



Research paper

RNA-seq reveals Lysyl oxidase as a potential biomarker of glomerular function in diabetic nephropathy in rats

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ABSTRACT

Purpose: We downloaded the gene expression profiles of patients with diabetic nephropathy from the GEO database and combined it with differential gene analysis of rat transcriptome, our study employed animal models to examine the role of key hub genes in diabetic nephropathy and to pinpoint significant gene regulation in this disease.

Methods: An examination of differential expression was performed using the online analysis tool GEO2R and the DN-related datasets GSE30528 and GSE1009 obtained from the GEO database. A comparison of gene expression between the normal and diabetic nephropathy groups was conducted using the RNA-seq technique. We further examined body weight changes and detected the levels of blood glucose, 24-hour urine microalbumin, and expression of IL-6 and TNF- α . We also measured the levels of Lysyl oxidase (LOX) using quantitative real-time PCR and western blotting.

Results: We found that LOX was among the top 10 significantly differentially expressed genes in both the GEO database and transcriptome. Moreover, the levels of fasting blood glucose, 24-h urine microalbumin, and expression of TNF- α and IL-6 were significantly increased in the DN than in the normal group ($P < 0.05$).

Conclusions: Our study demonstrates that the LOX gene is extensively expressed in diabetic nephropathy, with significantly upregulated expression and accompanying notable physiological markers such as TNF- α , IL-6, fasting blood glucose, and 24-hour urine microalbumin. The observed alterations indicate that the LOX gene has a potential biomarker function in the advancement of the disease.

1. Introduction

Among the various diabetic complications, diabetic nephropathy (DN) is the most common and severe cause of end-stage renal failure, affecting 30–45 % of diabetic patients. The presence of albuminuria, glomerulosclerosis, and a gradually increasing decline in renal function defines diabetic nephropathy (Samsu, 2021). Therefore, it is of utmost importance to comprehend the molecular mechanisms underlying DN and discover new targets that can be potentially used for treatment.

Lysyl oxidase (LOX) is a copper-dependent enzyme that catalyzes the last enzymatic process necessary for covalently connecting collagen and elastin molecules in the extracellular matrix. The main function of Lysyl

oxidase is to catalyze the cross-linking of collagen and elastin, thereby increasing the stability and strength of ECM. Evidence suggests that elevated levels of intracellular copper ions stimulate LOX to enhance the process of collagen and elastin crosslinking, therefore contributing to the progression of kidney fibrosis (Laczko and Csiszar, 2020).

Sequencing methods such as RNA-seq have been widely used in revealing pathogenic alterations associated with diabetic nephropathy (DN). Brennan et al. identified gene expression profiles related to TGF- β 1 in renal epithelial cells, which were also observed in human diabetic nephropathy, highlighting the relevance of these findings to the disease pathology (Brennan et al., 2012). In a similar vein, Dominguez et al. utilized RNA-seq to obtain a comprehensive profile of renal transcripts

Abbreviations: LOX, Lysyl oxidase; DN, diabetic nephropathy; RAAS, renin-angiotensin-aldosterone system; AngII, angiotensin II; TGF- β , Transforming growth factor- β ; ACL, anterior cruciate ligament; MCL, medial collateral ligament; TNF- α , tumor necrosis factor- α ; GEO, Gene Expression Omnibus; PPI, protein-protein interaction; STZ, streptozotocin.

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in ZS rats, a diabetic nephropathy model, revealing that the activation of specific renal genes might contribute to iron accumulation in diabetes (Dominguez et al., 2015). Furthermore, Chen et al. investigated the role of long non-coding RNAs (lncRNAs) in mesangial cell proliferation within diabetic nephropathy-associated ceRNA networks using computational analysis and bioinformatics approaches, providing insights into the regulatory mechanisms of lncRNAs in the pathophysiology of DN (Chen et al., 2019).

Similarly, Wang et al. analyzed the podocyte translome in type 2 diabetic nephropathy (T2DN) and compared it with type 1 diabetic nephropathy (T1DN) using translating ribosome affinity purification (TRAP) combined with RNA sequencing. This study revealed significant differences in podocyte mRNA translation profiles between the two forms of diabetic nephropathy (Wang et al., 2021). Moreover, Hu et al. conducted a bioinformatics analysis to identify genes associated with

ferroptosis in DN, emphasizing the importance of understanding cell death mechanisms in the context of DN pathophysiology (Hu et al., 2021).

In a recent study, Liao et al. highlighted the anti-inflammatory effects of mclRBP derived from Momordica Charantia in alleviating diabetic nephropathy, with RNA-seq data showing that mclRBP modulated inflammation-related pathways, particularly the NF- κ B signaling pathway (Liao et al., 2022).

The research on DN and the use of RNA-seq techniques underscore the diverse applications of this technology in delineating gene expression profiles, regulatory processes, and pathogenic mechanisms associated with DN. These studies significantly advance our understanding of the molecular underpinnings of diabetic nephropathy and may pave the way for the development of novel therapeutic strategies to combat this debilitating disease.

