Identification of Hub Genes Associated With Clinical Characteristics and Potential Therapy of Diabetic Nephropathy by Bioinformatics Analysis.

Si Xu
Wuhan University Zhongnan Hospital
Xiaoning Li (xiliusa2000@126.com)
Wuhan University Zhongnan Hospital https://orcid.org/0000-0003-4596-798X
Sha Wu
Wuhan University Zhongnan Hospital
Min Yang
Wuhan University Zhongnan Hospital

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Abstract

Background: To provide theoretical basis for the molecular mechanism of the development of diabetic nephropathy and targeted molecular therapy by screening expressed genes based on bioinformatic analysis.

Methods: We analyzed diabetic nephropathy microarray datasets derived from GEO database. Perl and R programming packages were used for data processing and analysis and for drawing. STRING online database and Cytoscape software were utilized for protein-protein interaction network analysis and screened for hub genes. Also, WebGestalt was used to analyze the relationship between genes and microRNAs. Nephroseq online tool was used to visualize the correlation between genes and clinical properties.

Results: We found 91 differentially expressed genes between diabetic nephropathy tissues and normal control tissues. Protein-protein interaction network analysis screened out 5 key modules and a total of 14 hub genes were identified by integration, also11 microRNAs were associated with hub genes. Especially mir29 could regulate COL6A3 and COL15A1.

Conclusions: The internal biological information in diabetic nephropathy can be revealed by integrative bioinformatical analysis, providing theoretical basis for further research on molecular mechanism and potential targets for diagnosis and therapeutics of diabetic nephropathy.

1. Background

Diabetic nephropathy (DN) is one of the most common microvascular complications of diabetes mellitus and also the main cause of end-stage renal disease (ESRD) [1, 2]. In terms of pathophysiology, diabetic nephropathy can be divided into glomerular lesion, tubules and interstitial atrophy and fibrosis. The clinical features of diabetic nephropathy is characterized by a persistent decrease in glomerular filtration rate (GFR), accompanied by a sustained increase in proteinuria and serum creatine(SCR)[3]. Renal tubulointerstitial and glomerular pathology have crucial roles in the progression of DN. However, studies providing renal gene expression profiles of diabetic nephropathy are relatively rare. At present, the treatment of diabetic nephropathy can only provide symptomatic support for treatment, which is limited for preventing DN progression such as blood glucose control and blood pressure lowering, etc [4, 5]. At the same time, there is a lack of clinical indicators for the diagnosis of diabetic nephropathy. Thus, it is an urgent task to identify molecular biomarkers and explore underlying mechanisms involved in the process of DN, which could help seek new diagnostic and therapeutic approaches.

Genome-wide transcriptome analysis using expression arrays can be used to identify biomarkers for disease progression and to gain insights into the disease pathogenesis and molecular therapy[6].With the wide application of genome transcriptome analysis, a large amount of core slice data has been produced, most of which is already stored in a public database. Reanalyzing these data can provide significant clues for other researches [6, 7]. In recent years, some microarray data analysis studies on DN have been conducted, and numerous differentially expressed genes (DEGs) have been identified [8]. However, solid results may be difficult to acquire due to the small sample size and false-positive rate in a single microarray analysis. We combine integrate bioinformatics methods and expression profiling techniques to address these disadvantages. In this work, we downloaded five original microarray datasets from the NCBI-Gene Expression Omnibus (NCBI-GEO) database (available online: https://www.ncbi.nlm.nih.gov/geo) for further analyses. DEGs between renal tissues of DN patients and normal controls were screened to determine key biomarkers. Then, we developed a Gene Ontology (GO) analysis, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment and protein-protein interaction (PPI) network analysis, DEGs was integrated to identify hub genes in DN through Cytohubba and MCODE module analysis. Nephroseq v5 online platform was used to conduct correlation analysis and subgroup analysis on the clinical characteristics of hub genes and DN.

MicroRNA(miRNA) is a kind of highly conservative single small non-coding RNA that can regulate the expression level and function of their target genes in the process of protein synthesis[9, 10]. In recent years, studies have found that the miRNA can participate in the development regulation, cell growth, differentiation and apoptosis, and identification of maintenance of cells and tissues, and other important life processes[11]. Mirnas play the role of oncogenes or tumor suppressor genes and play an important regulatory role in the occurrence and development of tumors [12]. Some mirnas are low expressed in tumor tissues, and these mirnas play the role of tumor suppressor genes to prevent tumor action, which are called tumor suppressor genes. On the contrary, other mirnas are high expressed in tumor tissues, and these mirnas play the role of tumor suppressor genes to promote tumor development, and they are called oncogenes [13].Therefore, mirnas can achieve a new targeted molecular therapy by regulating the expression of targeted hub genes.

2. Methods

2.1. Acquisition and processing of microarray data

Gene expression data related to diabetic nephropathy was downloaded from GEO online database (https://www.ncbi.nlm.nih.gov/geo/). By searching the following search terms: “diabetic nephropathy,” “Homo sapiens,” and “Expression profiling by array,” five microarray data including GSE30122, GSE1009, GSE47185-GPL11670, GSE47185-GPL14663 and GSE104954-GPL22945 were screened out by searching the following search terms: “diabetic nephropathy,” “Homo sapiens,” and “Expression profiling by array” .There were a total of 140 samples in these five microarray data, including 61 diabetic nephropathy samples and 79 normal control samples.

The probe data in the matrix file was transformed into gene symbol data through affymetrix platform file, if a single gene corresponds to multiple probes, the average expression value is calculated. After the integration of five matrix data through Perl, batch normalization was conducted through the SVA (surrogate variable analysis) package of R language (version 3.6.1)

2.2. Screening of differential genes

Differential genes (DEGs) were calculated and screened by limma (Linear Models for Microarray Data) package of R language. Only when the FDR < 0.05, and |logFC|>1, can it be identified as the DEGs for subsequent analysis.

2.3. The gene ontology (GO) function and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses

Gene Ontology (GO, which consists of three independent ontology, namely biological processes, molecular function and cellular component), was also used for pathway, disease and functional analysis (Mao Yudi et al., 2018). KEGG is an integrated database resource for the biological interpretation of genome sequences and other high-throughput data(Zhang lijie et al., 2019). GO (gene ontology) function and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment analysis was performed using ClusterProfiler package of R language. Cut-off conditions for GO enrichment analysis: P = 0.05, q = 0.05, while the cut-off conditions for KEGG enrichment analysis were P value = 1 and q value = 1.

2.4. Screening of key differential genes

To analyze the interactions between gene-encoded proteins, the screened differential genes were introduced into the search tool of the Interaction genes database (STRING11.0) to construct a protein-protein interaction (PPI) network, and then the results were imported into Cytoscape. In Cytoscape visualization software, CytoHubba was used to calculate the value of each DEG according to 12 topological algorithms. Download the data and sort it, selecting the first 15 hub nodes. The public hub nodes calculated by ≥ 5 topological algorithms were finally designated as hub genes. Besides, sub-networks were screened and the node degrees were calculated using Molecular Complex Detection (MCODE), and then the high relevant modules were obtained.

2.5. Correlation analysis of hub genes and microRNAs.

WEB-based GEne SeT AnaLysis Toolkit (WebGestalt) is a functional enrichment analysis web tool, which supports multiple enrichment analysis algorithms, and covers a comprehensive database of functional annotations. In my research, WebGestalt was used to predict miRNAs that targeted the hub genes associated with DN. By introducing the hub genes into WebGestalt, the miRNAs targeted by WebGestalt were predicted. Differentially expressed microRNAs were selected according to the top ten P values of Significance Level.

2.6. Genes correlations with clinical properties

Nephroseq is a free platform to the academic community for integrative data mining of genotype/phenotype data, which is supported by the University of Michigan O'Brien Renal Center. The primary goal of the Applied Systems Biology Core is to provide to the renal research community a platform for integrative data mining of comprehensive renal disease gene expression data sets. By inputting hub genes, correlation data between relevant genes and clinical characteristics were obtained in Nephroseq (https://www.nephroseq.org), and then the data was imported into GraphPad Prism 6 to make relevant analysis diagram.

3. Results

3.1. Identification of DEGs between DN samples and normal control samples

After pre-processing and normalizing, expression values of 7564 genes in 140 kidney samples were obtained from merged five microarray data sets. Overall, 91 DEGs were involved in DN as shown in the heatmap (Fig. 1a), of which 49 were down-regulated and 42 were up-regulated (Fig. 1b).

3.2. PPI network of DEGs and screening of key genes of DN

Search tool for the retrieval of interacting genes (STRING 11.0; https://string-db.org) was applied to establish a PPI network of DEGs. Interaction with a combined score > 0.7 was set as the cut-off point (Fig. 2a). Cytoscape software was used to visualize significant modules and hub genes by MCODE, After MCODE detection, a total of 30 genes were found to participate in 5 modules. The parameters of DEGs clustering and scoring were set as follows: MCODE score ≥ 3, degree cut-off = 2, node score cut-off = 0.2, max depth = 100, and k-score = 2. Besides, the first 15 hub nodes were selected from the 12 algorithms of CytoHubba, and the topological algorithm was finally designated as the hub gene after calculation of ≥ 5 by common hub nodes. 16 genes were identified as key gene, VEGFA, COL1A2, ALB, FOS, EGR1, EGF, COL3A1, CLU, CTGF, SST, EGR2, LUM, FOSB, COL6A3, PROM1, COL15A1 through integration in 12 algorithms of Cytohubba(Table 1). Ultimately, these 16 genes were intersected with 30 genes in the 5 modules of MCODE to obtain the 14 hub genes, including VEGFA, COL1A2, ALB, FOS, EGR1, EGF, COL3A1, CLU, SST, EGR2, LUM, FOSB, COL6A3 and COL15A1(Fig. 2b-f).

Table 1
Hub genes of diabetic nephropathy provided by 12 algorithms of CytoHubba

Groups	Genes	Gene title	Number of crosses
Up	COL1A2	Collagen type I alpha2	11
	COL3A1	Collagen type IIII alpha1	9
	CLU	Clusterin	9
	LUM	Lumican	6
	COL6A3	Collagen type VI alpha3	6
	COL15A1	Collagen type XV alpha1	5
	PROM1	Prominin 1	5
Down	VEGFA	Vascular endothelial growth factor B	11
	ALB	Albumin	11
	EGR1	Early growth response 1	10
	FOS	Fos proto-oncogene	10
	EGF	Epidermal growth factor	9
	CTGF	Connective tissue growth factor	8
	SST	Somatostatin	7
	EGR2	Early growth response 2	7
	FOSB	FosB proto-oncogene	6
Number of crosses: Each number represents represent a number of intersection algorithm of the 12 algorithms.			

