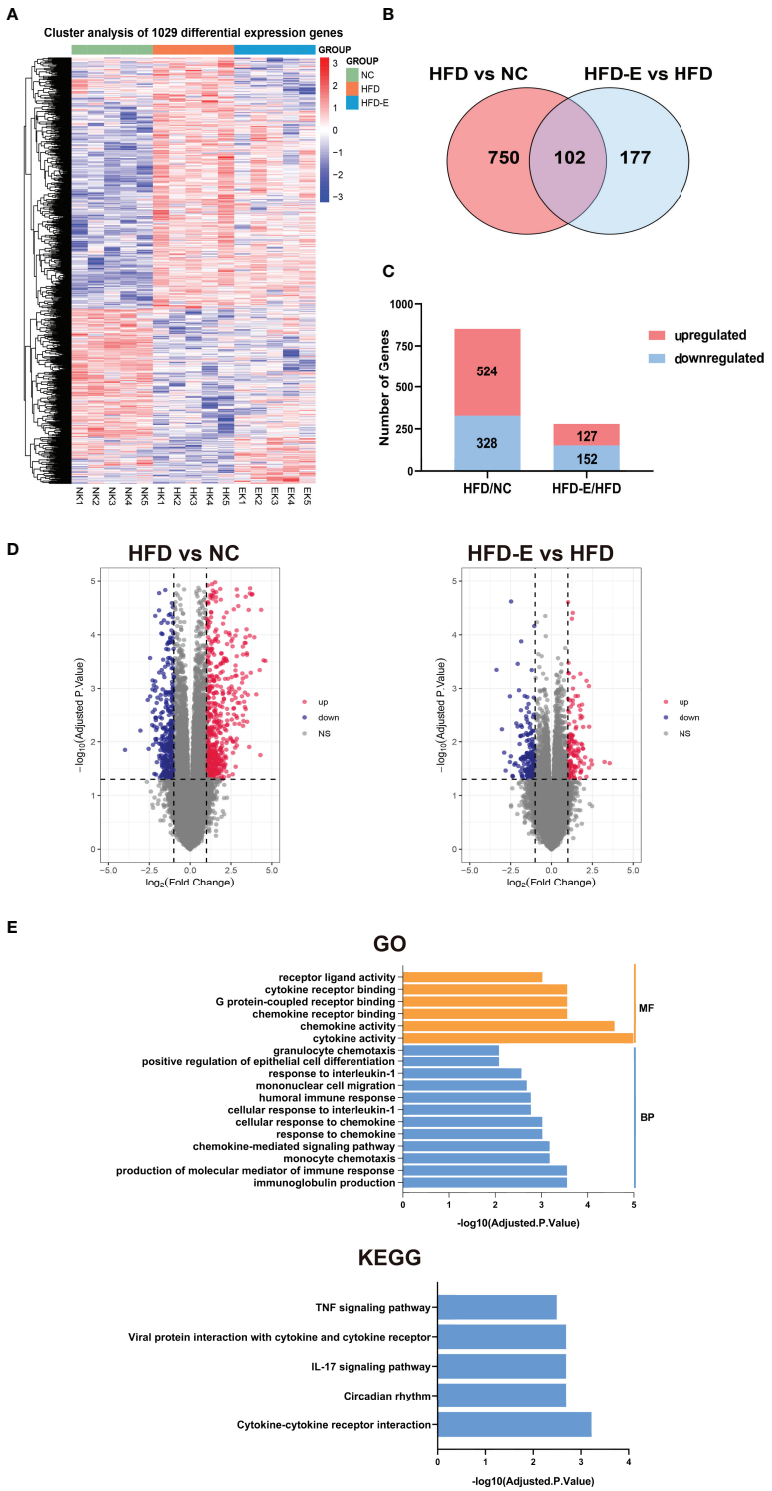

FIGURE 3 | Kidney tissue were collected and then subjected to RNA-seq analysis. **(A)**The DEGs of HFD vs. NC and HFD-E vs. HFD. **(B)**. DEGs associated with HFD vs. NC and HFD-E vs. HFD (light red and blue). **(C)** The number of upregulated and downregulated genes from the HFD vs. NC, and HFD-E vs. HFD in the mice renal genome. **(D)** Volcano plot for the distribution of DEGs between the HFD vs. NC and HFD-E vs. HFD. Blue represents a down-regulation in expression, red represents upregulation and gray represents no significance compared to control. **(E)** Main GO terms and KEGG pathways based on shared DEGs between HFD vs. NC and HFD-E vs. HFD.

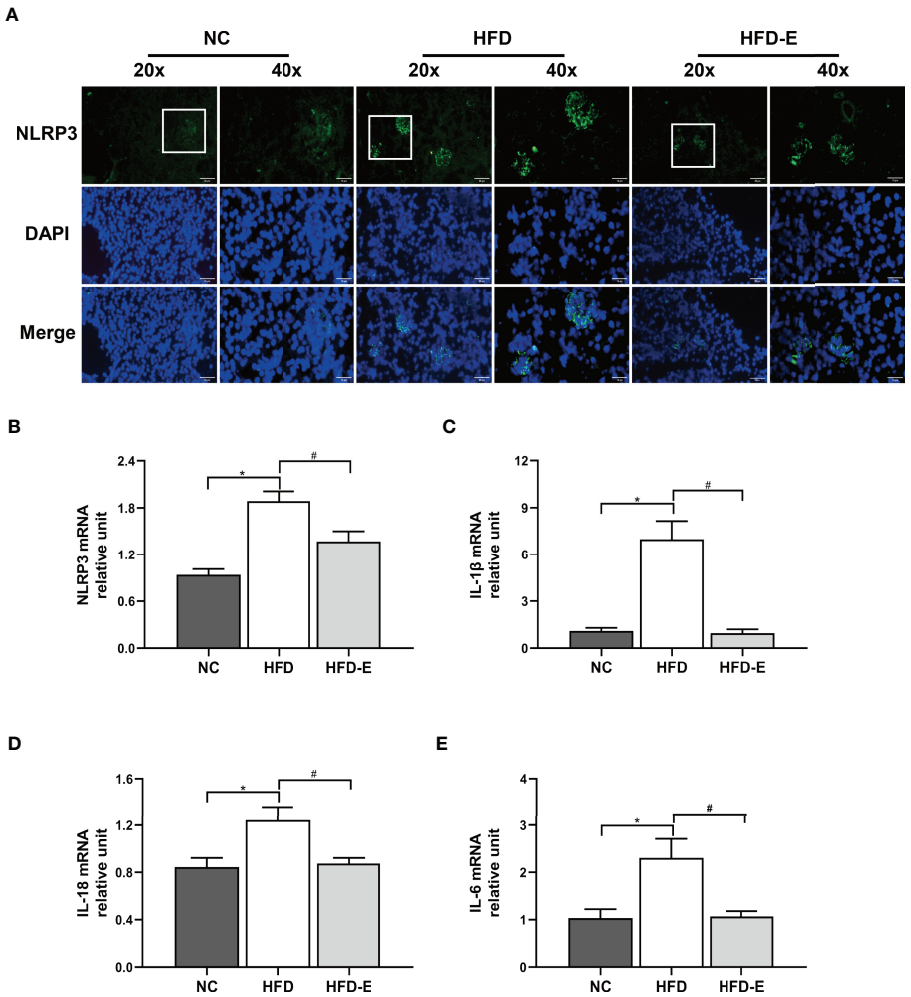

FIGURE 4 | EMPA attenuated the NLRP3 inflammasome. **(A)** Immunofluorescence of NLRP3 inflammasome. Scale bar = 20 μm and 10 μm. **(B–E)** mRNA levels of NLRP3, IL-1β, IL-6, IL-18 (n = 6/group). Data are means ± SEM. *P < 0.05 NC; #P < 0.05 vs. HFD.

HO-1 is a rate-limiting enzyme that catalyzes heme degradation, with important anti-inflammatory and anti-oxidative properties; it is mainly synthesized by the spleen and "visceral adipose tissue macrophages (31). HO-1 has been shown to reduce NLRP3 inflammasome activity in mice (32); it also reduces visceral fat accumulation, normalizes metabolic profiles, and prevents obesity, thereby reducing cardiovascular and renal complications (7, 33–35). Notably, these beneficial effects were partly mediated through impacts on the adiponectin-dependent pathway (36, 37). Adiponectin is mainly secreted by white adipose tissue; however, its levels are usually lower in the context of obesity and metabolic syndrome, despite adipose accumulation (38). A lower adiponectin level is inversely associated with insulin resistance. Adiponectin has various beneficial effects and modulates many metabolic processes, including anti-atherosclerotic and anti-inflammatory effects (39). We previously showed that HO-1 induction could increase serum adiponectin, thus reducing urinary albumin levels and protecting against OKD by improving endothelial dysfunction (8). These results indicate that HO-1 activation may be a useful treatment for obesity-related renal damage. However, no studies have reported whether renal HO-1 and adiponectin participate in OKD. Here, we found that both of them in kidney tissue were decreased after HFD induction. However, EMPA treatment could increase these levels, indicating the HO-1–adiponectin axis was activated by EMPA. In addition, HO-1 overexpression may protect the D-Galactosamine and lipopolysaccharide-induced hepatic malfunction through suppression of the NLRP3 (40). Adiponectin could inhibit NLRP3 inflammasome activation in nonalcoholic steatohepatitis or cerebral ischemia-reperfusion injury (41, 42). Thus, HO-1 and adiponectin are implicated in NLRP3 inflammasome activation. These findings support our hypothesis that EMPA treatment increased HO-1–adiponectin axis activity and decreased NLRP3 inflammasome activity.

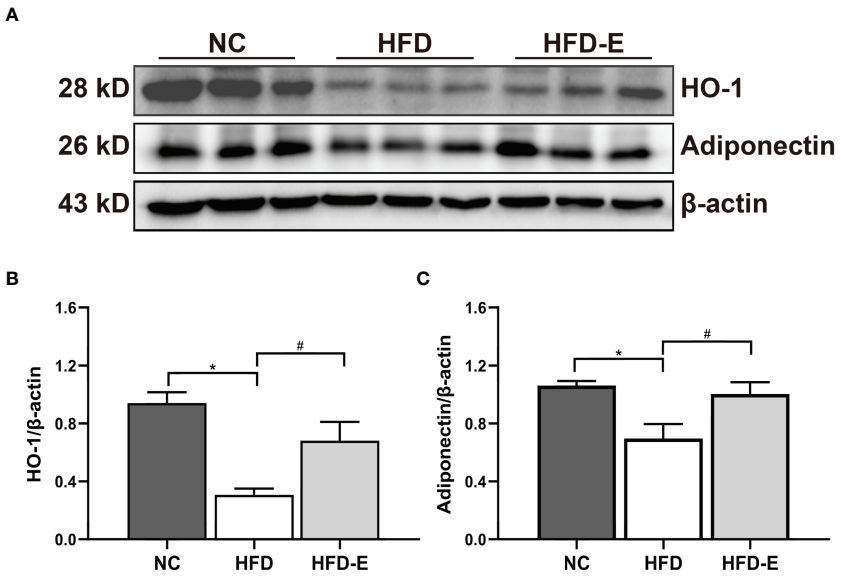

FIGURE 5 | EMPA induced HO-1-adiponectin protein expressions. **(A–C)** Immunoblotting analysis and quantification of HO-1 and adiponectin. n = 6/group; Data are means ± SEM. *P< 0.05 vs. NC; #P < 0.05 vs. HFD.

CONCLUSION

In conclusion, our study demonstrated that EMPA can protect against OKD by activating the HO-1–adiponectin axis and reducing NLRP3 inflammasome activity in HFD mice. Kidney transcriptome analysis revealed that EMPA affects essential genes closely associated with inflammation. Our findings provide new knowledge concerning the mechanism by which EMPA exhibits protective effects in OKD.

DATA AVAILABILITY STATEMENT

The data presented in the study are deposited in the NCBI repository, accession number BioProject ID: PRJNA835250.

ETHICS STATEMENT

The animal study was reviewed and approved by Animal Ethics Committee of Weifang Medical University.

AUTHOR CONTRIBUTIONS

TY and JZ collected the data, conducted the analysis, and drafted the manuscript. XS and FH designed the entire study and revised the manuscript. Other authors participated in the data collection and analysis.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.907984/full#supplementary-material

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Shear Wave Elastography in the Diagnosis of Peripheral Neuropathy in Patients With Chronic Kidney Disease Stage 5

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Objective: To observe the feasibility of shear wave elastography (SWE) in the diagnosis of peripheral neuropathy in patients undergoing hemodialysis [chronic kidney disease stage 5 dialysis (CKD5D)].

Methods: Forty patients with CKD5D were divided into a uremic peripheral neuropathy (UPN) group (n = 25) and a non-UPN group (n = 15) according to the results of a neuro-electrophysiological examination. Sixteen healthy control subjects were also enrolled in this study. Two-dimensional ultrasound examination was conducted, and SWE was then performed to measure Young's modulus of the tibial nerve. The left and right diameters (D1), anterior and posterior diameters (D2), perimeter (C), cross-sectional area (CSA), and Young's modulus (E) were measured three times at the same non-entrapment site. The average values were recorded and calculated. The following evaluation indices were also analyzed: sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and area under the receiver operating characteristic curve (AUC).

Results: D1, D2, C, and CSA were not significantly different among the three groups ($P > 0.05$). However, the difference in the E value among the three groups was statistically significant ($P < 0.05$). The AUC was 0.889 based on the E value. Using a tibial nerve E value of 48.35 kPa as the cutoff value, the sensitivity, specificity, PPV, and NPV were 86.0%, 84.0%, 81.1%, and 88.1%, respectively.

Conclusions: SWE is useful for the diagnosis of peripheral neuropathy in patients with CKD5D. Young's modulus of 48.35 kPa for the tibial nerve is the optimal cutoff value and has the best diagnostic efficiency for peripheral neuropathy in CKD5D patients.

Keywords: chronic kidney disease, peripheral neuropathy, cutoff value, tibial nerve, elasticity imaging techniques

INTRODUCTION

Chronic kidney disease (CKD) is a global health problem that reduces quality of life and disrupts economic development. One of the most common neurological complications of CKD, especially CKD stage 5 dialysis (CKD5D), is peripheral neuropathy, which affects approximately 60% to 90% of patients with CKD (1). Uremic peripheral neuropathy (UPN) has characteristic symptoms or can be detected by clinical examinations. The most common and earliest symptoms are mainly sensory dysfunctions, such as pain and paresthesia; these are followed by limb weakness and atrophy (2). However, some patients with impaired nerve function exhibit no clinical symptoms (3, 4). Thus, a nerve conduction study (NCS) is usually performed to evaluate the function of peripheral nerves. However, the nerve conduction is not sensitive enough to detect peripheral neuropathy, especially in asymptomatic patients (5).

Ultrasound (US) elastography has become an important tool for the evaluation of nerve stiffness. This procedure takes less time to perform and causes less discomfort to patients. US examinations are used to assess the elasticity of the nerve in patients undergoing hemodialysis by measuring the nerve cross-sectional area (CSA) (6, 7). Two-dimensional shear wave elastography (2D-SWE) is a new complementary method to NCS that has been widely applied for the detection of diabetic peripheral neuropathy in recent studies (8, 9). It can reveal minor peripheral nerve lesions that cannot be detected by electrophysiology (8) and has higher sensitivity and specificity than US, which is mainly based on CSA measurement (6). Although research has revealed changes in nerve elasticity in patients undergoing hemodialysis, few studies have used 2D-SWE for the detection of peripheral nerve damage in these patients. Thus, the diagnostic performance of 2D-SWE in patients undergoing hemodialysis was evaluated in the present study.

MATERIALS AND METHODS

Ethics and Consent

This study was approved by the ethics committee of the Affiliated Hospital of Weifang Medical University. All participants provided written informed consent.

Participants

Forty patients (15 women, 25 men) undergoing hemodialysis were recruited from the Affiliated Hospital of Weifang Medical College dialysis center. All patients underwent electrophysiological tests. Sixteen healthy volunteers with no clinical signs or symptoms were enrolled as the control group. All participants underwent US and 2D-SWE examinations. The following basic data were collected for all participants: sex, age, hemoglobin (Hgb), hematocrit (HCT), albumin (Alb), total protein (TP), blood lipid indices, blood urea nitrogen (BUN), serum creatinine (Scr), parathyroid hormone (PTH), beta-2 microglobulin, blood sodium, blood potassium, blood calcium, blood phosphorus, and duration of dialysis. The inclusion criteria were treatment with hemodialysis in our dialysis center and good cognitive function and communication skills; and the age ranged from 18 to 78 years. The exclusion criteria were polyneuropathy caused by diabetes, hereditary factors, alcohol intake, metabolic factors, inflammatory factors, a malignant tumor, or toxic factors; skin lesions or swelling of the ankle; leg or ankle fractures; and damage to the liver, brain, heart, lung, or other important organs.

Diagnosis of UPN

The diagnosis of UPN is based on symptoms and clinical examination findings (10). Patients may have one or more of the following clinical symptoms: paresthesia, restless leg syndrome, increased pain sensation, impaired deep tendon reflexes, imbalance, numbness, and atrophy of the lower limbs (11–15). An NCS is currently regarded as the most effective method to diagnose peripheral neuropathy. Thus, it is used as the gold standard for the diagnosis of UPN (11, 12). In the present study, one neurologist with 10 years of experience in NCSs performed an NCS for all patients. The patients were grouped according to the results.

Electrodiagnostic Studies

Electromyography was performed using a conventional procedure on a standard system (Nicolet EDX; Natus Medical, Middleton, WI, USA). All examinations were performed in the same room at an ambient temperature of 25°C. They were begun 1 hour after a hemodialysis session because normalization of nerve excitability parameters can occur after hemodialysis (16, 17). An NCS of the bilateral tibial nerves was performed in every patient. We obtained all relevant data including latencies, amplitudes, and conduction velocity. The case definition for UPN was based on the results of electromyography, which is the gold standard method (18).

Patient Positioning

All patients were placed in the supine position during the examination. Their ankles were relaxed. To prevent the effect of ankle soft tissue pressure, ankle movement was avoided during the examination.

US and SWE Measurements

The whole study population underwent SWE and US examinations by Sonographer 1 (Zhaoguang Zhang, with 10 years of experience in US and 3 years of experience in elastography). Six of the participants were randomly selected for a second SWE examination by Sonographer 1 after 24 hours and they were performed by Sonographer 2 (Qian Luo, with 8 years of experience in US) by SWE. Both Sonographers were blinded to the participants' information, including their clinical history, previous examination findings, and NCS results. All SWE examinations were completed within 1 week after the NCS.

The examinations were performed using a device with an L2-9-D transducer (LOGIQ E20; GE Healthcare, Chicago, IL, USA). The transducer was gently placed onto the skin surface. Care was taken to perform the examination with light contact using ample

coupling gel. The tibial nerve was scanned upward from the medial malleolus to examine the cross section of the tibial nerve, determine the tibial nerve boundary, observe the internal structure of the tibial nerve, and assess the echo of the nerve bundle. The tibial nerve CSA was measured 4 cm above the medial malleolus. The transducer was then rotated 90° to view the longitudinal imaging plane. The depth of the image was adjusted to find the tibial nerve, and SWE was then performed. Three iterative measurements were obtained at 2-min intervals. The validated measurements of each tibial nerve were taken by each sonographer. Young's modulus (E) was expressed in kilopascals (kPa).

Statistical Analysis

SPSS version 17.0 software (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. Categorical variables are presented as percentage. Continuous data are presented as mean ± standard deviation or median and interquartile range. An independent t-test and One-way analysis of variance were performed for statistical comparisons, and then the LSD test was used for multiple comparisons. Non-parameter test was used for non-normal distribution data. Kruskal-Wallis test and Mann-Whitney *U* test were performed to compare variables. The best cut-off value of the nerve E value was obtained by plotting receiver operating characteristic curve. The interclass correlation coefficient (ICC) was used to evaluate the intra- and inter-observer reliability. Statistical significance was accepted at $P < 0.05$.

RESULTS

Clinical Baseline Characteristics

Clinical baseline data were collected from 56 participants (40 patients undergoing hemodialysis and 16 healthy control individuals). These baseline characteristics are displayed in **Table 1**. The statistical analysis showed no significant differences in age, HCT, Alb, TP, cholesterol, low-density lipoprotein cholesterol, or calcium among the three groups ($P > 0.05$). BUN and Scr were significantly higher in the CKD5D patients than in the controls ($P < 0.05$). CKD5D patients had lower Hgb than the controls ($P < 0.05$). The UPN group had more severe hyponatremia and hyperkalemia than the non-UPN and the control groups ($P < 0.05$). Triglycerides in the UPN group were higher than in the control group ($P < 0.05$). However, there were no significant differences in Hgb, BUN, Scr, PTH, phosphorus, triglycerides and beta-2 microglobulin between the UPN group and non-UPN group ($P > 0.05$). The UPN group had a longer duration of dialysis than the non-UPN group ($P < 0.05$).

US Features of Tibial Nerve

A total of 80 ankles of 40 CKD5D patients and 32 ankles of 16 healthy controls were enrolled in our study. The measurement indices of the tibial nerve in all three groups are displayed in **Table 2**. There was no significant difference in the left and right diameters, anterior and posterior diameters, perimeter, or CSA of the tibial nerve among the three groups ($P > 0.05$). The stiffness of the tibial nerve was measured with 2D-SWE and the shear elasticity index was displayed as Young's modulus(E). The stiffness of the tibial nerve in the UPN group (**Figure 1**) was significantly different from that in the non-UPN group and control group ($P < 0.05$) and was significantly different between the non-UPN group (**Figure 2**) and control group ($P < 0.05$) (**Figure 3**).

Determination of Optimal Cut-Off Value of E for Diagnosis of UPN Based on US and SWE Measurements

To determine the best cut-off value of E, we performed a receiver operating characteristic curve analysis based on the E value of the tibial nerve as shown in **Figure 4**. The optimal cutoff value of E

TABLE 1 | The general characteristics of three groups.

	UPN (n=25)	Non-UPN(n=15)	Healthy control(n=16)	*P* value
Male [n(%)]	16(64.0%)	9(60.0%)	8(50.0%)	
Age(years)	56.21 ± 11.85	50.73 ± 16.21	55.25 ± 10.15	0.414
Years on dialysis	3.25(2.00,6.13)[c]	1.00(0.16,2.50)	–	0.009
Hgb(g/l)	112.04 ± 23.37[a]	104.13 ± 22.64[b]	126.88 ± 14.95	0.013
HCT(L/L)	0.34 ± 0.07	0.32 ± 0.08	0.38 ± 0.05	0.061
Alb(g/l)	39.07 ± 7.61	38.31 ± 5.27	38.49 ± 6.09	0.932
TP(g/l)	65.15 ± 7.48	61.43 ± 8.37	63.88 ± 7.65	0.355
Cholesterol (mmol/l)	4.23 ± 1.31	4.14 ± 1.32	4.62 ± 0.95	0.524
Triglycerides(mmol/l)	1.70(1.29,1.90)[a]	1.29(0.76,1.92)	1.09(0.83,1.28)	0.021
LDL-cholesterol (mmol/l)	2.45 ± 1.11	2.12 ± 1.02	2.30 ± 1.00	0.658
Sodium (mmol/l)	138.97 ± 4.23[a,c]	141.71 ± 3.60	141.77 ± 2.15	0.023
Potassium (mmol/l)	5.22 ± 1.37[a,c]	4.55 ± 1.21	4.05 ± 0.44	0.007
Calcium(mmol/l)	2.19 ± 0.34,	2.20 ± 0.34	2.24 ± 0.12	0.860
Phosphorus(mmol/l)	2.17 ± 0.81	2.18 ± 0.46	–	0.949
BUN(mmol/l)	27.21 ± 10.34[a]	26.65 ± 8.31[b]	4.72 ± 1.02	< 0.001
Scr(umol/l)	892.68 ± 285.23[a],	972.06 ± 218.45[b]	0.25 ± 14.61	< 0.001
PTH(pmol/l)	143.00(65.90,410.80)	191.20(109.00,320.00)	–	0.544
Beta2 microglobulin (mg/l)	22.53 ± 3.83	22.68 ± 3.65	–	0.934

[a,b]*Compared with Healthy control,* [c]*Compared with non-UPN nephropathy, P < 0.05; P < 0.05 is considered statistically significant. Hgb, hemoglobin; HCT, hematocrit; Alb, albumin; TP, the total protein; LDL, cholesterol low density lipoprotein-cholesterol; BUN, blood urea nitrogen; Scr, serum creatinine; PTH, parathyroid hormone.*

TABLE 2 | The values in the tibial nerve for each group.

	UPN (n=50 ankles)	Non-UPN (n=30 ankles)	Healthy control (n=32 ankles)	*P* value
D1(mm)	5.56 ± 0.86	5.73 ± 0.66	5.67 ± 0.56	0.556
D2(mm)	3.87 ± 0.76	4.05 ± 0.64	3.94 ± 0.56	0.522
C(cm)	1.79 ± 0.17	1.82 ± 0.16	1.75 ± 0.13	0.244
CSA(cm^2)	0.22 ± 0.05	0.22 ± 0.04	0.20 ± 0.04	0.417
E(kPa)	65.16 ± 17.82[a,c]	48.44 ± 10.24[b]	33.63 ± 6.43	< 0.001

[a,b]*Compared with Healthy control,* [c]*Compared with non-UPN nephropathy, P < 0.05; P < 0.05 is considered statistically significant. D1, left and right diameters; D2, anterior and posterior diameters; C, perimeter; CSA, cross-sectional area; E, Young's modulus values.*

for diagnosing UPN was 48.35 kPa. The sensitivities, specificities, positive predictive values, and negative predictive values are summarized in **Table 3**.