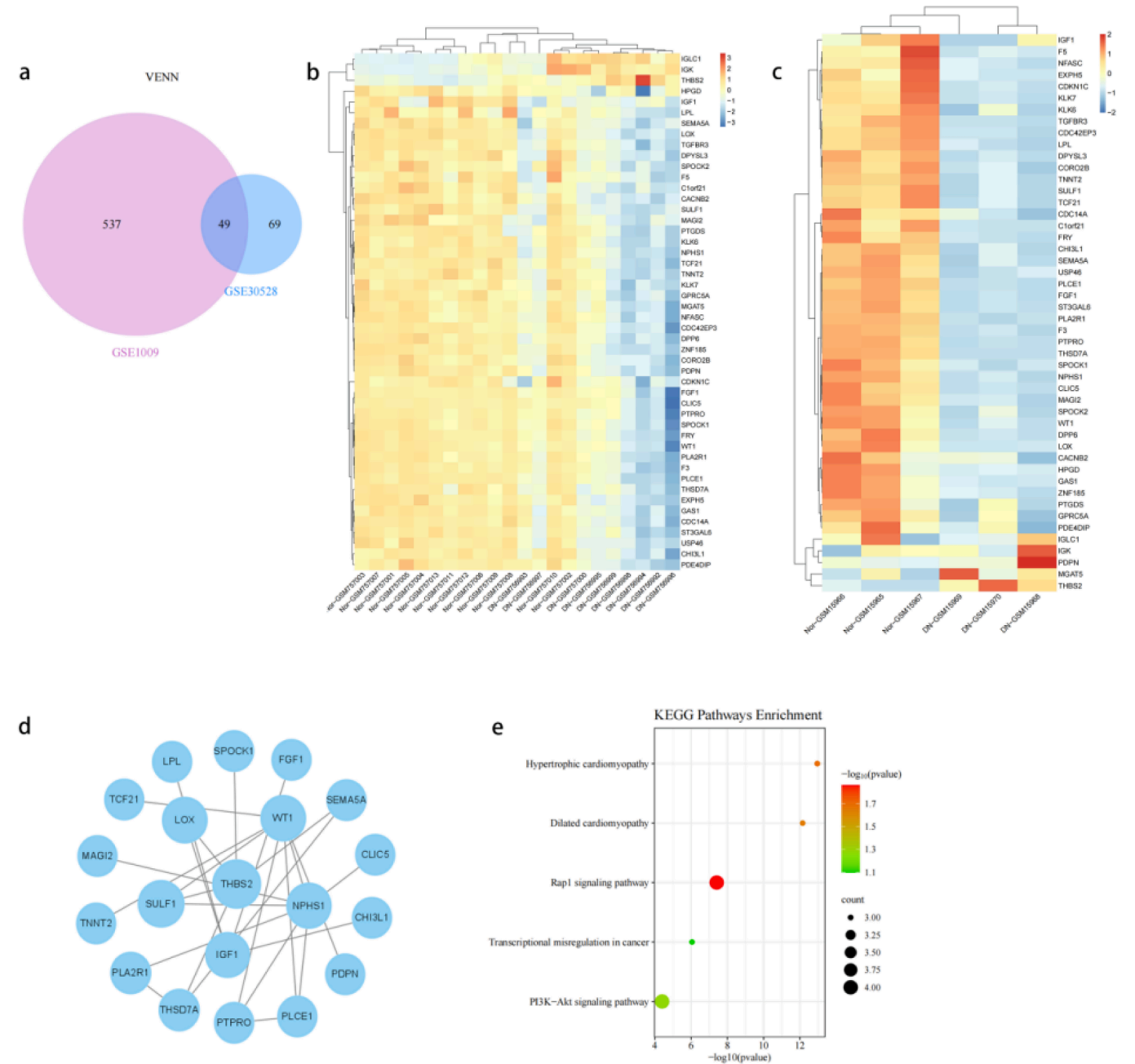


Fig. 1. DEG of Nor and Mod. (a) Venn diagram of therapeutic targets. (b) The heat map of the GSE30528 hub genes. (c) The heat map of the GSE1009 hub genes. (d) PPI network visualization analysis. (e) KEGG pathway enrichment analysis for targets of Nor and Mod.

In this study, we aimed to explore new biomarkers with diagnostic and therapeutic and elucidate the underlying mechanisms in DN development using bioinformatic approaches.

2. Results

2.1. Bioinformatics analysis of diabetic nephropathy using the GEO database

In the differentially expressed gene (DEG) analysis for Nor (normal group) and Mod (diabetic nephropathy group), we identified 589 DEGs in GSE30528, and 118 DEGs in GSE1009, including 49 common differentially expressed target genes. As shown in Fig. 1a and Table 1, we created a Venn diagram to identify the common DEGs in these 2 datasets. The heat map of the expression of hub genes in the 2 GEO datasets is shown in Fig. 1b-c. We then imported a hub of 49 common DEGs into the STRING online database to construct the PPI network, which was visualized using Cytoscape(Fig. 1d, Table 2). As shown in Fig. 1e, KEGG pathway enrichment analysis indicated that these genes were mainly associated with the Rap1 signaling pathway, hypertrophic cardiomyopathy, dilated cardiomyopathy, PI3K-Akt signaling pathway, and transcriptional misregulation in cancer.