TABLE 2 Hub genes-related signaling pathways

KEGG pathway	Genes	pValue
hsa04974: Protein digestion and absorption	CPA3/COL6A3/COL1A2/COL15A1/MME/COL3A1	2.62E-05
hsa04151: PI3K-Akt signaling pathway	COL6A3/THBS2/EGF/COL1A2/GHR/VEGFA/MAGI2/G6PC/FGF1	0.000313
hsa04933: AGE-RAGE signaling pathway in diabetic complications	COL1A2/EGR1/VEGFA/COL3A1/PLCE1	0.000389
hsa04510: Focal adhesion		
hsa04926: Relaxin signaling pathway	COL6A3/THBS2/EGF/COL1A2/VEGFA	0.008017
hsa04512: ECM-receptor interaction	COL1A2/FOS/VEGFA/COL3A1	0.008631
hsa05205: Proteoglycans in cancer	COL6A3/THBS2/COL1A2	0.017772
hsa05165: Human papillomavirus infection	LUM/COL1A2/VEGFA/PLCE1	0.038997
hsa04611: Platelet activation	COL6A3/THBS2/EGF/COL1A2/VEGFA	0.055614
hsa04610: Complement and coagulation cascades	COL1A2/COL3A1	0.183730
hsa05133: Pertussis	C1QB/CLU/C7	0.016208
hsa05020: Prion diseases	C1QB/CXCL6/FOS/LY96	0.001284
hsa04015: Rap1 signaling pathway	C1QB/C7/EGR1	0.001338
hsa05323: Rheumatoid arthritis	EGF/VEGFA/MAGI2/FGF1/PLCE1	0.009990
hsa04928: Parathyroid hormone synthesis, secretion and action	CXCL6/FOS/VEGFA	0.020562
hsa04935: Growth hormone synthesis, secretion and action	FOS/EGR1/CYP27B1	0.028883
hsa05166: Human T-cell leukemia virus 1 infection		
	GHR/FOS/SST	0.038742
	MMP7/FOS/EGR1/EGR2	0.048486

3.3. GO enrichment analysis of DEGs

GO functional enrichment analysis was performed on DN-related DEGs from the aspects of molecular function (MF), cell composition (CC) and biological process (BP). The most significant enrichment of DEGs MF in the figure we made included extracellular matrix structural constituent, SMAD binding, heparin binding, serine-type peptidase activity, sulfur compound binding, serine hydrolase activity, serine-type endopeptidase activity, extracellular matrix structural constituent conferring tensile strength(Fig. 3).

3.4. KEGG enrichment analysis of DEGs

To explore enriched pathways of DEGs, KEGG pathway analysis was done using ClusterProfiler package of R language. Results revealed that DEGs were mainly enriched in PI^3K-Akt signaling pathway, AGE-RAGE signaling pathway in diabetic complications, Focal adhesion, Relaxin signaling pathway, Rap1 signaling pathway, VEGF signaling pathway and Ras signaling pathway, and so on. (TABLE2).

3.5. Relationship between hub genes and targeted microRNAs of DN.

The top ten of key miRNAs that targeted the hubgenes were predicted: the miR-29 family (miR-29a/b/c), miR-196a/b, miR-199A, miR-377, miR-380-5P, miR-191, miR-186, miR-1, miR-206, miR-383 and miR-134. A total of eight groups were identified as FDR (False Discovery Rate) < 0.05(Table 3). In this study, the samples were also divided into renal tubules and glomeruli for analysis, and corresponding hub genes were obtained respectively. Then, the common hub genes were obtained by Using the Venn diagram: COL1A2, COL6A3, COL15A1, LUM, CLU, EGF, ALB, FOS, FOSB (Fig. 4).

Table 3
Hub genes-related MicroRNAs

MicroRNA	Genes	EnrichmentRatio	FDR
MIR-29A,MIR-29B,MIR-29C	COL15A1,COL3A1,COL6A3,VEGFA,FOS	8.836705	0.000081293
MIR-196A,MIR-196B	COL1A2,COL3A1	12.91901	0.009646
MIR-199A	VEGFA,FOS	10.07967	0.015526
MIR-377	VEGFA,EGR1	9.31218	0.018049
MIR-380-5P	COL1A2	41.69318	0.023746
MIR-191	EGR1	31.62931	0.031197
MIR-186	COL3A1,VEGFA	6.769373	0.032840
MIR-1,MIR-206	VEGFA,FOSB	6.156040	0.039137
MIR-383	VEGFA	18.34500	0.053253
MIR-134	VEGFA	17.63942	0.055331

3.6. Association between hub genes and clinical features of DN.

To verify potential roles of hub genes in DN, correlation analysis and subgroup analysis between hub genes and clinical features were performed using Nephroseq v5 online tool. First, the results showed that mRNA expression of VEGFA, ALB, EGR1, EGF, EGR2, FOS, FOSB, CTGF and SST positively correlated with GFR in tubulointerstitium of DN patients (Fig. 5), while mRNA expression of VEGFA, ALB and SST positively correlated with GFR in glomerulus of DN patients (Fig. 6), suggesting most of the down-regulated hub genes may delay or inhibit the development of DN. In addition, it also suggests that the effects of the same gene in different sites may be opposite. Meanwhile, the mRNA expression of COL1A2, COL3A1, COL6A3, COL15A1, CLU, LUM and PROM1 reversely correlated with GFR in DN patients (Fig. 7), indicating that those up-regulated hub genes may contribute to the progression of DN, and confirming that the roles of these up-regulated genes COL1A2, COL6A3, COL15A1, CLU and LUM were consistent in the glomeruli and tubules. Second, the up-regulation of hub genes COL1A2, COL3A1, CLU and LUM mRNA expression was positively correlated with SCR in DN patients (Fig. 8a–d), suggesting that those up-regulated hub genes may contribute to the progression of DN. The down-regulation of VEGFA and EGF mRNA expression was negatively correlated with SCR (Fig. 8e, h, i), while EGR1 and EGR2 mRNA expression were positively correlated with SCR in DN (Fig. 8f, g), indicating that VEGFA and EGF may have protective roles in DN, while EGR1 and EGR2 may promote the development of DN. Third, the mRNA expression of VEGFA, LUM and CTGF reversely correlated with proteinuria (Fig. 9a–c). In addition, the expression of ALB in nephrotic proteinuria group was lower than that of subnephrotic proteinuria group (Fig. 9d), while the expression of COL1A2 in nephrotic proteinuria group was higher than that of subnephrotic proteinuria group(Fig. 9e). However, there was no difference in COL3A1 and PROM1 expression between nephrotic proteinuria group and subnephrotic proteinuria group (Fig. 9f, g). In conclusion, VEGFA and EGF have an absolute inhibitory effect on the progression of diabetic nephropathy in this study, and FOS, FOSB and SST may have an inhibitory effect on the progression of diabetic nephropathy. However, COL1A2, COL3A1, COL6A3, COL15A1, CLU, LUM and other genes may promote the development of diabetic nephropathy.

4. Discussion

DN is the main cause of renal failure worldwide, it is quite clear that glomerulopathy and tubulopathy played a central role in the progression of DN. Recently, diabetic nephropathy has been pay more attention to acquire earlier diagnosis and additional targeted invention [14]. Increasing evidence indicated that autophagy[15], inflammation[16], oxidative stress and apoptosis[17], mitochondrial dysfunction [18], and abnormal activation of the renin angiotensin system [19] were closely involved in diabetic nephropathy. Although extensive efforts have been made, the underlying mechanisms of diabetic nephropathy remain elusive. The development of high throughput microarray technology and bioinformatic methods allows us to identify important genes associated with diabetic nephropathy, thus gaining a deeper understanding of its pathogenesis, and to seek molecular diagnostic markers and new therapeutic approaches through relevant important genes.

In this study, a total of 7564 genes were included after data consolidation and normalization. Limma package of R could provide valuable information on large-scale genes with a relatively normal fold change value. We identified 91 DEGs between renal tissues of DN patients and normal controls based on five microarray expression data sets. Among them, 42 differentially expressed genes were up-regulated and 49 were down-regulated. Protein-protein interraction coexpression network showed closely connected genes among DEGs. GO annotation result of DEGs demonstrated that extracellular matrix structural constituent, glycosaminoglycan binding, heparin binding, serine-type peptidase activity, sulfur compound binding, SMAD binding and serine hydrolase activity were chiefly enriched. The GO enrichment annotation results are 23 of the 42 up-regulated genes with differentially expressed genes, such as the extracellular matrix structural constituent, the extracellular matrix structural constituent conference ring tensile strength, etc. No GO enrichment annotation results were found among 49 down-regulated genes with differentially expressed genes. Most of the enrichment results were about molecular function. As an important lesion of DN, tubulointerstitial fibrosis is featured by accelerated deposition of extracellular matrix [20]. Many of extracellular matrix structural constituents have been implicated in the pathogenesis of renal disease [21] such as COL4A3 [22], decorin, biglycan and lumican[23].Enrichment analysis of DEGs by KEGG showed that many of these genes were mapped to protein digestion and absorption, Rap1 signaling pathway, ECM-receptor interaction, platelet activation, PI^3K-Akt signaling pathway, complement and coagulation cascade pathway, etc. In 42 up-regulated differentially expressed genes in KEGG enrichment has 5 results. Such as, Protein digestion and absorption, pertussis, Complement and coagulation cascades, ECM -receptor interaction and viral protein interaction with cytokine and cytokine receptor. The pathogenesis of diabetes is due to the abnormal insulin action, and then the long-term blood glucose abnormality leads to the occurrence of glucose kidney. Abnormal insulin is a typical protein digestion and absorption disorder, indicating that protein digestion and absorption must be involved in the process of DN. However, no studies have shown that collagen genes such as COL6A3/COL1A2/COL15A1 are related to protein digestion and absorption. There is also considerable evidence that activated complement systems and coagulant events may contribute to the development and progression of DN, such as complement C3, C1, and C7 [24–26].The expression of C7 gene was significantly up-regulated in the renal tissue of patients with DN [24]; renal immunohistochemistry detection of C1q and C3c deposition was associated with severe renal damage in DN[26]. Clusterin (CLU), an extracellular chaperone, is a ubiquitously expressed protein that can be identified in various body fluids and tissues. Expression of CLU can lead to various processes including suppression of complement system [27]. Clusterin is involved in apoptosis, inflammation, lipid transportation, cell-to-cell interactions and aging [28]. Additionally, it plays a role in diabetic conditions. Several previous reports showed that clusterin attenuates angiotensin-induced fibrosis and unilateral urethral obstruction (UUO) induced renal fibrosis [29, 30]. However, it has not been shown that CLU gene is related to the complement system related pathways in DN, thus providing us with research thought. The ECM-receptor interaction is a micro-environmental pathway that maintains cell and tissue structure and function. Usually, the ECM consists of a complex mixture of structural and functional macromolecules, which mainly include collagen, fibronectin (FN) and laminin[31]. KEGG enrichment analysis was not found among the 49 down-regulated genes with differential expression.