Inter- and Intra-Observer Consistency Analysis

The ICC of the intra-observe was 0.950(95% CI 0.350, 0.990). The inter-observe ICC was 0.931(95% CI 0.786, 0.979). SWE showed very good inter- and intra-observer consistency.

DISCUSSION

Peripheral neurological complications are very common in patients with CKD, with an estimated prevalence of up to 90% in the CKD5D population (19). Such complications present as a slowly progressive sensorimotor neuropathy. The clinical manifestations may include pain, paresthesia, numbness in the distal lower limbs, or loss of sensitivity. Peripheral neuropathy results from a variety of mechanisms. Diabetic neuropathy is one neurological manifestation of end-stage renal disease (ESRD) (12). The mechanisms of diabetic neuropathy are well known and widely reported. With respect to CKD-induced neuropathy, many substrates have been investigated as potential uremic neurotoxins. The retention of neurotoxic solutes with a molecular weight of 300 to 2000 Da, including PTH and beta-2 microglobulin, has been discussed as a cause of UPN because such solutes are slowly dialyzable (20). However, urea, creatine, and uric acid have shown no evidence of causality. Many nerve excitability studies in uremic neuropathy have provided evidence that hyperkalemia is related to nerve dysfunction and contributes to the development of neuropathy (21). Similarly, it showed more sever hyperkalemia in the UPN group than the non-UPN and the control groups in our study. The UPN group had lower sodium than the non-UPN and the control groups. Previous studies showed that hyponatremia may be related to the central nervous system toxicity *via* multiple pathways (22). It was uncertain whether lower serum sodium levels promoted injury to the peripheral neuropathy, which needed further research. In the present study, the sex distribution among the three groups was not revealed significant difference. However, Said et al. (15) found that uremic neuropathy is more common in male than in female patients. Additionally, Hojs-Fabjan et al. (23) found that polyneuropathy was associated with the patient's age and duration of dialysis treatment. In our study, the years of dialysis were longer in the UPN group than in the non-UPN group, which is consistent with the study by Hojs-Fabjan et al. (23). No significant difference in age was found among the three groups. The BUN and Scr concentrations were significantly higher in the CKD5D patients than in the healthy controls. Abnormal lipoprotein profile has been reported in CKD5D patients (24). Triglycerides were much lower in the UPN group than in the control group. However, no significant

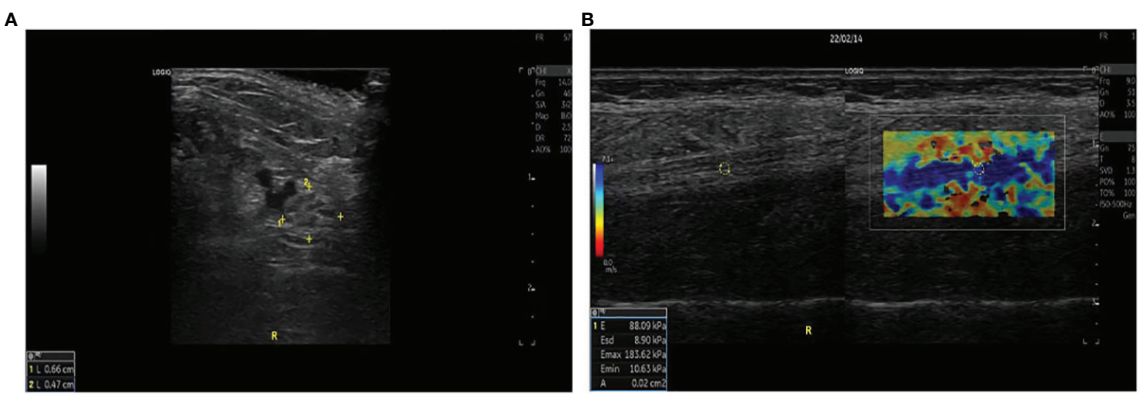

FIGURE 1 | **(A)** The left and right diameters, anterior and posterior diameters, perimeter, and CSA of the tibial nerve were measured 4 cm proximal to the medial level in a 57-year-old woman with UPN. **(B)** Two split US and SWE images were obtained at the same longitudinal level in a 39-year-old man with UPN. Quantitative SWE measurements showed that the mean nerve stiffness was 88.09 kPa. R, Right.

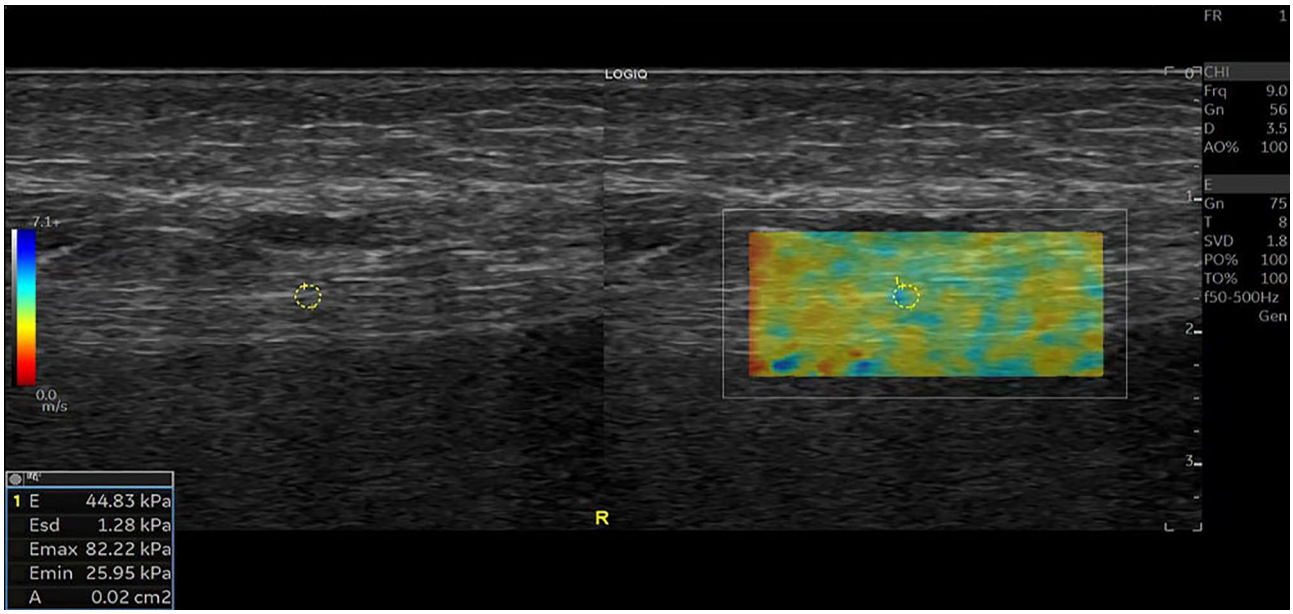

FIGURE 2 | SWE image of the tibial nerve in a 52-year-old man with non-UPN. Quantitative SWE measurements showed that the mean nerve stiffness was 44.83 kPa. R, Right.

differences were in Hgb, HCT, Alb, TP, cholesterol, low-density lipoprotein cholesterol, calcium, phosphorus, Scr, BUN, PTH, or beta-2 microglobulin between the UPN group and non-UPN group.

The gold standard method for the diagnosis of peripheral neuropathy is an NCS, which is time-consuming and invasive. High-resolution US has recently become more widely used in the detection of neuropathy because of its low cost, noninvasiveness, and ability to depict the location and range of the lesion. Our study showed no significant difference in the anterior and posterior diameters, left and right diameters, perimeter, or CSA of the tibial nerve among the three groups, indicating that

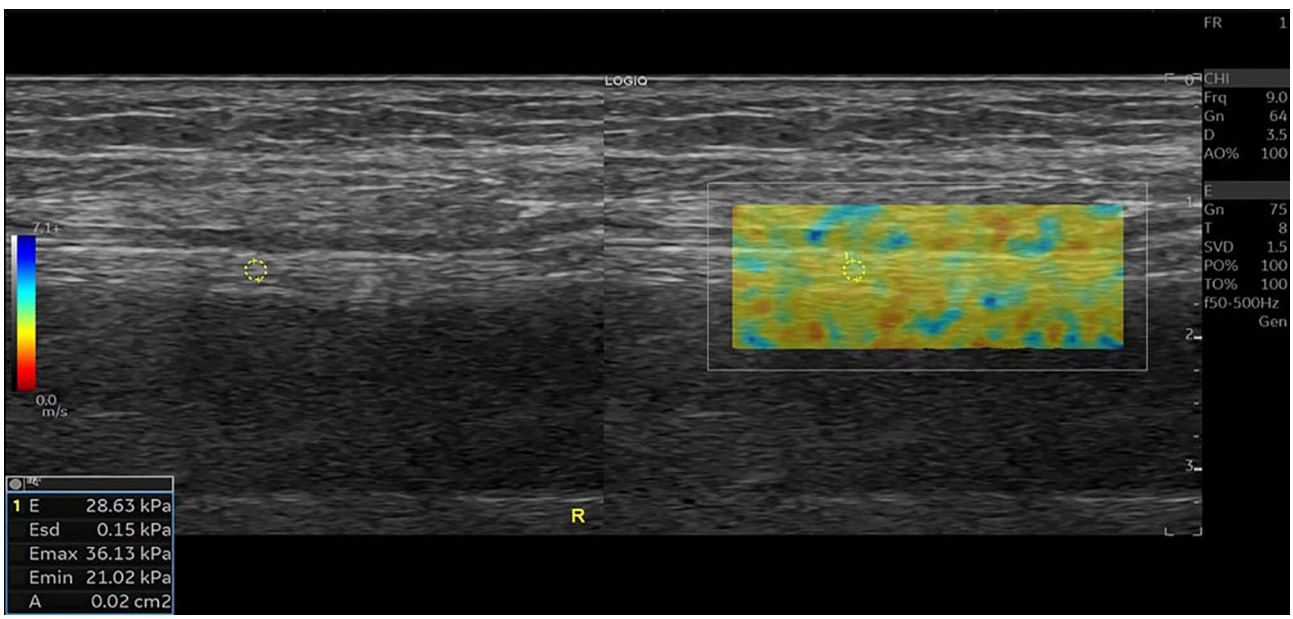

FIGURE 3 | SWE image of the tibial nerve in a 56-year-old healthy man. Quantitative SWE measurements showed that the mean nerve stiffness was 28.63 kPa. R, Right.

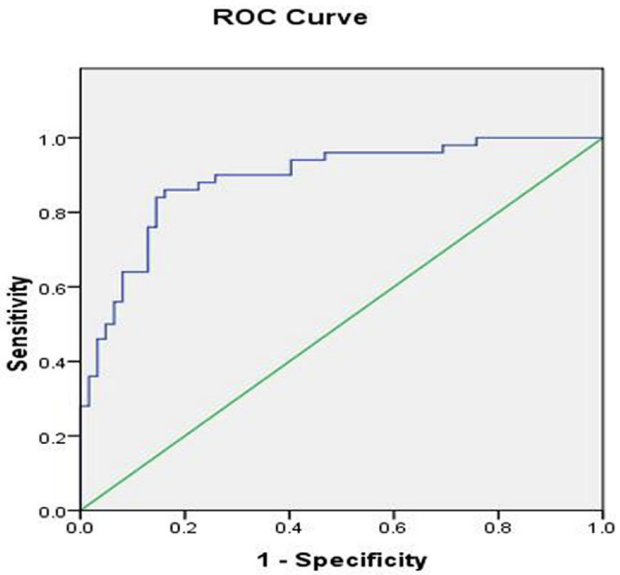

FIGURE 4 | Receiver operating characteristic curve based on SWE measurement values.

TABLE 3 | The efficiency of the cut-off values in diagnosing UPN.

	Cutoff	Sensitivity,%	Specificity,%	PPV,%	NPV,%	Youden index	AUC
E(kPa)	48.35	86.0(43/50)	84.0 (52/62)	81.1 (43/53)	88.1(52/59)	0.699	0.889

E, Young's modulus values; AUC, area under the receive operating characteristic curve; PPV, positive predictive value; NPV, negative predictive value.

nerve damage has little effect on the morphological characteristics of nerves. Consistent with the findings reported by Dikici et al. (8), the morphological changes were not obvious when the nerve was uncharacteristically damaged.

Through the measurement of Young's modulus, SWE can quantitatively reflect the elasticity of tissue. Harder tissue has a greater Young's modulus. This measurement index has been widely applied in liver disease (25), thyroid disease (26), diabetic peripheral neuropathy (8), and other conditions. However, few studies have been performed to assess the peripheral nerve elasticity in CKD5D patients. In this study, we evaluated the tibial nerve stiffness in such patients. The inter-observer and intra-observer reproducibility of SWE were excellent. The E value was statistically significant among the three groups. These findings suggest that more severe lesions are associated with greater nerve stiffness. Our group is currently performing animal studies to elucidate the mechanism of this finding. We demonstrated that the E value had high accuracy for identifying UPN. When 48.35 kPa was taken as the optimal value, the sensitivity was high (86.0%), specificity was high (84.0%), Youden's index was 0.699, and area under the curve was 0.889. Consistent with previous studies, SWE had better sensitivity and specificity than the CSA for diagnosis of neuropathy (27).

This study had some limitations. First, multiple neuropathies may occur in patients with ESRD undergoing hemodialysis; however, we only evaluated the tibial nerve at one point. More nerves and more measurement points of one nerve are needed. Second, we only evaluated patients at a single hemodialysis center, and these patients represent only a fraction of all patients with ESRD. These findings must be validated by large-sample multicenter prospective studies. Finally, we did not investigate the mechanism underlying the increased nerve stiffness in patients with ESRD. The cause of the change in nerve stiffness with time after dialysis is not clear. Therefore, studies in which different time points after dialysis are examined are needed to observe the changes in nerve stiffness.

In conclusion, SWE is useful for the diagnosis of peripheral neuropathy in CKD5D patients. Young's modulus of 48.35 kPa of the tibial nerve was taken as the optimal cutoff value for the diagnosis of peripheral neuropathy in CKD5D patients, with a sensitivity of 86.0%, specificity of 84.0%, and area under the curve of 0.889.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Affiliated Hospital of Weifang Medical University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

XL and HS: writing-original draft preparation, formal analysis and visualization. ZZ, JL, and QL: methodology and investigation. XC and ZG: conceptualization, methodology, funding acquisition and writing-review and editing. HX, LM, HZ, and JLL: resources and data curation. XW and MG: supervision and funding acquisition. All authors have read and agreed to the published version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.899822/full#supplementary-material

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REVIEW
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Metabolic Syndrome-Related Kidney Injury: A Review and Update

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Metabolic syndrome (MetS) includes visceral obesity, hyperglycemia, dyslipidemia, and hypertension. The prevalence of MetS is 20-25%, which is an important risk factor for chronic kidney disease (CKD). MetS causes effects on renal pathophysiology, including glomerular hyperfiltration, RAAS, microalbuminuria, profibrotic factors and podocyte injury. This review compares several criteria of MetS and analyzes their differences. MetS and the pathogenesis of CKD includes insulin resistance, obesity, dyslipidemia, inflammation, oxidative stress, and endothelial dysfunction. The intervention of MetS-related renal damage is the focus of this article and includes controlling body weight, hypertension, hyperglycemia, and hyperlipidemia, requiring all components to meet the criteria. In addition, interventions such as endoplasmic reticulum stress, oxidative stress, gut microbiota, body metabolism, appetite inhibition, podocyte apoptosis, and mesenchymal stem cells are reviewed.

Keywords: metabolic syndrome, chronic kidney disease, diagnosis, pathological characteristics, therapeutics

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1 INTRODUCTION

MetS called "Syndrome X" at first, it refers to a pathological state of metabolic disorders of proteins, fats, carbohydrates, and other substances in humans. It is including hypertension, hyperlipidemia, hyperuricemia, hyperglycemia, central obesity and insulin resistance. MetS is a common risk factor for the morbidity and mortality of cardiovascular events such as myocardial infarction, stroke, sudden cardiac death and thrombosis (1–6), it is also an important cause of new-onset CKD and progression of CKD. With the increasing prevalence of MetS, its effects on the kidneys have

Abbreviations: MetS, Metabolic syndrome; RAAS, Renin Angiotensin Aldosterone System; GFR, glomerular filtration rate; eGFR, estimated glomerular filtration rate; CKD, chronic kidney disease; ESRD, end-stage renal disease; ACEI, angiotensin converting enzyme inhibitor; OS, oxygen species; Nrf2,erythroid 2-related factor; HSL, Hibiscus sabdariffa; HDL, high-density lipoprotein; DPP-4, dipeptidyl peptidase-4; SGLT2,sodium-glucose cotransporter 2; GLP-1,glucagon-like peptide 1; CVD, cardiovascular disease (CVD); ARB, angiotensin receptor antagonist; TMAO, trimethylamine N-oxide; LPS, lipopolysaccharide; MSCs, Mesenchymal stem cells; WHO, World Health Organization; NCEP-ATPIII, National Cholesterol Education/Adult Treatment Panel III; CDC, Chinese Diabetes Society; WC, waist circumference; TG, triglycerides; IR, insulin resistance; AHA/NHLBI, American Heart Association/National Heart, Lung, and Blood Institute; JIS, Joint Interim Statement; FBG, fasting blood glucose; CDA, China Diabetes Association; NHANES, National Health and Nutrition Examination; TGF-β1,transforming growth factor-β1; ORG,obesity-Related Glomerulopathy; RPF, renal plasma flow; ANG II, angiotensin II; TNF-α, tumor necrosis factor α; MCP-1, macrophage chemoattractant protein-1; ROS, reactive oxygen species; NADPH, nicotinamide adenine dinucleotide phosphate oxidase. GFB, glomerular filtration barrier; GDF-11, growth differentiation factor- 11; GDF-15, Growth differentiation factor 15; pEVs, podocyte-derived EVs; 11β-HSD11,11beta-Hydroxysteroid dehydrogenase type 1; MSCs, Mesenchymal stem cells.

attracted an increasing amount of attention from nephrologists. The prevalence of MetS and MetS-related renal injury are also different due to different races, lifestyles, and MetS diagnostic criteria. Therefore, we will summarize the epidemiology, pathogenesis, diagnosis and treatment progress of MetS-related renal injury.

2 METS DEFINITION AND PREVALENCE

There is no uniform diagnostic standard for MetS in the world, and the World Health Organization (WHO, 1998) (7), National Cholesterol Education/Adult Treatment Panel III (NCEP-ATPIII,2001) (8), Modified NCEP-ATP III,2010 (9), American Heart Association (AHA, 2005) (10), International Diabetes Federation (IDF, 2005) (11), and the Chinese Diabetes Society (CDS,2020) (12) definitions are currently used. Components of the several diagnostic criteria include central adiposity, an impaired glucose tolerance, waist circumference (WC); blood pressure, high density lipoprotein, triglycerides (TG), and serum glucose. The WHO believes that the basic condition for diagnosing MetS is an abnormal glucose metabolism, diabetes, or insulin resistance (IR). The IDF believes central adiposity is a prerequisite for the diagnosis of MetS. Due to the different focus of several diagnostic criteria, the incidence of MetS in the same group is different.Therefore, to unify the diagnostic criteria of MetS, IDF, American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI), and other institutions published the MetS Joint Interim Statement (JIS) criterion (13). This standard no longer regards WC as a necessary condition for the diagnosis of MetS. The JIS criterion for defining MetS includes: (1) raised fasting blood glucose (FBG) or the use of hypoglycemic drugs; (2) increased blood pressure or the use of antihypertensive drugs; (3) increased plasma TG or the use of hypolipidemic drugs; (4) a decreased HDL-C; and (5) the Chinese criterion for central obesity (or visceral obesity) with a waist circumference of >85 cm for men and >80 cm for women. MetS was diagnosed if three or more of the above criteria were met (13). The diagnostic criteria of the China Diabetes Association (CDA) MetS of the Chinese Medical Association has a high similarity and coincidence rate with the JIS and NCEP-ATPIII (Kappa values are 0.730 and 0.774, respectively) (14). **Table 1** shows the common definitions of MetS.

It is estimated that the worldwide prevalence of MetS is 20 – 25% (15), but there are great differences in the prevalence of MetS in various countries. According to the literature, the lowest prevalence of MetS patients is only 6.1% and the highest is 55.6%. This may be due to different gender, age, racial, eating habits, education, medical security, nature of work, and living environment. From 1980 to 2012, the prevalence of MetS in the United States increased to 35% (16), however, the prevalence of MetS in the United States has been gradually decreasing in recent years. The data released by the National Health and Nutrition Examination (NHANES) in 2020 showed that the incidence rate of MetS was 24% in men and 22% in women (17). A meta-analysis from China showed that the prevalence of MetS was 24.5% between 2007 and 2015. By sex, the prevalence was 19.2% in men and 27.0% in females. The older the age, the higher the prevalence of MetS. The prevalence of MetS in aged ≥ 60 was 2.33 times higher than those in aged 15-39 (32.4% *vs.* 13.9%). The prevalence of MetS was 1.3 times higher in individuals living in urban areas than those living in rural

TABLE 1 | Criteria for diagnosing MetS.

	WHO,1998	IDF,2005	NCEP ATP III,2004	Modified NCEP ATP III,2010	AHA,2005	CDS,2020
	Presence of impaired glucose tolerance with any 2 of the following criteria	**Presence of central adiposity with 2 or more of the following criteria**	**Presence of 3 or more of the following criteria**			
Serum glucose	plasma glucose at 2h after glucose load ≥7.8 mmol/L	FPG ≥100 mg/dL (5.6 mmol/L) or previously diagnosed type 2 diabetes.	FPG≥110 mg/dL (6.1 mmol/L)	FPG≥100 mg/dL (5.6 mmol/L)	FPG ≥100 mg/dL (5.6 mmol/L)	FPG ≥6.1 mmol/L or plasma glucose at 2 h after glucose load ≥7.8 mmol/L
WC	–	M: > 90cm; F: > 80cm	M: >102 cm; F: >88 cm	M: >102 cm; F: >88cm(Asian origin, M: >90 cm and F: >80 cm)	M: >102 cm; F: >88 cm	M: ≥ 90cm; F: ≥ 85cm
BMI	>30 kg/m2					
WHR	M>0.90 ;F >0.85					
Hyperrension	≥140/≥90 mmHg	≥130/≥85 mmHg	≥130/≥85 mmHg	≥130/≥85 mmHg or current use of antihypertensive drugs	≥130/≥85 mmHg	≥130/≥85 mmHg
HDL Cholesterol	M: < 35 mg/dL (0.9 mmol/L); F: < 39 mg/dL (1 mmol/L)	M: < 40 mg/L (1.03 mmol/L); F: < 50 mg/L (1.29 mmol/L) or receiving treatment	M: <40 mg/dL (1.03 mmol/ L) ;F: <50 mg/dL (1.29 mmol/L)	M: <40 mg/dL (1.03 mmol/ L) ;F: <50 mg/dL (1.29 mmol/L)	M: <40 mg/dL (1.03 mmol/ L) ;F: <50 mg/dL (1.29mmol/L)	<40 mg/dL (1.04mmol/L)
Triglycerides	≥150 mg/dL (1.7 mmol/L)	≥150 mg/dL (1.7 mmol/L) or receiving treatment	≥150 mg/dL (1.7 mmol/L)	≥150 mg/dL (1.7 mmol/L)	≥150 mg/dL (1.7 mmol/L)	≥150 mg/dL (1.7 mmol/L)

areas (24.9% *vs.* 19.2%) (18). A joint survey study between the United States and China showed that the prevalence of MetS in the two countries was 36.6-37.3% and 23.0%, respectively. The highest prevalence of MetS is in aged 40-50 (19). MetS is also common in South Asian countries, including Afghanistan, Bangladesh, India, Maldives, Nepal, Pakistan, and Sri Lanka. The lowest prevalence of MetS was only 8.6%, and the highest was 46.1% (20). All were in South Asian, using the ATP III diagnostic criteria, Pakistani males had a higher prevalence of MetS (55.6% vs. 45.9%) (21), while Indian females had a 1.30-fold higher prevalence of MetS (50.9% vs. 39.2%) (22). From 2009 to 2013, the prevalence of MetS in Japan was 14.6% (648/4446). By sex, the prevalence was 20.6% in males and 6.1% in females (23). It can be seen that the prevalence of MetS is greatly affected by dietary habits, medical insurance, gender and age, especially postmenopausal women have a higher prevalence of MetS.

