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Bioinformatics on Screening and Pathway Analysis of Early Diabetic Nephropathy Related Genes

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Abstract. Diabetic nephropathy (DN), a severe complication arising from diabetes, necessitates timely diagnosis and therapeutic intervention to mitigate the disease's progression. The objective of this article is to identify pivotal genes linked to the early stages of DN through the application of bioinformatics techniques. Furthermore, this this article endeavors to elucidate the signaling pathways associated with these genes, thereby offering novel therapeutic targets for the preemptive identification and management of DN. Methods: this article obtained the relevant gene chip data from the public dataset Gene Expression Omnibus (GEO) database, and obtained the information derived from the analysis of the complete set of genes being expressed of kidney tissue of DN patients and healthy controls. The R programming language, in conjunction with the Bioconductor package, was employed to perform an analysis of gene expression variations, with the aim of identifying obvious differential expressed genes (DEGs). Functional annotation and enrichment analysis: Functional categorization and enrichment of DEGs were conducted utilizing the Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) resources, offering a deeper understanding of the molecular functions and pathways related to these genes. Protein-protein interaction (PPI) network construction: PPI networks of DEGs were constructed adopting search tool for the retrieval of interacting genes (STRING) database, and network visualization and key gene screening were performed by Cytoscape software. Other independent gene expression datasets and literature reports were used to verify the selected key genes. The results suggested that GSE176230 dataset was retrieved and downloaded from CEO database. After screening, there were 1,715 DEGs in DN and control samples, of which 1,026 were up-regulated and 689 were down-regulated. DEGs markedly associated with early DN (PBMCs, CXCL8, MMP9, 1L1B, C-C chemokine receptor type 2 (CCR2), TLR10, CX3CR1, P2RY14, APAF1, GREM1, FAM30A, PRKY, TMSB4Y, GDF3) were screened out. GO analysis indicated that a visible enrichment of these genes was observed in key biological processes, including inflammatory response, oxidative stress, apoptosis. KEGG pathway analysis suggested that the main signaling pathways involved included TGF-β, MAPK, and PI3K-Akt. PPI network analysis identified multiple key genes (CXCL8, MMP9, and CCR2). The mRNA levels of CXCL8, MMP9, and CCR2 in patients with early DN were markedly higher as against normal healthy people ($P < 0.05$). Utilizing bioinformatics techniques, this article effectively identified pivotal genes and associated signaling pathways that are linked to the early stages of DN. The insights gained from this article offer novel therapeutic targets and a foundational framework for the preemptive identification and management of DN. Upcoming research endeavors are poised to confirm the particular roles and mechanisms of these genetic elements and molecular pathwaysy.

Keywords: DN; gene screening; pathogenesis; bioinformatics; signaling pathways; DEGs.

1. Introduction

DN, a prevalent and severe microvascular complication of diabetes [1], predominantly leads to end-stage renal disease (ESRD). With the rising prevalence of diabetes worldwide, the incidence of DN is also increasing year by year. Data from the WHO indicates that the global diabetic population currently stands at approximately 460 million individuals, with projections forecasting a rise to 640 million by the year 2030 [2]. Commonly, diabetes is characterized by the presence of insulin resistance, recognized as a pivotal element in the disease's etiology [3]. DN poses a serious threat to patients' quality of life and public health system. Early diagnosis and intervention serve as a critical

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component in delaying the progression of DN, but the current understanding of its molecular mechanism is still incomplete, and there is a lack of effective early diagnostic markers and therapeutic targets.

In recent years, the development of genomics and bioinformatics technology has provided new tools to reveal the molecular mechanisms of complex diseases [4,5]. Through in-depth analysis of gene expression profiles, disease-related DEGs can be screened out, and their role in disease occurrence and development can be revealed through functional annotation and pathway enrichment analysis [6]. The pathogenesis of DN involves the interaction of metabolic and hemodynamic factors, such as hyperglycemia, renin-angiotensin system, and advanced glycation end products. Inflammation plays an important role in the early pathogenesis of DN. Hyperglycemia and AGEs activate inflammatory signaling pathways such as nuclear factor-kappa B (NF-κB) and induce the expression of a variety of inflammatory factors, such as tumor necrosis factor-α (TNF-α), interleukins (IL-1β, IL-6), monocyte chemoattractant protein-1 (MCP-1) [7,8]. These inflammatory factors aggravate renal inflammation and injury by pro-inflammatory response and attracting immune cell infiltration. The pathogenesis of early DN is the result of the joint action of multiple factors and pathways. Hyperglycemia, inflammation, oxidative stress, extracellular matrix (ECM) accumulation, and glomerular hyperfiltration interact with each other, which together lead to kidney injury [9]. Previous studies have shown that biological processes such as inflammatory response, oxidative stress, and apoptosis act in the occurrence of DN, but most of these studies focus on the middle and late stages of the disease, and the molecular mechanism of early DN is relatively less studied [10,11].

