



Identification of the molecular mechanism and candidate markers for diabetic nephropathy

Chun Chen[#], Liping Liu[#], Jia Luo

Department of Cardiology and Endocrinology, The Guangxi Zhuang Autonomous Region Workers' Hospital, Nanning, China

Contributions: (I) Conception and design: C Chen; (II) Administrative support: C Chen; (III) Provision of study materials or patients: L Liu; (IV) Collection and assembly of data: L Liu; (V) Data analysis and interpretation: J Luo; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work and should be considered as co-first authors.

Correspondence to: Jia Luo. Department of Cardiology and Endocrinology, The Guangxi Zhuang Autonomous Region Workers' Hospital, Nanning 530021, China. Email: jialuo@gxzarwh.org.cn.

Background: Diabetic nephropathy (DN) is one of the most common complications in diabetic patients. New strategies are needed to delay the occurrence and development of this pathology.

Methods: Differentially expressed genes (DEGs) in glomeruli and renal tubules were identified using the GSE30122 dataset, and a co-expression network was constructed to identify the hub genes of modules. The biological function and signaling pathway of the module genes were also analyzed. In addition, the expression of 24 immune cells and the area under the receiver operating characteristic (ROC) curve (AUC) values of the hub genes were also calculated.

Results: A total of 1,778 DEGs were isolated from glomeruli and 1,996 DEGs were isolated from renal tubules. Nine modules and their hub genes were identified using the co-expression network. Enrichment analysis showed that the module genes were mainly enriched in immune inflammation and oxidative stress. The expressions of B cells, activated dendritic cell, and T cells in the glomeruli and renal tubules of DN patients were higher than those in the controls, and the correlation between these immune cells was the strongest. Collagen type I alpha 2 chain (COL1A2), the hub gene of the brown module, had the highest AUC values and may have a better clinical diagnostic ability.

Conclusions: In conclusion, the module genes and related biological functions and signaling pathways found in this study can deepen our understanding of the molecular mechanism of DN progression. COL1A2 may be a potential biomarker for DN.

Keywords: Diabetic nephropathy (DN); immunity; oxidative stress; collagen type I alpha 2 chain (COL1A2); differentially expressed genes (DEGs)

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Introduction

Diabetes mellitus (DM) is a systemic disease characterized by the inability of the body to produce or effectively respond to the glucose regulatory hormone, insulin (1). The resulting hyperglycemia can disrupt hemodynamics and metabolize homeostasis enzymes, leading to serious complications (2). Diabetic nephropathy (DN), a kind of microvascular complication, is the most common cause of

end-stage renal disease (3). It is characterized by continuous proteinuria, a gradual decrease of the glomerular filtration rate, and increased blood pressure (4), and its diagnosis has always been based on the presence of microalbuminuria (MA) (5). However, MA does not adequately predict DN, especially in young patients or non-albuminuria patients. Therefore, new biomarkers are needed to identify individuals who will develop this life-threatening disease.

Oxidative stress and inflammation caused by chronic elevation of blood glucose in diabetic patients have increasingly been considered risk factors for DN (6). Cell and molecular experiments have shown that mitochondrial dysfunction, endoplasmic reticulum stress, and abnormal activation of the intracellular signaling pathway are closely related to DN (7). Without adequate oxygen supply, hyperglycemia may impair the stability of the hypoxia-inducible factor (HIF) and promote renal fibrosis (8). In both animal models and DN patients, increased infiltration of circulating inflammatory mediators and immune cells into renal tissue has been observed (9). Inflammation also plays an important role in the pathogenesis of DN, such as through cell adhesion molecules, growth factors, chemokines, and proinflammatory cytokines (10,11). A low dose of interleukin-17A (IL-17A) can prevent DN in type 1 and type 2 diabetic models (12). Although several effective treatments can delay the occurrence and development of DN, its incidence rate remains high.