2.2. Transcriptional profiles in the kidney of DN rats

Our PCA results showed that there was a certain distance between Nor and Mod, indicating that Nor and Mod samples differed between them(Fig. 2a). We then performed differential analysis and profiled the expression of transcripts from all tested samples. We found that the expression of 839 genes was significantly altered (≥ 2 -fold, $P < 0.05$), with 340 genes being upregulated, whereas 499 genes were down-regulated(Fig. 2b, Table 3). A heatmap of the expression of these DEGs is shown in Fig. 2c. Subsequently, we performed GO annotation to further investigate the functions of the identified DEGs. As shown in Fig. 2d, DEGs were annotated into 3 categories: molecular function, biological process, and cellular component. We observed that the 3 most enriched molecular function terms were retinoic acid 4-hydroxylase activity, catalytic activity, and active transmembrane transporter activity. We also determined that organic acid metabolic process, carboxylic acid metabolic process, response to organic substance, and extrinsic component of plasma membrane, apicolateral plasma membrane, plasma membrane region-basolateral plasma membrane ranked as the 3 most enriched GO terms in the ontologies of biological process and

Table 1

Twenty most significantly up- or down-regulated genes.

group	GSE30528		GSE1009		Up/down
Gene	logFC	pvalue	logFC	pvalue	
SPOCK1	2.7240	0.0000	3.4543	0.0014	Up
LPL	2.1655	0.0001	2.3340	0.0008	Up
TCF21	2.5398	0.0000	3.3949	0.0011	Up
MAGI2	2.6175	0.0000	3.8202	0.0103	Up
TNNT2	3.2378	0.0000	3.3737	0.0009	Up
PLA2R1	2.5850	0.0000	2.1320	0.0305	Up
THSD7A	2.4936	0.0000	2.5810	0.0001	Up
PTPRO	2.8369	0.0000	3.9222	0.0000	Up
PLCE1	3.1025	0.0000	3.9804	0.0000	Up
PDPN	2.6944	0.0000	2.0589	0.0062	Up
CHI3L1	3.5874	0.0000	3.1756	0.0015	Up
CLIC5	2.2199	0.0001	4.3810	0.0000	Up
SEMA5A	2.4304	0.0000	3.9569	0.0000	Up
FGF1	2.5178	0.0002	3.7434	0.0000	Up
LOX	2.9772	0.0000	2.2262	0.0010	Up
SULF1	2.1610	0.0000	3.9564	0.0013	Up
IGF1	2.3269	0.0002	2.2621	0.0181	Up
NPHS1	2.9627	0.0000	2.6165	0.0003	Up
WT1	2.3169	0.0000	2.7639	0.0012	Up
THBS2	-2.0140	0.0000	-3.2084	0.0013	Down

Table 2

Details of intramodular hub proteins.

Protein	Degree	Betweenness	Closeness
CHI3L1	1.0	0.0	0.23
CLIC5	1.0	0.0	0.30
FGF1	1.0	0.0	0.23
IGF1	4.0	102.0	0.29
LOX	2.0	120.0	0.36
LPL	1.0	0.0	0.23
MAGI2	1.0	0.0	0.30
NPHS1	7.0	110.7	0.42
PDPN	1.0	0.0	0.30
PLA2R1	2.0	18.3	0.37
PLCE1	3.0	0.0	0.33
PTPRO	3.0	0.0	0.33
SEMA5A	2.0	0.0	0.32
SPOCK1	1.0	0.0	0.31
SULF1	3.0	143.7	0.45
TCF21	1.0	0.0	0.30
THBS2	5.0	187.7	0.44
THSD7A	3.0	20.3	0.36
TNNT2	1.0	0.0	0.30
WT1	7.0	119.3	0.41

cellular component, respectively. Based on the results of our KEGG pathway enrichment analysis and using a threshold of $P < 0.05$, we selected a total of 16 significantly enriched pathways. We found that these genes were mainly associated with the regulation of the circadian rhythm, arachidonic acid metabolism, PPAR signaling pathway, glycerophospholipid metabolism, regulation of lipolysis in adipocytes, maturity-onset diabetes of the young, TGF-beta signaling pathway, biosynthesis of amino acids, and pentose phosphate pathway(Fig. 2e). We next used the 839 DEGs identified in this study to generate a PPI network using the STRING database(Fig. 2f). Using the PPI network correlation, we obtained the core target genes in the PPI network, and screened the first 10 core targets. The main core targets included Fras1, Cfr, Cyp7a1, Cav2, Vdr, Apoa4, Fgf21, Krt19, Lox, and Gck. Among them, we noticed that LOX was identified as a hub gene in both the GEO database and transcriptome analysis. Therefore, we assumed that this gene might be the key target for DN progression.

2.3. STZ induced increased levels of LOX mRNA in DN rats and was associated with renal alterations

We detected that at 8 weeks after STZ injection, the level of blood glucose of DN rats was increased, whereas the body weight was significantly decreased ($P < 0.05$, Fig. 3a and b), compared with those in the normal group. In addition, we found that DN rats presented albuminuria in their 24-h urine samples, and their levels of IL-6 and TNF- α were significantly increased compared with those in the normal group ($P < 0.01$, $P < 0.05$, $P < 0.01$, Fig. 3c and e-f). We also observed that DN rats developed significant capillary basement membrane thickening, mesangial matrix expansion, reduction of nephron number, glomerular hypertrophy, and inflammatory cell infiltration, as indicated in histological examinations (Fig. 3d). Importantly, these observed changes in kidney tissues were validated using both western blotting and PCR analyses ($P < 0.05$, Fig. 3g and h).

3. Discussion

Diabetes is the main etiology of end-stage renal disease and a well-seen outcome of type 2 diabetes. As DN affects a large worldwide population, with approximately one-third of diabetic individuals presenting nephropathy, the impact that DN causes on a personal and socio-economic level is enormous (Zhang et al., 2020). Hence, elucidating the molecular mechanism of DN is essential for the treatment and prevention of DN.