After combining the two analysis methods, a total of 14 DEGs were distinguished as hub genes, including COL1A2, COL3A1, COL6A3, COL15A1, LUM, CLU, EGF, VEGFA, ALB, SST, FOS, FOSB, EGR1 and EGR2. The upregulation of key genes, including collagen type I α2 chain (COL1A2) and collagen type III α1 chain (COL3A1) are closely related to the severity of renal fibrosis in DN[32, 33]. In other diseases, over-expression of miR-29 has been found to inhibit COL1A1/COL3A1 mRNA and Collagen I/III protein, but not in kidney diseases [34, 35]. The down–regulation of key genes such as Epidermal Growth Factor (EGF) is an active polypeptide used to promote the repair and regeneration of the damaged epidermis [36]. Soluble EGF binds to EGFR which leads to inner kinase activation and phosphorylation of tyrosine residues[37, 38], and then excessive activation of EGFR start multiple intracellular signaling pathways, including extracellular signal conditioning TGF-β1-smad signaling pathways, phosphoinositide 3 kinase (PI^3K)/Akt pathway and extracellular regulated protein kinases(ERK) pathway[38, 39], so as to regulate kidney diseases such as obstructive nephropathy, diabetic nephropathy and hypertensive nephropathy[40].Vascular endothelial growth factor A (VEGFA), an important endogenous angiogenic factor ,which is synthesized and secreted by tubular cells. Also, previous studies identified that vascular endothelial growth factor (VEGF) A is thought to be associated with glomerular endothelial cell dysfunction and tubular cell damage in diabetic nephropathy[41, 42]. Here we found that mRNA expression of VEGFA and EGF was positively correlated with GFR, negatively correlated with SCR, and negatively correlated with VEGFA and proteinuria. This suggests that VEGFA and EGF may be a potential treatment for diabetic nephropathy. Moreover, VEGFA has targeted effects with Mir-29, Mir-199a, Mir-377, Mir-186, Mir-1, Mir-206, Mir-383 and Mir-134. Albumin (ALB) encodes serum albumin, which regulates the plasma colloid osmotic pressure. It is clear that albumin as a basic indicator of renal disease is not specific in clinic. Somatostatin(SST), a natural cyclic peptide inhibitor, which plays the same beneficial role as angiotensin converting enzyme inhibitor(ACEI) in most parameters of diabetic nephropathy[43]. FOS and FOSB are members of the FOS family genes [44].Under the condition of high glucose, FOS, proto-oncogenes, significantly up-regulated and Jun protein form a dimer with activator protein-1(AP-1) which serve an important role in DN. However, the specific exact mechanism is yet unclear [45].Over-expression of microrNA-29b can inhibit the activation of hepatic stellate cells by inhibiting the expression of C-FOS mRNA in liver fibrosis [46]. Therefore, whether mir29 can alleviate renal fibrosis by inhibiting FOS expression in diabetic nephropathy? Early growth response proteins (EGRs), as a transcriptional regulatory family, are involved in the process of cell growth, differentiation, apoptosis, and even carcinogenesis. EGR1 and EGR2 are members of the EGRs family

genes [47]. It has been proved for the first time that EGR1 is a transcriptional activator of oxidative stress in diabetic nephropathy [48]. Meanwhile, early growth reactive protein-1 (EGR1) plays a key role in DKD by promoting mesangial cell proliferation and the production of extracellular matrix (ECM) [49].

So far, there is no report on the association between such hub genes and DN as EGR2, FOSB, COL15A1, COL6A3 and LUM. EGR2, which has the most extensive role in the body, also reduces the expression of fibroblast genes in human fibroblast cell lines [50].Among them, FOSB is an ap-1 transcription factor. In small intestinal neuroendocrine tumors, down-regulated expression of Mir-1 upregulates the expression of mir-1 gene targeted oncogene FOSB, leading to disease progression [51]. At present, there have been no relevant reports about Mir-206 and FOSB. Studies have shown that COL6A3 and COL15A1 may be involved in tubulointerstial injury, but whether they cause glomerulopathy is unknown [52]. And how to participate in the mechanism of tubule injury is not clear. In our study, COL6A3 and COL15A1 were reversely correlated with GFR. In addition, both COL6A3 and COL15A1 were in targeted correlation with the MIR29 family. Therefore, it may be possible to inhibit the expression of COL6A3 and COL15A1 by interfering with the content of MIR29 in vivo, so as to delay the progression of DN and play a role of targeted therapy. Lumican(LUM) is a small leucine-rich proteoglycan (SLRPs) that, like decorin, binds TGF-β in the extracellular matrix to modulate renal fibrosis [53, 54]. In our work, the mRNA expression of LUM in DN patients was negatively correlated with GFR and positively correlated with SCR. Of course, there are many shortcomings of this study. First, although 5 original microarray data sets of diabetic nephropathy were integrated and the total number of samples was sufficient, diabetic nephropathy did not account for the majority of the samples. Second, the lack of detailed demographic information makes it difficult to obtain a more convincing correlation between the selected genes and the severity of diabetic nephropathy using the same samples. Third, due to the lack of online data, the information of selected genes and related clinical indicators is insufficient, resulting in the unclear specific role of some genes. Therefore, further clinical and basic studies are needed in the future to verify our results and clarify the biological role of these genes and related microRNA in diabetic nephropathy.

In summary, the purpose of this study was to identify key molecules associated with diabetic nephropathy and to seek targeted therapies. Through transcriptome data analysis, 91 DEGs, 14 hub genes and 11 micrornas were screened out, among which MIR29 can target COL6A3 and COL15A1 to regulate their expression, thereby delaying the progress of DN. This may provide new ideas for the treatment of DN in the future.

Abbreviations

DN : diabetic nephropathy; ECM : extracellular matrix; GEO : Gene Expression Omnibus; GO : gene ontology; KEGG : kyoto encyclopedia of genes and genomes; miRNAs : microRNAs; PPI : protein–protein interaction; WebGestalt : WEB-based GEne SeT AnaLysis Toolkit.

Declarations

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Authors' contributions

XS conceived and designed the study. XS and WS performed the bioinformatics pipeline and processed data for the analysis. XS performed correlation analysis. XS wrote the manuscript .WS and YM put forward reasonable Suggestions for the discussion section of the study.LXN revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The Human Diabetic nephropathy transcriptome data set is downloaded from The GEO DataSets browser of The National Center for Biotechnology Information (NCBI) [https://www.ncbi.nlm.nih.gov/gds]. Data supporting the correlation between clinical features and hub-genes in this conclusion can be found in nephroseq[https://www.nephroseq.org].

Ethics approval and consent to participate

This study does not require ethical approval and consent due to the use of data in an online database for analysis.

Consent for publication

Not applicable.

Competing interests

The authors declare that there is no conflict of interests.

Author details

[1]Department of Nephrology, Zhongnan Hospital, Wuhan University, Hubei, China.

[2]Department of Nephrology, Zhongnan Hospital, Wuhan University, Hubei, China.

[3]Department of Nephrology, Zhongnan Hospital, Wuhan University, Hubei, China.

[*]Department of Nephrology, Zhongnan Hospital, Wuhan University, Hubei, China.

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Figures

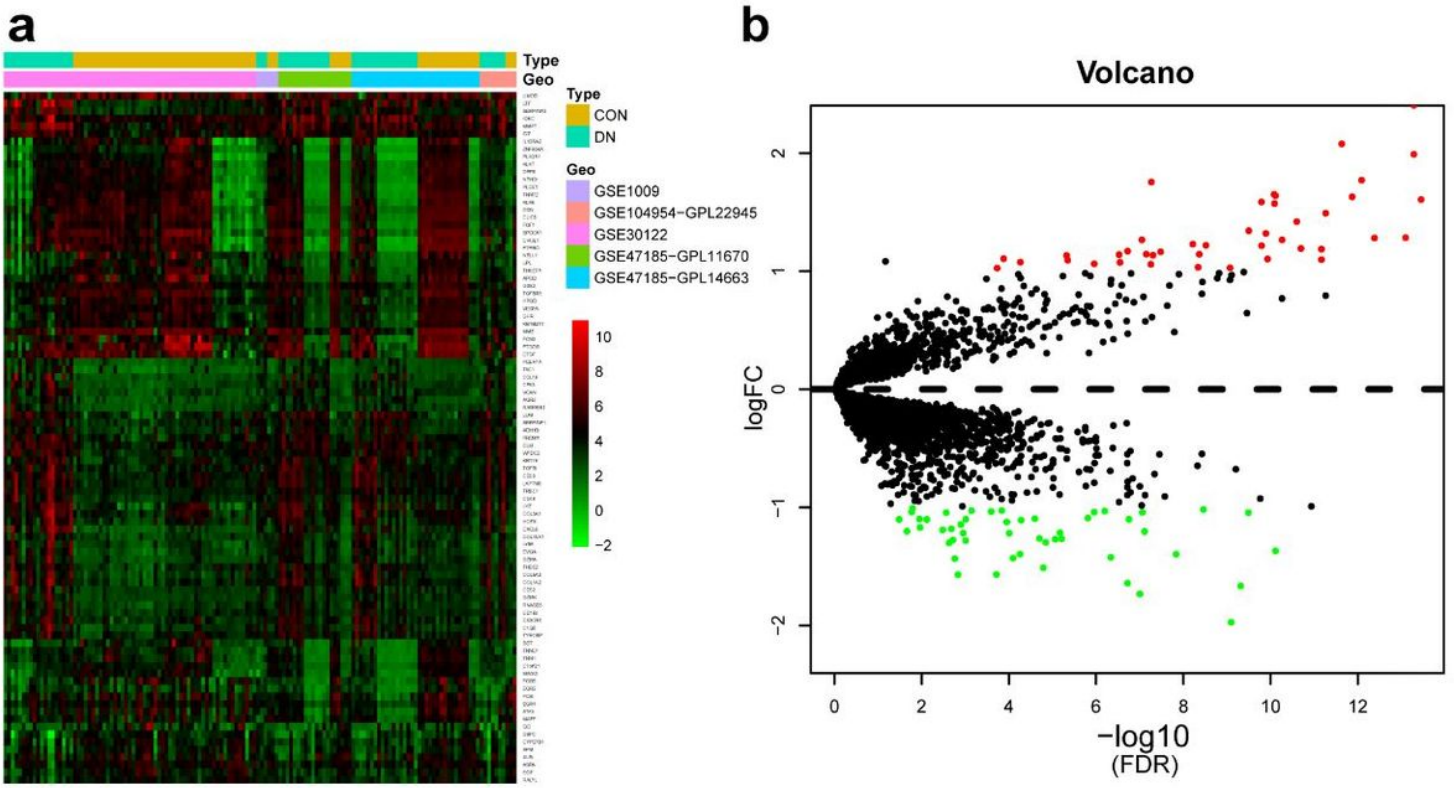
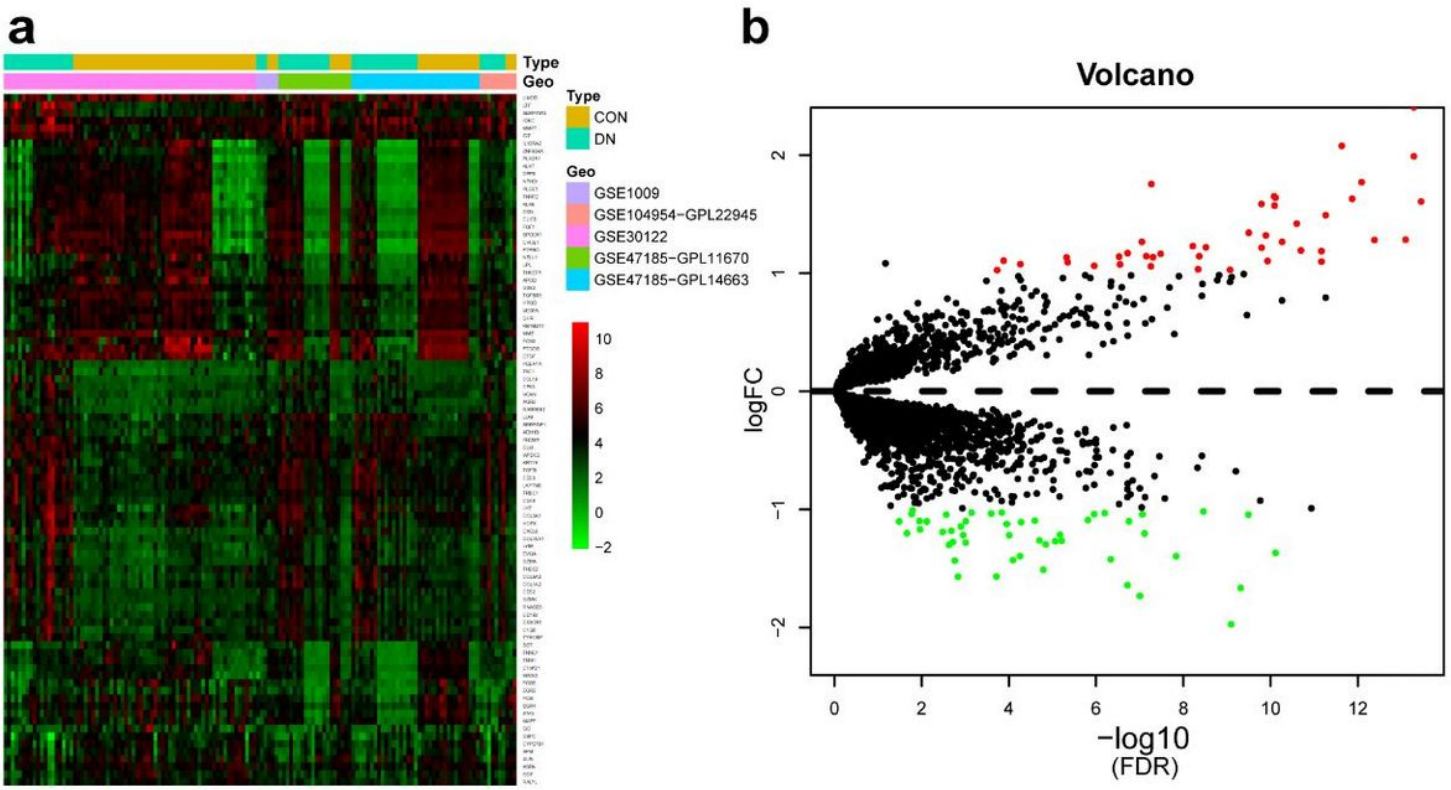