DN is characterized by glomerular and tubulointerstitial damage (13). This study aimed to investigate the molecular mechanism of DN by bioinformatics analysis of the transcriptome data of renal tubules and glomeruli in DN. Identifying new predictive biomarkers will provide opportunities for prevention and/or treatment interventions. We present the following article in accordance with the STREGA reporting checklist (available at <https://atm.amegroups.com/article/view/10.21037/atm-22-5128/rc>).

Methods

Data collection and difference analysis

The gene expression profiles of glomeruli and renal tubules

samples from DN donors and normal controls in the GSE30122 dataset were obtained from the Gene Expression Omnibus (GEO) database (<https://www.ncbi.nlm.nih.gov/geo>). This dataset included nine glomerular DN samples, 26 glomerular normal samples, 10 tubular DN samples, and 24 tubular normal samples. The demographic characteristics of the donors were shown as in Woroniecka *et al.* (14). There were obvious differences in the clinical indexes between DN patients and controls. The Affymetrix human genome U133A 2.0 array (GPL571) platform was used. The limma package was used to obtain the differentially expressed genes (DEGs) between DN and controls, and the conditions of $|\log_2 \text{fold change (FC)}| \geq 0.5$, $P < 0.05$ were used to screen the DEGs.

Co-expression analysis

The co-expression of the two groups of DEGs was analyzed through weighted gene co-expression network analysis (WGCNA). The co-expression similarity matrix of the genes was calculated, and the soft threshold was used to maintain the correlation between genes. The topological overlap difference was calculated from the adjacency matrix, and the hierarchical clustering method was used to define gene clusters, with each cluster representing a module. Using Pearson correlation, the first mock exam was associated with the characteristics of each module. The highly correlated genes in each module were classified as hub genes. The receiver operating characteristic (ROC) curve was drawn using the pROC package (15), and the area under the ROC curve (AUC) value of the hub genes was calculated.

Enrichment and Gene Set Enrichment Analysis (GSEA)

The ClusterProfiler package was used to analyze the Gene Ontology (GO) function and the Kyoto Encyclopedia of Genes and Genomes (KEGG) signaling pathway. A threshold value $P < 0.05$ was set. KEGG pathways involved in the expression of genes in glomeruli and renal tubules were determined using GSEA method (16), and $P < 0.05$ was set as the threshold.

Assessment of immune levels

The expression profiles of 24 immune cells (17) in the DN and control groups were calculated by single sample gene set enrichment analysis (ssGSEA) using GSVA package (18).

Highlight box

Key findings

- COL1A2 may be a potential biomarker for DN.

What is known and what is new?

- Immune inflammation and oxidative stress were involved in DN.
- COL1A2 may affect T cells and aDC, then subsequently participate in DN.

What is the implication, and what should change now?

- Identifying new predictive biomarkers will provide opportunities for prevention and/or treatment interventions. Future research focusing on COL1A2 is necessary.

The correlation was calculated by Pearson correlation analysis.

Ethical statement

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Statistical analysis

All analyses in this study were performed using the Bioinformcloud platform (<http://www.bioinformcloud.org.cn>).

Results

Co-expression of DEGs

We analyzed the DEGs between the DN samples and the controls in the GSE30122 dataset. A total of 1,778 DEGs were selected from the glomeruli and 1,996 DEGs from the renal tubules (*Figure 1A*). These DEGs may be related to the pathogenesis of DN. Through co-expression analysis of the union genes of the two DEGs groups, 3,130 genes were identified in nine modules (*Figure 1B,1C*). Among them, the brown module was most highly correlated with the glomerular and tubular DN samples (*Figure 1D*). Each module may represent different molecular maladjustment mechanisms, and the up- and down-regulated genes were distributed in each module (*Figure 1E,1F*). In addition, there was considerable up-regulation of the expression proportion of DEGs in the brown module in the glomeruli or renal tubules (*Figure 1G,1H*). The hub genes within each module were identified (*Table 1*).