The LOX family consists of 5 members, namely lysyl oxidase and

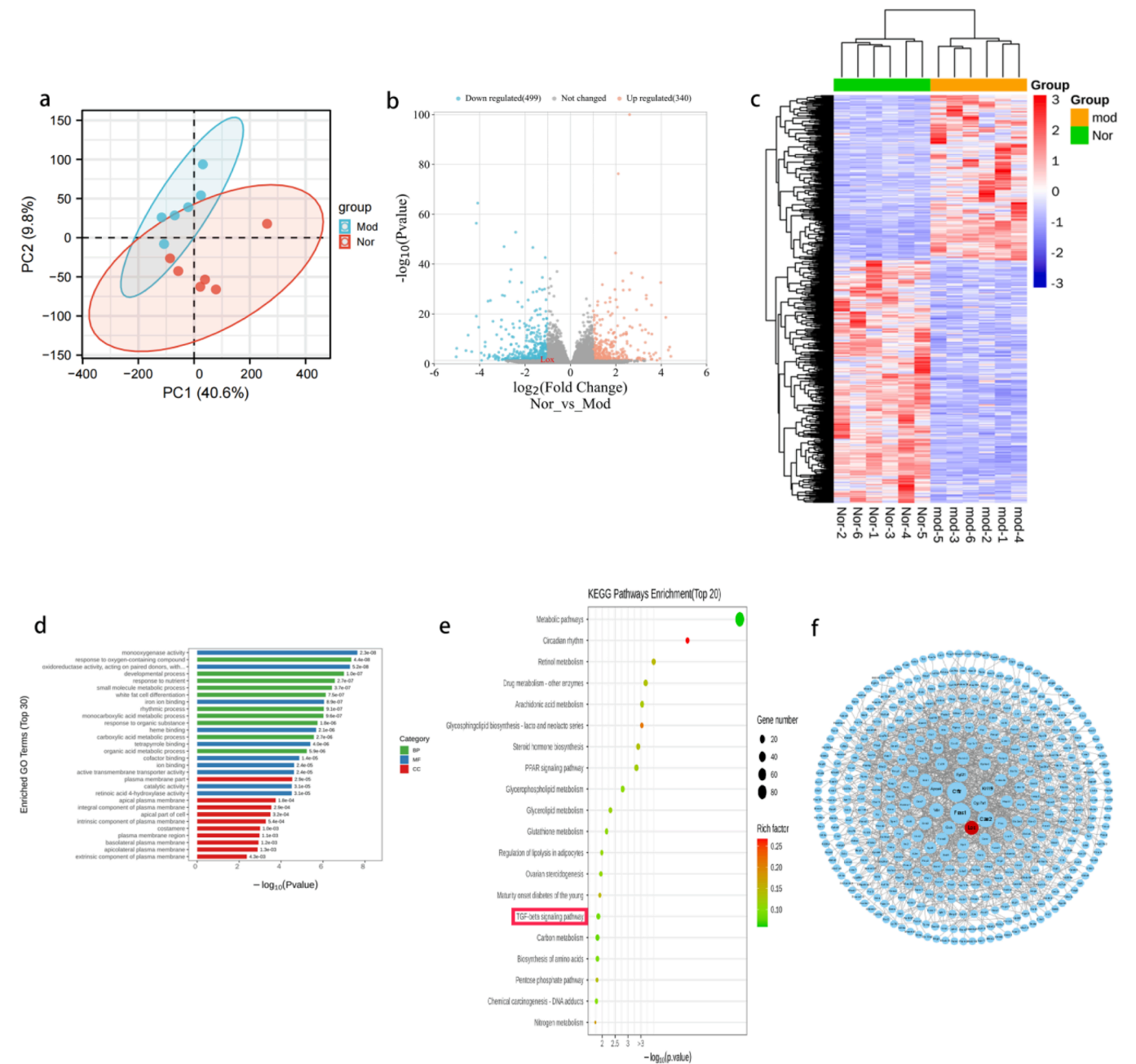


Fig. 2. (a) Principal component analysis of the DEGs. (b) Volcano plot of differentially expressed genes. Genes with a significant change of more than two-fold were selected. Green dot represented up regulated significant genes and red dot represented down regulated significant genes. (c) Heat map of differentially expressed genes. (d) GO annotation results of DEGs. The vertical axis represents the corresponding annotations on the most significantly enriched genes, including biological process, cellular component, and molecular function. (e) Kyoto Encyclopedia of Gene and Genomes (KEGG) pathway enrichment analysis of the DEGs. (f) PPI network visualization analysis.

lysyl oxidase-like 1–4 (LOXL 1–4) (Boufragech et al., 2016). Studies have shown that changes in the expression and function of LOX are correlated with kidney diseases. Studies have shown that changes in the expression and function of LOX are correlated with kidney diseases (Reiser et al., 1992). The renin-angiotensin-aldosterone system (RAAS), particularly angiotensin II (AngII), is critically involved in the development and advancement of diabetic nephropathy DN (Ames et al., 2018). AngII is the main effector molecule of RAAS; Ang II promotes glomerulosclerosis not only by altering glomerular hemodynamics, but also by promoting the proliferation of thylakoid, endothelial, and tubular epithelial cells, as well as the expression and extracellular matrix accumulation of growth factors (Phipps et al., 2021). Stimulation of

AT1R by AngII leads to contraction, hypertrophy, and fibrosis in kidney vessels (Wågsäter et al., 2016). Our study demonstrated various morphological changes in the kidneys, such as the thickening of the DN glomerular basement membrane and increased thylakoid matrix. These changes might be related to the AngII-induced degradation of elastin, damaging the integrity and function of the basement membrane. The LOX protein mediates the transformation of lysin in collagen and pre-elastin to highly active aldehydes that form cross-links and stabilize ECM proteins (especially type I collagen and elastin), hence regulating cell adhesion and invasion (Kagan and Li, 2003). The study by Hong et al. demonstrated that transcription factors, such as C-fos and C-jun in activating cells led to an increase in ECM synthesis, and this