3 ASSOCIATION BETWEEN METS AND CKD

MetS and CKD are causal and influence each other. A large number of studies have confirmed that MetS can lead to changes in renal structure and function, such as a decreased glomerular filtration rate (GFR) and increased urinary microalbumin (24–27). A meta-analysis showed that the risk of CKD in MetS was 1.34 times higher than those without MetS (28). Another meta-analysis showed that MetS increased the risk of CKD by 50% (29). Many studies found that each component of MetS was associated with CKD. The more components there were, the higher the risk of CKD (odds ratio, 1.96; 95%: 1.71,2.34) (26, 29). However, a few reports have shown a nonsignificant association, which may have been related to the different diagnostic criteria used for MetS (30). Similarly, due to impaired renal function, microenvironment changes and disorder of glucose and lipid metabolism in patients with CKD, the incidence of MetS in patients with CKD is significantly higher than that in the general population. With the progress of CKD, the incidence of MetS gradually increases (31). A study in Thailand found that the prevalence of MetS in patients with CKD was 71.3%, and the prevalence of met in patients with ckd3a to 5 was 70.1%, 72.3%, 73.4% and 72.7% respectively, which was significantly higher than that in patients without CKD (32).

Recently, after using the "MetS score" and the "MetS factor" to refine MetS and its components, it was found that regardless of gender and racial, the higher the score of MetS, the higher prevalence of CKD, including GFR decline and microalbuminuria increase (33–35). When each component of MetS was evaluated separately, it was found that hypertension and increased LDL metabolism were associated with microalbuminuria and estimated glomerular filtration rate (eGFR), hyperglycemia and hypertriglyceridemia were associated with microalbuminuria (36). Sixty percent of the study population had MetS in CKD3-4. After more than 2 years of follow-up, CKD stage 3-4 patients with MetS had a 1.33 times increased risk of progression to ESRD. Among the components of MetS, hyperlipidemia and elevated blood pressure are more likely lead to the progression of CKD to ESRD; Low HDL cholesterol increases the risk of death; Impaired glucose metabolism is an important risk factor for ESRD and death (37).

4 THE PATHOGENESIS OF METS-RELATED RENAL INJURY

The pathogenesis of MetS-related renal damage is complex, including insulin resistance, obesity, hypertension, dyslipidemia, inflammation, oxidative stress, and endothelial dysfunction. The pathogenesis of MetS-related kidney injury is shown in **Figure 1**.

4.1 Insulin Resistance

Insulin resistance is essential factor of MetS, it is able to induce sodium retention and vascular endothelium vasoconstriction by antinatriuresis, which leads to RAAS activation and renal tubular lipid accumulation finally. This may be the important cause of MetS-related renal damage caused by insulin resistance (38, 39). Insulin resistance-related renal injury also includes transforming growth factor-β1 (TGF-β1), which is increased in the adipocytes of obese patients with insulin resistance and is responsible for the proliferation of mesangial cells and ultimately CKD, a potent initiator of disease (40–43). The sterol regulatory element binding protein-1 (SREBP-1) increases lipid droplet deposition in renal tubular cells and interstitial extracellular mechanisms (44–46), leading to tubular atrophy and interstitial fibrosis. Insulin like growth factor-1 (IGF-1) and dedifferentiation of vascular smooth muscle cells can induce connected tissue growth factor (CTGF), thus promoting renal tubular fibrosis Hyperglycemia in rat mesangial cells inhibits the degradation of the extracellular matrix by metalloproteinase 9, resulting in mesangial extracellular matrix proliferation and fibrosis (47–50). In addition, insulin resistance damages the microvessels of patients, especially the fundus, kidneys, muscles and cardiac arteries, and eventually damages the corresponding target organs (51).

4.2 Obesity

Hemodynamic changes, abnormal lipid metabolism, and dysregulations of the hormone response are the primary pathogeneses of obesity-Related Glomerulopathy (ORG) (52). A study found that aldosterone levels were significantly elevated in obese patients, hypertension and high WC were positively correlated with the level of aldosterone and negatively correlated with HDL (53). Activation of the RAAS and enhanced sympathetic activity in obese patients lead to increased levels of aldosterone, which reflexively lead to increased renal tubular reabsorption of sodium salts, resulting in water and sodium retention (54). The activation of RAAS in obese patients leads to hemodynamic changes such as increased of GFR and renal plasma flow (RPF), which causes

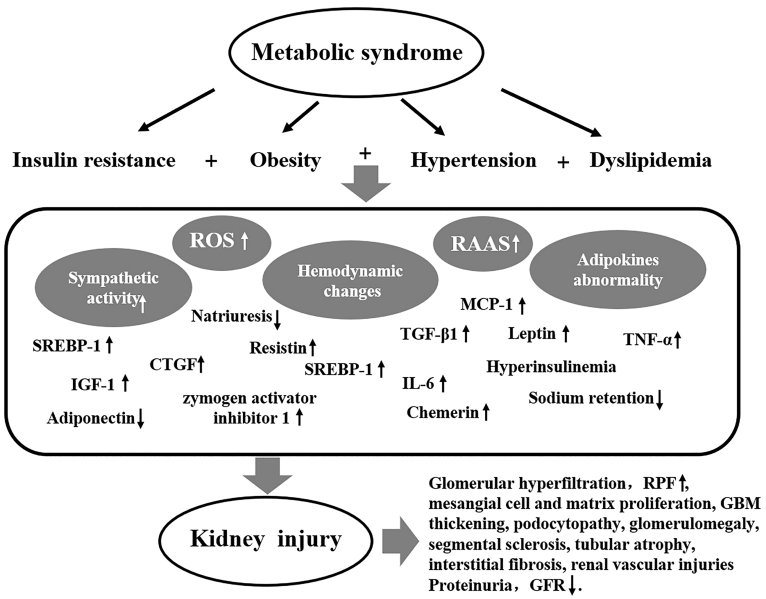

FIGURE 1 | The pathogenesis of MetS-related kidney injury.

glomerular hyperfiltration, compensated glomerulomegaly, segmental sclerosis, and promoting the progression of MetS-related renal damage (55–57). Hormone regulation disorder and ectopic lipid deposition in obese patients can directly or indirectly affect the structure and function of renal intrinsic cells (52). Hyperinsulinemia promotes the secretion of leptin by adipocytes, and leptin levels are significantly elevated in MetS patients (58, 59). Increased leptin secretion in MetS patients leads to kidney damage through the following two pathways, including (1) promoting the expression of TGF-β1 in renal parenchymal cells, increasing the production of type IV collagen, leading to tubular atrophy, interstitial fibrosis and glomerulosclerosis, (2) Leptin causes sodium reabsorption, leading to changes in renal hemodynamics (60, 61).

4.3 Hypertension

Hypertension is an important condition for the diagnosis of Mets. Hypertension in MetS patients is closely related to plasma aldosterone levels and sympathetic nerve activity (53). First, visceral adipocytes secrete a large number of substances such as angiotensinogen, which leads to the activation of RAAS (62–64). Second, the accumulation of excessive fat in and around the kidneys of patients with MetS compresses renal parenchymal cells, resulting in impaired pressure natriuresis (65–68). Thirdly, other hormone secretion disorders in MetS patients (including increased leptin, reduced adiponectin, etc.) lead to increased sympathetic nerve activity in the body (69–71), which are important reasons for hypertension in MetS patients. For a long time, the changes in renal structure and function caused by hypertension cannot be ignored, and it is also one of the important secondary causes of ESRD. Hypertension primarily causes kidney damage due to ischemia. Ischemia causes renal tubular, renal vascular, and glomerular damage, primarily renal tubular damage (72, 73). Ischemia can also increase the synthesis and secretion of angiotensin II (ANG II), which can further constrict blood vessels and lead to the proliferation of renal parenchymal cells, damaging the kidney through hemodynamic and non-hemodynamic effects (74).

4.4 Another Pathogenesis

During MetS, adiponectin, leptin, chemerin, resistin, IL-6, and tumor necrosis factor α(TNF-α) and other adipokines are abnormally secreted and released, or dysfunctional, which induces oxidative stress, endothelial dysfunction, inflammatory effects, and increased sympathetic activity, and finally lead to changes in renal function and structure (75–79). Furthermore, during Mets, adipose tissue secretion of pro-inflammatory factors increased, including macrophage chemoattractant protein-1 (MCP-1), macrophage migration inhibitory factor, chemokine ligand 5, and macrophage colony-stimulating factor. These pro-inflammatory factors can lead to inflammatory response, resulting in proteinuria and impaired renal function (80–82). The production of reactive oxygen species (ROS) in renal tissue increases due to inflammatory cell infiltration. ROS may cause damage to proximal tubules by interfering with renal tubular ion transport by altering renal and hemodynamics. ROS can also induce TGF-β1 and fibrinolysis through the activation of the nuclear factor-κ light chain enhancer and nicotinamide adenine dinucleotide phosphate oxidase (NADPH) pathways of activated B cells. The expression of pro-fibrotic molecules, such as zymogen

activator inhibitor 1, thus aggravates the progress of renal fibrosis (83).IR is an independently risk factor in many diseases, it affects renal podocytes. Podocyte foot loss causes partial shedding of the glomerular filtration barrier (GFB), resulting in macromolecular leakage and proteinuria (84, 85). The adipose tissue secretes all components of the RAAS. During Mets, excessive activation of RAAS will lead to increased renal volume load and hyperfiltration, thus damaging the GFB, including endothelial cells, basement membrane, especially the podocytes (67, 86). In obese patients, a large number of lipid droplets can be found in renal innate cells, especially in podocytes. The deposition of lipid droplets leads to the depletion of renal cell energy, and ultimately the apoptosis of intrinsic renal cells, resulting in CKD and even ESRD (85, 87, 88).

5 DIAGNOSIS OF METS-RELATED RENAL INJURY

MetS-related kidney disease is renal impairment in patients with MetS that includes glomerular hyperfiltration, eGFR < 60/mL/min per 1.73 m^2, proteinuria and/or microalbuminuria, renal tubular dysfunction, ultrasound abnormalities (increased intra-renal resistive indices) (89), and histopathological abnormalities (90). Of course, MetS with CKD is not exactly MetS-related kidney disease. In addition, there is consistent evidence in the literature regarding the association between MetS and kidney stones (91, 92).

Several components of MetS may affect the kidney, or they may be combined to damage the kidney. Therefore, the damage of MetS to the renal tissue structure is also diverse. Kidney pathological characteristics in patients with MetS include glomerulomegaly, podocytopathy, mesangial cell and matrix proliferation, GBM thickening, global sclerosis, segmental sclerosis, tubular atrophy, interstitial fibrosis, and renal vascular injuries (arterial sclerosis and hyalinosis) (93–97). Kidney pathological characteristics for patients with MetS is shown in **Figure 2** (98).

Recent studies have found that some new biomarkers are significantly altered in the blood and urine of MetS patients, such as growth differentiation factor -11 (GDF-11). GDF- 11 is an important endocrine factor involved in the metabolic process of the body (99, 100), was found to be negatively correlated with body mass index and WC of MetS patients (101, 102). Growth differentiation factor 15 (GDF-15) is an endocrine factor involved in metabolism, and GDF-15 levels have been found to be significantly increased in elderly MetS patients and has been independently correlated with MetS (103, 104). A study from the Japanese found that urinary A-megalin is associated with the clustering number of MetS traits including hyperhomocysteinemia (105, 106). At present, Urinary podocyte-derived EVs (pEVs) is widely recognized as a specific biomarker for podocyte injury, and studies have found that it is significantly increased in secondary podocytes, such as early diabetic nephropathy, hypertensive nephropathy, and eclampsia (107–109). An animal study found that the urine pEVs of MetS model pigs increased significantly, Meanwhile, kidney histology confirmed the existence of podocyte and mitochondrial damage in MetS pigs. Therefore, urinary pEVs may be an early biomarker for MetS-related kidney injury (88). Whether the above indicators can be used as markers for the diagnosis of MetS-related nephropathy requires further study.

6 MANAGEMENT OF METS-RELATED KIDNEY DISEASE

A study found that patients with uncontrolled MetS had a 3.28 times higher risk of rapid decline in renal function than those who were controlled (110). Therefore, early treatment of MetS is beneficial to prevent and delay the progression of kidney disease. Specific measures include lifestyle change, managing weight control, hypertension, hyperlipidemia, and abnormal blood sugar. If each component of MetS is treated, each component can meet the standard. In recent years, studies have found that intervention targeting gut microbiota, oxidative stress, and inflammatory responses, and stem cell transplantation may help to alleviate Mets related nephropathy.

6.1 Lifestyle Interventions

Lifestyle interventions have always been important means of control MetS, including changing dietary patterns, focusing on a veggie–fruit–grains dietary pattern (111), adhering to aerobic exercise, a diet rich in medium-chain fatty acids and short-chain fatty acids, reasonable sleep, smoking cessation, and avoiding excessive intake of coffee. A study found that excessive coffee consumption (≥3 cups/day) increased the incidence of MetS by 1.5 times (112).

6.2 Weight Loss

Lifestyle interventions such as dietary restriction, change dietary Patterns, aerobic exercise, a diet rich in medium-chain fatty acids and short-chain fatty acids, reasonable sleep, and smoking cessation have always been important means of weight control. Studies have found that after dietary restriction or dietary restriction combined with aerobic exercise, serum creatinine and albuminuria decreased, GFR increased, renal hemodynamics improved, and the risk of kidney stone formation reduced with weight loss (81, 113–115). A study from Taiwan showed that compared with other dietary patterns, MetS patients with veggie–fruit–grains dietary pattern had better parameters of kidney function, including lower serum creatinine, blood urea nitrogen, and serum uric acid levels, and higher eGFR (111). A study of bariatric surgery in adolescents showed that eGFR increased by an average of 26ml/min/1.73m2 three years after surgery. Participants with albuminuria at baseline had improved significantly after operation (116). Similarly, after 6 years of follow-up for weight loss by gastric bypass, it was found that the patient's metabolic-related indicators were well controlled, cardiovascular events were significantly reduced, and the risk of moderate to severe kidney disease was reduced by 45% (117). However, the existing literature demonstrated that despite its perioperative risks and short-term complications, surgical weight loss has a better long-

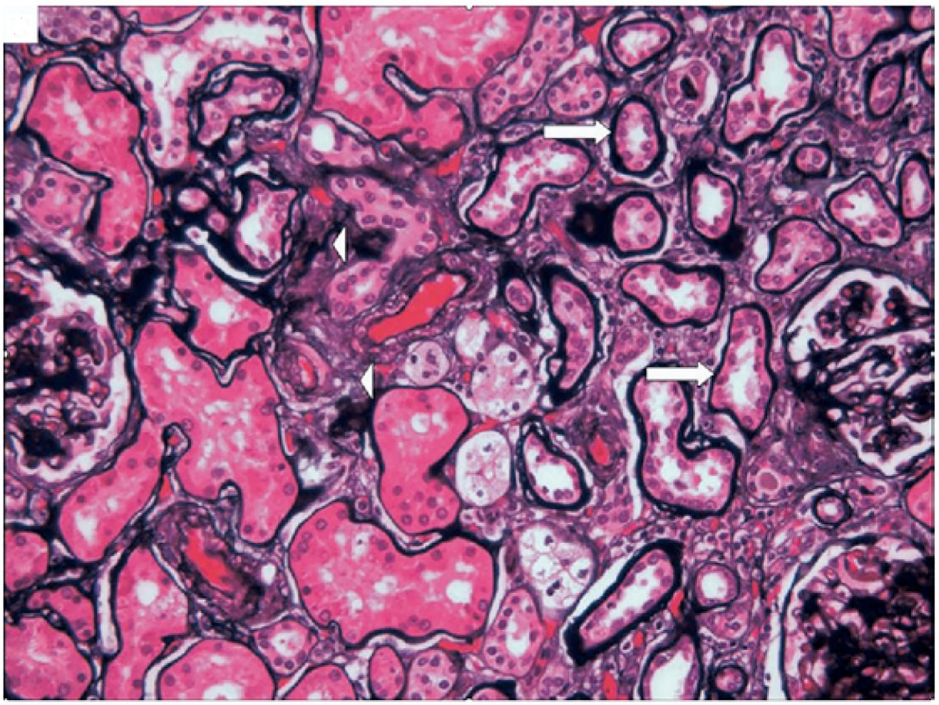

FIGURE 2 | Kidney pathological characteristics for patients with MetS. Tubular atrophy (arrows) and interstitial fibrosis (arrowheads), PASM. This picture is from Mariam P et al., 2009 (98).

term prognosis for cardiovascular and renal disease (116, 118–121).

6.3 Antihypertensive Therapy

Patients with hypertension should first adhere to a salt restriction diet. The second is the application of antihypertensive drugs. It is recommended to use angiotensin converting enzyme inhibitor (ACEI) and angiotensin receptor antagonist (ARB) to control hypertension in MetS patients, and this is beneficial to reduce Renin Angiotensin Aldosterone System(RAAS) activation, relieve glomerular hyperfiltration, and reduce proteinuria and hyperuricemia (122).RAAS blocker combined with exercise training can better reduce the hypertension, urine albumin-to-creatinine ratio, and serum creatinine of MetS patients (39, 123). Losartan can stably reduce blood pressure in MetS patients, while maintaining the normal circadian rhythm of blood pressure and achieve renal protection (124).

Animal studies have found that long-term administration of telmisartan can reduce the release of leptin from adipose tissue, thereby reducing proteinuria (125). Calcium channel blocker drugs, benidipine can also reduce the mean arterial pressure and resistance of the renal arterioles in MetS patients, and the urinary protein excretion rate is reduced by 1.5 times (126).

6.4 Lipids Adjustment

Statins have very solid evidence in controlling hyperlipidemia, stabilizing atherosclerosis, and reducing the risk of cardiovascular disease, and have become the first-line drugs for hyperlipidemia in MetS patients (127). Fibrates are also effective in hyperlipidemia, especially in the control of atherosclerotic dyslipidemia (128). In addition, the anti-inflammatory, antioxidant, antithrombotic, anti-fibrotic, and endothelial cell function improvement effects of statins can reduce proteinuria in patients with MetS and delay the progression of kidney disease (129). A study found that the benefit of cardiovascular protection in patients with MetS treated with statins was significantly higher than that without MetS. The eGFR in the MetS increased by 13.7mi/min/1.73m2, which was 2.36 times higher than that in MetS patients without statins (130). In MetS patients with CKD, it is safe to control hyperlipidemia with statins or fibrates. Is the combination of the two drugs safe for MetS with a variety of dyslipidemia? A study found that in MetS patients with CKD treated with low-dose statins combined with fibrate still have high safety and can significantly reduce proteinuria (131). Studies have found that the expression of 11beta-Hydroxysteroid dehydrogenase type 1 (11β-HSD1) is significantly increased in obesity and glucose and lipid metabolism disorders (132). 11β-HSD1 inhibitors can improve the lipid metabolism disorder in MetS (133–135).

6.5 Glucose Control

Metformin, pioglitazone, dipeptidyl peptidase-4 (DPP-4) inhibitors, glucagon-like peptide 1 (GLP-1) receptor agonists,

and the sodium-glucose cotransporter 2 (SGLT2) inhibitor have been studied in patients with MetS. Metformin has good safety in the treatment of hyperglycemia and can enhance insulin sensitivity at the same time. It is the best solution for the treatment of MetS glucose metabolism disorder in children (136). Thiazolidinedione increases insulin sensitivity, improves IR and glycemic control, and also significantly reduces blood pressure, increases high-density lipoprotein (HDL), improves endothelial cell function and fibrinolytic activity, and reduces inflammation.

DPP4 inhibitors not only have definite hypoglycemic effect, but also play a protective role in cardiovascular, kidney and important target organs through the following mechanisms. Including regulating the levels of adenosine monophosphate-activated protein kinase and adiponectin levels in MetS mice, reducing inflammatory response and fatty liver (137), DPP-4/incretin axis reducing cardiovascular events (138), and improving hyperglycemia induced vascular lesions through endothelium-dependent Manner (139).

In recent years, GLP-1 receptor agonists have been used more and more widely. They not only exert the hypoglycemic effect, but also do not increase the risk of hypoglycemia. At the same time, they also have the effects of reducing weight, controlling hyperlipidemia, hypertension, and reducing inflammatory reaction, so as to prevent cardiovascular events (140). Liraglutide is a widely used GLP-1 receptor agonist, which has definite efficacy in reducing plaque formation and anti-atherosclerotic action (141).

Similarly, in addition to controlling hyperglycemia, SGLT2 also plays a role in reducing weight loss, controlling hypertension, increasing urinary sodium excretion and reducing edema by reducing sympathetic activity, improving insulin resistance (142), regulating renal sodium and urate transport and excretion in MetS patients (143, 144). It has a definite protective effect on cardiovascular and renal function in type 2 diabetes and MetS patients.

6.6 Chinese Herbal Medicines

In recent years, many Chinese herbal medicines and their extracts have achieved significant efficacy in the treatment of MetS and MetS- related kidney damage. They mainly improve components of the MetS and protect the kidneys by exerting anti-endoplasmic reticulum stress, antioxidant and anti-inflammatory activities.

Endoplasmic reticulum stress is an important pathogenesis of MetS kidney damage. Berberine can reduce urinary microalbumin, the body mass index, and postprandial blood glucose and triglyceride levels in MetS patients by regulating glucose and lipid metabolism, endoplasmic reticulum stress, inflammatory factors, insulin resistance, oxidative stress, and intestinal microbiota (145–148).

Osthol and Hibiscus sabdariffa L (HSL) have significant antioxidant effects. The mechanisms include inhibiting ketohexokinase activity and regulating adipogenesis; reducing oxidative stress by activating nuclear factor-erythroid 2-related factor (Nrf2) (149); promoting an increase in the enzymatic and non-enzymatic antioxidant systems, leading to a reduction in oxygen species (150). Ultimately, it promotes renal repair and delay renal progression in MetS patients.

Pycnogenol is an extract of pine bark, which is rich in bioactive substances such as procyanidins and catechins, has strong anti-inflammatory activities (151), affects endothelial function and reduces blood pressure (152). A randomized controlled study of ramipril alone and ramipril combined with pycnogenol in the treatment of MetS found that the combined treatment group decreased urinary albumin more significantly than the control group, and the renal cortical blood flow rate and renal function improved more significantly (123).

6.7 Other Treatments

6.7.1 Probiotics

Gut microbiota can interfere with host metabolism, and the taxonomic species or abundance of gut microbes are affected by dietary patterns, lifestyles, and drugs. A study found changes in gut microbial taxonomic species in obese patients (153), and gut microbial-derived metabolites may induce subclinical inflammatory processes in MetS patients, leading to target organ damage (154).. Studies have found that probiotics have great benefits for obese and MetS patients by improving the body's inflammatory state and reducing homocysteine and blood glucose levels (155). A randomized controlled study of patients with MetS found that the severity of MetS was significantly reduced after probiotic supplementation (156). Whether probiotics have a protective effect on MetS-related renal damage needs further study.

6.7.2 Glycine

Glycine is a non-essential amino acid in the human body, but it participates in the important process of metabolism, as well as the process of immune regulation and anti-inflammatory. A study found that plasma the glycine level in MetS patients were lower than that in healthy. Glycine supplementation can improve a variety of clinical symptoms of MetS, such as abnormal glucose metabolism, overweight, hypertension, and hyperlipidemia (157). A study of 60 patients with MetS found that daily supplementation glycine (15 g/day) for 3 months, significantly reduced oxidative stress responses in the treatment group, including superoxide dismutase-specific activity and thiobarbituric acid reactive substances. The patient's hypertension was significantly improved (158).

6.7.3 Stem Cell Therapy

Mesenchymal stem cells (MSCs) can improve the various disorders of MetS such as abdominal obesity, hyperglycemia, hypertriglyceridemia, and hypertension. MSCs promote renal injury in diabetic mice (159, 160). A superior source of MSCs is from the umbilical cord -MSCs, where the cells can be obtained conveniently, less invasively, and have excellent regenerative and immunosuppressive properties (161). However, whether stem cell therapy can improve the renal

tissue structure and function of MetS related renal damage has not been studied.

7 DISCUSSION

With improvements in living standards and changes in lifestyle, the prevalence of MetS is increasing annually. In the future, MetS-related renal damage will become one of the key diseases for CKD prevention and treatment. We should focus on prevention and pay attention to early comprehensive intervention by including the control of body weight, hypertension, hyperglycemia, and hyperlipidemia, requiring all components to meet the criteria. Recently, studies have demonstrated that many new intervention measures, such as anti-inflammatories, antioxidants, the regulation of intestinal microorganisms, inhibiting appetite, stem cell transplantation, and other treatment methods, have achieved preliminary results and are expected to become new treatment targets for MetS related renal injury. This will reduce the prevalence of MetS renal damage and improve the prognosis.

AUTHOR CONTRIBUTIONS

LL, WT, xP, ET and ZW collected all the literature, carried out the analysis of data and outcome. LL mainly drafted the manuscript. JY revised and approved the final manuscript. Each author contributed important intellectual content during the drafting and revision of the manuscript. All the authors read and approved the final version of the manuscript to be published.

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REVIEW
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The Emerging Role of Bone-Derived Hormones in Diabetes Mellitus and Diabetic Kidney Disease

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Diabetic kidney disease (DKD) causes the greatest proportion of end-stage renal disease (ESRD)–related mortality and has become a high concern in patients with diabetes mellitus (DM). Bone is considered an endocrine organ, playing an emerging role in regulating glucose and energy metabolism. Accumulating research has proven that bone-derived hormones are involved in glucose metabolism and the pathogenesis of DM complications, especially DKD. Furthermore, these hormones are considered to be promising predictors and prospective treatment targets for DM and DKD. In this review, we focused on bone-derived hormones, including fibroblast growth factor 23, osteocalcin, sclerostin, and lipocalin 2, and summarized their role in regulating glucose metabolism and DKD.