Figure 1

Heatmap and Volcano plot analysis. (a)Heatmap of 91DEGs screened. Red areas represent highly expressed genes and green areas represent lowly expressed genes in DN patients compared with normal controls. DEG: differentially expressed gene. (b) Volcano plot analysis identifies DEGs. Red dots represent up-regulated genes, green dots represent down-regulated genes, and black dots represent normally expressed genes in DN patients compared with normal controls.



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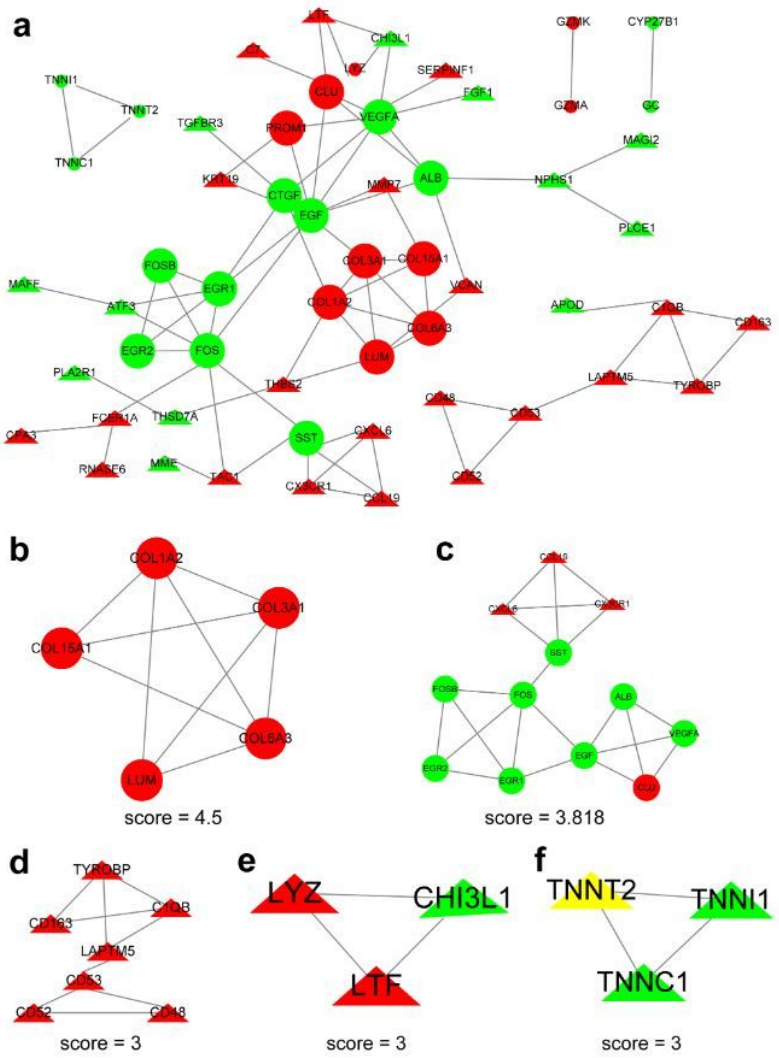
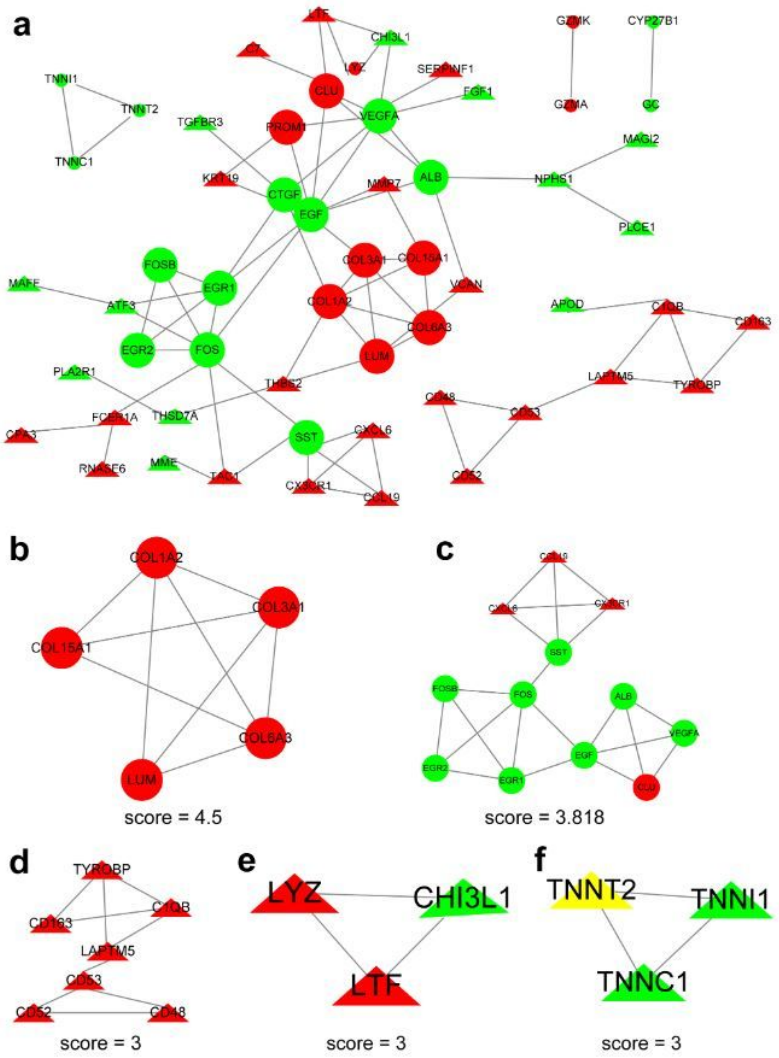


Figure 2

PPI network and five significant modules of DEGs. Red represents up-regulated genes and green represents down-regulated genes in DN patients compared with normal controls. Circles represent hub genes and Triangles represent DEGs (a) PPI network of DEGs created by STRING. The nodes stand for DEGs and the lines stand for the interactions between two proteins. (b)The most significant module identified by MCODE. (c) The second significant module identified by MCODE. (d) The third significant module identified by MCODE. (e) The forth significant module identified by MCODE. (f) The fifth significant module identified by MCODE.DEG: differentially expressed gene.