Table 3
Genes up- or down-regulated with significant differences (selected examples).

symbol	log2FoldChange	pvalue	Adjusted P-value
Gcnt2	2.6055	2.59E-104	1.50738E-101
Lmo7	2.1084	5.87E-77	1.70817E-74
Npas2	-4.0833	3.47E-65	6.7318E-63
Arntl	-4.1468	4.34E-57	6.3147E-55
Cyp4f39	-2.4123	1.75E-53	2.037E-51
Csad	-2.926	9.86E-48	9.5642E-46
Pld1	-1.7715	3.23E-18	2.80576E-17
Slc41a3	1.8992	5.25E-08	1575E-07
Gck	-3.7622	1.23E-07	3.5264E-07
Fabp4	1.5492	6.03E-07	1.5127E-06
Phf19	-1.0198	0.0030521	0.00454302
Trim24	1.0588	0.0031526	0.00468065
Lhx1	1.0029	0.0057331	0.00796340
Cnkrs1	1.2179	0.0057478	0.00796481
Twist1	-1.1639	0.0085453	0.01117610
Slc2a4	1.6667	0.0085981	0.01121994
Lox	-1.0157	0.034869	0.03765076
Btg4	-2.0275	0.048631	0.04896755
Ube2c	-1.3962	0.049096	0.04935038
Fut2	-1.4449	0.04969	0.04969

accumulation of ECM was shown to be a sign for kidney physiological changes (Hong et al., 2014). Reduction in the number of damaged nephrons, and increased single-nephron glomerular filtration rate due to individual glomerular hypertrophy also increased the susceptibility of hypertrophied glomeruli to progressive injury. Miller et al. used transgenic rats to demonstrate that a reduction in the number of nephrons was an important factor affecting hypertension onset and susceptibility to diabetes (Miller et al., 2021). The study by chen et al. utilized a type 2 diabetic rat model and found by immunohistochemical staining that the expression of LOX and LOXL2 was significantly higher in renal tissues of the diabetic group than that of the control group, which suggests that LOX and LOXL2 may be key factors, which are pathologically related to inflammation and fibrosis (Chen et al., 2018).

Among the involved pathways identified in our study, the TGF-beta signaling pathway drew our attention. Transforming growth factor-β (TGF-β) has a role in tissue fibrosis by increasing the production of matrix proteins, inhibiting matrix degradation, and altering cell-to-cell junctions (Frangogiannis, 2020).

LOX presents coregulated expression facilitated by TGF-β response promoter elements. Studies have shown that in the fibrotic process, TGF-β communicates through its transmembrane serine/threonine kinase receptors, specifically type I and type II receptors. The signaling pathway results in the phosphorylation of receptor-regulated Smad3, which then moves into the nucleus. Within the nucleus, it has been observed that Smad3 can bind to DNA, thereby exerting control over the transcription process of genes associated with fibrosis. As a result, there has been a significant rise in the expression of LOX mRNA and protein levels (Xu et al., 2019). The purpose of Xie et. al. was to identify the markers of the LOX family in the reaction of the anterior cruciate ligament (ACL) and medial collateral ligament (MCL) to TGF-β (Xie et al., 2012). Xia et. al. show that the levels of mRNA and protein expression of LOX were significantly elevated in response to hypoxia-induced TGF-β activation. Furthermore, they observed that these effects could be reversed by knocking down TGF-β (Xia et al., 2019).

Inflammation is a complex physiological response that includes processes such as the release of inflammatory mediators, the infiltration of immune cells, and tissue repair (Rayego-Mateos et al., 2020). Some studies have shown that the expression and activity of Lysyl oxidase also play an important role in inflammation. For example, some inflammatory mediators (such as TGF-β, IL-1β, and TNF-α, among others) can induce the expression of Lysyl oxidase, thereby increasing the cross-linking of collagen fibers. The researchers, Yang et. al., conducted a study to examine the regulation of tumor necrosis factor-α (TNF-α)-induced lectin-like oxidized low-density lipoprotein receptor-1 (LOX-

1) expression in cultured human aortic smooth muscle cells by 1,25D3 (Yang et al., 2015).

In our investigation, the blood serum concentrations of proinflammatory cytokines TNF-α and IL-6 were markedly elevated in rats with diabetic nephropathy. Similarly, other researchers demonstrated that LOX increased the levels of IL-6 by inducing c-Fos and IL-6 promoter binding. LOX-1 is a receptor of ox-LDL; accordingly, excessive levels of ox-LDL will increase the secretion of proinflammatory adipocytokines (TNF-α and IL-6), in turn exacerbating inflammatory reactions (Nguyen et al., 2021; Santiago-Fernández et al., 2020). In DN animal models, the expression of LOX in kidneys has been reported to be significantly increased at both the protein and mRNA levels, suggesting that LOX might mediate the inflammatory function of pro-inflammatory factors, such as TNF-α and IL-6. The recent study by Ebersson et al. demonstrated that activation of the AT1 receptor by AngII stimulated the expression of TGF-β and its downstream mediator and led to an increase in the levels of LOX (Ebersson et al., 2015). As reported, the LOX inhibitor decreased the AngII-induced inflammation in C57BL/6J mice (Nguyen et al., 2021). NF-κB is an important transcription factor involved in the regulation of inflammatory and immune responses. Studies have shown that activation of the NF-κB signaling pathway can upregulate LOX expression, especially in inflammatory environments. High-sugar or high-fat environments promote the release of pro-inflammatory cytokines through the activation of NF-κB, which increases LOX synthesis and further promotes fibrosis and kidney injury (Sobeh et al., 2020). Feng et al. demonstrated that the TLR4/NF-κB signaling pathway plays a crucial role in the up-regulation of oxLDL-induced expressions of LOX-1 in human umbilical vein endothelial cells (HUVECs) (Feng et al., 2014).