Keywords: bone-derived hormone, fibroblast growth factor 23 (FGF23), osteocalcin (OCN), sclerostin, diabetes mellitus (DM), diabetes kidney disease (DKD)

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INTRODUCTION

The incidence and prevalence of diabetes mellitus (DM) are rapidly growing worldwide (1). With advances in medical therapies and the increase in life expectancy, the prevalence of DM complications is also expected to rise substantially. Diabetic kidney disease (DKD) is one of the most devastating microvascular complications of DM. DKD manifests with albuminuria regression, rapid decrease in glomerular filtration rate (GFR), or non-proteinuric or non-albuminuric DKD (2). The initiation of DKD is hyperglycemia associated, whereas the promotion of DKD is strongly related to hyperglycemia, albuminuria, hypertension, insulin resistance, and so on (3). Because of the lack of effective prevention and treatment, DKD leads to the major cause of end-stage renal disease (ESRD) and mortality in patients with DM (4). Thus, a deeper mechanistic understanding of DKD is needed.

The bone is classically considered a structural organ for supporting the human body and physical movement, safeguarding internal organs, and storing and maintaining mineral homeostasis. In recent years, bone has been established as an endocrine organ, and bone-derived hormones are involved in regulating glucose and energy metabolism (5, 6). The bone-derived hormones, as part of endocrine systems, reinforce the tight link between bone and other organs and maintain homeostasis (7).

Abbreviations: DKD, diabetes kidney disease; ESRD, end-stage renal disease; DM, diabetes mellitus; FGF23, fibroblast growth factor 23; OCN, osteocalcin; LCN2, lipocalin-2.

It is conceivable that abnormalities of bone-derived hormones may lead to disorders in glucose metabolism and dysfunctions in glucose regulatory organs, such as the pancreas, liver, adipose, and kidney. From another perspective, the higher fracture risk in patients with DM also suggests that bone health and endocrine functions can be strongly affected by long-term exposure to a hyperglycemia environment (8). Among the DM complications related to bone metabolism, DKD stands out for its direct effects on mineral homeostasis (9). In this review, we focus on classical bone-derived hormones, summarize the physiological regulation of glucose metabolism, and discuss how those factors influence the DKD population. We propose that bone-derived hormones are promising therapeutic targets. In addition, in-depth studies could contribute to the prevention of DKD and improvement of patients' quality of life.

BONE-DERIVED HORMONES AND DKD

Fibroblast Growth Factor 23

Physiological Function and Regulation of FGF23

Fibroblast growth factor 23 (FGF23), the first bone-derived hormone to be discovered, is primarily produced by osteoblasts and osteocytes (10). FGF23 mainly acts on the kidney *via* the FGF receptor (FGFR)–Klotho complex co-receptor and plays a key role in regulating calcium and phosphate homeostasis (11). In the renal distal convoluted tubule, FGF23 reduces renal calcium excretion by upregulating the expression of calcium-selective channel protein TRPV5 (transient receptor potential vanilloid channel subfamily member 5) (12). In the renal proximal tubules, FGF23 induces phosphaturia by inhibiting the expression of type IIa and IIc sodium–phosphate cotransporters (Na-Pi IIa/IIc) (13). At the same time, FGF23 also suppresses the production of 1,25-dihydroxy vitamin D3 [$1,25(OH)_2D_3$] by inhibiting renal 1α-hydroxylase activity and switching from 24-hydroxylase (13). The reduction of $1,25(OH)_2D_3$ synthesis downregulates calcium and phosphate absorption in the intestine, leading to further decreased serum levels (13). The parathyroid glands also express the FGFR-Klotho complex, and the binding of FGF23 and FGFR-Klotho complex activates the mitogen-activated protein kinase/extracellular signal-regulated kinase 1/2 signaling pathway to inhibit parathyroid hormone (PTH) synthesis and secretion (14). In addition, FGF23 suppresses the secretion of PTH by Klotho-independent calcineurin-mediated signaling pathway (15). Renin–angiotensin–aldosterone system (RAAS) plays important role in the progression of CKD. Moreover, the RAAS inhibitors show clinical evidence to slow disease progression, not only rely on the blood pressure control but also reduce proteinuria, and inhibit RAAS activity. The effect of FGF23 on the RAAS is also noteworthy. FGF23 could suppress renal angiotensin-converting enzyme 2 (ACE2) and facilitates the production of angiotensin II (Ang II), which is both prohypertensive and proinflammatory (16). However, the direct association between FGF23 and blood pressure remains unclear. Mice with X-linked hypophosphatemic rickets (XLH) characterized by high FGF23 levels do not show hypertension, suggesting that FGF23 may not affect blood pressure directly (17). Calcitriol, PTH, and dietary phosphate are the major systemic regulators of FGF23 levels (13). The high blood levels of calcitriol and PTH stimulates biosynthesis of FGF23 in bone (13, 18). Only high-phosphate diets result in FGF23 secretion, and phosphate infusion did not affect FGF23 levels in healthy humans (19, 20). It is reported that other factors, such as iron deficiency, hypoxia, chronic inflammation, adipokines, leptin, and acidosis metabolic acidosis, also affect the circulating FGF23 level (13). However, the accurate mechanism of FGF23's local regulation *via* paracrine remains unclear (13).

FGF23 in Glucose Metabolism

Serum phosphate is an important mediator between FGF23 and blood glucose. In an animal study, hypophosphatemia impairs adenosine triphosphate (ATP) production in pancreatic islet cells and results in decreased insulin secretion (21). In healthy individuals, low serum phosphate levels are associated with reduced insulin resistance (IR) (22); phosphate supplementation, especially when co-ingested with glucose, can, in turn, improve insulin sensitivity (23). Moreover, serum phosphate level is disturbed in the early progression of diabetes and the phosphate deregulation adversely affects glucose metabolism (24). In Fgf23 gene-deficient mice, hypoglycemic and increased peripheral insulin sensitivity is observed, and subcutaneous glucose tolerance is improved (25). However, the effects of FGF23 on glucose metabolism in humans are less known. A clinical trial in healthy adults reported that the increased FGF23 concentrations induced by diet did not affect fasting glucose or insulin levels (26). In addition, in vitamin D–deficient patients with impaired glucose metabolism, oral glucose loading decreased the secretion of FGF23 (26). On the other hand, the association of FGF23 with IR is controversial. Wojcik et al. indicated that FGF23 contributes to insulin sensitivity and negatively correlates between FGF23 and homeostatic model assessment of IR (HOMA-IR) in adolescents with obesity (27, 28). Hanks et al. showed that FGF23 was positively associated with HOMA-IR in community-dwelling adults (29). A large cohort of 3,115 elderly subjects with diabetes demonstrated that FGF23 levels were not related to the IR (30). More investigations are needed to explain the causal association between FGF23 and glucose metabolism in humans.

FGF23 in DM and DKD

The fluctuations of blood FGF23 levels are complex in patients with DM. Recently, it is reported that insulin is a negative regulator of FGF23 (31). Several studies demonstrated that blood FGF23 levels are increased in patients with T2DM (32, 33). Chronic inflammatory conditions in T2DM may result in raising FGF23 levels by overruling the suppressive effect of hyperinsulinemia (34). Furthermore, leptin, advanced glycation end products (AGEs), early renal tubular dysfunction, and the application of sodium-glucose cotransporter-2 inhibitors (SGLT-2is) may also contribute to the elevated serum levels of FGF23 (24). SGLT-2is shows significant glucose-lowering and cardiovascular-renal protective effect (35). The Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes (EMPA-REG OUTCOME) and the CANagliflozin cardioVascular Assessment Study (CANVAS) program showed improvements in renal outcomes, and SGLT-2is are helpful in

the prevention of the development and progression of DKD (35). However, randomized studies in the last several years have discovered that the treatment of Canagliflozin, Dapagliflozin, and Empagliflozin increases serum phosphate, PTH, and FGF23 (36–38). In the CANVAS trial, it was even observed that a possible association between canagliflozin and increased fracture risk (39). SGLT-2is inhibits SGLT-2 in renal proximal tubules, directly upregulate the Na-Pi reabsorption *via* Na-Pi IIa/IIc cotransporter, and then increase serum phosphate and circulating FGF23 levels. Thus, the increase in the FGF23 levels generated by SGLT2i is noticeable, as it may result in adverse diabetes outcomes including fracture and cardiovascular events. FGF23 levels started to increase early in the course of chronic kidney disease (CKD), which occurs before the increase of blood phosphate level (40). In parallel with declining kidney function and decreasing phosphate clearance, FGF23 levels will elevate further and be more than a thousand-fold higher in end-stage renal disease (ESKD), compared with normal value (41). In addition, Carlson et al. reported the intradialytic clearance of FGF23 occurs in patients undergoing chronic hemodialysis, and the clearance of FGF23 is related directly to the ultrafiltration rate (42). However, the intradialytic plasma concentrations of FGF23 remained unchanged (42). A cross-sectional study revealed that patients with DM with IR exhibited higher FGF23 levels in the CKD stages 3–5 (43). Osteoporosis is also common in the DM population. A prospective study of 126 patients with T2DM with CKD stages 2–3 suggested that patients with a fracture event displayed higher levels of FGF23, and FGF23 could independently affect the occurrence of fracture (44). On the other hand, increased serum FGF23 levels increase all-cause and cardiovascular mortality in patients with T2DM, especially under the CKD conditions (45). Some studies revealed possible mechanisms by which FGF23 affects CKD progression. In wild-type mice, elevating FGF23 levels increase hepatic and circulating cytokines and drive inflammatory states, which is associated with poor clinical outcomes of CKD (46). FGF23 signaling also impairs leukocyte recruitment *in vitro* and *in vivo* during CKD, and the disordered leukocyte recruitment increased predisposition to infections by weakening host response (47). However, in patients with CKD with hemodialysis, high FGF23 is not the cause of infection or systemic inflammation but is positively associated with vascular calcification (VC) (48, 49). VC is highly prevalent in DM and is deemed to participate in the pathogenesis of uremic VC (50). Because elevated circulating FGF23 level is associated with cardiovascular mortality and progression in CKD, the effect of phosphorus restriction diet–induced FGF23 reduction was investigated. The result suggested that a standard low-phosphorus diet reduced circulating FGF23 level in both early and advanced CKD. In addition, serum PTH was decreased in the advanced CKD group, and $1,25(OH)_2D_3$ levels was increased in the early CKD group (51). Another randomized controlled crossover study suggested that very low–protein diet with a consequently low intake of phosphorus could rapidly reduce FGF23, serum phosphate, and urinary phosphate excretion within 1 week (52). In nephrectomized (Nx)–induced uremia rat model, serum phosphate, urinary phosphate excretion, serum FGF-23, and PTH were significantly lower in the low dietary phosphate group. Modification of phosphorus concentration in the diet affected the apoptosis of enterocytes and type IIb sodium–phosphate cotransporters (Na-Pi IIb) and phosphate inorganic transporter-1/2 (PiT-1/2) expression in jejunum mucosa (53). Those studies suggest that early control of phosphorus intake prevent FGF23 increasing and improve the VC and CKD progression. Further studies are warranted to clarify the potential role of high plasma FGF23 levels in CKD. FGF23 could be new hope for the prevention and treatment of DKD.

Osteocalcin

Physiological Function and Regulation of OCN

OC is an osteoblast-secreted and vitamin K–dependent protein and is comprised of two forms: undercarboxylated osteocalcin (ucOCN) and carboxylated osteocalcin (cOCN) (54, 55). Circulating OC is accessible to measurement, and ucOCN is considered to be the bioactive form of OC that plays a role in regulating energy metabolism and glucose homeostasis (56). OCN maintains calcium homeostasis and facilitates bone mineralization and growth (55). The specific receptor of ucOCN is G protein–coupled receptor class c group 6 member A (GPRC6A), expressed broadly in various organs except for the brain. In mice models, ucOCN affects adipocyte gene expression and reduces fat mass (57). In addition, ucOCN is also required and sufficient to strengthen the exercise capacity of skeletal muscle (58). More importantly, OCN could directly stimulate β-cells proliferation, insulin secretion, and insulin sensitivity (59, 60). Mizokami et al. further found that ucOCN also induces glucagon-like peptide-1 (GLP-1) release from the gut that plays a main role in insulin secretion stimulated by ucOCN (61). Beyond that, OCN makes it possible that a more mixed regulation between bone and islet β cells. Insulin could increase OCN activity and suppress osteoprotegerin (OPG) expression that enhances bone resorption *via* osteoclasts (62). On the other hand, Delta-like 1 (DLK1) produced by pancreatic β cells could be stimulated by ucOCN and negative feedback regulate the OCN production in osteoblasts (63). In addition, ucOCN modulates reproductive function by situating testosterone secretion from the testis (64). G protein–coupled receptor 158 (GPR158) acts as the receptor for ucOCN in the brain, and through binding to which ucOCN enhances the brain's cognitive function (65).

OCN in Glucose Metabolism

The above summary of OCN functions, based on the experimental models, provides preliminary evidence for the connection between OCN and glucose metabolism. The evidence of OCN that directly impacts glucose metabolism is also accumulating in humans. A cross-sectional study of 2,353 participants showed that the serum OCN level was highest in the normal glucose tolerance (NGT) group and gradually reduced in the impaired glucose tolerance (IGT) group and T2DM participants (66). After this, 1,049 participants with no diabetes and 983 participants with NGT were follow-up for 4 years, and researchers reported that the low serum OCN level group

(<23.33 ng/ml) exhibited an increased risk of T2DM, impaired fasting glucose (IFG), and IR (66). A prospective community-based cohort study, which consists of 6,595 middle-aged to elderly Chinese participants, demonstrates that high circulating OCN was significantly associated with decreased blood glucose level, IR, triglyceride (TG), and body mass index (BMI) (67). Thus, OCN may correlate positively with glycemic metabolism status, and lower serum OCN concentration is associated with incident T2DM, which was also justified in different populations by the subsequent studies by Urano et al. and Ye et al (68, 69). However, a prospective investigation showed that there was no evidence of an association between ucOCN and incident T2DM in older participants (70). Aside from this, OCN is a medium through which some medication affects glucose metabolism. Randomized clinical implementation trials have proved that the glucocorticoid through decreasing OCN concentrations reduces hepatic insulin sensitivity and induces basal and postexercise IR (71, 72). Interestingly, the decline of OCN caused by medication is not always influencing blood glucose levels. Lewis et al. discovered glycated hemoglobin (HbA1c) did not alter clearly although OCN was fall in older women after 1 year of calcium supplementation (73). Future research may assess whether treatment with more profound effects on OCN interferes with glucose metabolism.

OCN in DM and DKD

Many basic experimental studies have proved OCN engages in different stages of DM development and play a protective role through influencing adipose tissue metabolism, pancreatic function, and oxidative stress (74). In a clinical trial involving 75 middle-aged to aged Japanese without any anti-diabetic agent administration, it was observed that ucOCN is correlated with HbA1c and insulinogenic index (IGI) in the DM group, and ucOCN plays more vital roles in insulin secretion than in insulin sensitivity in patients with DM (75). In a study of children with newly diagnosed DM, serum C-peptide levels are related to a higher ucOCN and ucOCN/cOCN ratio (76). The abovementioned research suggested OCN favors insulin secretion in patients with DM, but the relationship between OCN and glucose homeostasis, which is crucial for controlling the progression of DM complications, is uncertain. A previous study investigated the community-based adults with type 1 DM (T1DM), finding that OCN is unrelated to any glucose homeostasis marker (77). Nevertheless, the relationship between OCN and DKD has been well documented. A 4.6-year prospective study of 1,174 patients with DM with normal kidney function concluded that lower OCN levels were relevant to an increased risk of incident DKD (69). A cross-sectional study induced 374 men and 364 postmenopausal women showed that patients with T2DM with micro or macro-albuminuria had lower OCN levels compared with patients with normal albuminuria (78). In addition, the decreased OCN levels could affect osteogenesis in T2DM with proteinuria (78). Similar to FGF23, OCN also independently affected the occurrence of bone fracture in patients with DKD and was lower in patients with a fracture event compared with patients without fructure (44). Hemodialysis is an important therapeutic choice for patients with ESKD. Carlson et al. observed that OCN blood concentrations declined during hemodialysis but rebounded within 6 h, and the intradialytic plasma concentrations of OCN did not change significantly (42). Fusaro et al. found that patients with DM undergoing hemodialysis had a higher risk of all-cause mortality and total OCN and ucOCN were lower, compared with patients without DM undergoing hemodialysis, which might indicate that OCN plays a potential protective role in patients with ESKD (79). A lower OCN level is unfavorable for blockading the onset and progression of DKD. Given that ucOCN is the active form of OCN, more clinical research studies are necessary to evaluate the role of ucOCN in the DKD population.

Sclerostin

Physiological Function and Regulation of Sclerostin

Sclerostin is a glycoprotein predominantly produced by mature osteocytes, and it can inhibit bone formation by occupying Wnt coreceptors low-density lipoprotein receptor-related proteins 5 (Lrp5) and Lrp6 to suppress Wnt signaling pathway (80). The latest study supplemented that the binding of sclerostin to Lrp4 enhances this suppression by facilitating sclerostin-Lrp6 binding (81). The changes in Wnt signaling also stimulate bone resorption by repressing the expression of OPG, a downstream target of the Wnt signaling pathway that can inhibit bone resorption (82). Therefore, sclerostin effectively reduces bone mass and volume, and antisclerostin monoclonal antibody has gradually held an important place in the treatment of osteoporosis (83). In addition, sclerostin can affect mineral metabolism by altering mineral homeostasis-related hormones. Sost is the gene encoding sclerostin, and $Sost^{-/-}$ mice display lower FGF23 levels, reduced calcium excretion, and elevated serum phosphorus (84). On the other hand, the Wnt pathway is also been linked to adipogenesis (85). $Sost^{-/-}$ mice exhibit a notable increase in bone formation and a decrease in visceral and subcutaneous adipose, which are explained by the sclerostin deficiency, blocking the differentiation of adipocyte progenitors to mature adipocytes (86). In addition to white adipose tissue, the major component of visceral and subcutaneous adipose, sclerostin, also increased the brown adipose tissue (BAT)–specific gene expression and the bone marrow adipose tissue (BMAT) formation that were confirmed in other experiments (87, 88). Thus, inhibiting sclerostin also contributes to the treatment of obesity. In addition, it has been proposed that sclerostin's regulation of adipogenesis also affects the immune cell maintenance, and sclerostin depletion is disadvantageous for B lymphopoiesis and myelopoiesis, even hematopoiesis (89). Mechanical force can promote bone formation, and the Wnt signaling pathway represents a critical role in the regulation of mechanical stress-induced bone formation (90). Hence, sclerostin then becomes an obligatory step for this process and can be downregulated by mechanical force to increase bone formation (90). Furthermore, Sost transcription is negatively regulated by PTH, and Lrp4 plays an integral role in this process (91).

Sclerostin in Glucose Metabolism

As stated above, sclerostin promotes adipogenesis. Adipose tissue has endocrine function and exerts an impact on energy metabolism. Numerous studies showed that sclerostin could influence glucose metabolism. Lrp4 is necessary for normal sclerostin function and is expressed in both adipocyte and osteoblast (92). Kim et al. found that mice with Lrp4-deficient adipocytes showed increased glucose and insulin tolerance and that mice with Lrp4-deficient osteoblasts developed impairments in glucose tolerance and insulin sensitivity (92). Following this experiment, Kim et al. conducted another study in $Sost^{-/-}$ mice and observed improvements in glucose metabolism (86). Similar research was also performed on children and adolescents. Wedrychowicz et al. identified that sclerostin correlated negatively with HOMA-IR, and this correlation was stronger in obese children and adolescents (93). These investigators also found an inverse association between sclerostin and insulin in the obese group and an inverse association between sclerostin and C-peptide in the health cohort (93). These results are complemented by a recent study that sclerostin was also inversely related to fasting glucose in obese children and adolescents, and the negative relationship between sclerostin and fasting insulin levels has been also observed (94). Consequently, sclerostin is closely correlated to blood glucose level and insulin resistance. In addition, to better understand how sclerostin affects glucose metabolism in the human body, further in-depth research focusing on the potential mechanism is required.

Sclerostin in DM and DKD

In an *in vitro* experiment, investigators found high glucose (HG) and AGEs significantly increased sclerostin expression in osteocytes, and this function can be antagonized by PTH (95). The increased expression of sclerostin is also observed in streptozotocin-induced DM rats, which further confirmed the detrimental effects of sclerostin on bone in patients with DM (96). A case-control study including 40 T1DM cases and 28 healthy controls showed that serum sclerostin levels were negatively associated with HbA1c in patients with T1DM and the sclerostin levels were significantly greater compared with healthy participants (97). Another clinical study involving T2DM postmenopausal women found that T2DM upregulates the expression of Sost and AGEs, contributing to the impairment of bone microarchitecture (98). A prospective cohort that included 1,778 individuals revealed no clear association between sclerostin and T2DM risk (99). However, in the cross-sectional study, Napoli et al. and Shalash et al. suggested that serum sclerostin levels in patients with T2DM were noticeably higher than those subjects without DM (100, 101). In addition, the positive correlation between sclerostin and VC, sclerostin and atherosclerosis, and sclerostin and arterial stiffness in patients with T2DM was well-proved by cross-sectional studies (101–103). In addition, a protective role of sclerostin in VC development was demonstrated in $Sost^{-/-}$ mice (104). Moreover, Jean et al. found higher sclerostin levels are associated with a better survival rate in patients undergoing hemodialysis (105). Like the two mentioned hormones, the concentrations of sclerostin also remained nearly constant, although it can be cleared during hemodialysis (42). In patients with DKD, Kim et al. detected the sclerostin level begin to elevate in CKD stage 3 and dramatically elevate in CKD stage 4/5 (106). In addition, Wu et al. found that urinary sclerostin is positively related to fractional excretion of magnesium in patients with DKD or patients with T2DM without CKD (107). The above results implied that the increased sclerostin level is probably a protective phenomenon and that urinary sclerostin also plays a potential role in renal electrolyte excretion in patients with DKD. Additional research is warranted to shed light on this phenomenon.

Lipocalin-2 (LCN2)

Physiological Function and Regulation of LCN2

LCN2 is a 198–amino acid adipocytokine, also termed neutrophil gelatinase–associated lipocalin (NGAL) (108). It exists in a wide variety of cells, such as neutrophils, hepatocytes, adipose tissue, renal cells, and bone marrow (108). Megalin/glycoprotein (gp) 330 and solute carrier family 22 member 17 (SLC22A17) or 24p3 cell-surface receptor (24p3R) are two receptors that bind human LCN2 and LCN2 mouse protein, respectively (108). LCN2 plays an essential role in normal bone formation and participates in the endocrine function of the bone (109, 110). Some experiments in mice have demonstrated that osteoblast-secreted LCN2 can promote adaptive β-cell proliferation, induce insulin secretion, improve insulin sensitivity, and inhibit food intake (110, 111). The melanocortin 4 receptor (MC4R) is a key receptor for controlling food intake (110). LCN2 can activate the MC4R-dependent anorexigenic (appetite-suppressing) pathway, decreasing fat mass and body weight (110). In addition, LCN2 has bacteriostatic properties that make it competent for combating infection, injury, and other cellular stress (108, 112). LCN2 also plays an important role in cell differentiation, apoptosis, cancer progression, and metastasis (108). Pathologic conditions such as inflammation and metabolic diseases can upregulate the expression of LCN2, and LCN2 can be found in the brain, heart, and skeletal muscle that do not express LCN2 under normal conditions (108, 113–115). Moreover, AGEs, insulin, and dexamethasone are strong facilitators of LCN2 expression and secretion (108).

LCN2 in Glucose Metabolism

LCN2 has been shown an intimate association with the metabolism of glucose. Capehorn et al. confirmed that improved insulin sensitivity and suppressed gluconeogenesis were present in LCN2 knockout (LCN2KO) mice (116). Currò et al. also reported that LCN2 was positively correlated to the homeostatic model assessment (HOMA) index in normal subjects, which suggests a regulatory role of LCN2 in IR (117). Furthermore, Capulli et al. observed that LCN2KO mice showed lower fasting glucose and higher glucose tolerance compared with wild-type mice (109). These investigators also found that insulin levels were increased and the insulin tolerance remained mostly unchanged in LCN2KO mice (109). In addition to this, Mosialou et al. considered that the elevated circulating LCN2 levels are a protective reaction to resist obesity-induced glucose

intolerance (110). In addition, a cross-sectional study involving 2,519 Chinese aged 50–82 years observed that the serum LCN2 was remarkably higher in subjects with IFG and/or IGT and newly diagnosed T2DM than in healthy individuals (118). Another notable finding is that the circulating levels of LCN2 are related to intrapancreatic fat deposition but not to fatty liver (119). The effect of this ectopic fat deposition on glucose metabolism merits further investigation.