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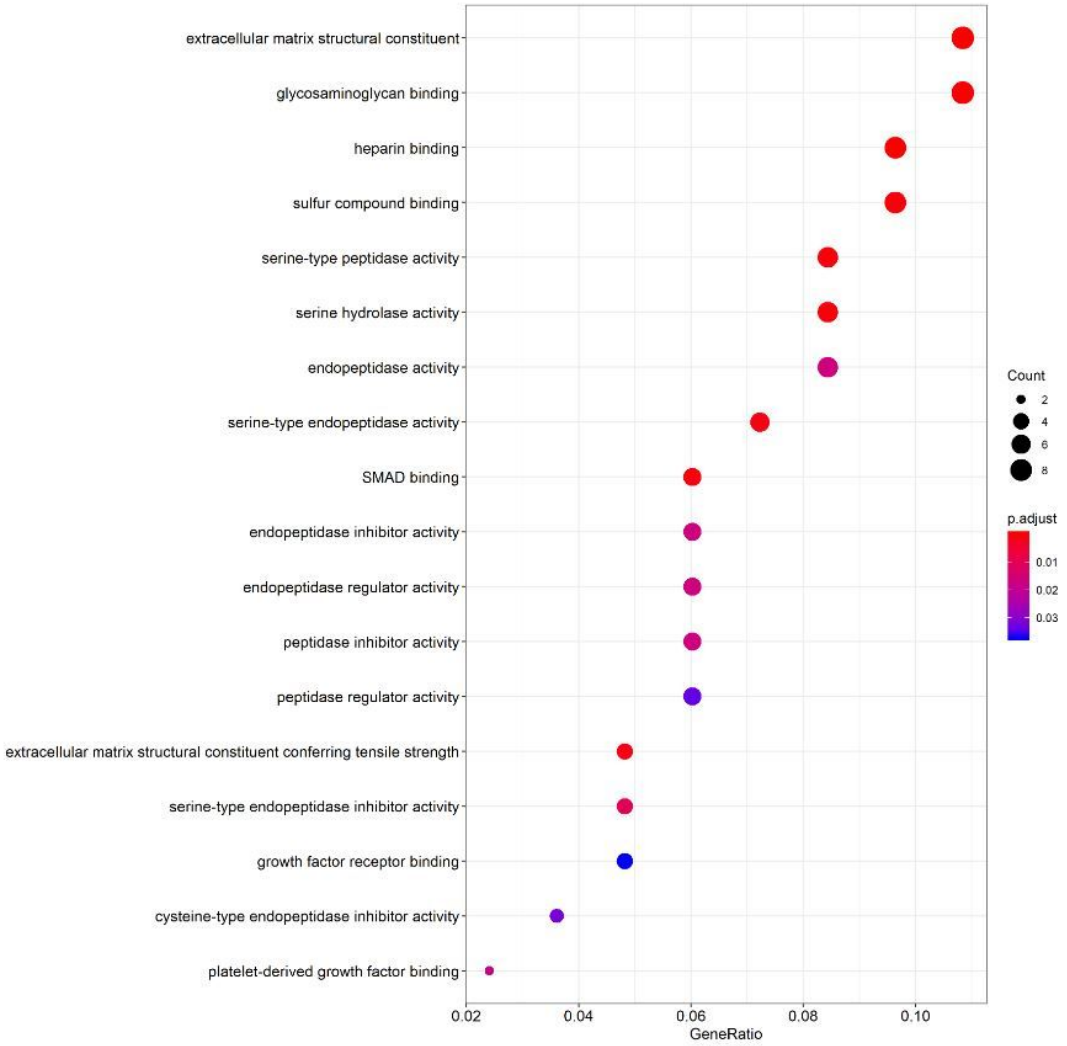
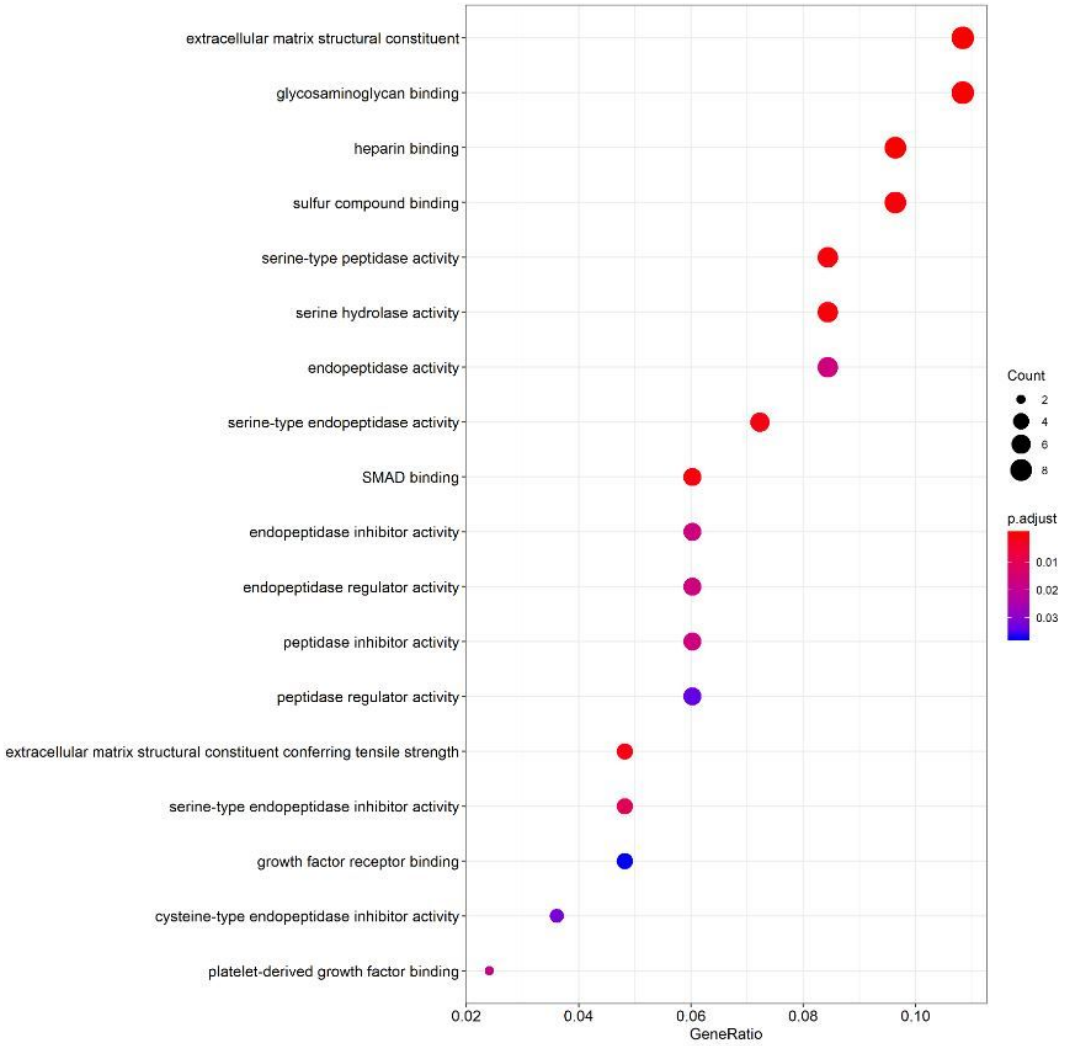


Figure 3

GO enrichment result of DEGs. The x-axis represents gene ratio and y-axis represents GO terms. The size of circle represents gene count. Different color of circles represents different adjusted p value.



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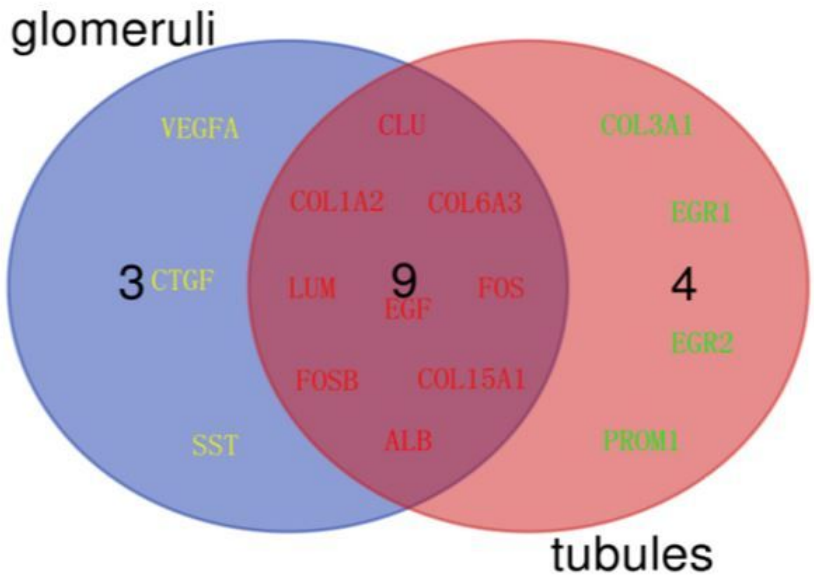
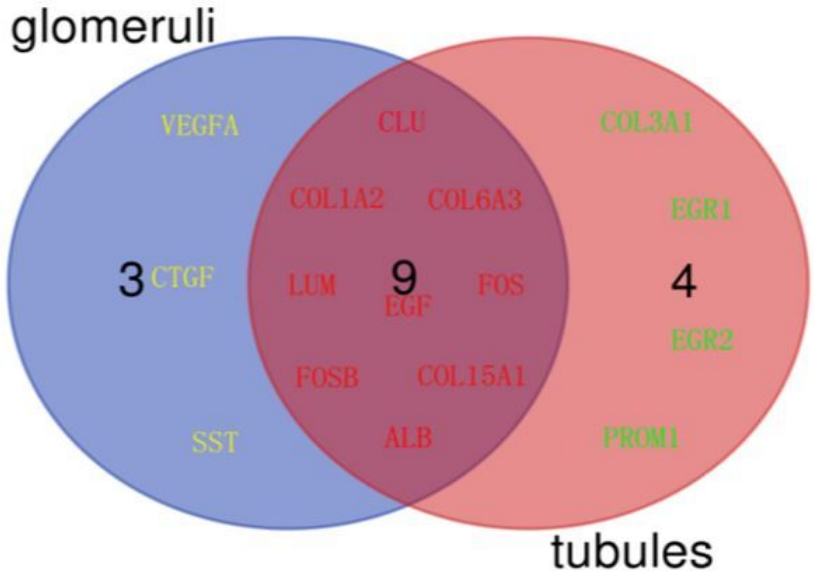


Figure 4

Venn diagram of hubgenes. Three genes were expressed only in glomerular tissue, four genes were expressed only in tubular tissue, and nine genes were expressed in both.



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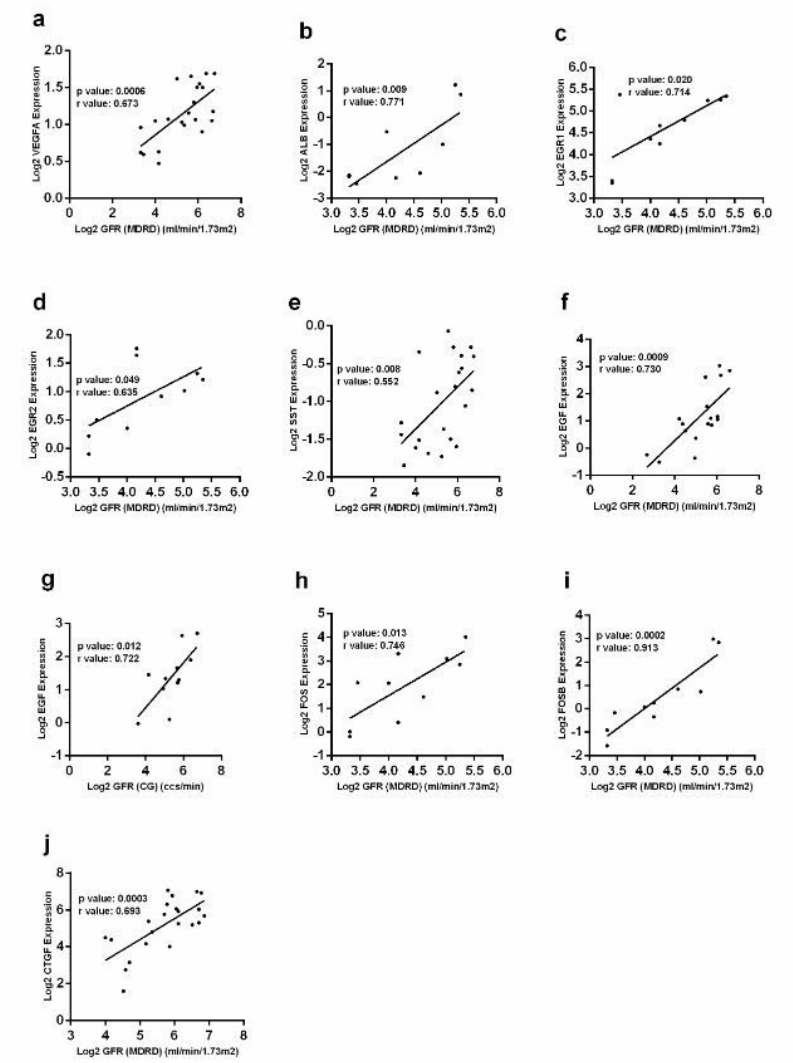
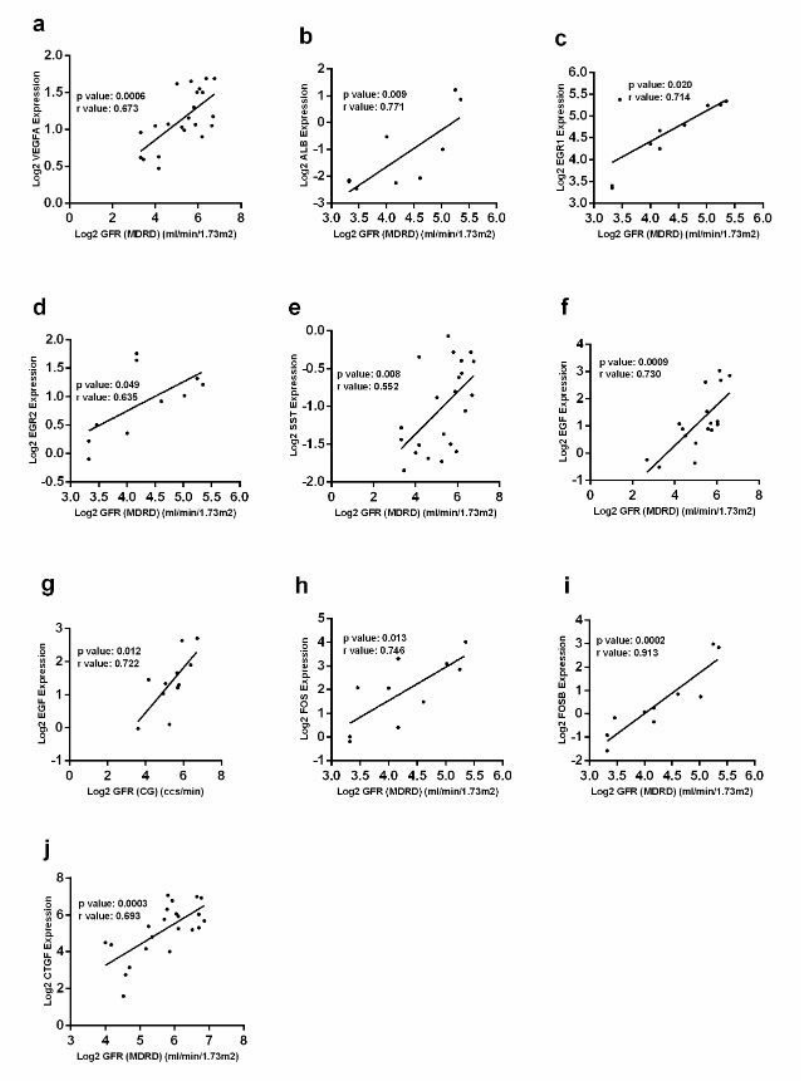