In this study, we performed a combined screening of the two datasets through the GEO database, from which we selected significant genes associated with diabetic nephropathy (DN). By further analyzing the screened genes, we noticed that LOX genes showed significant differential genes in the transcriptome data, which attracted our special attention. Currently, few studies have explored the mechanism of action of LOX in DN. We think that LOX affects both kidney fibrosis and inflammation to a certain extent. In order to validate the function of LOX in DN progression, we designed an experimental animal model for validation. Through this series of experiments, we confirmed that the upregulation of LOX in diabetic nephropathy is not only statistically significant at the molecular level, but also closely related to the pathological features of renal fibrosis. Increased levels of LOX promote the activation of the AngII receptor AT1R in RAAS, leading to ECM deposition and causing fibrosis in the kidney. The levels of IL-6 and TNF-α were increased in DN rats, suggesting that LOX might mediate the inflammatory role of IL-6 and TNF-α. In conclusion, using a combination of GEO and transcriptomic methods, we observed that the levels of expression of LOX were increased in DN rats. Hence, LOX was identified as a new potential treatment target and biological marker in DN. This study helped to further understand the mechanism of DN progression and provided a fundamental contribution to further research aimed at identifying novel therapeutic targets for overcoming DN. This study has certain limitations that need to be considered. Firstly, the sample size of the diabetic nephropathy (DN) rat model utilized in this research was relatively small. Although the findings obtained are valuable, a relatively small sample size might potentially have an impact on the statistical power and generalizability of the results. There is a possibility that a limited sample could lead to some degree of uncertainty and may not fully capture the complexity and diversity of diabetic nephropathy in a broader context. Additionally, the study did not comprehensively address potential confounding factors. For instance, environmental factors such as a high-fat diet, which are known to be associated with diabetic nephropathy and could potentially influence lysyl oxidase (LOX) expression and the progression of the disease, were not adequately accounted for. Future investigations could focus on increasing the sample size to enhance the robustness of the data and implementing more stringent control measures for these confounding