Biological functions and signaling pathways of the module genes

Enrichment analysis showed that the module genes participated in 6,769 biological processes (BP), 795 cell components (CC), 1,310 molecular functions (MF), and 212 KEGG signaling pathways (available online: <https://cdn.amegroups.com/static/public/atm-22-5128-1.xlsx>). Among them, oxidative stress and autophagy were the main biological functions (*Figure 2A*). The phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) and nucleotide-binding and oligomerization domain (NOD)-like receptor signaling pathways were the main signaling pathways (*Figure 2B*). In addition, by comparing the GSEA results,

it was found that three signaling pathways were consistent with those in the renal tubules, and 15 signaling pathways were consistent with those in the glomeruli (*Figure 2C,2D*). These pathways may be closely related to the immune system.

Module genes related to immune cells

In this study, the expression profiles of 24 immune cells were calculated by ssGSEA (*Figure 3A,3B*). Most of the immune cells were highly expressed in DN glomeruli, and B cells, activated dendritic cells (aDC), T cells, and T helper cells were highly expressed in the DN renal tubules. In addition, immune cells, especially T cells and aDC, were highly correlated (*Figure 3C*). T cells and B cells exhibited the strongest correlation in the glomeruli, while that between T cells and aDC was the strongest in the renal tubules (*Figure 3D,3E*). Among these, the brown module's hub gene, collagen type I alpha 2 chain (COL1A2), was highly correlated with the glomerular T cells of DN and the tubular aDC (*Figure 3F,3G*). These results suggested that COL1A2 might affect T cells and aDC, and subsequently participate in DN.

Biomarkers of DN

We found that COL1A2, SLC25A11, SORD, TRIM22, and TUBB exhibited the same expression trends in the glomeruli and renal tubules (*Figure 4A,4B*). To further identify the molecular markers of DN, we calculated the AUC values of the hub genes of the modules (*Figure 4C*). It was found that the AUC values of COL1A2 and TRIM22 were in the top 5 in both tissues (*Figure 4D,4E*). Based on our comprehensive analysis, we believed that COL1A2 exhibited better clinical diagnostic ability and may have potential as a biomarker of DN.

Discussion

This study explored the gene expression profiles of DN and control groups obtained from the GEO database and analyzed the DEGs. The number of DEGs in glomeruli and renal tubules was similar, and the distribution of modular genes in the two groups was also similar. It may be that both tissues have early damage in diabetes (19). We then performed WGCNA to investigate DN-associated co-expressed gene networks and identify the hub genes. Selective impairment of glomeruli and dysfunction of