This article aims to use bioinformatics methods to systematically screen the key genes associated with early DN, analyze the signaling pathways involved, and uncover novel potential targets and a theoretical framework that facilitate the early detection and therapeutic intervention of DN. By conducting a comprehensive analysis of gene expression and pathways, this article has identified new biomarkers and therapeutic avenues, laying a solid foundation for preemptive clinical diagnosis and treatment strategies.

2. Materials and methods

2.1 Gene chip data sources

NCBI website: https://www.ncbi.nlm.nih.gov/ was visited to enter the GEO database: https://www.ncbi.nlm.nih.gov/geo/, entering keywords (diabetic nephropathy). The acquisition dataset GSE176230 platform GPL24676 has 34 samples. The acquisition dataset GSE262414 platform GPL18573 has 33 samples. In the data set, 11 subjects as controls and 9 subjects in the DN group were selected.

2.2 DEGs screening

The GEO2R was used to analyze DEGs in the dataset. The GEO2R, an analytical tool within the GEO2 online repository, facilitates the identification of differentially expressed genes (DEGs) across multiple samples and ranks genes based on their significance. In this article, GEO2R was employed to analyze the expression differences of the subjects. Genes were considered to exhibit obvious differential expression if they met the criteria of a P-value less than 0.05 and |log FC| at least 1.5, indicating a substantial difference in expression levels of the subjects.

2.3 Enrichment analysis of DEGs

The Fisher exact probability method was used to calculate the P value, and the P value was sorted from small to large under the condition of $P < 0.05$. The R language “ClusterProfiler” data package was adopted to carry out GO enrichment analysis and KEGG pathway analysis on the screened DN DEGs.

2.4 PPI network analysis

STRING was used (https://string-db.org/). The database predicts the interaction between proteins, and the likelihood of PPI can be assessed through the outcome of the PPI score. The DEGs coding protein was input into the STRING database, and the screening threshold was set as: binding score >0.4. PPI network diagram was constructed using Cytoscape-v3.6.1 to screen the core targets in the PPI network, and the score predicted by the degree algorithm was indicated by the color depth.

3. Results

3.1 DEGs screening

The GSE176230 dataset was retrieved and downloaded from the CEO database, and analyzed by R software. After screening, there were 1,715 DEGs in DN and control samples. Up-regulation was observed in 1,026 genes, while 689 genes exhibited down-regulation (Figure 1).

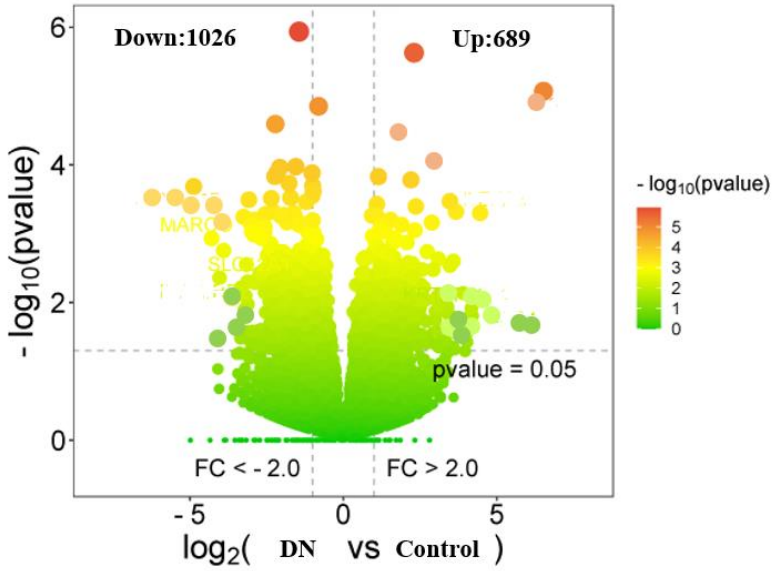

Figure. 1 Volcano plot of DEGs.