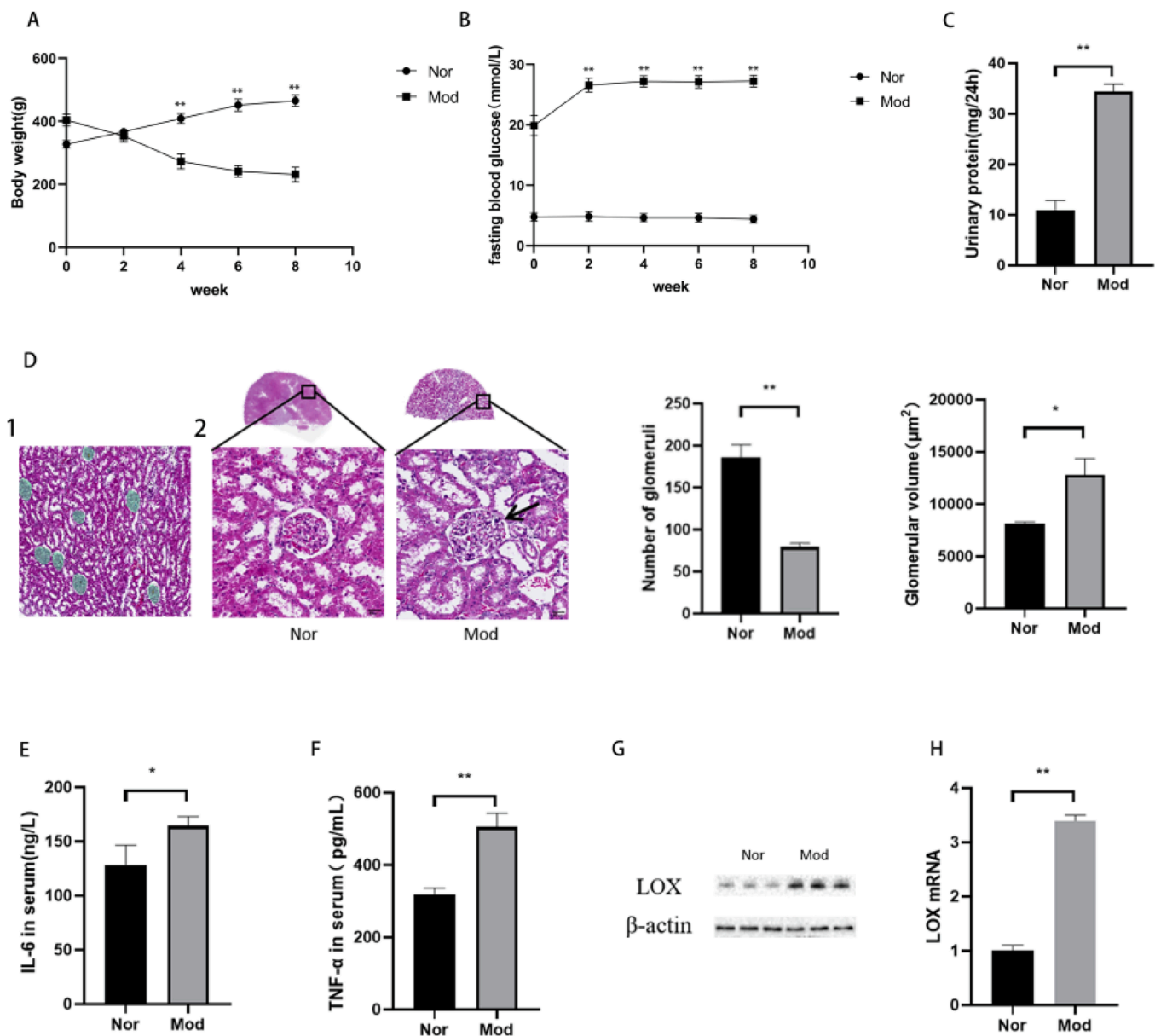


Fig. 3. (a) body weight changes. (b) Fasting Blood glucose. (c) 24 h proteinuria. (d) (1) Histological images. 1.Glomerulus. 2.Afferent arteriole. 3.Efferent arteriole. (2) HE staining of renal sections ($\times 40$). Black arrow: basement membrane. (e) IL-6 levels. (F) TNF- α levels. (g) Protein levels of LOX. (h) mRNA expression of LOX was detected by RT-qPCR. (* $P < 0.05$, ** $P < 0.01$).

variables. This would contribute to improving the precision, reliability, and clinical applicability of the research outcomes, providing a more comprehensive understanding of the mechanisms underlying diabetic nephropathy and facilitating the development of more effective therapeutic strategies and more clinical samples are warranted to validate these results.

4. Materials and methods

4.1. GEO bioinformatic analysis

The genomic sequences GSE30528 and GSE1009 were obtained by searching the Gene Expression Omnibus (GEO) database (GEO, <http://www.ncbi.nlm.nih.gov/geo/>). Comparative analysis of target gene expression in normal renal tissues and patients with diabetic nephropathy was conducted using the GEO2R program (<https://www.ncbi.nlm.nih.gov/geo/geo2r>) (Hunt et al., 2022). The cut-off criteria were $|\log_2 FC| > 2$ and $P < 0.05$. The analysis of differentially expressed genes

(DEGs) was performed using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) database (DAVID, <https://david.ncifcrf.gov/>). The research involved the annotation of Gene Ontology (GO) and the analysis of pathway enrichment using the Kyoto Encyclopedia of Genes and Genomes (KEGG). The analysis of protein-protein interactions (PPIs) of differentially expressed genes (DEGs) was performed using Cytoscape (v3.8.0) within the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database (STRING, <http://string-db.org/>), and the centrality analysis was employed to identify the hub genes in the protein-protein interaction (PPI) network.

4.2. Animal experiments

16 Male Sprague-Dawley rats weighing 260 ± 20 g were used in this study. The animals were accommodated and acclimated in an air-conditioned room at 22°C maintaining a 12-hour light/dark cycle and having unrestricted access to food and water. Suitable feeding for one week. Eight rats were randomly selected as the normal group and the

rest as the model group. The normal group was fed maintenance chow, and the model group was put into high-fat chow. Four weeks after the induction of a high-fat diet, diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ, 35 mg/kg) (Noshahr et al., 2020), freshly dissolved in citrate buffer (0.1 mol/L). Blood was collected from the tail vein 48 h after the STZ intraperitoneal injection, followed by random blood glucose testing for 3 consecutive days to confirm that the levels of glucose were greater than 16.7 mmol/L (Wang et al., 2021). In addition, the 24 h urine volume was recorded and 24 h urine protein was measured in rats continuing with high-fat feeding for 8 weeks. The successful generation of the DN model was verified when the urine protein volume was > 30 mg/24 h (Yi and OuYang, 2019). Rats were then randomly divided into 2 groups: Normal (n = 8) and diabetic nephropathy model (n = 8). Monitor their weight and fasting blood glucose every 2 weeks. At the end of the study, rats were euthanized using an anesthetic overdose of isoflurane, and blood samples were obtained through the abdominal aorta. Subsequently, the kidneys of each rat were promptly extracted. The right kidney was preserved in a 4 % paraformaldehyde solution for histological examinations, while the left kidney was stored at -80°C for biochemical investigations. Additionally, the liver of each rat was dissected and immediately placed in liquid nitrogen for subsequent RNA-seq analysis. The feces samples were also collected and stored at -80°C for 16S rRNA gene sequencing to assess the gut microbiota composition. All experimental protocols and studies were approved by the animal experiment committee of North China University of Science and technology University(2022028).

4.3. Transcriptomics analysis

The liver was used to isolate total RNA using TRIzol reagent (15596026, manufactured by Thermo Fisher Scientific). After validation using an Agilent 1260 Bioanalyzer system from Agilent Technologies, the total RNA was subjected to sequencing library construction using the NEBNext Ultra RNA Library Prep Kit from Illumina. The preprocessing of raw readings was conducted using the FastQC program. The transcript expression levels were determined by analysis of fragments per kilobase per million reads (FPKM) values and quantified using the RSEM program. The EdgeR software was utilized in this study to identify differentially expressed genes (DEGs). The functional enrichment study was conducted by mapping all differentially expressed genes (DEGs) to terms in the Gene Ontology (GO) databases. Following that, we identified significantly enriched Gene Ontology (GO) concepts among the differentially expressed genes (DEGs) using a threshold of $P < 0.05$. The categorization of GO terms was conducted into three subgroups: biological process (BP), cellular component (CC), and molecular function (MF). By applying a significance threshold of $P < 0.05$, we mapped all genes that showed differential expression (DEGs) to the KEGG database. Subsequently, we searched to identify KEGG pathways that were highly enriched with these DEGs.