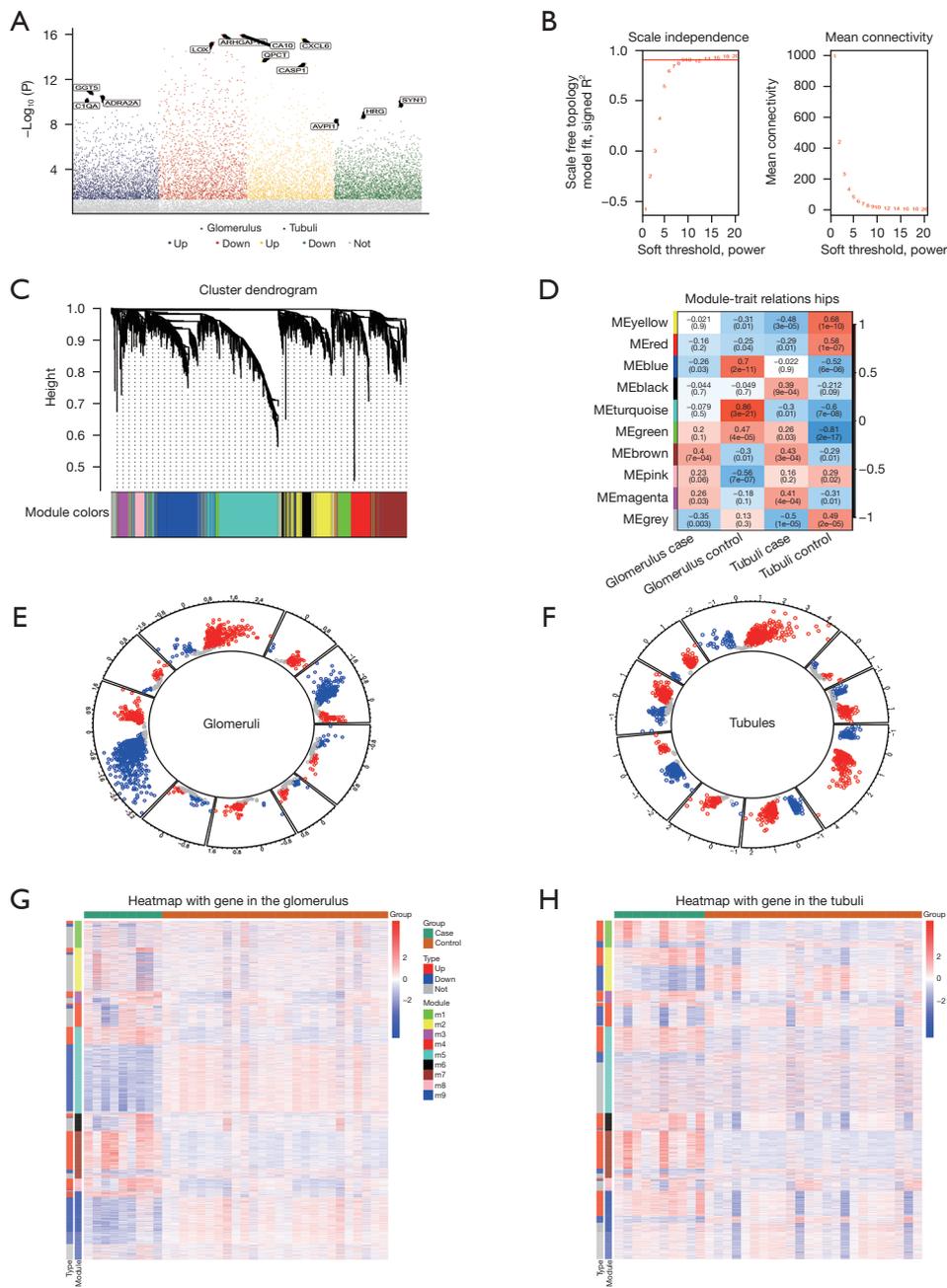


Figure 1 Differentially expressed genes of diabetic nephropathy. (A) Differential gene expression in the glomeruli and renal tubules of diabetic nephropathy patients. (B) Soft threshold power analysis was used to obtain the scale-free fit index of network topology. (C) Hierarchical cluster analysis was conducted to detect co-expression clusters with corresponding color assignments. The different colors represent different modules. (D) The correlation between module and phenotype. Red denotes a positive correlation; blue represents a negative correlation. DEGs of the glomeruli (E) or renal tubules (F) in modules. Red is up-regulated expression and blue is down-regulated expression. The expression of module genes in the glomeruli (G) or renal tubules (H). ME, module eigengene; DEG, differentially expressed gene.

Table 1 Hub genes of the modules

Color	Hub genes	Module
Black	<i>TM9SF2</i>	m6
Blue	<i>NPTN</i>	m9
Brown	<i>COL1A2</i>	m7
Green	<i>TRIM22</i>	m1
Magenta	<i>TUBB</i>	m3
Pink	<i>SLC25A11</i>	m8
Red	<i>SORD</i>	m4
Turquoise	<i>ARHGAP19</i>	m5
Yellow	<i>ERF</i>	m2