Figure 5

Correlation between down-regulated hub gene mRNA expression and GFR in tubulointerstitium of DN patients, r is the correlation coefficient. (a) The expression of VEGFA positively correlated with GFR (p = 0.0006, r = 0.673). (b) The expression of ALB positively correlated with GFR (p = 0.009, r = 0.771). (c) The expression of EGR1 positively correlated with GFR (p = 0.02, r = 0.714). (d) The expression of EGR2 positively correlated with GFR (p = 0.049, r = 0.635). (e) The expression of SST positively correlated with GFR (p = 0.008, r = 0.552). (f) The expression of EGF positively correlated with GFR (p = 0.0009, r = 0.730). (g) The expression of EGF positively correlated with GFR (p = 0.012, r = 0.722). (h) The expression of FOS positively correlated with GFR (p = 0.013, r = 0.746). (i) The expression of FOSB positively correlated with GFR (p =0.0002, r =0.913). (j) The expression of CTGF positively correlated with GFR (p = 0.0003, r = 0.693).



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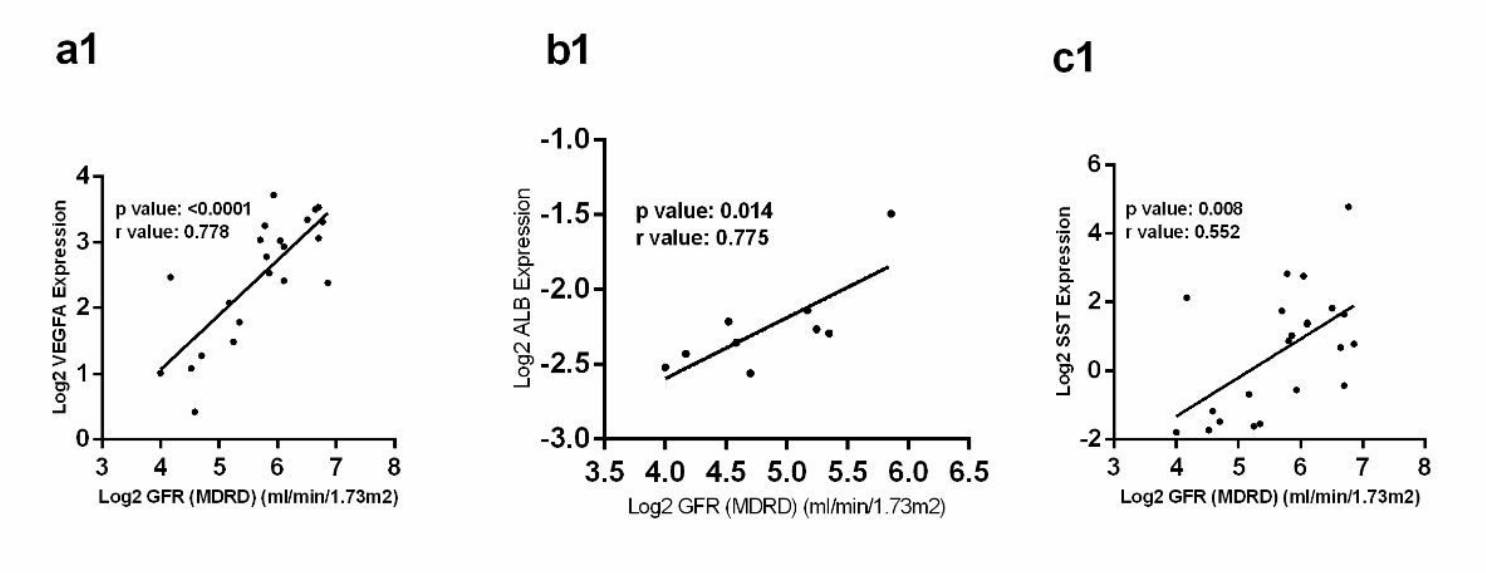
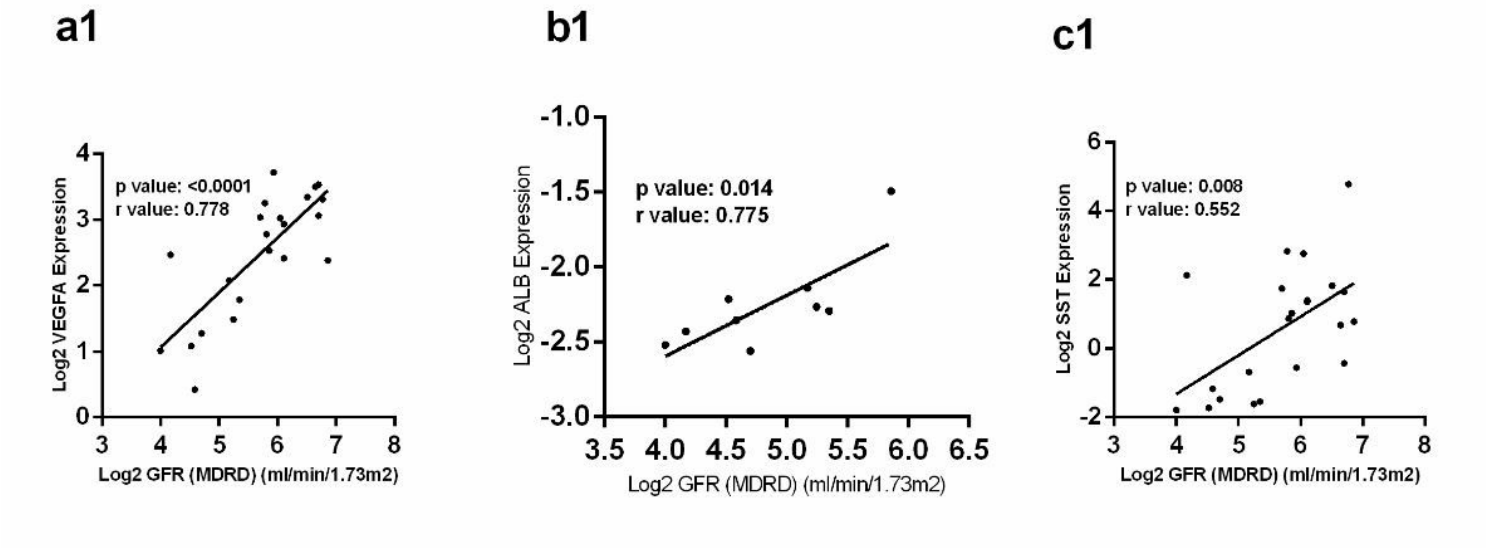


Figure 6

Correlation between down-regulated hub gene mRNA expression and GFR in glomerulus of DN, r is the correlation coefficient. (a1)The expression of VEGFA positively correlated with GFR ($p < 0.0001$, $r = 0.778$). (b1) The expression of ALB positively correlated with GFR ($p = 0.014$, $r = 0.775$). (c1) The expression of SST positively correlated with GFR ($p = 0.008$, $r = 0.552$).$p < 0.05$ was considered statistically significant. CG: Cockcroft Gault; DN: diabetic nephropathy; GFR: glomerular filtration rate; MDRD: modification of diet in renal disease; mRNA: messenger RNA.