4.4. Data processing methods

Heatmaps and volcano plots were used in this work to visualize significantly differentially expressed genes (DEGs). The web platform <https://www.bioinformatics.com.cn> was used to construct all of the graphics. After importing the gene names, log2FC values, and P values to create volcano plots, the platform was used to create heatmaps using the FPKM values of the two sets of samples. In the meantime, the Venn diagram tool in the “Weishengxin” platform was utilized to import the datasets and draw the intersection in order to determine which genes were shared by the two datasets. Gene ontology (GO) annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were performed for DEGs in order to functionally annotate them. The GO annotation encompassed molecular functions (MF), cellular components (CC), and biological processes (BP). A $P < 0.05$ was established as the threshold of substantial enrichment after gene names

and log2FC values were loaded into the “Weishengxin” platform for enrichment analysis. For enrichment analysis, the gene names and log2FC values were imported into the “Weishengxin” platform.

4.5. Determination of biochemical parameters

At the end of 8 weeks, rats were housed in individual metabolic cages for the collection of 24-h urine samples. The amount of protein in the urine sample was measured using a BCA protein quantification kit (AR0146, BOSTER, China). Plasma samples were used to detect the levels of IL-6 (YD-30219, lunchangshuo, China) and TNF- α (JM-02415 M1, jingmei, China).

4.6. Renal Histology

The renal tissues were preserved in a 4 % paraformaldehyde solution and then embedded in paraffin for further analysis. The tissue slices, which were four micrometers thick, were stained using the hematoxylin and eosin (HE) method. Image acquisition and analysis were performed using the TissueFAXS fluorescence and brightfield microscope system and StrataQuest software, respectively.

4.7. Western blotting

The BCA Protein assay kit was utilized for the biochemical analysis of total proteins. The electrophoresis of sodium dodecyl sulfate (SDS) was performed using a polyacrylamide gel. The resulting protein bands were then transferred onto a polyvinylidene difluoride membrane. The membranes were blocked with 3 % BSA(BS102, biosharp, China) followed by primary antibodies recognizing LOX (1:500, AF7392, Beyotime Biotech, Shanghai, China), GAPDH(1:5000, AF1186, Beyotime Biotech, Shanghai, China) and incubated at 4°C overnight. After incubation with the secondary antibodies(SA00004-2, proteintech, China) at room temperature for 2h, the membranes were soaked with enhanced chemiluminescence (ECL)(AP34L024-B, iLAB BIO, Shanghai, China) reagent and exposed to X-ray film. Quantitative protein analysis was performed using ImageJ(NIH, Bethesda, MD, USA).

4.8. RNA isolation and quantitative real-time PCR (qRT-PCR)

The extraction of total RNA from liver tissues was conducted using an RNAeasy™ Animal RNA Isolation Kit with Spin Column (R0027, Beyotime Biotech, Shanghai, China). The reverse transcription of the cDNA was conducted by employing a BeyoRT™ First Strand cDNA Synthesis Kit (RNase H minus) (D7168, Beyotime Biotechnology, Shanghai, China) with 1 μg of the isolated RNA. The cDNA was used as a template for a quantitative reverse transcription-PCR (qRT-PCR) assay using a TB Green Premix Ex Taq (RR820A, Takara, Beijing, China), following the manufacturer's instructions. The primer sequences used in this study were as follows:

Rattus LOX Forward primer (5'to 3'):CAAGTTGGTTTCTCGCCGTC
Reverse primer (5'to 3'):TGTACGTACGTGGATGCCTG
Rattus β -actin Forward primer (5'to 3'):CCGCGAGTACAACCTTCTT
Reverse primer (5'to 3'):AGGGTCAGGATGCCTCTCTT

4.9. Statistical analysis

All experimental data were analyzed using SPSS (version 25.0; IBM). Data were presented as the mean $\pm$ standard deviation (SD). The Student's t-test was used to analyze the differences between groups. Values of $P < 0.05$ were considered statistically significant.

Contributors

Liu JX and Bai SF contributed equally to this work. Fu QR performed experiments. La XJ designed the study, supervised the project and

contributed to the preparation of the manuscript. Gao XJ supervised the project and contributed to the preparation of the manuscript. All authors reviewed and approved the final version of the manuscript.

CRedit authorship contribution statement

Jiaxuan Liu: Writing – original draft, Visualization, Data curation. **Sufen Bai:** Project administration, Methodology, Conceptualization. **Chenxi Wu:** Writing – review & editing, Software. **Chunyu Tian:** Formal analysis, Data curation. **Qianru Fu:** Methodology. **Xiujuan Gao:** Writing – review & editing, Visualization, Supervision. **Biwei Zhang:** Visualization, Validation. **Ji'an Li:** Resources, Project administration. **Xiaojin La:** Writing – review & editing, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Ji'an li reports financial support was provided by Science and Technology Partnership Program, Ministry of Science and Technology of China. Ji'an li reports financial support was provided by Tangshan Science and Technology Innovation Team Training Program. Ji'an li reports financial support was provided by Science and Technology Planning Project of Hebei Province, China. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data sharing statement

This study benefited from the GEO databases. We appreciate the data platform, and the authors uploaded their data.

Data availability

Data will be made available on request.

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