3.2 Heat map

14 DEGs in the DN group were revealed using the cluster plot and the heat map. PBMCs CXCL8, MMP9, 1L1B, CCR2, TLR10, CX3CR1, P2RY14, APAF1, GREM1, FAM30A, PRKY, TMSB4Y, GDF3 (Figure 2).

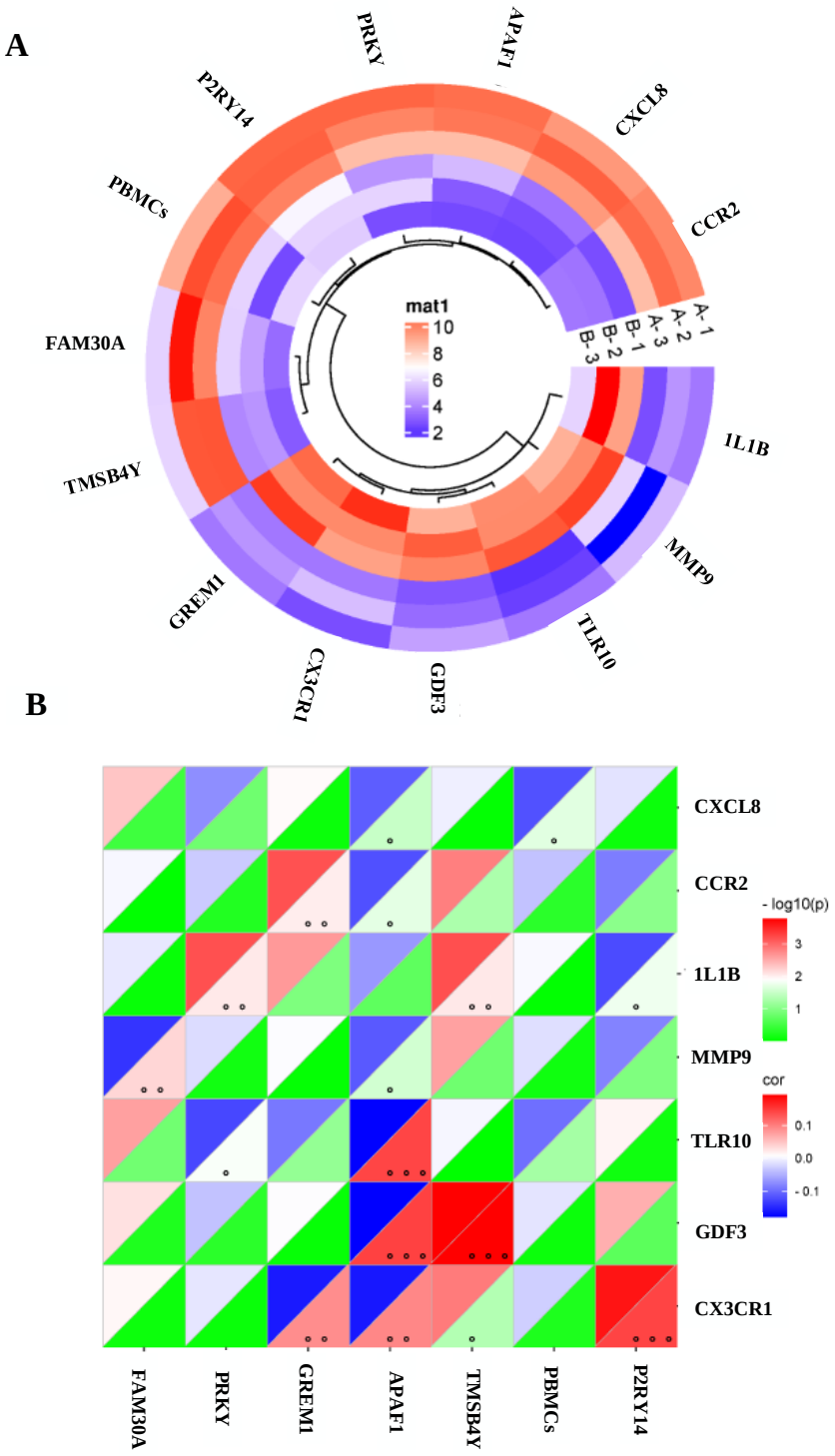

Figure. 2 Cluster plot and the heat map of DEGs.