LCN2 in DM and DKD

DM, considered a circumstance of metabolic inflammation, could lead to a certain impact on plasma LCN2 concentrations. Takaya et al. reported that the levels of LCN2 were higher in adolescents with T2DM compared with the normal control group (120). Shahnawaz et al. stated that LCN2 was significantly increased in subjects with T2DM with chronic hepatitis B infection (121). The findings from Huang et al. suggested that the elevated serum LCN2 is independently correlated with T2DM in middle-aged and elderly Chinese patients (118). A 5-year prospective study in postmenopausal women with prediabetes found that there was a strong positive association between circulating LCN2 levels and insulin levels, HOMA-IR, homeostatic model assessment of β-cell function (HOMA-B), and BMI (110). However, De la Chesnaye et al. and Wang et al. observed that the levels of LCN2 were decreased in individuals with long-term T2DM and inversely related to HbA1c and BW in diabetes (122, 123). From this, the actual changes in LCN2 levels are more complicated than expected. Interestingly, the relationship between LCN2 and the neuropathy of diabetes is well demonstrated. In mouse models of DM, LCN2 plays a critical role in the pathogenesis of diabetic encephalopathy (124). In addition, a recent study of subjects with T2DM revealed the role of LCN2 in diabetic peripheral neuropathy (DPN) and highlighted the value of LCN2 in the evaluation of DPN severity (125). Otherwise, LCN2 is also identified as the biomarker for acute and chronic kidney injury (108). Capulli et al. found that LCN2KO mice exhibited polyuria, glycosuria, proteinuria, and renal cortex vacuolization (109). Li et al. reported that the variants of LCN2 in human urine were correlated with renal dysfunction (126). In the kidneys of obese prediabetic rats, the elevated LCN2 expression occurred earlier than the biomarkers of inflammation, oxidative stress, and fibrosis, which means that LCN2 is an important predictor of early kidney injury (127). Whether the clearance of LCN2 is affected in DKD is not clear, and more comprehensive studies determining the role of LCN2 in human DKD are urgently needed.

FUTURE PROSPECTS

With the aid of bone-derived hormones, an intimate relation between bone and glucose metabolism has been noticed (**Figure 1**). Bone-derived hormones play an emerging role in the treatment, prevention, and prediction of DM. In addition, we summarized the relevant therapeutic studies around the bone-derived hormones in DM and DKD.

The description of FGF23 hinting to reduce its serum level is a reliable treatment option. The reduction of FGF23 levels was observed in the studies on pharmacological treatments (for example, dapagliflozin) of DKD (37). On the other hand, anemia is common in patients with CKD, and targeted therapy against it, for example, erythropoietin (EPO)–stimulating agent treatment, can induce increased FGF23 serum levels (128). Thus, more therapy strategies for patients with DKD should be

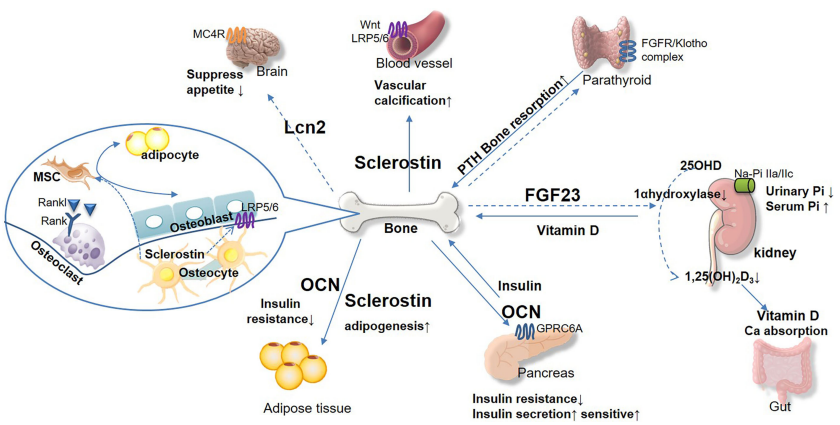

FIGURE 1 | The emerging role of bone-derived hormones in glucose metabolism and cross-talk in kidney, pancreas, and other diabetic complication target organs. Bone acts as the endocrine organand links kidney and glucose metabolism. Bone-derived hormone FGF23 is secreted from osteocytes and regulates urinary phosphorus excretion from kidney. FGF23 suppresses 1 α-hydroxylase activity, inhibits 1,25(OH)2D3, and reduces parathyroid hormone (PTH) level. Osteocalcin (OCN) acts on pancreas and adipose tissue to regulate glucose and energy metabolism, insulin secretion, and insulin resistance. Sclerostin plays important role in vascular calcification, which promotes chronic kidney disease progression directly and indirectly. Osteoblast secreted lipocalin-2 (LCN2) regulates central food intake and pancreatic β-cell proliferation, to maintain glucose and energy homeostasis. The bone-derived hormones would be potential therapeutic target for DM and complications based on their effect on maintaining glucose homeostasis and bone health.

considered associated with FGF23. Burosumab, the monoclonal antibody that targets and blocks the activity of FGF23, has been studied comprehensively in the treatment of mineral disorder and is expected to provide a potential choice for improving mineral metabolism in patients with DKD (129, 130). It leads an important future direction that evaluating the efficacy and safety of the anti-FGF23 monoclonal antibody in patients with DKD, which is beneficial in reducing the risk of fracture and lowering the incidence of adverse cardiovascular events. In addition, deity phosphorus restriction in patients with CKD reduces circulating FGF23 level and then improves the VC, cardiovascular outcomes, bone metabolism, and disease progression. Previous studies of mice showed that OCN appears to be a viable therapeutic method in obesity and insulin resistance. A recent study provided further evidence that the short- and long-term treatment of decarboxylated OCN (dcOCN), a kind of uncarboxylated OCN, can increase glucose uptake in MG63 cells (human osteoblast-like osteosarcoma cells), which implies that dcOCN may be a potential approach for T2DM (131). Otherwise, the clinical application for OCN as a predictor of DM complications is also underway. Zhu et al. found that circulating OCN can emerge as a predictor of ketosis in T2DM (132). In recent years, the role of OCN in gestational DM (GDM) is also arousing attention. Song et al. demonstrated that the synthesis of OCN can occur in the placenta and that a lower OCN concentration in umbilical vein serum is related to GDM (133). Inhibition of sclerostin is an effective way to lower T2DM-associated fracture risk. However, a meta-analysis indicated this therapeutic approach may lead to increased cardiovascular events (134). Thus, it calls for utmost vigilance that the cardiovascular safety of the application of sclerostin inhibitors in patients with DM and DKD. LCN2, as a novel bone-derived hormone, plays an active role in energy metabolism. The administration of exogenous LCN2 can reduce food intake and fat mass (110). On the other hand, it is also noticeable that LCN2 is a promising diagnostic biomarker and drug target in neuropathy of diabetes (135). The small-molecule LCN2 inhibitors and neutralizing antibodies against LCN2 are important future directions for the treatment of diabetic neuropathy (135). Moreover, the activation of epidermal growth factor receptor (EGFR) and the expression of LCN2 are often found in the same pathologic conditions, such as CKD (136). In addition, in the CKD model, the inactivation of the LCN2 gene can prevent EGFR recycling to the plasma membrane, which is related to a dramatic reduction of renal lesions (136). Thus, the therapeutic suppression of LCN2 may be useful to counteract kidney damage.

In short, studies on bone-derived hormones have just begun, and large prospective studies are still necessary to infer more causal relationships. In future work, more novel agents for the treatment of DM will emerge by focusing on the endocrine function of bone.

CONCLUSION

Bone has long been known for its supportive and protective function. However, the endocrine function of bone deserves more attention in recent DM studies. Bone-derived hormones correlate with insulin secretion, insulin resistance, and glucose metabolism and are implicated in the development and outcomes of DM and DKD. Bone-derived hormones would be promising therapeutic targets for DM and complications based on their potential effectiveness in maintaining glucose homeostasis and bone health.

AUTHOR CONTRIBUTIONS

YL wrote the manuscript. ZG, JW, and YW provided helpful suggestions. XC conceived and designed the study. BD designed this study, created and prepared the figures, supervised the project, and takes responsibility for this study. All authors have read and agreed to the published version of the manuscript.

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REVIEW
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Excess Uric Acid Induces Gouty Nephropathy Through Crystal Formation: A Review of Recent Insights

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Uric acid (UA) is the final product of purine metabolism in the human body, and impaired purine metabolism can increase the uric acid in serum, finally resulting in hyperuricemia (HUA). Current evidences suggest that urates might have antioxidant properties under certain circumstances, but most evidences suggest that urates promote inflammation. Hyperuricemia leads to the formation of urate crystals, which might be recognized as a red flag by the immune system. Such a response stimulates macrophage activation, leads to the activation of NOD-like receptor protein 3 (NLRP3) inflammasome vesicles, and ultimately the production and liberation of interleukin-1b (IL-1b) and interleukin-18 (IL-18), which can mediate inflammation, apoptosis and necroinflammation and cause an inflammatory cascade response. The kidney is one of the most commonly affected organs in HUA, which promotes the development of chronic kidney disease (CKD) by damaging endothelial cells, activating the renin-angiotensin system (RAS), and promoting inflammatory responses. Pharmacological interventions and lifestyle modifications are the primary means for controlling gout and lowering UA. The febuxostat is safe for CKD patients in the UA lowering therapy. Although dialysis can reduce UA levels, the application of drug is also necessary for dialysis patients. This article reviews the synthesis and metabolism of UA, etiology of HUA, the relationship between HUA and kidney disease, the treatment of gout and gouty nephropathy (GN).

Keywords: uric acid, hyperuricemia, gout, gouty nephropathy, treatment

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INTRODUCTION

Uric acid is the final metabolic product of purines in humans. As the final product of exogenous purines from food and endogenous purines from damaged and dead cells, uric acid is synthesized mainly in the liver, intestine and vascular endothelium (1). The kidney plays a leading position in the excretion of uric acid, with about 70% of the uric acid produced daily being excreted by the kidneys; as the remaining 30% is excreted from the intestines (1). After filtration by the glomerulus, uric acid is absorbed, secreted and reabsorbed by the proximal tubule, and the unabsorbed portion is excreted in the urine. In the proximal tubule, reabsorbed urate is secreted into the tubular lumen, about 10% of the filtered urate is excreted in the urine, and the rest 90% is reabsorbed (2).

Under physiological conditions, the synthesis and excretion of uric acid in our body is in balance. Hyperuricemia results when this balance is disturbed. Typically, levels of serum uric acid >6.8 mg/dl are considered to be hyperuricemia (3). The overall prevalence of hyperuricemia in China is 13.3%, and the prevalence of gout is 1.1%, Hyperuricemia is more common in men than in women; UA levels in women of reproductive age are lower than their male counterparts due to the inhibition of renal urate reabsorption with an increased renal urate clearance by estrogenic compounds (4). Elevated plasma uric acid is caused by either overproduction or decreased excretion. Overproduction is usually idiopathic and may also occur as a result of increased purine release due to massive tissue destruction, such as tumor lysis syndrome, crush injuries or intractable epilepsy. Overproduction may also be caused by genetic enzyme defects and reduced excretion may be idiopathic and related to drugs (e.g., thiazide diuretics, cyclosporine A). The dietary factor plays an important part in the development of hyperuricemia (5), as purine eventually degrades into uric acid; excessive consumption of alcohol and purine-rich foods (such as red meat, seafood, some vegetables, and animal proteins) is associated with the development of hyperuricemia. Dairy product intake is negatively associated with serum urate concentration (3). The development of hyperuricemia has been shown to be associated with multiple genetic factors and the uric acid transporter protein genes SLC2A9 (encoding GLUT9), SLC22A12 (encoding URAT1), SLC17A1 (encoding NPT1) and ABCG2 were most strongly correlated with changes in serum uric acid levels (6–9). Therefore, by detecting pathogenicity associated with urate crystals, gene assay can screen for high risk of gout in hyperuricemia patients.

When the level of serum uric acid exceeds the solubility threshold, uric acid precipitates into crystalline urate crystals, which manifest as acute episodes of painful arthritis, forming gout (10). Studies have found that a variety of factors influence the information of urate crystals, such as temperature, sodium ion concentration, pH, mechanical stress, cartilage composition, uric acid binding antibodies, cartilage and synovial fluid composition (11). Although some patients do not relapse after the first episode, the majority progress naturally, showing chronic inflammation, frequent attacks, gout stone formation and joint destruction (12). Only about 2-6% of patients with hyperuricemia progress to gout (3), but the mechanism by which most patients with hyperuricemia do not develop gout is not yet understood (13). A multi-stage genome-wide association study (GWAS) identified three loci, 17q23.2 (rs11653176, BCAS3), 9p24.2 (rs12236871, RFX3) and 11p15.5 (rs179785, KCNQ1), which contain inflammatory candidate genes and are likely to be associated with the development from hyperuricemia to gout (14). Another GWAS study showed that three loci (CNTN5, MIR302F and ZNF724) were related to the mechanism of gout development (15), which is different from the gout risk loci that raise serum uric acid levels we know now. In this review, we focus on the role that uric acid and gout play in kidney disease and the problems currently encountered in the treatment of gouty nephropathy.

URIC ACID - ANTI-INFLAMMATORY, OR PRO-INFLAMMATORY?

It has been shown that urate has properties to scan for free radicals and has strong antioxidant capacity in human body (16). Uric acid positively affects neurological function by inhibiting the accumulation of oxygen free radicals, stabilizing calcium homeostasis, maintaining mitochondrial function and protecting neurons from glutamate-related toxicity. Against the data supporting the anti-inflammatory effects of urate, urate has been found to be a pro-oxidant, forming free radicals in the reaction with other oxidants that appear to target mainly lipids (e.g., low-density lipoprotein (LDL)) (16). Uric acid stimulates nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-dependent reactive oxygen species (ROS), leading to mitogen-activated protein kinase (MAPK) kinase p38 and extracellular regulated protein kinases (ERK) 1/2 activation, decreased nitric oxide bioavailability, and increased protein nitrosylation and lipid oxidation (17). The limited prospective data do not clearly support the potential antioxidant and organ-protective effects of urate. On the contrary, when the pro-inflammatory effects of urate exceed the anti-inflammatory effect, especially as its dissolution exceeds the limit (>6.8 mg/dL), gout occurs. The results of several observational studies have shown that hyperuricemia is associated with hypertension (18) and heart failure (19).

GOUT AND KIDNEY DISEASE

Gouty nephropathy, also known as uric acid nephropathy, is a series of kidney disorders caused by an increase in uric acid in the human serum, which accumulates in the renal tubules and interstitium over a long period of time. Renal damage is a common comorbidity of gout and as kidney function declines, uric acid excretion through the urine is reduced, leading to hyperuricemia. Persistent hyperuricemia leads to the formation of urate crystals in joints and tissues (20). A recent meta-analysis estimated that 24% (95% confidence interval 15 - 28) of gout patients exhibited chronic kidney disease beyond stage 3 (21). Hyperuricemia also often occurs in advanced CKD, with a prevalence of 64% in patients with stage 3 CKD and 50% in patients with stage 4 or 5 CKD (22). In a representative national study in the USA, 19.9% of gout adults had CKD ≥ stage 3, in contrast to 5.2% in adults without gout (23). Uric acid induces hypertension by affecting endothelial function and impaired nitric oxide production, and hypertension may be the initial trigger for subclinical renal damage. UA significantly increased the production of reactive oxygen species and angiotensin II, inducing senescence and apoptosis of endothelial cells at concentrations above 6 and 9 mg/dL, respectively. Hyperuricemia may also lead to microvascular injury by stimulating the renin-angiotensin system (RAS) (24), inhibiting endothelial-type nitric oxide and vascular smooth muscle proliferative effects (**Figure 1**). Hyperuricemia increases renin expression in glomerular cells and (pro)renin receptor expression

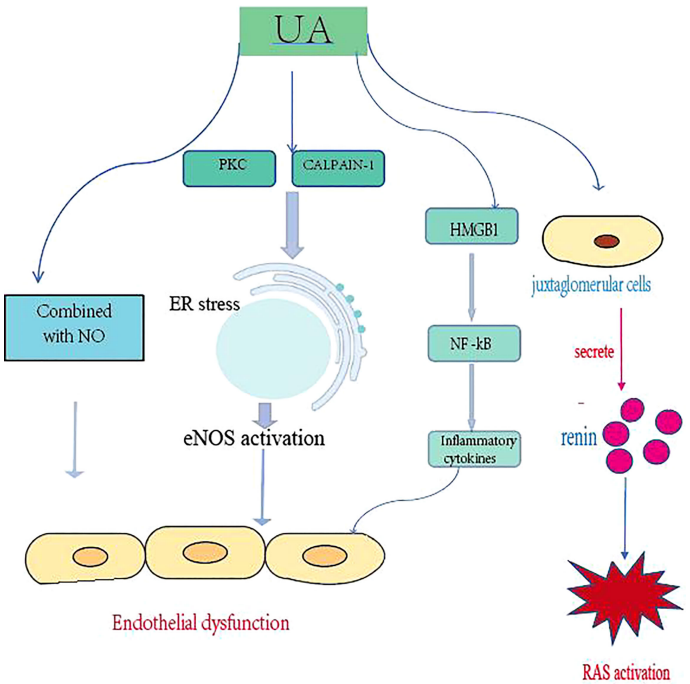

FIGURE 1 | Uric acid promotes the development of CKD by damaging endothelial cells, activating the RAS, and promoting inflammatory responses. PKC, protein kinase C; ER, endoplasmic reticulum; NOS, endothelial nitric oxide synthase; HMGB1, high mobility group box chromosomal protein 1; NF-kB, nuclear factor kB; RAS, renin-angiotensin system.

in endothelial cells, while decreasing nitric oxide synthase-1 expression in the macula. The formation of urate crystals in hyperuricemia causes gout attacks largely through activation of monocytes and macrophages, generates NLRP3 inflammatory vesicle-mediated interleukin (IL)-1β release, and leads to many other local and systemic high-level pro-inflammatory responses and joint neutrophil in-flow and activation (25). Consistent with the findings of urate crystals, Braga et al. showed that soluble uric acid salts also activate NLRP3 inflammatory vesicles and induce IL-1β production. This proinflammatory effect of uric acid on tubular cells works through High mobility group box chromosomal protein 1 (HMGB1) release and nuclear factor kappa-B (NF-κB) signaling activation (26). In this context, the hypo-inflammatory phenotype in CKD has been confirmed by several studies, which are associated with increased concentrations of serum C-reactive protein, multiple pro-inflammatory cytokines, prostaglandins and leukotrienes, and dysbiosis of the intestinal flora (27). In addition, cumulative data suggest that treatment to reduce UA may slow the progression of these diseases.

PATHOGENESIS OF GOUTY NEPHROPATHY

The mechanism of GN is mainly related to hyperuricemia and the deposition of monosodium urate crystals in the body. Survival conditions comprise high purine intake, excessive obesity and high dietary fructose concentration drinks, combined with the abuse of some drugs that affect the metabolic process of acid, such as thiazide diuretics, salicylates, and other metabolic substances such as lactic acid, ketone bodies and angiotensin. Excess uric acid deposited in the capillaries greatly increases the burden on the kidneys, leaving them in a state of long-term compensatory work, which eventually leads to a decrease in the filtration function of the kidneys and the deposition of urate crystals in the kidneys, causing lesions. Monosodium urate crystals precipitate in the renal tubules (usually the collecting ducts), causing acute gouty nephropathy. Uric acid stones may develop in 15-20% of patients with acute gouty nephropathy (28). Chronic gouty nephropathy is associated with urate crystal deposits and is mainly seen in patients suffer from gout, hypertension. The characteristic histological features of uric acid nephropathy are the presence of urate deposits in the interstitium and tubules, which can be seen as birefringent, needle-like urate crystals. Microcalcifications in the collecting ducts can cause dilatation of the collecting ducts and predispose to secondary bacterial infections. It is also associated with endothelial cell damage, activation of the renin-angiotensin system (RAS), induction of inflammatory responses by monosodium urate crystals, and activation of the cyclooxygenase (COX-2) system. A cross-sectional study of 502 patients found that the renal medulla of patients with severe gout was diffusely hyperechoic (29). This

finding supports the idea that the renal medulla of patients with long-term untreated gout is echogenic, which may be associated with the development of gout stones within the renal medulla. This nephropathy is not the only mechanism of hyperuricemia and gouty kidney damage; other factors, such as vascular involvement and non-steroidal anti-inflammatory drugs occur in 15-20% of patients with acute gouty nephropathy. Acute gouty nephropathy shows clusters of urate crystals in the aggregated tubular lumen with acute tubular damage. Needle-like birefringent crystals of sodium urate are seen on alcohol-fixed or frozen sections. These crystals dissolve during paraffin-embedded tissue processing and form needle-like fissures. Chronic gouty nephropathy presents as intra-tubular and/or interstitial microliths consisting of a central needle-like cleft surrounded by cellular reactions including epithelioid macrophages, lymphocytes and eosinophils, accompanied by tubular interstitial fibrosis. Glomerular changes include thylakoid stromal hyperplasia and double contouring of the glomerular basement membranes (28).

TREATMENT OF GOUT AND GOUT NEPHROPATHY

The main goal of gout treatment is to remove all urate crystals by lowering uric acid levels below 6 mg/dL. The process of deposition of urate crystals is reversible; crystals continue to form in gout patients and persist in patients with hyperuricemia, but dissolve when serum uric acid is lowered below the saturation point and the associated inflammation subsides with the disappearance of urate crystals (30). Importantly, lower uric acid levels lead to an accelerated rate of crystal dissolution: serum uric acid values below 4 mg/dL reduce the diameter of gout stones at a rate twice as fast as serum uric acid values above 5 mg/dL (31). Early treatment can lead to easier improvement. The 2020 American College of Rheumatology (ACR) guidelines recommend colchicine, non-steroidal anti-inflammatory drugs (NSAIDs), and non-gut/oral glucocorticoids as first-line treatment options for gout (32). However, treatment options for gout attacks with minimal or no residual renal function are limited, with the potential risk of further renal impairment. Corticosteroids have been recognized as the safest option for most patients with gout attacks and CKD (25). Short-term use of glucocorticoids is considered an acceptable risk and long-term use of glucocorticoids can lead to an increased risk of associated adverse events, particularly infections (33). The use of NSAIDs is a very common treatment for gout attacks but is not indicated for patients with renal injury (RI) and many comorbidities in older adults. In the AGREE clinical trial, low-dose colchicine was comparable to high-dose colchicine for the treatment of gout attacks with minimal side effects; therefore, low-dose colchicine has been recommended for the prevention and treatment of gout attacks (34). However, the use of colchicine for the treatment of gout attacks in RI patients has been banned (35). There are several randomized controlled trials (RCT) on colchicine to treat gout attacks, none of which have results stratified by renal function (36). In case reports and case series, we see different results on the effects of gout attack treatment on renal function. For example, 12 studies reported worsening renal function with colchicine, while another seven studies reported stable renal function with colchicine (37). All NSAIDs are widely regarded as contraindications in advanced CKD, and those patients with renal failure are usually given intraarticular or systemic steroids.

For gout sufferers, dietary control is advocated. Reduce the consumption of high purine foods such as animal offal, red meat, sugar, seafood, soda and alcoholic beverages. Eat plenty of vegetables, vitamin C, skimmed milk, low-fat yoghurt, soy products, drink plenty of water (keep daily urine output above 2000ml), and avoid full meals. Alkalinize urine to pH 6.2-6.5 (excessive alkalinization tends to form calcium phosphate or calcium carbonate stones). Lifestyle modifications: exercise properly, lower body mass, drink green tea, maintain proper hunger (1 hour of hunger rests organs and increases longevity genes), promote early dinner, and in addition, educate patients properly to improve compliance and treatment outcomes.

THE USE OF URIC ACID-LOWERING DRUGS IN PATIENTS WITH GOUT

There are two main types of drugs that are commonly used clinically to lower blood uric acid (ULT): XO inhibitors that inhibit uric acid synthesis, such as allopurinol and febuxostat, and drugs that improve the excretion of uric acid (benzbromarone, etc.). Compared with allopurinol, previous studies have shown febuxostat to be more effective and safer. Recent studies have shown that ULT has no effect on the occurrence of the primary endpoint events (cardiovascular death, non-fatal myocardial infarction, non-fatal stroke, or unstable angina combined with emergency revascularization). XO inhibitors have been reported to be beneficial in CKD. A meta-analysis showed that XO inhibitors reduced the risk of end stage renal disease significantly and also improved estimated glomerular filtration rate (eGFR) from data from randomized controlled trials with long-term follow-up (>3 months) (38). Further studies are needed to elucidate the effect of non-purine XO inhibitors on the development and progression of CKD. However, current meta-analyses do not demonstrate a nephroprotective effect of ULT. CKD is shown to lead to an increased risk of cardiovascular disease (CVD) in the general population (39). The use of ULT in patients with advanced CKD varies considerably among rheumatologists, nephrologists and general practitioners (40), and the proper use of ULT in patients with gout and CKD is controversial. The ACR, the European Rheumatism Association and the British Society for Rheumatology have published the updated guidelines with difference in some important areas, such as allopurinol dosing in patients with CKD (32, 41, 42). Allopurinol is metabolized in the liver to active allopurinol and excreted by the kidneys. When the kidneys are not functioning properly, allopurinol tends to accumulate in the body, increasing the risk of drug toxicity. There are two main reasons for avoiding the use of ULT in patients with CKD: lack of efficacy and increased risk of adverse events. In

general, patients with eGFR <30 ml/min/1.73 m^2 are reluctant to use allopurinol because of the potentially fatal allopurinol hypersensitivity syndrome (AHS) and the poor prognosis for patients with impaired renal function who develop AHS (43). Febuxostat is a non-purine selective xanthine oxidase inhibitor, metabolized mainly in the liver by glucosylation, and its use in CKD has become more widely accepted. In one of the largest studies of febuxostat for CKD, which included 96 patients with glomerular filtration rates (eGFR) in the range of 15 - 50 ml/min/1.73 m^2, febuxostat 60 - 80 mg/day was associated with a reduction in serum urate concentrations (compared with placebo) but no reduction in renal function (44), which may suggest that febuxostat is effective in reducing serum uric acid and is well tolerated in patients with moderate to severe renal insufficiency in gout. It is not clear whether the rate of gout attacks in patients with CKD at the time of initiation of ULT is the same as in those without ULT and whether prophylaxis is always required (45).