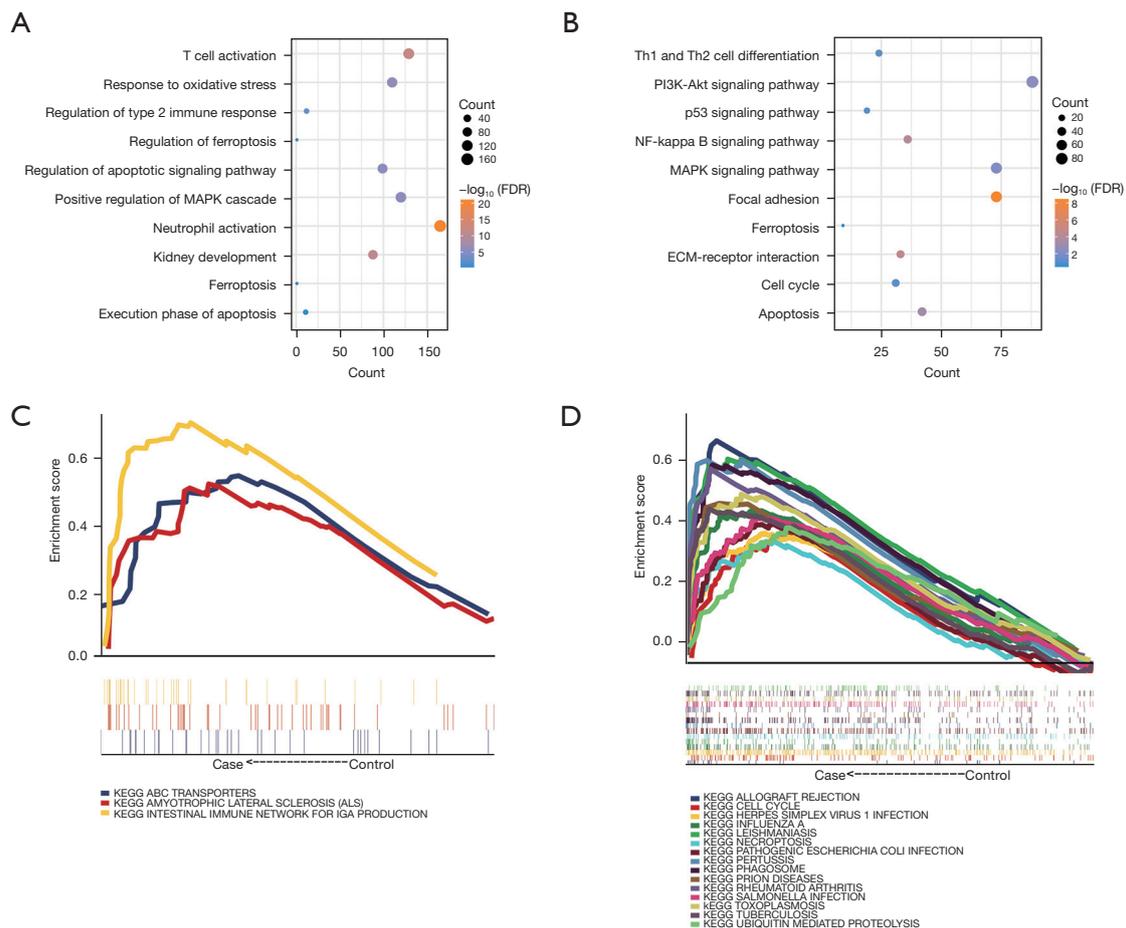


Figure 2 The molecular mechanism involved in module genes. (A) The biological function of module genes. (B) The KEGG pathway of the module genes. The results of GSEA were the same as those of KEGG enrichment in the glomeruli (C) and renal tubules (D). MAPK, mitogen-activated protein kinase; NF, nuclear factor; ECM, extracellular matrix; FDR, false discovery rate; KEGG, Kyoto Encyclopedia of Genes and Genomes; GSEA, Gene Set Enrichment Analysis.