(A: circular cluster plot; B: triangular heat map of correlation coefficient)

3.3 KEGG enrichment pathway analysis

The functional enrichment of 1,715 DEGs is presented in Figure 3. The functional enrichment pathways include: inflammatory response, cytokines, chemokines, smooth muscle cell proliferation, chemokine signaling pathway, JAK-STAT pathway, NF-κB, macrophage migration regulation, cell adhesion molecule pathway, ECM receptor interaction. Inflammation-related pathways could be implicated in the onset and progression of early DN.

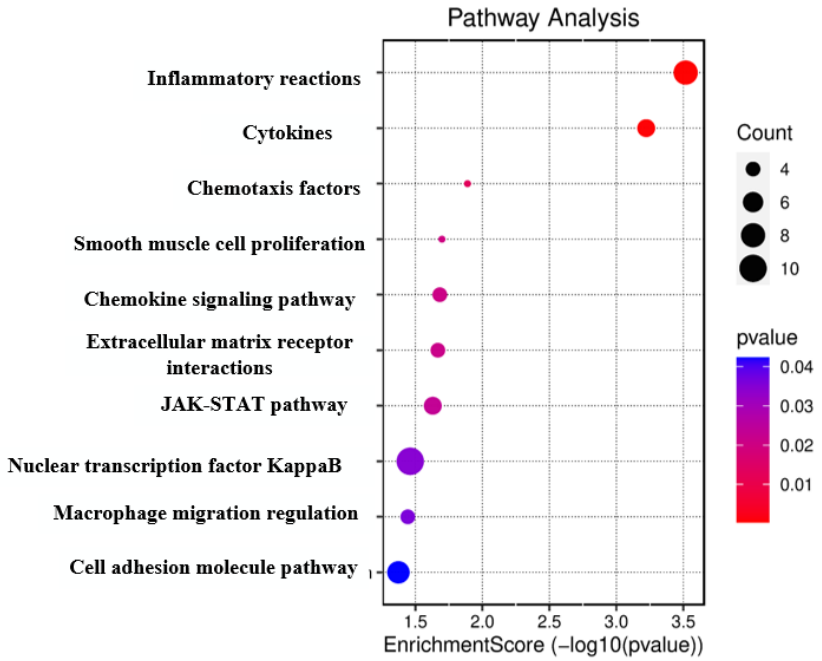

Figure. 3 KEGG enrichment pathway analysis.

3.4 Network diagram of DN DEGs

Figure 4A illustrates the PPI diagram. TLR10, CXCL8, CX3CR1, CCR2, and MMP9 gene proteins were closely connected. Figure 4B illustrates the PPI network of DN DEGs. The darker the color, the higher the node degree value of the network, and it was in the core position in PPT. CXCL8, CCR2, MMP9 genes had high connectivity in the network, and these factors were believed to exert a substantial influence on the initial presentation and subsequent course of DN.

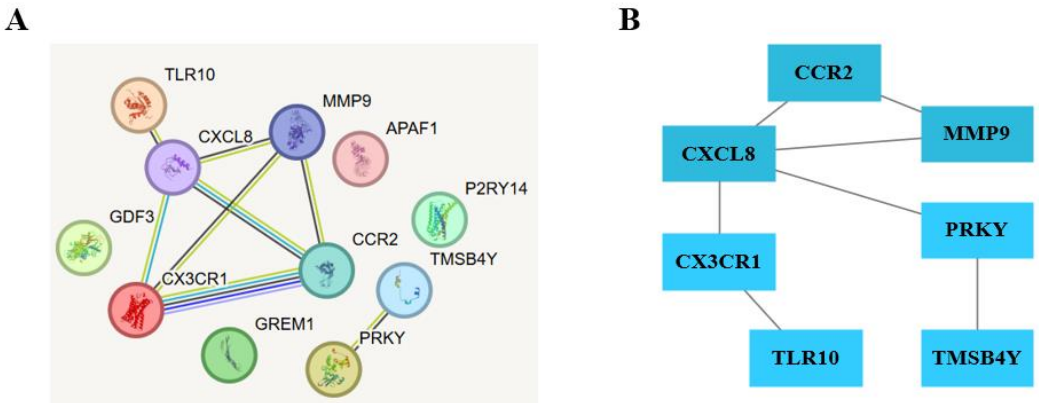

Figure. 4 Network diagram of DN DEGs.