Regarding the cardiovascular safety of febuxostat versus allopurinol in patients with gout and CVD, the use of febuxostat in CVD patients has been controversial. CARES conducted a large randomized controlled trial (RCT) in the United States in patients with gout and pre-existing cardiovascular disease. CARES randomly assigned 6190 patients with gout and cardio-vascular disease to receive febuxostat or allopurinol and patients were stratified according to kidney function. The CARES study found that all-cause and cardiovascular mortality were higher in the febuxostat group than in the allopurinol group (hazard ratio for death from any cause, 1.22 [95% CI, 1.01 to 1.47]; hazard ratio for cardiovascular death, 1.34 [95% CI, 1.03 to 1.73]) (46). FAST is another large randomized controlled trial conducted in European countries to compare the cardiovascular safety of febuxostat versus allopurinol in patients with gout. The primary endpoint of febuxostat was not lower than that of allopurinol. In contrast to the CARES trial, FAST found that febuxostat treatment was not associated with increased cardiovascular death or all-cause mortality, and mortality in the febuxostat group was lower than in the allopurinol group. In the febuxostat group, 222 (7.2%) of 3063 patients died and 1720 (57.3%) of 3001 in the safety analysis set had at least one serious adverse event (with 23 events in 19 [0.6%] patients related to treatment). In the allopurinol group, 263 (8.6%) of 3065 patients died and 1812 (59.4%) of 3050 had one or more serious adverse events (with five events in five [0.2%] patients related to treatment) (30). Although the two studies were of similar size, there were still several differences. Only 33% of patients in FAST had cardiovascular disease at baseline while all patients in CARES had established cardiovascular disease. FAST had more complete follow-up than CARES because they used telephone or personal contact and national hospitalization and death records. In consideration of these findings, we should reconsider the use of febuxostat in patients with cardiovascular disease.

DIALYSIS AND URIC ACID

Dialysis provides appropriate clearance of serum uric acid. Relevant studies have shown that the average uric acid in hemodialysis patients is less than 5 mg/dL and the average serum uric acid after dialysis is less than 1 mg/dL, suggesting that the initiation of hemodialysis leads to clearance of tophus (43). It has been shown that serum urate reaches target concentrations less frequently in hemodialysis patients than in peritoneal dialysis patients, possibly because dialysis removes urate intermittently rather than continuously (47). It is also shown that ULT should be considered as dialysis alone is not enough to achieve ideal serum urate levels for patients (48). Allopurinol, the active metabolite of allopurinol, has been shown to be effective in reducing serum uric acid in hemodialysis patients.

CONCLUSION

Much of the current knowledge of the biological role of uric acid comes from experimental studies that have revealed that uric acid is associated with immune system activation and inflammation hyperuricemia may play a key role in the development and progression of CKD. Available evidence suggests that uric acid reduction therapy may slow the progression of CKD, although the molecular mechanism of uric acid-induced gout nephropathy remains to be further understood. Gout patients should be screened for renal function and clinicians should be aware of the link between gout and impaired renal function. The use of ULT drugs remain controversial. Long-term dietary control, lifestyle modification and patient education are the cornerstones of treatment.

AUTHOR CONTRIBUTIONS

YM contributed to the conception and the writing of the article. BD performed the framework. ZG gave the constructive discussions to the article. LX revised important intellectual content critically for important intellectual content. All authors contributed to the article and approved the submitted version.

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Break-in Period ≤24 Hours as an Option for Urgent-start Peritoneal Dialysis in Patients With Diabetes

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Background: The optimal break-in period (BI) of urgent-start peritoneal dialysis (USPD) initiation for patients with end-stage renal disease (ESRD) and diabetes is unclear. We aimed to explore the safety and applicability of a BI ≤24 h in patients with ESRD and diabetes.

Methods: We used a retrospective cohort design wherein we recruited patients with ESRD and diabetes who underwent USPD at five institutions in China between January 2013 and August 2020. The enrolled patients were grouped according to BI. The primary outcomes were mechanical and infectious complication occurrences, whereas the secondary outcome was technique survival.

Results: We enrolled 310 patients with diabetes, of whom 155 and 155 patients were in the BI ≤24 h and BI >24 h groups, respectively. The two groups showed a comparable incidence of infectious and mechanical complications within 6 months after catheter insertion (p>0.05). Logistic regression analysis revealed that a BI ≤24 h was not an independent risk factor for mechanical or infectious complications. Kaplan–Meier estimates showed no statistically significant between-group differences in technique survival rates (p>0.05). Cox multivariate regression analysis revealed that a BI ≤24 h was not an independent risk factor for technique failure.

Conclusion: USPD initiation with a BI ≤24 h may be safe and feasible for patients with ESRD and diabetes.

Keywords: end-stage renal disease, urgent start peritoneal dialysis, diabetics, break-in period, complications

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INTRODUCTION

There is a global increase in the number of patients with end-stage renal disease (ESRD). Many of these patients require an urgent commencement of dialysis owing to late referral or an accidental deterioration of residual renal function (1, 2). Urgent-start hemodialysis (HD) *via* a central venous catheter is usually chosen in an unplanned dialysis method, but this technique could increase the

prevalence of central venous stenosis, bacteremia, and thrombosis (3–5). In contrast, peritoneal dialysis (PD) has more potential benefits than HD, including cost-effectiveness, the preservation of residual renal function, and lifestyle flexibility. Urgent-start PD (USPD) is defined as the initiation of PD therapy within 2 weeks (6, 7) or 3 days after catheter insertion (8, 9). Most recent studies have demonstrated that USPD may be an adoptable dialysis option (1, 10, 11).

Several studies have reported complications related to USPD (1, 7, 10, 12–14). Some scholars argued that there may be an increased risk of dialysate leakage and catheter migration when dialysis is initiated urgently after catheter insertion (1, 12–14), whereas others hold the opposite view (7, 10). Patients undergoing PD have an increased intra-abdominal pressure due to the volume of dialysate infused into the peritoneal cavity, which can lead to anatomical complications in the abdominal wall. Patients with ESRD and diabetes are more susceptible to infections and poor wound healing due to high blood glucose levels (15, 16). We speculate that if PD is initiated urgently, patients with diabetes may be more likely to have mechanical and infectious complications than patients with adequate break-in periods (BIs). However, since there are no reports of a BI ≤24 h in patients with diabetes, the optimal BI for diabetic patients with ESRD is unclear.

Therefore, in this study, we compared dialysis-related complications, and PD technique survival rates between patients with BI ≤24 h and BI >24 h in a large sample population. The aim was to determine the safety and applicability of a BI ≤24 h as an urgent method of initiating dialysis in patients with diabetes.

METHODS

Study Design and Patient Selection

This real-world study used a retrospective cohort design. The inclusion criteria were patients diagnosed with ESRD between January 2013 and August 2020 at five institutions (The Second Hospital of Jilin University, The First Hospital of Jilin University—the Eastern Division, Jilin City Center Hospital, Jilin FAM General Hospital, and Xing'anmeng People's Hospital,). The indications for USPD were as follows: uremia symptoms (such as gastrointestinal symptoms and consciousness alteration), severe volume overload or pulmonary edema, hyperkalemia (K >6.5 mmol/L), and severe acidosis (serum bicarbonate < 10 mEq/L) as we described previously (17). Patients were excluded if they exhibited any of the following: 1) non-USPD, 2) incomplete data, 3) age younger than 18 years, 4) those who received chronic HD therapy before and/or after PD initiation, 5) percutaneous catheter placement and laparoscopic surgery, and 6) patients without diabetes.

Catheter Implantation and Dialysis Prescription

Catheter implantation was performed in a standardized manner at each PD center. Double-cuffed Tenckhoff catheters were inserted under local anesthesia during an open surgery for all patients as described previously (18). First, a nephrologist made a left paramedian incision 9–13 cm above the pubic symphysis. Subcutaneous tissue was carefully detached to reach the anterior sheath of the rectus muscle, and a 2–4 cm incision was made over the anterior rectus sheath. Subsequently, the posterior sheath was incised, and the peritoneum was exposed using blunt dissection. Purse-string suturing was performed along the small opening in the peritoneum. The PD catheter was then inserted into the peritoneal cavity. The correct positioning of the catheter tip was tested by assessing patient sensations and the free flow of saline into and out of the abdominal cavity. Thereafter, the purse-string suture was tightened and tied. Finally, the catheter was pulled through the exit site *via* a subcutaneous tunnel (18). All clinicians performed the procedures had received specialized training in catheter implantation. The number of clinicians who performed PD catheter implantation was four, two, two, one and one in The Second Hospital of Jilin University, The First Hospital of Jilin University—the Eastern Division, Jilin City Center Hospital, Jilin FAM General Hospital, and Xing'anmeng People's Hospital, respectively.

During the first few days of dialysis, the exchange volume for both groups was 0.5–1.0 L. In the absence of PD-related complications, such as dialysate leakage, the exchange volume was gradually increased to 2 L within 2 weeks. Continuous ambulatory PD or automated PD was available during the initiation period. Patients were adequately educated on PD, including dialysate exchange and catheter care. Patients were followed up every 3–6 months to monitor the adequacy of PD, including weekly measurement of Kt/V_{urea} and weekly creatinine clearance, with targets of ≥1.7 and >50 L/week/1.73 m^2, respectively.

Primary outcomes were the occurrences of early mechanical and infectious complications. Complications were examined up to 6 months following PD catheter insertion. All patients with complications initially received conservative treatments. If the complications were not resolved, surgical interventions were performed with the patient's informed consent. Mechanical complications included dialysate leakage, bleeding, catheter migration, and omental wrap. Infectious complications included peritonitis, exit-site infection, and tunnel infection. The secondary outcome was technique survival.

Catheter migration was defined as a drainage outflow volume significantly less than the inflow volume and the location of the catheter tip outside the true pelvis, which was confirmed by abdominal radiography (7). Dialysate leakage was defined as the loss of dialysate from the peritoneal cavity, or the appearance of dialysate at the exit site. Anatomical dialysate leakage to other areas was confirmed by visual observation, computed tomography, ultrasonography, or the methylene blue method. Bleeding episodes were defined as blood loss into dialysate that required hemostatic drugs, blood transfusion, or surgical intervention for hemostasis. Omental wrap was proven by secondary surgery. Technique failure was defined as conversion from PD to HD for at least 30 days (19, 20). Chronic HD was defined as an HD program lasting for >3 months and >7 sessions of HD monthly (21). Temporary HD was defined as HD treatment within 3 months before and/or after PD initiation.

Data Collection

The following data were collected: 1) patients' demographics, including sex, age, presence or absence of temporary HD, cause of ESRD, comorbidities, the history of abdominal surgery, date of PD initiation, and date of catheter insertion; 2) preoperative laboratory indicators, including the levels of white blood cells (WBC), hemoglobin (Hb), blood albumin (Alb), triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), blood creatinine (Cr), blood uric acid (UA), blood urea nitrogen (BUN), estimated glomerular filtration rate(eGFR), blood potassium (K), blood sodium (Na), blood calcium (Ca), blood phosphorus (P), and blood glucose (BG); and 3) complications and outcome events, including date(s) of mechanical and infectious complications, treatment and outcome of complications, and date of technique failure.

Statistical Analysis

Statistical analyses were performed using SPSS Statistics version 25.0 (IBM Corp, Armonk, NY, USA). Measurement data were expressed as the mean ± standard deviation, and the *t*-test was used for between-group comparisons of normally distributed data; otherwise, the data were expressed as median (interquartile range), and the Wilcoxon rank sum test was used for between-group comparisons of non-normally distributed data. Comparisons between groups of count data were performed using the chi-square or Fisher exact test and expressed as numbers and percentages. Factors associated with complications were determined using logistic regression analysis. To avoid missing important risk factors in the multivariate logistic regression analysis, the *p*-value for significance was relaxed to 0.2. In statistical language, a *p*-value <0.2 is acceptable (22). Technique survival rates were assessed using the Kaplan–Meier method and the differences between the two groups were compared by the log-rank test. Factors associated with technique failure were determined using Cox multivariate regression analysis. Covariates with *p*-value < 0.2 in the univariate analysis were used for multivariate regression. Graphs were plotted using GraphPad Prism (GraphPad Software, Armonk, NY, USA). A *p*-value <0.05 was considered statistically significant. In this study, we performed consecutive sampling of patients in the five PD centers who met the eligibility criteria.

RESULTS

Patients' Characteristics

As shown in **Figure 1**, this study included 310 patients with diabetes who underwent PD, including 155 patients in the BI ≤24 h group (50%) and 155 patients in the BI >24 h group (50%). Patient baseline characteristics are presented in **Table 1**. The mean age of patients was 56.56 years, and 202 (65.2%) patients were men. Compared to the BI >24 h group, the BI ≤24 h group had more men (71.0% versus 59.4%, p=0.032) and fewer cases of temporary HD (23.2% versus 41.3%, p=0.001) "should be "Compared to the BI >24 h group, the BI ≤24 h group had more men (71.0% versus 59.4%, p=0.032) , fewer cases of temporary HD (23.2% versus 41.3%, p=0.001) and shorter BIs (1 vs 4, p=0.000) however, there were no significant between-group differences in the other measured parameters.

Mechanical Complications

Mechanical complications that occurred in the first 6 months after catheter insertion are presented in **Table 2**. At each follow-up time point, no significant between-group differences in the occurrence of mechanical complications were observed (p>0.05) (**Table 2**). The percentage of patients who experienced catheter leakage, bleeding, catheter migration, and omental wrap within 6 months in the BI ≤24 h and BI >24 h groups were 3.2% and 2.6%, 0% and 2.6%, 3.9% and 4.5%, and 1.3% and 0.6%, respectively.

Results of multiple logistic regression analysis showed that a BI ≤24 h was not an independent risk factor for mechanical complications after adjustment for PD center, age, temporary HD usage, and a history of abdominal surgery, as well as levels of WBC, Hb, Cr, BUN, K, and P (p>0.05) (**Figure 2A**). Similarly, after adjusting for PD center, temporary HD usage, hypertension,

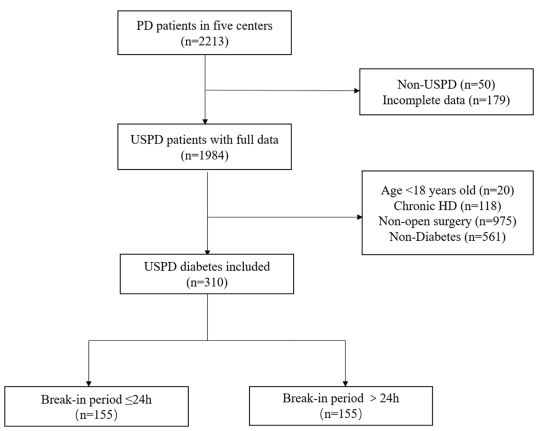

FIGURE 1 | Flowchart. PD, peritoneal dialysis; USPD: urgent start PD; HD, hemodialysis.

TABLE 1 | Baseline characteristics of patients in different BI groups.

	Overall (n=300)	BI ≤ 24 h (n=155)	BI > 24h (n=155)	x^2/z/t-value	*p*-value
Sex (men %)	202 (65.2%)	110 (71.0%)	92 (59.4%)	4.604	0.032
Age (years)	56.56 ± 12.16	55.25 ± 12.65	57.88 ± 11.55	-1.908	0.057
Temporary HD [n (%)]	100 (32.3%)	36 (23.2%)	64 (41.3%)	11.573	0.001
Cause of ESRD [n (%)]				6.002	0.409
CGN	22 (7.1%)	9 (5.8%)	13 (8.4%)		
Diabetes	253 (81.6%)	127 (81.9%)	126 (81.3%)		
hypertension	16 (5.2%)	11 (7.1%)	5 (3.2%)		
Interstitial nephritis	2 (0.6%)	0 (0.00%)	2 (1.3%)		
PKD	1 (0.3%)	0 (0.00%)	1 (0.6%)		
Others	3 (1.0%)	1 (0.6%)	2 (1.3%)		
Unknown cause	13 (4.2%)	7 (4.5%)	6 (3.9%)		
Comorbidities [n (%)]					
Hypertension	303 (97.7%)	151 (97.4%)	152 (98.1%)	0.000	1.000
Abdominal surgery history [n (%)]	37 (11.9%)	13 (8.4%)	24 (15.5%)	3.713	0.054
Break-in period (d)	1.5 (1,4)	1 (0,1)	4 (3,5)	-15.51	0.000
Laboratory indicators					
WBC (10*9/L)	7.05 (5.50,8.60)	6.91 (5.56,8.60)	7.10 (5.50,8.80)	-0.824	0.410
Hb (g/l)	87.00 (76.00,101.00)	89.00 (76.00,104.00)	86.00 (76.00,99.00)	-0.856	0.392
Alb (g/L)	32.50 (29.28,36.35)	32.10 (28.90,36.00)	32.70 (29.63,36.90)	-1.369	0.171
TG (mmol/L)	1.56 (1.34,1.90)	1.56 (1.21,2.02)	1.56 (1.44,1.83)	-0.468	0.640
TC (mmol/L)	4.43 (3.97,4.97)	4.43 (3.72,5.02)	4.43 (4.26,4.81)	-1.010	0.312
HDL (mmol/L)	0.94 (0.86,1.07)	0.94 (0.83,1.11)	0.94 (0.92,1.06)	-0.170	0.865
LDL (mmol/L)	2.62 (2.29,2.97)	2.62 (2.20,2.96)	2.62 (2.40,3.00)	-0.980	0.327
Cr (μmol/L)	650.00 (521.74,842.30)	647.00 (532.20,801.00)	666.70 (511.20,885.90)	-0.715	0.474
UA (μmol/L)	417.00 (340.75,498.08)	418.00 (348.00,505.00)	417.00 (327.00,484.00)	-1.565	0.118
BUN (mmol/L)	20.13 (13.92,27.28)	20.13 (13.52,25.81)	20.13 (14.00,27.65)	-0.377	0.706
eGFR	6.70 (5.10,8.77)	7.30 (5.35, 9.03)	6.24 (4.75, 8.50)	-1.953	0.051
K (mmol/L)	4.33 (3.88,4.97)	4.38 (3.87,5.00)	4.33 (3.89,4.92)	-0.449	0.654
Na (mmol/L)	140.30 (138.00,142.30)	140.40 (138.00,142.20)	140.18 (138.00,142.70)	-0.186	0.852
Ga (mmol/L)	2.01 (1.87,2.16)	2.01 (1.86,2.16)	2.01 (1.89,2.16)	-0.450	0.653
P (mmol/L)	1.68 (1.37, 2.05)	1.66 (1.29,2.03)	1.71 (1.43, 2.13)	-1.292	0.196
BG (mmol/L)	6.00 (4.92, 7.52)	6.00 (4.94, 7.57)	6.00 (4.91, 7.52)	-0.330	0.742

BI, break-in period; ESRD, end stage renal disease; CGN, chronic glomerulonephritis; PKD, polycystic kidney; WBC, white blood cells; Hb, hemoglobin; Alb, blood albumin; TG, triglyceride; TC, total cholesterol; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; Cr, creatinine; UA, blood uric acid; BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate; K, blood potassium; Na, blood sodium; Ca, blood calcium; P, blood phosphorus; BG, blood glucose.

TABLE 2 | Mechanical complications between different BI in diabetics with urgent PD within varies follow-up time.

	≤ 24 h (n=155)	> 24 h (n=155)	*p*-value
Within 2 weeks [n (%)]			
Leakage	4 (2.6%)	4 (2.6%)	1.000
Bleeding	0 (0.0%)	4 (2.6%)	0.131
Migration	6 (3.9%)	5 (3.2%)	0.759
Omental wrap	0 (0.0%)	1 (0.6%)	1.000
Within 1 month [n (%)]			
Leakage	4 (2.6%)	4 (2.6%)	1.000
Bleeding,	0 (0.0%)	4 (2.6%)	0.131
Migration	6 (3.9%)	7 (4.5%)	0.777
Omental wrap	1 (0.6%)	1 (0.6%)	1.000
Within 3 months [n (%)]			
Leakage	4 (2.6%)	4 (2.6%)	1.000
Bleeding	0 (0.0%)	4 (2.6%)	0.131
Migration	6 (3.9%)	7 (4.5%)	1.000
Omental wrap	2 (1.3%)	1 (0.6%)	1.000
Within 6 months [n (%)]			
Leakage	5 (3.2%)	4 (2.6%)	1.000
Bleeding	0 (0.0%)	4 (2.6%)	0.131
Migration	6 (3.9%)	7 (4.5%)	0.777
Omental wrap	2 (1.3%)	1 (0.6%)	1.000

BI, break-in period; PD, peritoneal dialysis.

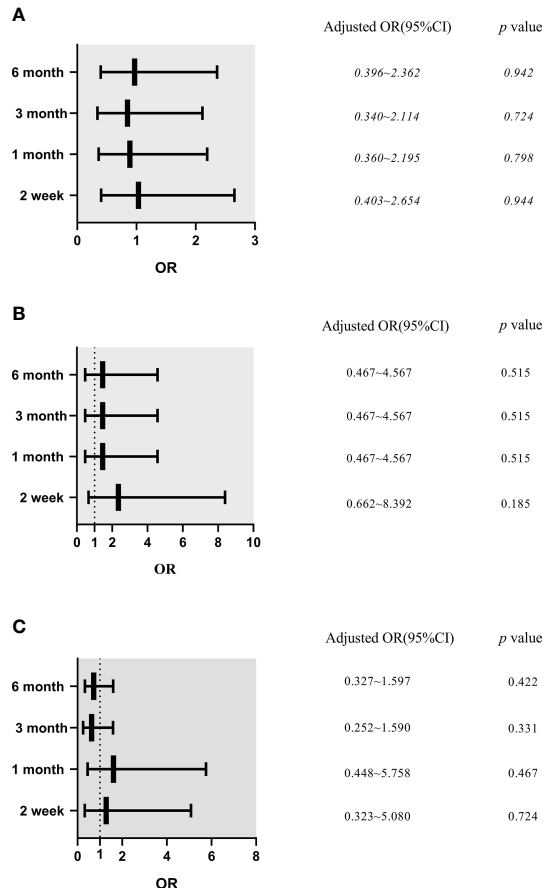

FIGURE 2 | The effects of break-in period on mechanical complications, catheter migration and infectious complications in different follow-up time (Logistic Multivariate Analysis). **(A)** Mechanical complications. Model was adjusted for peritoneal dialysis center, age, temporary hemodialysis usage, abdominal surgery history, white blood cells, hemoglobin, creatinine, blood urea nitrogen, blood potassium, blood phosphorus. **(B)** Catheter migration. Model was adjusted for peritoneal dialysis center, temporary hemodialysis usage, hypertension, abdominal surgery history, white blood cells, blood urea nitrogen and blood phosphorus. **(C)** Infectious complications. Model was adjusted for peritoneal dialysis center, temporary hemodialysis usage, sex, cause of end stage renal disease, hypertension, abdominal surgery history, white blood cells, hemoglobin, blood albumin, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, blood urea nitrogen, blood uric acid, creatinine, blood calcium, blood phosphorus and blood glucose. OR, odds ratio; CI, confidence interval.

and a history of abdominal surgery, as well as levels of WBC, BUN and P, a BI ≤24 h was not found to be an independent risk factor for catheter migration (p>0.05) (**Figure 2B**).

Infectious Complications

The percentage of patients diagnosed with a tunnel infection within 6 months in the BI ≤24 h versus BI >24 h groups were 0.6% versus 0%, respectively, whereas the percentage of patients diagnosed with peritonitis in the BI ≤24 h versus BI >24 h groups were 12.3% versus 14.2%, respectively. At each time point, there was no between-group difference in the occurrence of infectious complications. Peritonitis was the most common infectious complication (**Table 3**).

After adjusting for PD center, temporary HD usage, sex, cause of ESRD, hypertension, and a history of abdominal surgery, as well as levels of WBC, Hb, Alb, HDL, LDL, BUN, UA, Cr, Ca, P, and BG, a BI ≤24 h could not be considered as an independent risk factor for infectious complications (p>0.05) (**Figure 2C**).