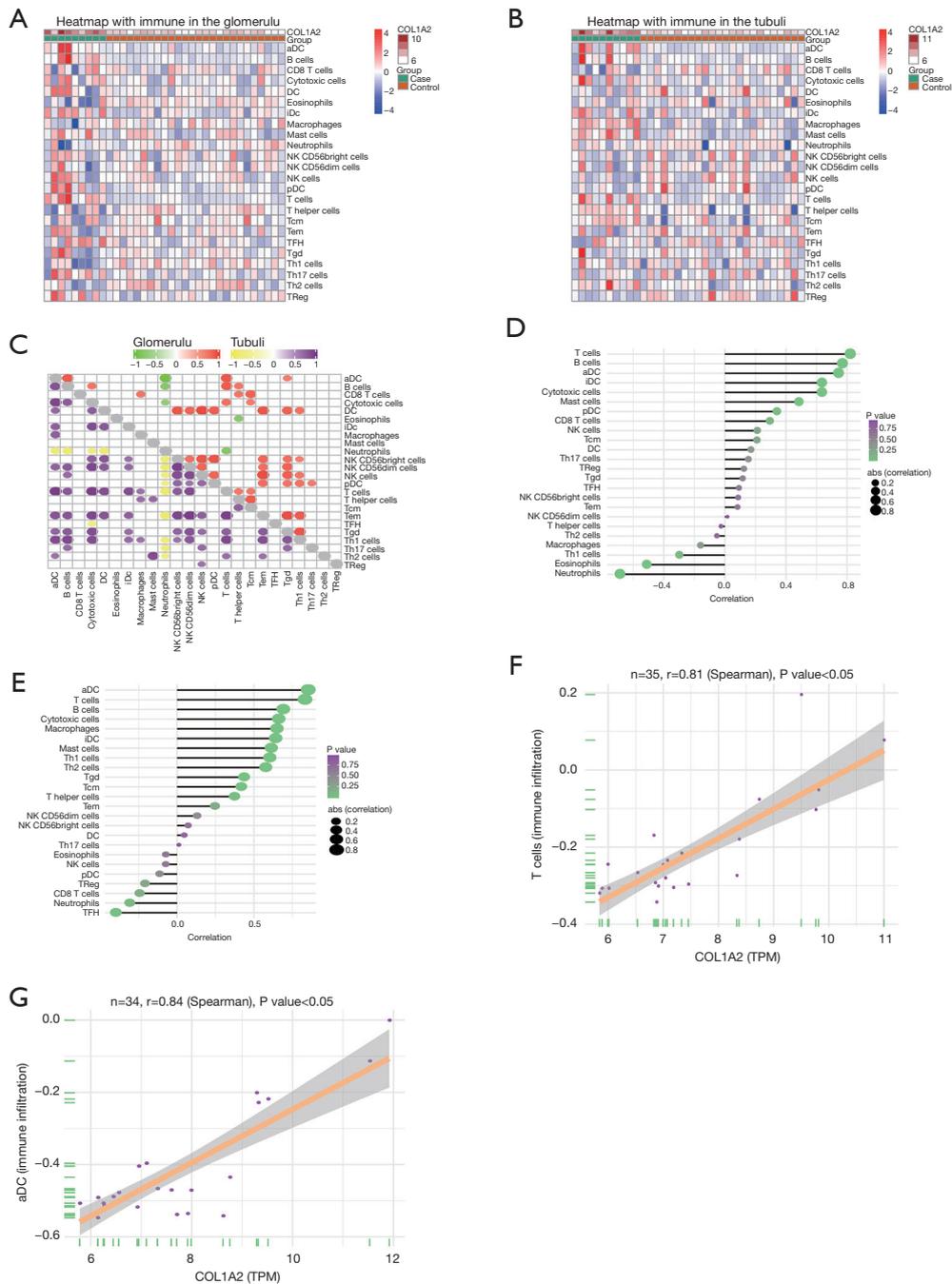


Figure 3 The correlation between modular genes and immune cells. Expression of 24 kinds of immune cells in the glomeruli (A) and renal tubules (B); red denotes up-regulation and blue represents down-regulation. (C) Estimated absolute scores for each immune cell type in the glomeruli or renal tubules. There was a high correlation between immune cells in the glomeruli (D) or renal tubules (E). (F) Correlation between COL1A2 and T cells in the glomerulus. (G) Correlation between COL1A2 and aDC in the renal tubules. COL1A2, collagen type I alpha 2 chain; aDC, activated dendritic cells; NK, natural killer; DC, dendritic cell; TFH, follicular helper T cell; TPM, transcripts per million.