3.5 mRNA level comparison

CXCL8 and MMP9 genes were up-regulated genes in DN, while CCR2 was down-regulated gene in DN. The mRNA levels of CXCL8, MMP9, and CCR2 in the NC group were markedly higher as against the controls ($P < 0.05$) (Figure 5).

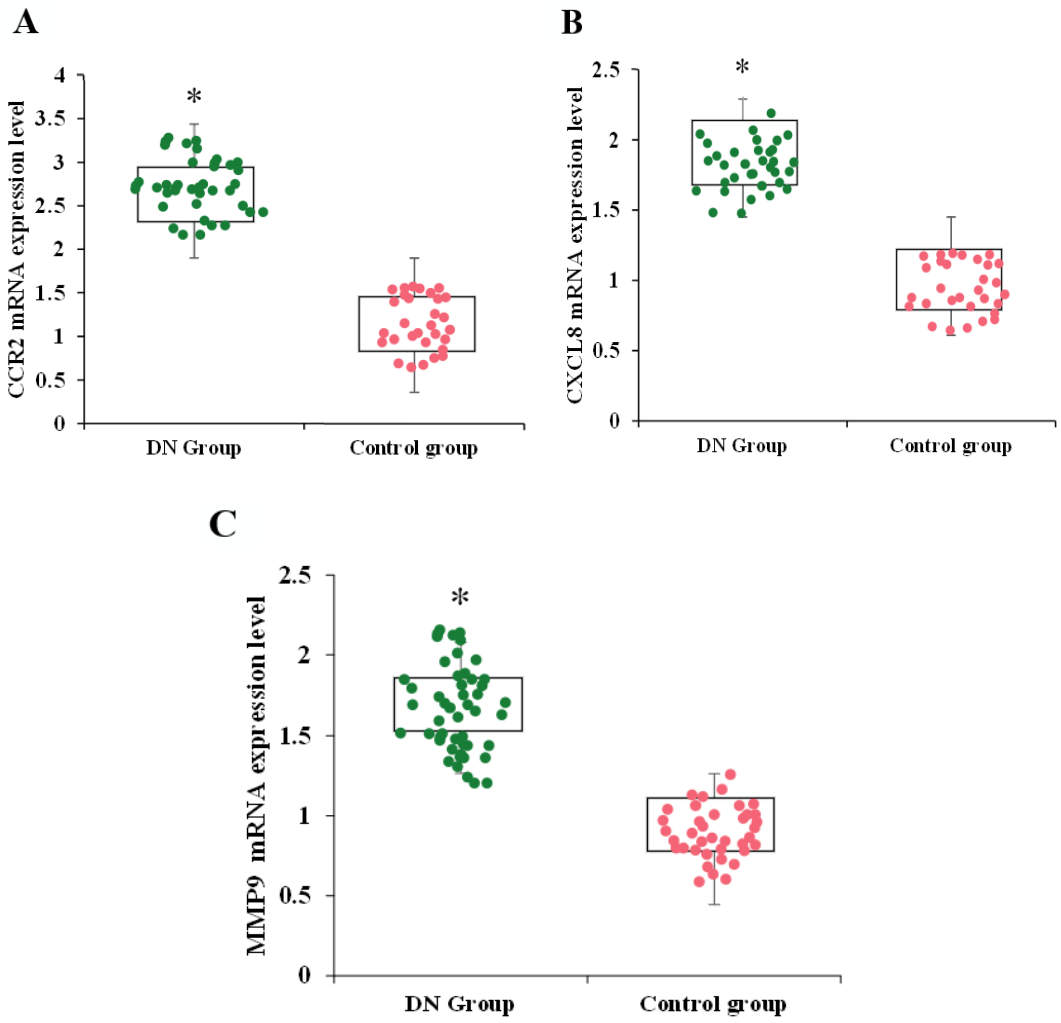

Figure. 5 mRNA level comparison.