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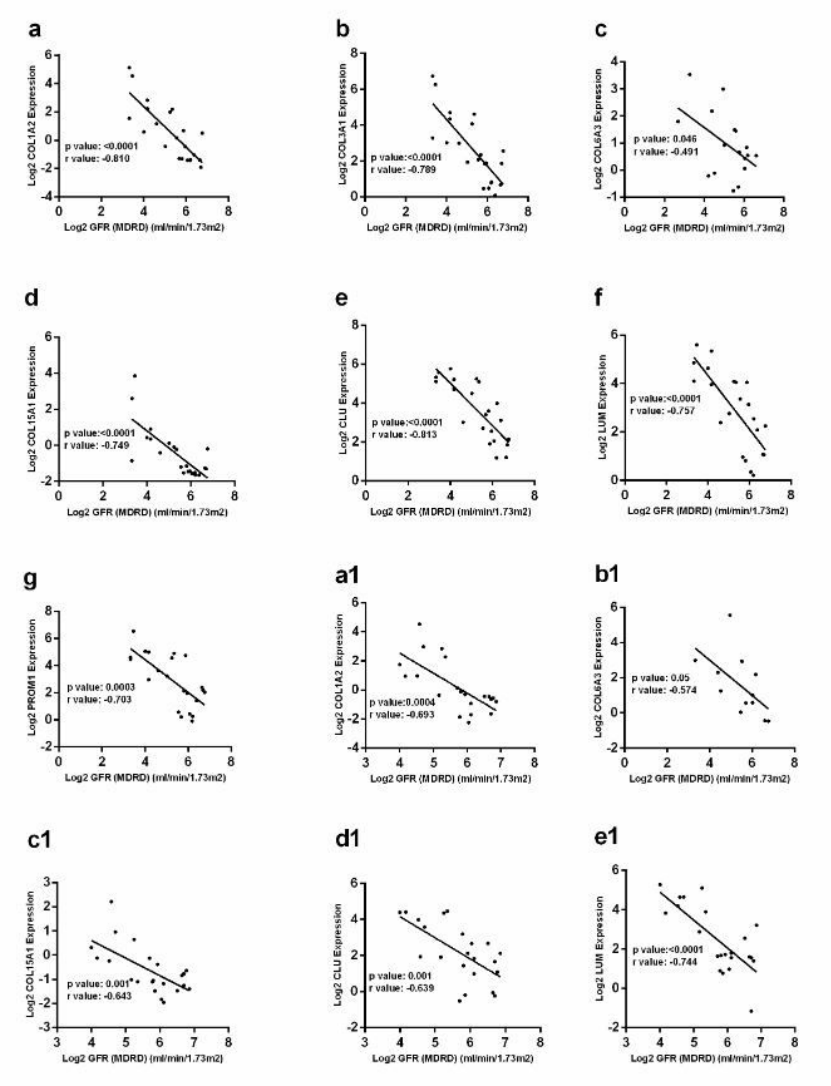
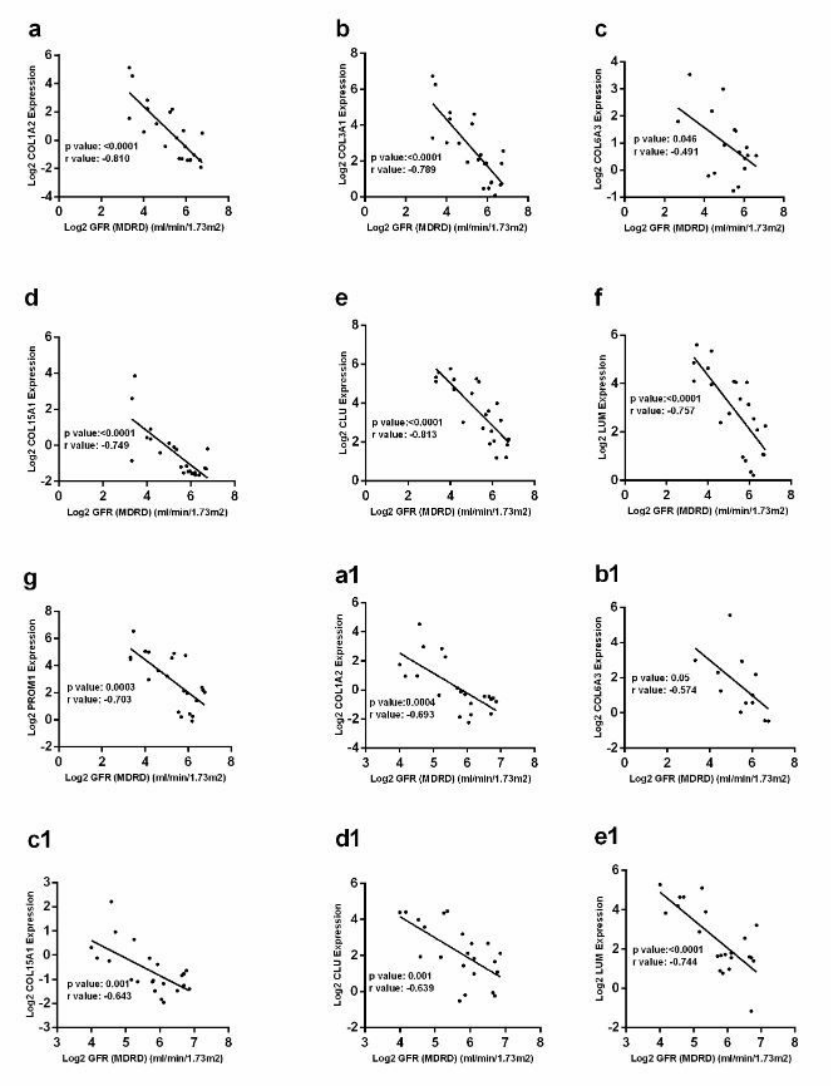


Figure 7

Correlation between up-regulated hub gene mRNA expression and GFR in renal tubulointerstitium of DN patients, r is the correlation coefficient. (a) The expression of COL1A2 negatively correlated with GFR ($p < 0.0001$, $r = -0.810$). (b) The expression of COL3A1 negatively correlated with GFR ($p < 0.0001$, $r = -0.789$). (c) The expression of COL6A3 negatively correlated with GFR ($p = 0.046$, $r = -0.491$). (d) The expression of COL15A1 negatively y correlated with GFR ($p < 0.0001$, $r = -0.749$). (e) The expression of CLU negatively correlated with GFR ($p < 0.0001$, $r = -0.813$). (f) The expression of LUM negatively correlated with GFR ($p < 0.0001$, $r = -0.757$). (g) The expression of PROM1 negatively correlated with GFR ($p = 0.0003$, $r = -0.703$).Correlation between up-regulated hub gene mRNA expression and GFR in glomerulus of DN patients. (a1) The expression of COL1A2 negatively correlated with GFR ($p = 0.0004$, $r = -0.693$). (b1) The expression of COL6A3 negatively correlated with GFR ($p = 0.05$ $r = -0.574$). (c1) The expression of COL15A1 negatively correlated with GFR ($p = 0.001$, $r = -0.643$). (d1) The expression of CLU negatively correlated with GFR ($p = 0.001$, $r = -0.639$). (e1) The expression of LUM negatively correlated with GFR ($p < 0.0001$, $r = -0.744$). $p < 0.05$ was considered statistically significant. CG: Cockcroft Gault; DN: diabetic nephropathy; GFR: glomerular filtration rate; MDRD: modification of diet in renal disease; mRNA: messenger RNA.



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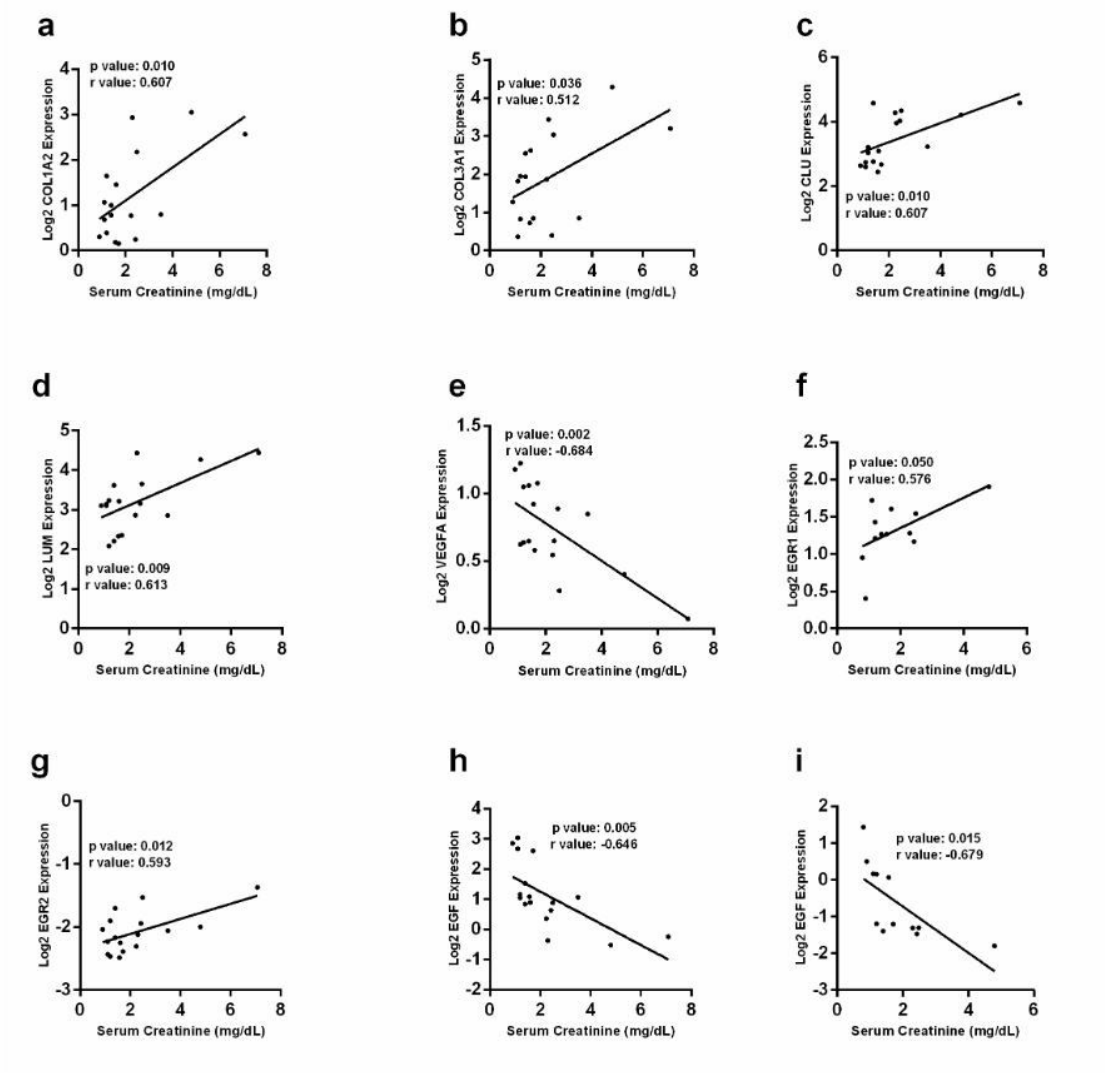
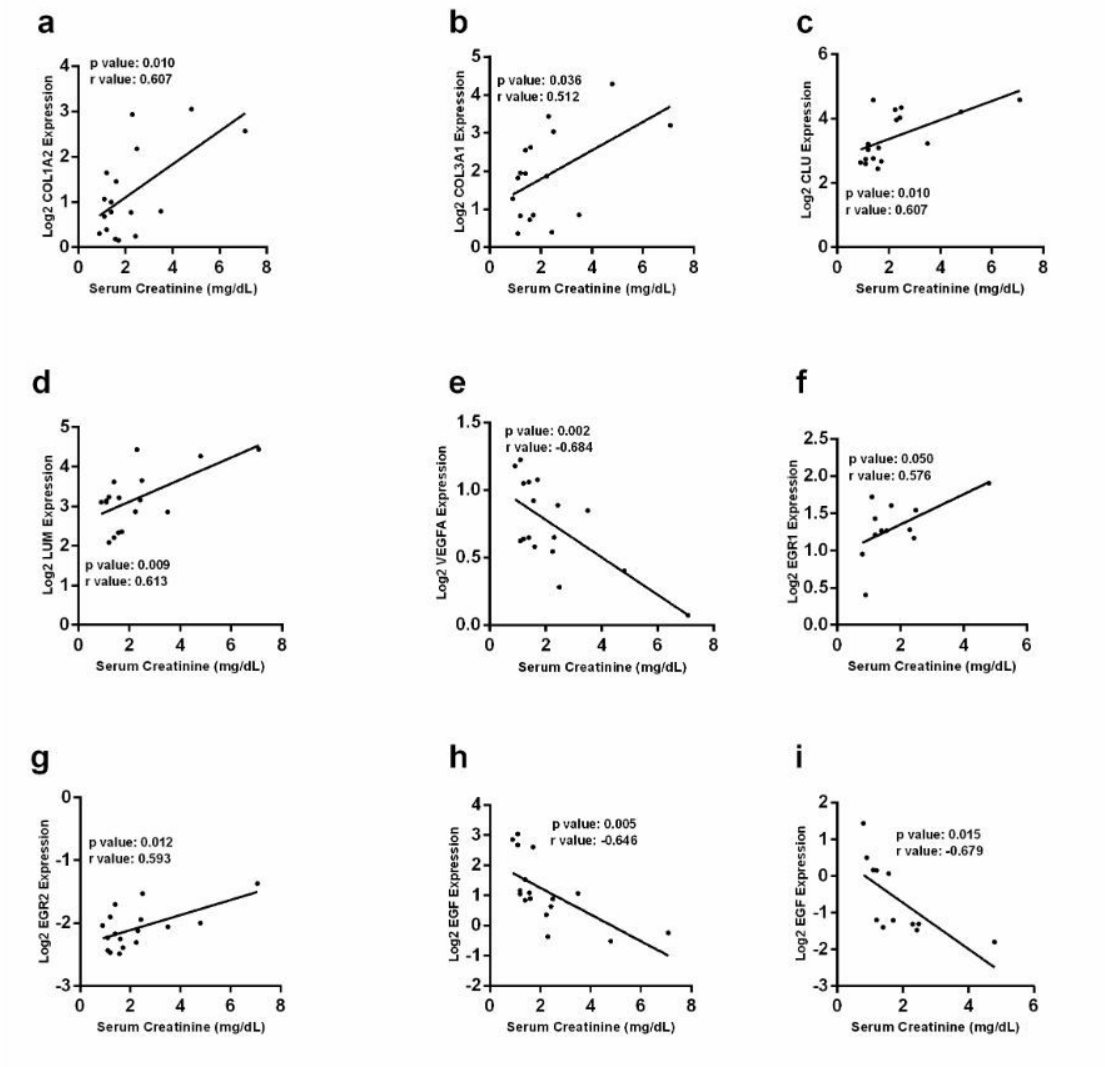