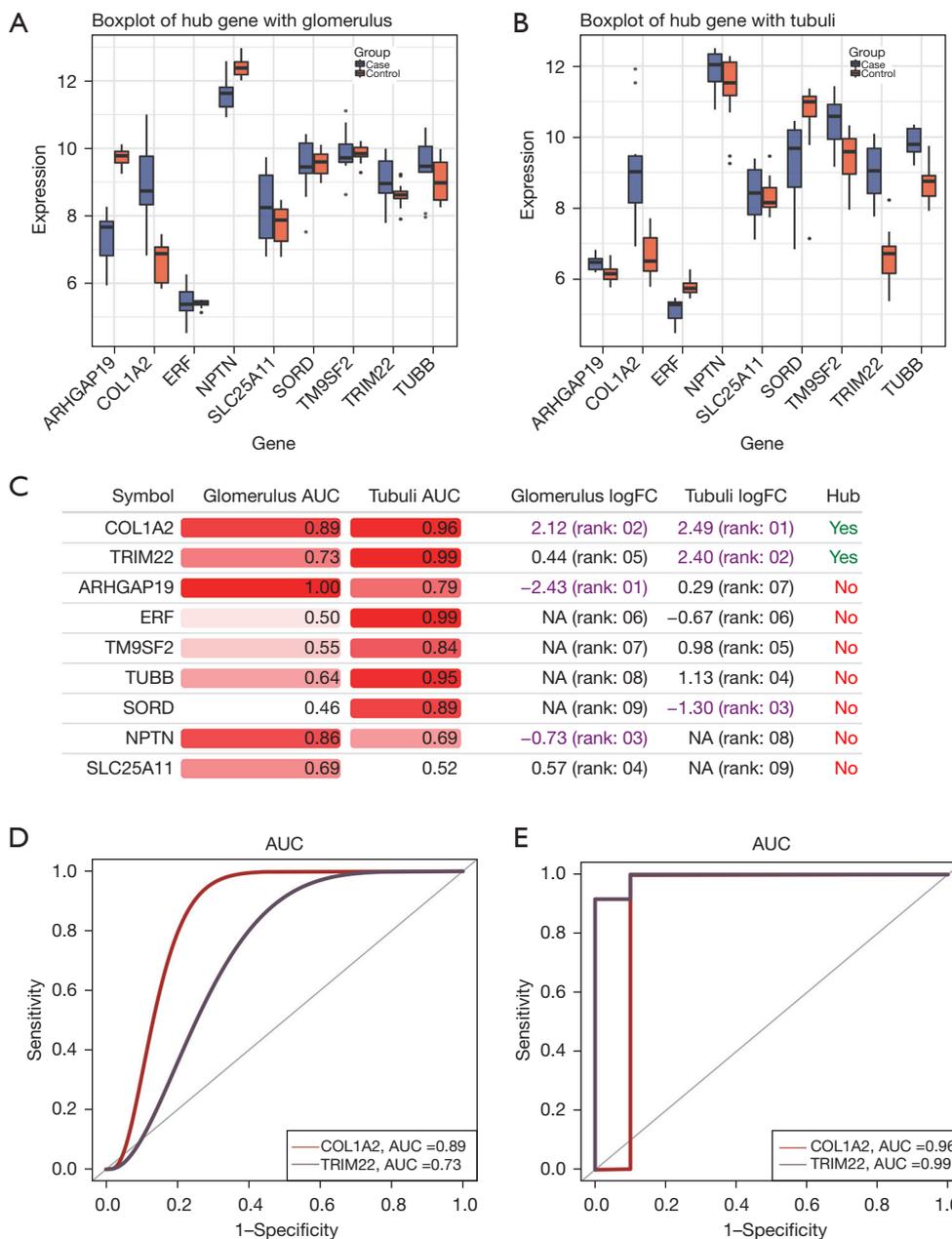


Figure 4 Identifying the biomarkers of DN. The expression of hub genes in the glomerulus (A) or renal tubules (B) of DN. (C) The AUC values of hub genes. The ROC curves of COL1A2 and TRIM22 in the glomerulus (D) or renal tubules (E) of DN. AUC, area under the ROC curve; COL1A2, collagen type I alpha 2 chain; DN, diabetic nephropathy; ROC, receiver operating characteristic; FC, fold change.

proximal renal tubule reabsorption contributes to the development of DN (20,21).