(Note: A: CCR2; B: CXCL8; C: MMP9, *P < 0.05)

4. Discussion

DN is not only an independent risk factor for the life and survival time of diabetic patients, but also a major cause of kidney disease. Studies have shown that the pathogenesis of DN is multifactorial and complex. Exploring the signaling pathways and molecules related to the pathogenesis of DN contributes substantially to the initial strategies aimed at averting and decelerating the onset of diabetes [12,13]. In this article, through bioinformatics analysis, we screened out some key genes associated with early DN, and focused on the mRNA expression levels of CXCL8, MMP9, and CCR2. The results suggested that the mRNA levels of these genes in patients with early DN were markedly higher as against normal healthy controls. These genes play important roles in inflammation and immune responses, and their elevation indicates that these processes have been activated in the early stages of DN. These DEGs are enriched in biological processes such as inflammatory response, oxidative stress, apoptosis. Through PPI network analysis, several core genes (CXCL8, MMP9, CCR2) with high connectivity in the network were identified. The reliability of these key genes was further verified by using independent data sets and literature support.

XCL8, also known as IL-8, this cytokine acts as a critical mediator in the inflammatory response. Its main functions include attracting and activating neutrophils [14]. Studies have shown that the level of CXCL8 is increased in DN patients, which is associated with the exacerbation of renal inflammation and injury [15]. High levels of CXCL8 may promote the infiltration of inflammatory cells into the kidney, resulting in enhanced local inflammatory response, thereby exacerbating kidney

injury. Matrix metallopeptidase 9 (MMP9) is a matrix metalloproteinase involved in ECM degradation and remodeling [16,17]. In DN, the elevated activity of MMP9 is closely related to the imbalance of matrix metabolism and glomerulosclerosis. Under hyperglycemic environment, overexpression of MMP9 may lead to excessive degradation of basement membrane and ECM, damage glomerular structure, and then trigger proteinuria and decline of renal function [18]. CCR2 is a chemokine receptor, and its main ligand is monocyte chemoattractant protein-1 (MCP-1). CCR2 plays a major role in inflammation and immune response, and is involved in the recruitment of monocytes and macrophages [19,20]. Du et al. (2021) [21] pointed out that in DN, in the context of macrophage response, Loganin has been shown to be effective in curtailing infiltration and inflammatory activation via the MCP-1/CCR2 axis. He et al. (2023) [22] put forward that CCR2 is an important chemokine in renal fibrosis. This article found that the expression of CCR2 was markedly elevated in DN patients, which may lead to more monocytes and macrophages infiltrating the kidney tissue, enhancing the local inflammatory response and fibrosis process. The DEGs and core genes screened provide new potential markers for the diagnosis of early DN. These genes were markedly upregulated in patients with early DN. By regulating TGF-β, MAPK, and PI3K-Akt signaling pathways, new therapeutic strategies can be developed to slow down or prevent the progression of DN. This article deepened the understanding of the molecular mechanism of early DN through functional annotation and pathway analysis. The important role of inflammation, oxidative stress, and recent studies have provided additional evidence supporting the involvement of ECM accumulation in both the onset and advancement of DN.

The elevation of CXCL8, MMP9, and CCR2 indicated the importance of inflammatory response in early DN. Liu et al. (2018) [23] indicated that CXCL8 level could be used as a marker for early diagnosis of DN. Tang et al. (2021) [24] emphasized the paramount importance of signaling pathways in the intricate regulation of metabolic and protective processes within the context of DN pathogenesis. The upregulation of CXCL8, MMP9, and CCR2 may lead to the aggregation and activation of inflammatory cells and exacerbate kidney injury. The mRNA level changes of these genes can be used as potential diagnostic markers of early DN. By inhibiting the expression or function of these genes, it is expected to slow down the inflammatory response and kidney injury. Li et al. (2024) [25], Moser and Loetscher (2021) [26] have demonstrated the importance of inflammation and oxidative stress in DN, and this article further confirmed the specific key genes and signaling pathways through systematic bioinformatics analysis. In addition, this article used a variety of databases and analysis methods to enhance the reliability and universality of the results. Although this article revealed the important roles of CXCL8, MMP9, and CCR2 in early DN, the results were mainly based on bioinformatics analysis and lacked experimental validation. Future studies should further verify the specific roles and mechanisms of these genes in DN through cell experiments and animal models.

5. Conclusion

This article systematically screened the key genes associated with early DN by the bioinformatics method, and revealed the signaling pathways involved. This article found that the mRNA levels of CXCL8, MMP9, and CCR2 in patients with early DN were markedly higher as against normal healthy people. The involvement of these genes in the development of DN suggests their utility as diagnostic indicators and therapeutic objectives. However, the results need to be confirmed and applied through further experimental validation and extended research. Future studies should persist in uncovering the detailed mechanisms of these genes and pathways in the genesis of DN, to drive medical implementations and augment the health of patients.

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