Technique Survival

After 1, 2, and 3 years, technique survival rates were 94.4% and 91.8%, 89.8% and 85.9%, and 83.8% and 84.3% in the BI ≤24 h and BI >24 h groups, respectively. No significant between-group difference in technique survival rate was demonstrated (log-rank: p=0.891) (**Figure 3A**).

In a multivariable Cox analysis including PD center, age, hypertension, WBC, BUN and K in the model, BI ≤ 24h was not an independent predictor for technique failure (HR= 0.518, 95% CI =0.227–1.186, p > 0.05) (**Figure 3B**).

DISCUSSION

To the best of our knowledge, this is the first study that focuses on the feasibility of applying a BI ≤24 h in patients with diabetes undergoing USPD. We found that, similar to patients who underwent PD initiation >24 h after catheter insertion, those who underwent PD initiation ≤24 h after catheter insertion did not have a higher risk of complications and had comparable technique survival rates.

It is particularly important to monitor the quality of catheter implantation procedures in different PD centers. In our research, professionally trained and experienced doctors performed the surgery. Additionally, the variable "PD center" was corrected for in our analysis to reduce the effect of different dialysis centers on the results. In addition, the patient's preoperative nutritional status is also an important factor affecting short-term postoperative complications. Baseline data of patients in this study showed that Hb, Alb and other indicators were comparable between the two groups. Similarly, we corrected for the above nutritional indicators in multivariate regression analysis, thus avoiding their influence on the results.

Many factors may contribute to mechanical complications in patients undergoing PD. For instance, the method of catheter implantation surgery, the initial dialysate volume, a history of abdominal surgery, and the BI can affect the incidence of mechanical complications (5, 9, 23). As previously suggested, a shorter BI is associated with a higher occurrence of mechanical complications in general patients undergoing PD (12, 13). Liu et al. (12) and Kim et al. (13) investigated the feasibility of USPD with a BI ≤7 days and BI ≤48 h, respectively. Both studies concluded that patients who underwent USPD presented a much higher risk of early mechanical complications, such as catheter malposition, in the shorter BI than in the longer BI group. Liu et al. explained that a shorter BI could lead to catheter floating and increased pressure in the peritoneal cavity, which might lead to catheter leakage and malposition (12). Unfortunately, the two aforementioned studies did not directly compare the

TABLE 3 | Infectious complications between different BI in diabetics with urgent PD within varies follow-up time.

	≤ 24 h (n=155)	> 24 h (n=155)	*p*-value
Within 2 weeks [n (%)]			
Tunnel infection	1 (0.6%)	0 (0.0%)	0.317
Peritonitis	5 (3.2%)	5 (3.2%)	1.000
Within 1 month [n (%)]			
Tunnel infection	1 (0.6%)	0 (0.0%)	0.317
peritonitis	8 (5.2%)	7 (4.5%)	0.791
Within 3 months [n (%)]			
Tunnel infection	1 (0.6%)	0 (0.0%)	0.317
peritonitis	14 (9.0%)	16 (10.3%)	0.701
Within 6 months [n (%)]			
Tunnel infection	1 (0.6%)	0 (0.0%)	0.317
peritonitis	19 (12.3%)	22 (14.2%)	0.615

BI, break-in period; PD, peritoneal dialysis.

complications in patients with diabetes. Ranganathan et al. (24) in Australia evaluated 122 patients who underwent PD, including 43 patients with diabetes. Patients who underwent PD initiation at 1, 2, and 4 weeks after the insertion of a PD catheter were assigned to groups 1, 2, and 3, respectively. Among the patients with diabetes, the incidence of catheter leakage in group 1 (46.7%) was significantly higher than that in groups 2 (14.3%) and 3 (7.1%). A shorter BI delays wound healing, which may increase the risk of catheter leakage (12). Furthermore, wound healing is more complicated in patients with diabetes (16). However, we found that patients with diabetes who underwent USPD initiation with a BI ≤24 h did not have an increased incidence of mechanical complications. Additionally, our study showed that the BI was not an independent risk factor for mechanical complication. In our study, the most common short-term mechanical complication among patients who underwent USPD was catheter tip migration, which corroborated with a previous study finding (13). We also found that the BI was not an independent risk for catheter migration. This finding may be related to the following reasons: First, open surgery has the advantage of direct visualization, which may reduce the risk of catheter malposition. Second, purse-string sutures were used to reduce the risk of leakage. Finally, a low initial dwell volume could reduce abdominal pressure, and thus reduce the incidence of catheter leakage.

Infection is another common complication in patients undergoing PD. Peritonitis associated with PD is the main reason for hospital admission and referral for HD (25, 26). In the literature,

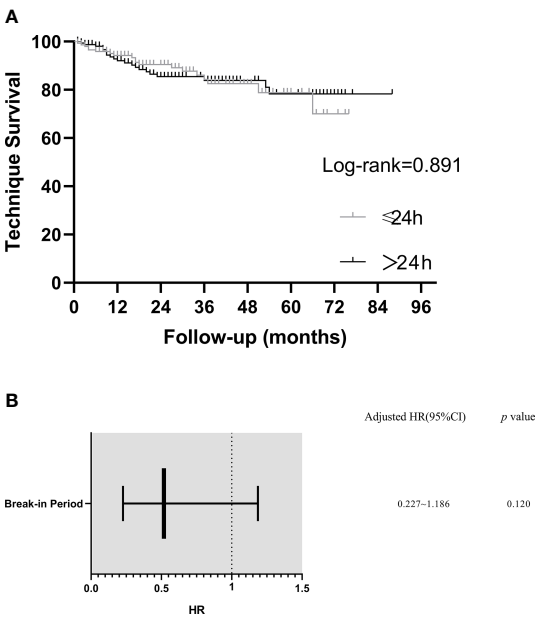

FIGURE 3 | Kaplan–Meier plot of technique survival analysis in different break-in period group and predictors of technique failure (Cox Multivariate Analysis). **(A)** Technique survival curves. **(B)** Predictors of technique failure. Model was adjusted for peritoneal dialysis center, age, hypertension, white blood cells, blood urea nitrogen and blood potassium. HR, hazard ratio; CI, confidence interval.

the proportion of patients diagnosed with peritonitis after USPD reportedly varies from 4.0% to 15.4% during the 6-month follow-up period (1, 7, 12, 13). Reduced residual renal function, low Alb and catheter leakage are considered risk factors for peritonitis (27, 28). Liu et al. and Kim et al. evaluated the relationship between the BI and incidence of infectious complications in patients undergoing PD; they found that the BI did not affect the incidence of infectious complication. Moreover, Ranganathan et al. (24) performed a subgroup analysis of ESRD patients with diabetes. In their experience, the proportions of infectious complications in groups with BIs of 1, 2, and 4 weeks were 13.3%, 0.0%, and 7.1%, respectively, over a 4-week follow-up period. The three treatment groups showed no difference in the infection risk. Consistent with the aforementioned studies, we found no difference in the incidence of infectious complications within 6 months between patients in the different BI groups. It is well established that PD center factors affect the risk of peritonitis (29). In multiple logistic regression analysis, we adjusted for the PD center as a covariate and determined that a BI ≤24 h was not a significant risk factor for infectious complications. For USPD patients, avoiding catheter leakage, prophylactic antibiotic administration, aseptic processing procedures, and operational process education are beneficial to reduce infection (28, 30).

It has been suggested that USPD may have no important implications on technique survival (12, 13). Jin et al. (31) evaluated 50 patients with diabetes who underwent USPD with a BI ≤14 days and reported that the technique survival rates at 12 and 36 months were 98.0% and 93.7%, respectively. Unfortunately, no subgroup analyses were performed in terms of the BI. In the current study, we observed technique survival rates that were similar to those reported by Jin et al. (31). Importantly, we found that a BI ≤24 h was not an independent risk factor for technique failure, probably because catheter insertion was performed by a seasoned nephrologist using open surgery, strong purse-string sutures, a low initial dwell volume, and prophylactic antibiotic administration perioperatively, which considerably lowered early technique failure resulting from catheter-related complications. Our patient population had a low incidence of mechanical and infectious complications. Therefore, a shorter BI did not affect technique failure.

We believe that USPD is an alternative treatment modality for late-presenting patients with ESRD and diabetes. A previous small sample, single-center study confirmed the feasibility of initiating dialysis within 14 days in ESRD patients with diabetes (31); however, our study found that it is also safe to initiate dialysis within 24 h after catheter insertion. The advantages of this retrospective study could be summarized as follows: First, we included the largest cohort of patients with diabetes who underwent USPD. Although different catheterization methods may have affected the incidence of PD-related complications (14), all patients were treated with open surgery. Second, in the multivariate analysis, we considered most of the recognized confounders simultaneously. Third, we included patients undergoing temporary HD, who usually have poor initial clinical statuses; hence, our study was different from previous studies (1, 31, 32) and was more reflective of real-world situations. Generally, patients undergoing temporary HD have worse baseline conditions than those not undergoing temporary HD, which may affect the incidence of short-term complications in patients undergoing USPD. By correcting for the variable "temporary HD usage" in our analysis, we found that a BI ≤24 h still did not affect the occurrence of short-term mechanical and infectious complications in patients with diabetes undergoing USPD, thereby indicating that the results of this study are widely applicable.

Nevertheless, our research has several limitations. First, some data were not recorded in this study, such as the levels of brain natriuretic peptide and cardiac markers. Therefore, it was not possible to perform a risk factor analysis of these parameters. Second, our research was conducted in Northeast China, and thus our findings may not be generalized to other parts of the world. Lastly, the generalizability of our study findings is limited by the non-randomized and retrospective design. Future prospective, randomized controlled trials are required to confirm the optimal timing of USPD initiation in patients with diabetes.

CONCLUSION

For most ESRD patients with diabetes, it may be feasible to commence dialysis immediately (BI ≤24 h) after catheter implantation.

DATA AVAILABILITY STATEMENT

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

Informed consent from the subjects was waived due to the retrospective aspect of the study. This study was approved by the Ethics Committee of The Second Hospital of Jilin University (No. 2020031, retrospectively registered). The research was conducted in compliance with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

WC designed this study. LY, ZS, XZ, XZ provided data. XH, XW, SL collected data. XH analyzed data and wrote the manuscript. WC and WZ reviewed the manuscript. All the authors read and approved the final manuscript.

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Effects of female obesity on conception, pregnancy and the health of offspring

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As we all know, female obesity has become a global epidemic, which is usually accompanied with endocrine and metabolic disorders. Obese women are more likely to experience reproductive problems, including infertility, embryonic developmental defects and abnormality in offspring. Female obesity is a complex multifactorial condition, where there are many mechanisms involved in the effects of overweight and obesity on the development of these reproductive disorders. The insulin resistance, hyperinsulinaemia and hyperandrogenism, lipotoxicity and inflammation are important mechanisms. However, the precise mechanism concerning their correlation is still unclear. Fortunately, weight loss methods have been found to reverse the effects of maternal obesity on the fertility, fetus and offspring.

KEYWORDS

obesity, infertility, embryonic development, offspring, nephropathy, cardiac disease

Introduction

Overweight and obesity are defined as abnormal or excessive fat accumulation that can damage health. The World Health Organization (WHO) recommends use of BMI to classify overweight and obesity in adults. BMI is defined as weight in kilograms divided by height in meters squared (kg/m^2). Adults with a BMI of ≥ 25 are regarded as overweight, whereas those with a BMI of ≥ 30 as obese (1). In recent decades, the incidence of obesity has risen at an alarming rate worldwide, and is reaching epidemic levels. In 2015, an estimated 1.9 billion and 609 million adults were respectively suffering from overweight and obese globally, accounting for about 39% of the world's population, and generally women have higher rates of obesity than men (2). Studies have shown that obesity increases the risk of many chronic disorders, including cardiovascular disease,

hypertension, diabetes and even several cancer types (e.g. colon, breast, endometrium cancers) (3). In addition, a growing body of research has revealed that obese women are at a high risk for reproductive health. Their disturbed reproductive health status tend to associate with poor fertilization, abnormal embryo development, poor offspring growth and vulnerability to disease (4–6). Thus, in present study, we provide an overview for current knowledge of the effect of obesity on fertility, pregnancy outcome and health status of offspring.

Obesity and female infertility

According to the literature, one out of every seven women of childbearing age is infertile in developed countries, and one out of every four women in developing countries. In some parts of the world, such as South Asia, the Middle East and Central Asia, infertility rates even reach 30% (7). Infertility is caused by various reasons, including tubal defects, malformations of the uterus, ovulatory dysfunction, genital infections, endometriosis and endocrine disorders (8). But one of the leading causes of infertility in females nowadays is obesity that is recognized as an independent risk factor for female infertility. The incidence of infertility in overweight women is three times higher than that of normal-weight women (9). Obesity is characterized by abnormal or excessive fat accumulation in women. Excessive fat, especially visceral adipose tissue, can lead to stimulation of the ovaries and adrenal glands, androgen excess, menstrual disorders, and ultimately lead to infertility. So obesity causes infertility in many ways.

Obesity in childhood or adolescence increases the risk of menstrual disorders in women of childbearing age. Obese women appear to have more menstrual irregularities than those of normal weight. 30% to 47% of overweight or obese women have been reported to have menstrual cycle disorders (10). Obesity is a metabolic disease that is usually associated with increased circulating levels of insulin, followed by increased ovarian androgen secretion (11). Next, excessive adipose tissue aromatizes the androgens into estrogen, leading to negative feedback on the hypothalamic-pituitary axis (HPO) and finally decrease in production of gonadotropins (12). Gonadotropins play distinct roles in follicle development, oocyte maturation and corpus luteum formation (13). As a consequence, the lower level of gonadotropins lead to inhibition of ovarian activity, as well as menstrual abnormalities and infertility.

In human pregnancies, the embryo first attaches to the uterine luminal epitheliuml, and then invades into the stroma of the endometrium, where the stromal cells differentiate into decidual cells and provide nutrients to the developing embryo (14). The adipose tissue may affect endometrium functions through the production of many factors such as leptin, free fatty acids (FFA) and cytokines. Studies have shown that endometrial decidualization is impaired in mice with diet-induced obesity (DIO) (15). Compared with normal diet mice, the DIO mice had significant reduction in the number of implantation sites and decreased response of endometrial stromal cells to hormonal stimulation. Similarly, human endometrial stromal cells in obese women experiences reduced ability to undergo normal decidualization, which could inhibit endometrial receptivity. As is known, the optimal endometrial receptivity ensures the successful embryo implantation (16). Therefore, the impaired endometrial receptivity in obese women is responsible for failure embryo implantation and infertility.

Polycystic ovary syndrome (PCOS) is a common endocrine abnormalities of women of reproductive age, and underpinned by insulin resistance and hyperandrogenism. The prevalence of PCOS in obese women is close to 30%, yet obesity is not necessarily the cause of PCOS (17). Actually, consistent data support a bidirectional link between obesity and PCOS. Obesity exacerbates the symptoms of PCOS because it leads to insulin resistance and adipokine release (18). Recent studies have implicated visceral fat as a contributor to insulin resistance by releasing specific adipokine and fatty acid, and thereby contributing to metabolic dysfunction in PCOS. On the other hand, the women with PCOS are more susceptible to weight gain than women without PCOS, which may be mediated by abnormal energy expenditure, excessive androgen secretion, PCOS-related emotional barriers and physical inactivity (19). However, in view that PCOS is a complex multifactorial condition, there is a lack of clear evidence supporting the role of PCOS in weight-gain, not to mention the underlying molecular mechanism.

PCOS is defined by a combination of three major symptoms of hyperandrogenism, ovarian dysfunction and presence of polycystic ovaries (20). The enhanced ovarian androgen production could impair follicular growth by stimulating atresia and apoptosis. The role of androgens in anovulation could be demonstrated by the ovulation restoration in PCOS patients treated with the antiandrogen for six months. During ovulation, the degradation of collagenous tissue in the follicle wall is necessary, in which matrix metalloproteinases (MMP) has a key role. A study in a dehydroepiandrosterone-induced rat model of PCOS demonstrated that MMP2 activity was significantly down-regulated whereas lysyl oxidase (LOX) activity was up-regulated in response to androgens, indicating that androgens could inhibit collagen breakdown and thus cause anovulation in PCOS. Furthermore, not only the number of small antral follicles but production of anti-Müllerian hormone (AMH) by each individual follicle significantly increase in women with POCS, compared with those without PCOS. The increased AMH concentration would lead to more secretion of GnRH by hypothalamic neurons, which would then stimulate luteinizing hormone (LH) production by the anterior pituitary gland and progesterone (rather than estradiol) production by ovary in the end. The premature progesterone rise at the end of

the follicular phase seems to advance endometrial maturation and impair endometrial receptivity, leading to embryo-endometrium asynchrony.

Obesity and embryonic developmental abnormalities

It is reported that one-fifth women start pregnancy as obese, while 20–40% of women gain more weight than recommended during pregnancy. According to the World Health Organization (WHO), reports that the prevalence of obesity in pregnancy varies from 1.8 to 25.3% (21). The maternal obesity poses a threat to the lives of mothers and babies. The fetal risks include preterm birth, macrosomia, congenital abnormalities, preterm births and perinatal death.

The maternal obesity is regarded as a stronger predictor of fetal macrosomia than maternal hyperglycemia (22). Obesity in pregnant women significantly increases the risk of fetal macrosomia, affecting about 20% of newborns. It has been documented that overweight pregnant women have a greater placental weight than those of normal-weight pregnant women (23). The placenta is a combination of a fetal portion (the chorion) and a maternal portion (the decidua), and provides an exchange interface for gas, nutrient and waste products between the mother and the fetus. Macrosomia is typically defined as a birth weight above the 90th percentile for gestational age or >4,000 g. There is a high correlation between the placenta weight and the birth weight (24). The pathophysiology of macrosomia can be explained by the maternal hyperglycemia caused by insulin resistance, leading to elevated placental glucose transport and endogenous fetal insulin secretion. Hence, there is increased utilization of glucose and hyperplastic growth of fetal adipose and protein tissues. However, the relationship between fetal growth restriction and maternal obesity were also demonstrated in obese pregnant women, yet the mechanisms of this relationship are not fully understood. The fetal growth is mainly determined by placental functions, nutrients transportation through the placenta and genetic factors. Therefore, the impaired placental functions by maternal obesity may be associated with the development of fetal growth restriction, and the fetus fails to reach its full growth potential.

Meanwhile, obese pregnant women have a 30% higher risk for congenital anomalies of neural tube, heart and limb than normal-weight pregnant women (25). The precise mechanism by which maternal obesity impacts fetal development is not known. Maternal obesity is known to be related to increased risk for gestational diabetes mellitus. However, Brite et al. reported that no obvious decrease was observed in the risk of congenital heart disease even after adjusting for glucose levels, suggesting that abnormal glucose metabolism could not fully explain the occurrence of congenital anomalies. In addition to glycemic dysregulation, a wide range of metabolic abnormalities are involved in the pathogenesis of obesity. Thus, the multiple adverse metabolic changes in obese pregnant women may contribute to adverse effects in the fetus.

Obesity and the offspring health

An increasing number of experiments have demonstrated that maternal obesity increases the prevalence of future metabolic dysfunction and malformation in the offspring. Children of obese mothers are more likely to develop obesity, type 2 diabetes, kidney disease, hypertension and cardiovascular disease as adults (26).

It was reported that maternal obesity increased risk of developing congenital kidney defects in the offspring by 22% (27). Moreover, the offspring of obese mothers had worse glomerular damage in comparison with offspring of normal-weight mothers (28). The mechanism of obesity-related nephropathy in offspring induced by maternal obesity is multifactorial. Lipid metabolism disorders have been reported to be associated with the occurrence of kidney disease (29). In the pathogenesis of chronic kidney injury, free fatty acids (FAs) and triglycerides could be freely filtered and reabsorbed by glomerular and tubulointerstitial cells (30). Free fatty acids functions as a link between adipose tissue activity and chronic inflammation, owing to their capability of promoting oxidative stress and inflammation (31). The excess lipid induces the production of reactive oxygen species, pro-fibrotic growth factors and pro-inflammatory cytokines, ultimately developing irreversible tubular damage and tubulointerstitial fibrosis (32). As expected, Yang et al. observed that the inflammation increases cellular uptake of fatty acids, finally triggering glomerular damage in animal experiments (33). Additionally, other metabolic risk factors also may underlie the obesity-related nephropathy such as hypertension, insulin resistance, and dyslipidemia.

Epidemiological studies have revealed a general association between maternal obesity and risk for congenital heart defects in the offspring (e.g. septal defects, conotruncal defects, aortic arch defects). Congenital heart defects, the most common type of malformation, accounts for one-third of all severe malformation (34). There is plenty of evidence showing that increased fat mass is associated with insulin resistance, hyperinsulinemia, lipotoxicity and inflammation, which may adversely impact the embryo development (35). It has also been suggested that maternal obesity may impair the self-renewal in stem cells and induce epigenetic alteration in the embryo, contributing to cardiac malformations (36). Meanwhile, some reported that the offspring of obese women also have an increased risk of being obese. Likewise, increased leptin secretion by excessive

adipose tissue leads to insulin resistance and inflammatory response, and thereby directly elicits adverse cardiovascular effects (37). In obese offspring, fatty tissue accumulation increases the stroke volume of the left ventricle, which places an additional burden on the heart. These changes would result in hypertrophy and enlargement of the ventricles, and even predispose patients to the development of heart failure (38).

Weight loss and the prevention of female reproductive disease

Based on the above, losing weight is important to reduce the risk of obesity-related reproductive dysfunction. In a clinical study involving 170 women undergoing *in vitro* fertilization, women with short-term weight loss have significantly higher production of mid-stage II oocytes than obese women (39). In obese women, reduced-calorie diet and exercise interventions are recognized as an effective way to lose weight, which is associated with improved ovulation and pregnancy rates for women. In a prospective study of 87 obese women, the subjects made changes in diet, exercise and lifestyle for 6 months. Finally, the women who completed the 6 months (total 67) lost an average of 10.2 kg/m^2. Among them, 90% resumed spontaneous ovulation, 77.6% conceived (32.7% spontaneously), 67% achieved a live birth (40). Conversely, none of these changes occurred in the women who did not complete the treatment. Thus, weight loss can help to improve ovulation, pregnancy and live birth, and thus women with a high BMI should be advised to lose weight prior to conceiving.

Conclusion

In summary, obesity in women carries the risk of infertility and negative effects on the fetus and offspring. Fortunately, these adverse outcomes can be avoided by moderate weight loss. Meanwhile, for the health of more women, more in-depth research is needed to further understand the relationship between obesity and female reproduction.

Author contributions

JT and JC for research project with conception, organization, and execution. WW, XZ, BZ, and BG for statistical analysis with design, execution, review, and critique. JT and JC for manuscript preparation with writing of the first draft, review, and critique. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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Primary cilium in kidney development, function and disease

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The primary cilium is a hair-like, microtubule-based organelle that is covered by the cell membrane and extends from the surface of most vertebrate cells. It detects and translates extracellular signals to direct various cellular signaling pathways to maintain homeostasis. It is mainly distributed in the proximal and distal tubules and collecting ducts in the kidney. Specific signaling transduction proteins localize to primary cilia. Defects in cilia structure and function lead to a class of diseases termed ciliopathies. The proper functioning of primary cilia is essential to kidney organogenesis and the maintenance of epithelial cell differentiation and proliferation. Persistent cilia dysfunction has a role in the early stages and progression of renal diseases, such as cystogenesis and acute tubular necrosis (ATN). In this review, we focus on the central role of cilia in kidney development and illustrate how defects in cilia are associated with renal disease progression.

KEYWORDS

primary cilium, kidney development, renal disease, renal function, ciliopathy

Introduction

Cilium emanates from the mother centriole, and it is classified into motile cilia (9 + 2 structure) and nonmotile cilia (9 + 0 structure). Motile cilia have nine peripheral doublets of microtubules with a central pair complex (9 + 2), and primary cilia lack the centrally located pair (9 + 0) (1, 2). In vertebrates, cilia are widely distributed. Motile cilia are distributed on the surface of cells of the cerebral ventricle, respiratory mucosa, and reproductive system, and primary cilia are mainly distributed in embryos, kidneys and retina. They play fundamental roles in the asymmetric development of organs, mucus clearance of the respiratory tract, hearing, neurogenesis, and sperm motility (3–7).