Figure 8

Correlation between mRNA expression of up-regulated hubgenes and SCR in DN patients, r is the correlation coefficient. (a) The expression of COL1A2 positively correlated with SCR (p = 0.010, r = 0.607). (b) The expression of COL3A1 positively correlated with SCR (p = 0.036, r = 0.512). (c) The expression of CLU positively correlated with SCR (p = 0.010, r = 0.607). (d) The expression of LUM positively correlated with SCR (p = 0.009, r = 0.613).Correlation between mRNA expression of down-regulated hubgenes and SCR in DN patients. (e) The expression of VEGFA negatively correlated with SCR (p = 0.002, r = −0.684). (f) The expression of EGR1 positively correlated with SCR (p = 0.050, r = 0.576). (g) The expression of EGR2 positively correlated with SCR (p = 0.012, r = 0.593). (h) The expression of EGF negatively correlated with SCR in tubulointerstitium of DN patients (p = 0.005, r = −0.646). (i) The expression of EGF negatively correlated with SCR in glomerulus of DN patients (p = 0.015 r = −0.679). p< 0.05 was considered statistically significant. DN: diabetic nephropathy; mRNA: messenger RNA; SCR: serum creatine.



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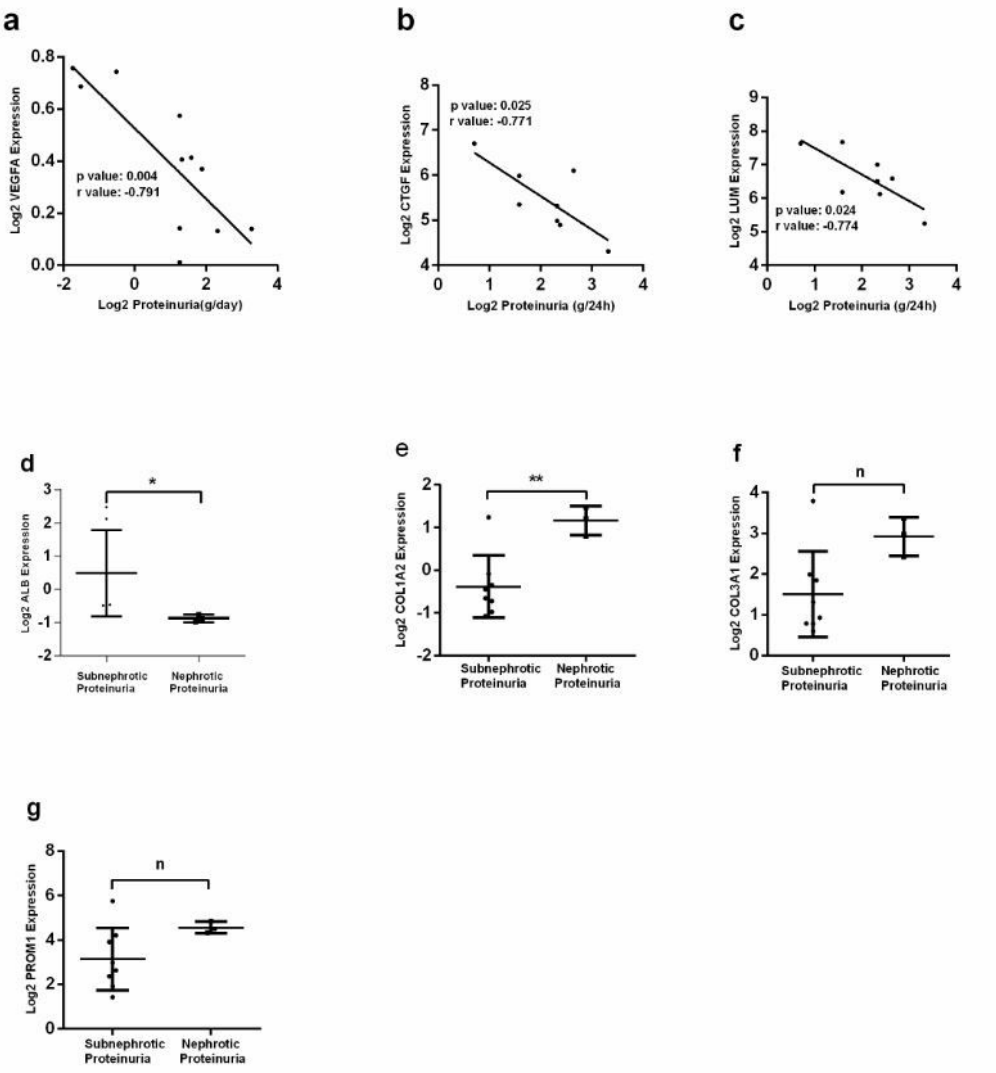

Figure 9

Association between mRNA expression of hub genes and proteinuria in DN patients. (a) The expression of VEGFA negatively correlated with proteinuria ($p = 0.004$, $r = -0.791$). (b) The expression of CTGF negatively correlated with proteinuria ($p = 0.025$, $r = -0.771$). (c) The expression of LUM negatively correlated with proteinuria ($p = 0.024$, $r = -0.774$). (d) The expression of ALB in nephrotic proteinuria group was lower than that of subnephrotic proteinuria group. (e) The expression of COL1A2 in nephrotic proteinuria group was higher than that of subnephrotic proteinuria group. (f) There was no difference in COL3A1 expression between nephrotic proteinuria group and subnephrotic proteinuria group. (g) There was no difference in PROM1 expression between nephrotic proteinuria group and subnephrotic proteinuria group. $p < 0.05$ was considered statistically significant. * $p < 0.05$, ** $p < 0.01$, n means no significant difference . DN: diabetic nephropathy; mRNA: messenger RNA.

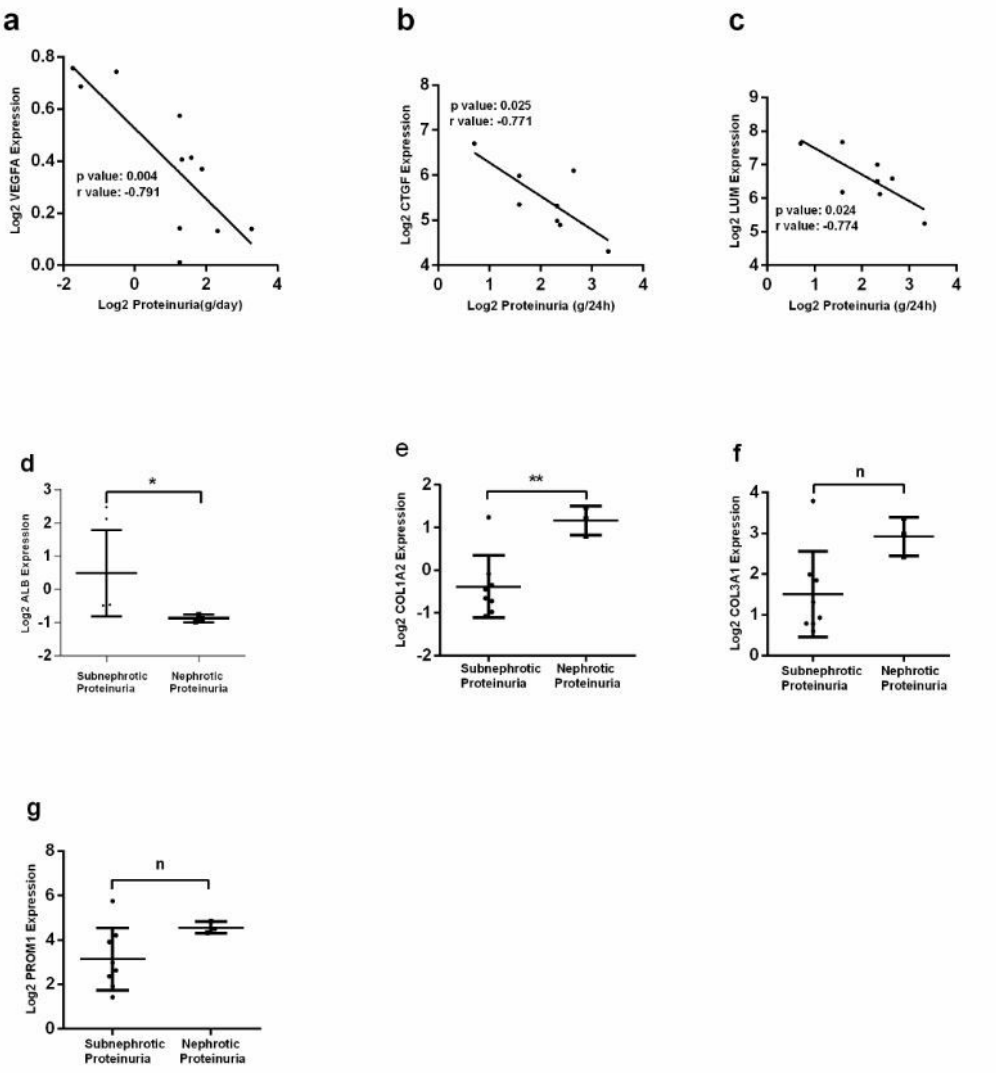

Figure 9

Association between mRNA expression of hub genes and proteinuria in DN patients. (a) The expression of VEGFA negatively correlated with proteinuria ($p = 0.004$, $r = -0.791$). (b) The expression of CTGF negatively correlated with proteinuria ($p = 0.025$, $r = -0.771$). (c) The expression of LUM negatively correlated with proteinuria ($p = 0.024$, $r = -0.774$). (d) The expression of ALB in nephrotic proteinuria group was lower than that of subnephrotic proteinuria group. (e) The expression of COL1A2 in nephrotic proteinuria group was higher than that of subnephrotic proteinuria group. (f) There was no difference in COL3A1 expression between nephrotic proteinuria group and subnephrotic proteinuria group. (g) There was no difference in PROM1 expression between nephrotic proteinuria group and subnephrotic proteinuria group. $p < 0.05$ was considered statistically significant. * $p < 0.05$, ** $p < 0.01$, n means no significant difference . DN: diabetic nephropathy; mRNA: messenger RNA.