Enrichment analysis showed that most of the DEGs were enriched in the immune inflammatory response and oxidative stress. KEGG pathway analysis determined that the PI3K/Akt and NOD-like receptor signaling

pathways were enriched. Inflammation played a key role in the progression of DN. Preclinical studies have identified several anti-inflammatory molecules that effectively reduce proteinuria and/or proteinuria (22). The study of a diabetic experimental animal model showed that oxidative stress was the main determinant of DN pathophysiology (23).

Activation of the PI3K/Akt signaling pathway could promote the development of DN while blocking this pathway seemed to improve DN (24). The intracellular NOD-like receptor comprises an inflammatory body, which could activate and release caspase-1, IL-1 β , and IL-18, thereby causing an inflammatory reaction, which is closely related to the occurrence and development of DN (25,26). Therefore, the DEGs in this study may participate in the DN progression through these biological functions and signaling pathways.

Numerous immune cells play an important role in DN, including key members of the innate and adaptive immune systems. In cases of severe DN, the renal infiltration of T cells, neutrophils, and macrophages increases (27). Moreover, the accumulation of macrophages or T cells has been confirmed in both a human DN model and a rodent model (28). Dendritic cells (DCs) are the key mediators of immune and antigen-specific immune tolerance (29). The change of specific DC subsets in the cornea may be an early indicator of immune activation in DM, and its function is also closely related to the DN progression (30,31). At present, the role of B cells in the pathogenesis of DN is not clear. The most likely mechanism for B cell participation in DN is through the production of antibodies, the formation of immune complexes, and depositing in the kidney via complement activation, leading to inflammation and glomerulonephritis (32).

Importantly, we identified nine pivotal genes, among which the brown module of COL1A2 was the most closely correlated with DN, T cells and aDC. COL1A2 showed a good diagnostic ability in the glomeruli and renal tubules. Previous studies have shown that COL1A2 is involved in renal tubulointerstitial injury in diabetes (33). Also, the high expression of COL1A2 promotes the differentiation of fibroblasts (34). In the process of tubulointerstitial fibrosis in DN, there is increased production of type I collagen, which is the main component of extracellular matrix (35). It has been suggested that the high expression of COL1A2 may promote the occurrence and development of DN through inflammation and fibrosis (36-38). In addition, TRIM22 affects the inflammation-related signaling pathway and participates in several diseases (39). The increased expression of TRIM22 in monocyte macrophages treated with high glucose may be related to DN induced by high glucose (40). Therefore, COL1A2 and TRIM22 are expected to be a new research target and provide a new direction for the diagnosis and treatment of DN.

Our study also has limitations that should be noted.

Firstly, the sample size used for analyzing was too small to allow for generalization of the results, a larger number of samples will be used in future. Also, we should conduct relevant experimental tests to determine the potential biological role of the pathways in DN. Additionally, experiments to study the detailed molecular mechanism and biological function of the COL1A2 will be performed in future. Finally, in follow-up studies, we should expand the experimental samples to verify the potential diagnostic effects of the hub genes on DN.

In conclusion, the biological functions through which DEGs participate may play a regulated role in DN. COL1A2, which was identified by the co-expression network, may be a potential biomarker of DN. Therefore, future research focusing on COL1A2 is necessary.

Conclusions

The molecular mechanisms of renal tubules and glomeruli in DN are similar and are related to immune inflammation and oxidative stress. T cells, B cells, and aDC play an important role in the immune process. Importantly, we determined that COL1A2 might serve as a potential biomarker of DN.

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Footnote

Reporting Checklist: The authors have completed the STREGA reporting checklist. Available at <https://atm.amegroups.com/article/view/10.21037/atm-22-5128/rc>

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <https://atm.amegroups.com/article/view/10.21037/atm-22-5128/coif>). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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