The cilia life cycle is tightly related to the cell cycle (8–11) and consists of cilium assembly and cilia disassembly. The diverse roles of the primary cilium depend on the well-established balance between cilia assembly and disassembly (Figure 1). Cilia assembly is a precise and orderly multistep process. In the absence of mitogen or stimulation by differentiation signals, cells escape from the mitotic phase and enter the G0 phase. Then, cilia begin to assemble. First, within a few minutes of mitogen deprivation, vehicles originating from the Golgi or recycling endosomes, distal appendage vesicle (DAV), cluster at the distal appendage of the mother centriole (MC). This initiates the conversion from MC to the basal body, building a platform for cilia assembly. DAVs aggregate and fuse at the mother centriole to form the ciliary vesicle (CV) (12). CV formation marks the maturation of the basal body. The distal accessory structure protein Cep164 helps to maintain the integrity of this structure and anchors its fusion with the ciliary vehicle by binding to the GTP enzymes Rab8 and Rabin8 (13, 14). In the second step of cilia assembly, Cep164 recruits TTBK2 to the mother centriole. Proper localization of TTBK2 is required for the disappearance of the key repressor CP110 from the mother centriole, which thereby recruits the intraflagellar transport protein (IFT) complex. This complex is responsible for bidirectional protein cargo transport along axonal microtubules and thus promotes axoneme assembly (13, 15, 16). In this process, kinesin 2 carries the cargo from the tip to the base of the cilium, while dynein functions in cargo transport on the opposite side. After CV maturation, the transition zone (TZ) begins to assemble. It then embeds into and enlarges the CV. Subsequently, the ciliary axoneme is wrapped by CV and extends longitudinally. It is covered by the ciliary sheath, and finally fuses with the cell membrane. Ultimately, the mature ciliary composition includes axonemes composed of microtubules and associated proteins, the ciliary membrane connected to the cell membrane, and various matrices between the axonemes and the membrane (8).

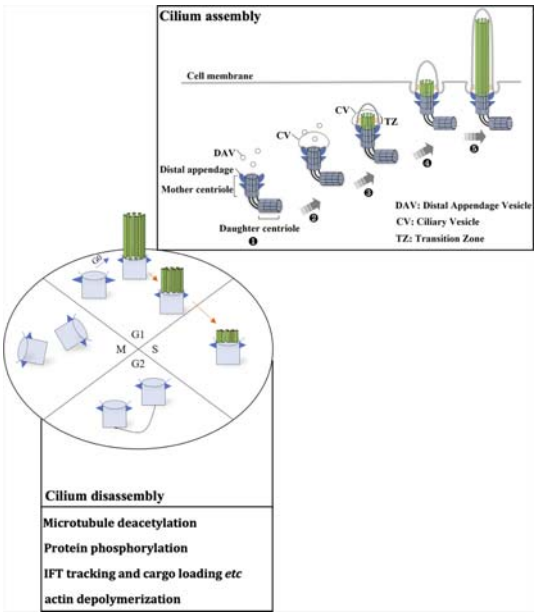

FIGURE 1
The ciliary life cycle is in tune with the cell cycle. Ciliogenesis occurs in the G0/early G1 phase or differentiation stage. Each stage of the cell cycle is as indicated (G1, S, G2 and M phase), and blue and orange arrows indicate cilium assembly and disassembly, respectively. The mother centriole (light blue cylinder) can initiate ciliogenesis (cilium assembly). (1) Distal appendage vehicles (DAVs, dark blue triangles) accumulate near the distal appendage of the mother centriole. (2) DAVs aggregate and fuse with the mother centriole, forming a ciliary vehicle. (3) Assembly of the transition zone (TZ). (4) The ciliary axoneme (indicated by parallel green rods) covered by CV elongates vertically and fuses with the cell membrane. (5) Extension of the ciliary axoneme and membrane. These microtubule structures disassemble as cells progress to S phase. Several key signaling pathways that mediate cilia disassembly are summarized in the box. During the later S phase, centrosomes begin to duplicate. After mitosis, centrosomes set out to assemble primary cilia.

Compared to cilium assembly, the signaling pathways for cilia disassembly are poorly understood (17). The Aurora A-HDAC6 and Nek2-Kif24 pathways and actin polymerization are the key signaling pathways that can induce cilia disassembly. It is widely accepted that Aurora A is the major pathway for the direct induction of ciliary microtubule deacetylation through the activation of HDAC6 (18). Nek2 ensures Kif24 activation in cells that lack cilia, and Nek2 is mainly expressed during the S and G2 phases (19). Imbalance between cilium assembly and disassembly leads to the loss of cell cycle regulation, and the loss of cilia may be an initiating factor of the oncogenesis of renal cancer, melanoma, and breast, pancreatic and prostate cancer (20–22).

Cilia in kidney development and function

Ciliary membranes are rich in receptors and ion channels that can be activated by mechanical or chemical stimuli (23). The proper spatiotemporal localization of receptors and the coordinated transportation of related signal modules that localize to the cilium lay the foundation for cilia sensory function (1, 24). The cilium is an important nexus for Hedgehog signaling, Wnt signaling, GPCR signaling and transforming growth factor-β (TGF-β)/bone morphogenetic protein (BMP) signaling (1, 25, 26). In nephrogenesis, Wnt signaling is of great importance (26). The Wnt signaling branches to β-catenin-dependent (canonical) and β-catenin-independent (noncanonical) pathway.

Wnt9b is expressed in the stalk of the ureteric bud (UB) as it invades and branches into the metanephric mesenchyme (MM) and acts as a paracrine signal to induce the expression of

tubulogenic pathway markers, such as fibroblast growth factor-8 (FGF8), Wnt4 and Pax8 (27).. Wnt9b is required for the planar cell polarity (PCP) signaling pathway (28). Wnt4 was detected in condensing mesenchyme, pre-tubular aggregates (Figure 2) (29). Inactivation of FGF8 block formation of Wnt4-expressing pre-tubular aggregates, which led to S-shaped bodies, the precursor of nephron cannot develop (30, 31). Wnt9b and Wnt4 primarily employ the canonical, β-catenin-dependent pathway. Studies have shown that β-catenin activation is necessary and sufficient to initiate the tubulogenic program and induce MM in *Wnt9b*$^{-/-}$ and *Wnt4*$^{-/-}$ mice. However, it is important to maintain a proper balance of canonical Wnt signaling activity, and constitutive β-catenin activation results in cyst formation in all tubular segments (32). *Ksp-cre* conditional inactivation of *APC*, which enhances β-catenin activity, results in cystic kidneys in all tubular segments and a hyperproliferative epithelium (33). Module component jouberin (JBN)-deficient mice show cystic kidneys and malformations of the central nervous system caused by dysregulated Wnt-β-catenin signaling (34).

Simon et al. suggested that Wnt functions primarily *via* β-catenin-dependent pathways in the absence of flow (35). Flow sensing by the primary cilium is thought to function as a switch from canonical pathway to noncanonical pathway (Figure 3). Studies have suggested that the primary cilium inhibit the activity of canonical Wnt signaling (perhaps promoting noncanonical signaling pathway) in mouse embryos, primary fibroblasts and embryonic stem cells (36).

Activation of GPCR signaling, functionally coupled to calcium channels, led to an increase in calcium concentration in the cilia, and cilium was lengthened by mediating actin depolymerization (1). TGF-β signaling induces the shortening of primary cilia in mouse renal tubular epithelial cells (RTECs) and is related to epithelial and mesenchymal transition (37).

In addition, shear stress stimulates lipophagy and mitochondrial biogenesis in RTECs to produce fatty acids that provide substrates for mitochondrial β-oxidation to generate ATP. This ensures an energy supply for the reabsorption of glucose in RTECs, and this process is dependent on the primary cilium (38). Unilateral ureteral obstruction (UUO) in mice reduced fluid flow, and the authors found defects in lipophagy. These defects resulted in lipid droplet accumulation in kidney cortical cells, intensifying the central role of the primary cilium in sensing mechanical stress to regulate mitochondrial activity and lipophagy (38). Miceli and Roccio et al. hypothesized that the primary cilium–autophagy axis plays a key role in the response to shear stress induced by fluid flow (39–41).

Mutations leading to ciliary structure and function defects give rise to multiple organ-involved disorders termed ciliopathies (42–44). These ciliopathies are accompanied by the following phenotypes: retinal degradation, hearing loss, malformation of the central nervous system, and polycystic kidney (45, 46). The significance of the renal cilium is reinforced by the fact that defects in this organelle lead to polycystic kidney disease, Meckel-Gruber syndrome (MKS), Bardet–Biedl syndrome (BBS), nephronophthisis (NPHP) and renal cell carcinoma (RCC) (5, 45–48) (Table 1).

Cystic kidney disease

Cystic kidney diseases are classified into two broad groups (49): (1) polycystic kidney disease (PKD), which includes

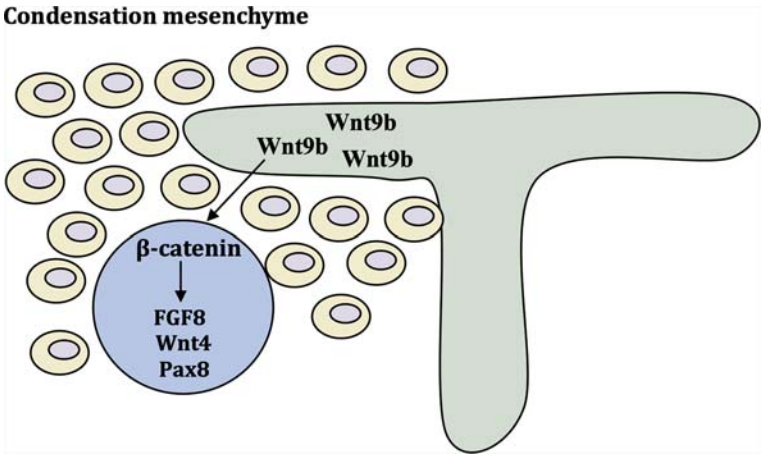

FIGURE 2
Molecular control of early kidney development. Wnt9b is expressed in ureteric bud and turns on differentiation markers FGF8, Wnt4 and Pax8 *via* β-catenin pathway. These cells form the pre-tubular aggregate highlighted by the expression of critical differentiation factors Pax8 FGF8 and Wnt4.

10.3389/fendo.2022.952055

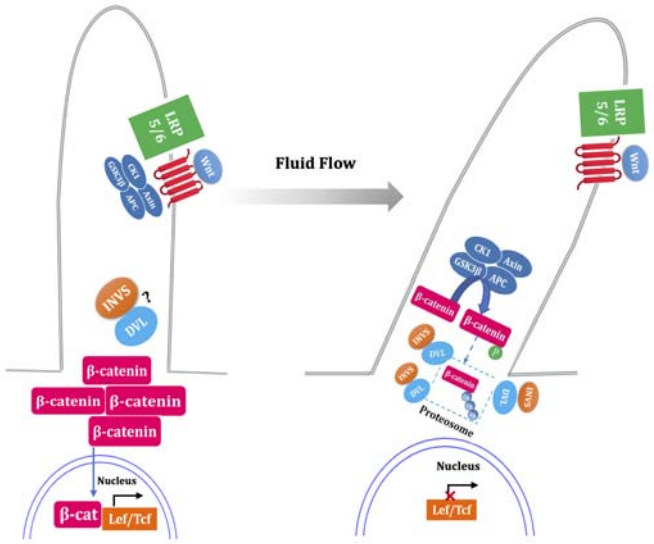

FIGURE 3
Fluid flow flip the switch from canonical to noncanonical Wnt signaling pathway. In the absence of flow, ligand binding with the Frizzled and LRP5/6 complex results in the inactivation of the β-catenin destruction complex (GSK3β, CK1, APC, Axin), led to stabilization of cytoplasmic β-catenin, which translocate to nucleus and coactivate the transcription level of LEF-TCF family. Fluid flow stimulated primary cilia, which is thought to increase the expression of inversin (INVS) and then reduce the cytoplasmic levels of dishevelled (DVL) by increasing its proteasomal degradation pathway. This process is deemed as switch off canonical signal pathway due to the activation of β-catenin destruction complex. The question mark indicate uncertainty as to the INVS-DVL complexes localization. The dotted box mark indicate that whether β-catenin is phosphorylated and ubiquitylated within the cilium is unknown.

autosomal dominant polycystic kidney disease (ADPKD) and autosomal recessive polycystic kidney disease (ARPKD), is characterized by large, polycystic kidneys; (2) the group of hereditary cystic diseases with interstitial nephritis that are characterized by small- to normal-size kidneys with tubular atrophy and interstitial fibrosis, including nephronophthisis (NPHP), Bardet-Biedl syndrome (BBS), and Meckel-Gruber syndrome (MKS). The cysts are lined by epithelial cells and filled with fluid and amorphous material.

Polycystic kidney disease

ADPKD is a genetic disease with a prevalence of 1:1000; it is caused by mutations in *PKD1* and *PKD2* (50). *PKD1* and *PKD2* encode polycystin-1 (PC1) and polycystin-2 (PC2), respectively. Colocalization of GPCR PC1 and ion channel PC2 mediates flow-sensitive mechanotransduction in primary cilia and responds to flow by increasing calcium influx (51). Loss of cilia causes PC1 and PC2 to fail to localize to cilia to perform

TABLE 1 Cilia phenotype in kidney disease.

Kidney disease		Cilia phenotype	Related gene
Acute Kidney Injury		Increase in cilium length at the early stage	—
Cystic Kidney Disease	Polycystic kidney disease(PKD): ADPKD and ARPKD	Absence of cilium in most cases	*Pkd1,Pkd2, PKHD1, Kif3a etc.*
	Cystic Diseases with Interstitial Nephritis: (NPHP, BBS, MKS, Alström syndrome *etc.*)		*NPHP- BBS- MKS-related gene*
Primary Glomerular Disease	IgA nephropathy	—	—
	Membranous Nephropathy	—	—
	Focal segmental Glomerulosclerosis (FSGS)	(1) *TTC21B*$^{-/-}$lead to cilia defect (2) Increase in cilium length compared to healthy control	*TTC21B*
Secondary Kidney Disease	Lupus Nephritis	Morphological alterations (from 9+2 to atypical 8+2 pattern)	—
	Diabetic Kidney Disease	—	—

—: not reported.

their functions. This results in excessive proliferation and enlargement of kidney epithelial cells, leading to polycystic kidneys. Inactivation of *Pkd1* and other ciliary proteins in adult animals can cause cystic disease. This suggests that cilia are required not only for proper kidney development but also for maintenance of normal function and morphology (52).

ARPKD occurs mainly in infants and young children, with a prevalence of 1:20,000 (53). It is caused by mutations in *PKHD1* and is characterized by cystic dilatations predominantly of the collecting duct. *PKHD1* encodes polyductin/fibrocystin (PD).

Cystic diseases with interstitial nephritis

Nephronophthisis (NPHP) is an autosomal recessive disease that accounts for 10%-20% of cases of renal failure in children. It is characterized by cystic kidney tubules and interstitial fibrosis with inflammatory infiltrate. NPHP-related genes (*NPHP1, NPHP2/inversin, NPHP3, NPHP4, NPHP5, NPHP6/CEP290, NPHP7/GLS2, NPHP8/RPGRIP1L, NPHP9/NEK8*) have been implicated in NPHP. Unidentified mutated genes still need to be explored in 70% of cases (54).

BBS is a rare autosomal recessive syndrome characterized by postaxial polydactyly, retinitis pigmentosa, intellectual disability, obesity, hypogonadism in men, and a variety of renal abnormalities that include cysts, calyceal clubbing and blunting, tubulointerstitial nephropathy, and dysplastic kidneys. More than 12 genes have been implicated in BBS.

Alström syndrome is a rare autosomal recessive disease caused by mutations in *ALMS1*. It is mainly characterized by retinitis pigmentosa, hearing loss, insulin resistance, and obesity in children. In adult patients, the presentation of hyalinization of tubules and interstitial fibrosis in kidneys are observed. The ALMS1 protein is located at the base of cilia and centrosomes.

Primary cilium and cystic kidney disease

The first functional evidence linking primary cilium to cystic disease was derived from *Caenorhabditis elegans* IFT88 and its mouse homologue, polycystic kidney disease gene tg737 mutant mice. *Ift88*$^{Orpk/Orpk}$ (Tg737) mutant mice have shorter and blunted primary cilia in collecting ducts (55). Genetic repairment of *Ift88*$^{-/-}$ cells may possibly restore ciliary length and normalize ciliary function. The *Kif3A* conditional knockout mouse model also suggests that cystic disease can result from disrupting ciliary function and increasing canonical Wnt-β-catenin activity (36, 56). Evidence linking ciliary function to kidney cyst formation and PCP signaling was demonstrated using *Ift20 Hoxb7Cre* conditional mice (57). Wnt signaling activation and a misoriented axis of cell division account for cyst formation. *Ksp-Cre; Wnt9b* mutant mice have few cysts at P1 but many at P10. This is mainly because of a misoriented and random mitotic axis along the tubule in mutants compared to controls, suggesting that the *Wnt9b* mutant links noncanonical Wnt signaling to cyst formation (28). In summary, these findings support that defective PCP signaling plays a key role in cyst formation during kidney development. However, how these processes deregulate cellular orientation to prompt cystogenesis remains elusive.

An overwhelming abundance of data linking to cilia to cystic kidney disease but the causal relationships between them still need to be defined. A possible explanation for the primary cilia anomalies that cause cyst formation is that ciliogenesis is a multistep process. In this process, the NPHP complex, BBSome and over 20 cystoproteins are localized to the centrosome or the base body of cilium (58, 59), and any gene mutation that causes a loss/gain of function leads to defective ciliogenesis. It disables the function of the canonical Wnt-β-catenin or noncanonical Wnt-PCP signaling pathway, or certain proteins fail to localize to the cilium to induce downstream signaling pathways. Such disruptions lead to the overproliferation of epithelial cells and cystogenesis. Recently Hansen et al. elucidated the contribution of ciliary-derived cAMP signalosome to renal cystogenesis, ciliary cAMP signaling activates mTOR signaling and drives cell proliferation, countering the level of cAMP inhibits cyst formation (60). The study unravels a new molecular mechanism promoting PKD and provides new therapeutic targets to the treatment of PKD.

Acute kidney injury

Acute kidney injury (AKI) is a clinical syndrome of rapid decline in kidney function over a short period of time (a few hours or days), resulting in the retention of metabolic waste products, urea and creatinine (61, 62). Acute tubular necrosis (ATN) represents only one of multiple causes of AKI; it results largely from ischemia–reperfusion injury (IRI) (62). Deficiency of cilia promotes TGF-β-induced EMT and exacerbates the signaling under its pro-fibrotic signals (37), so restoring cilium length or occurrence may be a promising therapeutic target to anti-fibrosis in IRI. Cilia are critical for epithelial repair in renal IRI, indicating a relationship between the change in cilium length and sensitivity in the altered environment of the injured kidney (63, 64).

Elizabeth Verghese et al. identified that acute tubular necrosis causes an increase in the length of renal cilia, modifying their sensory sensitivity during repair (63). Biopsies from human renal transplants suffering ATN showed a dramatic increase in cilium length at 7 days post transplantation and a trend toward the normalization of cilium length at the later stage. A mouse model of ischemia–reperfusion injury (IRI) showed a similar trend. There was an increase in renal cilium length 1 week post-IRI and a return to normalization at 6 weeks. In addition, Jee In Kim et al. reported that the average length of the cilium in the proximal tubule initially shortened after IRI,

and the length of cilia increased at 4 and 7 days, facilitating the initiation of the repair mechanism (64). Thus, we summarized the role of the renal cilium in response to injury and repair of damaged tubular epithelial cell reconstruction.

Primary glomerular disease

Focal segmental glomerulosclerosis (FSGS) is a pathologic diagnostic term that is mainly characterized by sclerosis of part of the glomerular (focal) or part of the glomerular capillary loops (segmental) (65). Evelyne Huynh Cong et al. reported that a homozygous missense mutation in the ciliary gene *TTC21B* causes familial primary FSGS, and knockdown of the *TTC21B* gene product IFT139 in podocytes leads to primary cilia defects and abnormal cell migration (66). Nevertheless, mutations of the ciliary gene TTC21B that lead to primary FSGS indicate a novel cilia function in primary glomerulus disease.

Ivana Solic et al. recently summarized the length of the primary cilium between healthy control and pathologically-changed kidney tissues, including FSGS, CSF and MCDK. In MCDK, CNF and FSGS, cilia were significantly elongated compared to healthy controls (67).

There are still many cilia-related phenotypes and functions that need to be explored in the near future in IgA nephropathy and membranous nephropathy.

Secondary kidney disease

Lupus nephritis

Lupus nephritis represents the most severe clinical manifestation of systemic lupus erythematosus (SLE) and leads to a high percentage of morbidity and mortality in patients. The etiology of SLE is characterized by interactions between genetic susceptibility, immune system abnormalities and hormone regulation disorders that result in tolerance disorders and sustained autoantibody production (68). Numerous 9 + 2 cilia and atypical 8 + 2 pattern cilia were found in the kidney biopsy from a patient with lupus nephritis, suggesting that the frequent occurrence of cilia in the lupus kidney results from metabolic and chemical derangements (69).

Diabetic kidney disease

Diabetic kidney disease (DKD) is one of the most serious complications of diabetes and has become the most common cause of ESRD worldwide (70). Studies have suggested that the pathogenesis of DKD includes an early increase in glomerular ultrafiltrate velocity leading to an increase in mechanical force in the renal tubule lumen, damage to and reduction in podocytes, thickening of the glomerular basement membrane, and expansion of the mesangial membrane (71, 72). Glomerular hyperfiltration is the initial factor contributing to kidney disease in diabetes (71). The high glucose environment also stimulates the activation of the following metabolic pathways (73): (1) the pentose phosphate pathway; (2) the polyol pathway; (3) the hexosamine pathway; (4) the protein kinase C (PKC) pathway; and (5) the advanced glycation end (AGE) pathway. Subsequently, ROS, diglyceride, pyruvaldehyde and lactic acid accumulate and cause cell damage (74). These changes in energy supply and metabolites are collectively termed metabolic reprogramming. A recent study showed that shear stress is transmitted into the cell *via* "antennas" on the surface of RTECs and primary cilia, which direct the metabolic reprogramming of cells to adapt to the environment (38). However, the function of primary cilia in diabetic kidney disease is still unknown.

The primary cilium of RTECs is an important mechanical force sensor for the shear stress, regulates the energy metabolism homeostasis in RTECs to ensure the energy supply for reabsorption function (38). Diabetic kidney disease is characterized by an increase in luminal shear stress induced by a high ultrafiltrate flow rate originating from the glomerulus. we hypothesized that elongated cilium were observed in the RTECs from DKD. It has been shown that glomerular expression of Sirtuin-1 (SIRT1), an NAD+-dependent protein/histone deacetylase, is reduced in human diabetic glomeruli (75), the expression and acetylation of HDAC6 is regulated by Sirt1 (76), so expression of HDAC6 exhibit down-regulated and activity of tubulin deacetylation was inhibited, so we speculated that glomerular hyperfiltration induced the key cilia disassembly regulator HDAC6 down-regulation, promoting cilium elongation and accelerates the progression of diabetic kidney disease. Lipid nanoparticles targeting renal cilium to remote control of cilia movement will be a possible therapeutic target to the diabetic kidney disease.

Renal cell carcinoma

In patients with RCC with mutations in the VHL (von Hippel–Lindau disease tumor suppressor) gene, primary cilia have been lost, and the re-expression of VHL proteins restored cilia occurrence (77). Ciliogenesis is inhibited in many types of cancer, including renal cell carcinoma (78), prostate cancer (79), pancreatic cancer (80), breast cancer and ovarian cancer (81, 82). Primary cilia are essential for Hedgehog signaling activation during development (1). Abnormal activation of Hedgehog can lead to a variety of tumorigenesis (83–85). The importance of cilium loss in tumorigenesis, maintenance, and progression, as well as chemotherapeutic resistance emerged, which suggesting that restoration of primary cilia in tumor cells may be a potential therapeutic approach.

Ciliary-targeted therapy technology

Since proper cilium assembly and disassembly are required for embryogenesis and organ function, agents regulating cilia-associated proteins to control cilium length and number may become promising treatments for ciliopathy. The need to develop specific cilia-targeted treatments is urgent. Rajasekharreddy Pala. et al. designed an iron oxide nanoparticle-based and cilia-targeted delivery system to deliver agents specifically to the primary cilia, The hearts of ciliopathic displayed hypertrophy with declined functions in left ventricle because of prolonged hypertension. Magnetic field or fluid flow control cilia and then lead to the increase of Intraciliary and cytosolic Ca^{2+}. The CT-Fe_2O_3-NPs significantly improved cardiac function in the ciliopathic hypertensive models (86).. Techniques for tissue-specific mRNA delivery and CRISPR-Cas gene editing nanoparticles have been developed (87), so we can specifically rescue the compromised cilia phenotype in ciliopathy by tissue-specific gene editing.

Histone deacetylase 6 (HDAC6), a cytoplasmic enzyme, is the major driver of cilium disassembly, and small molecules that inhibit HDAC6 have been demonstrated to restore the ciliary defective phenotype (88–90). Anti-proliferating agents could also be candidates for polycystic kidney disease due to defective cilia-induced cell overproliferation. In addition, tissue-specific mRNA delivery and the CRISPR-Cas gene editing system could be applied to edit cilia-related genes and may be possible therapeutic targets for ciliopathies.

Conclusions

In summary, the primary cilium is the center platform that regulates diverse developmental signaling pathways, and its function relies on the control of the precise dynamic balance between cilia assembly and disassembly. More details of these signaling pathways and their involvement in kidney disease remain to be explored. How cilia sense mechanical stimuli is still an ongoing research topic. Ciliary-targeted technology urgently needs to be developed. Insights into ciliary defects in kidney disease will help us identify therapeutic targets for kidney injury relief and provide novel insights into disease mechanisms and ciliopathy intervention.

Author contributions

YB conceptualized and wrote the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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