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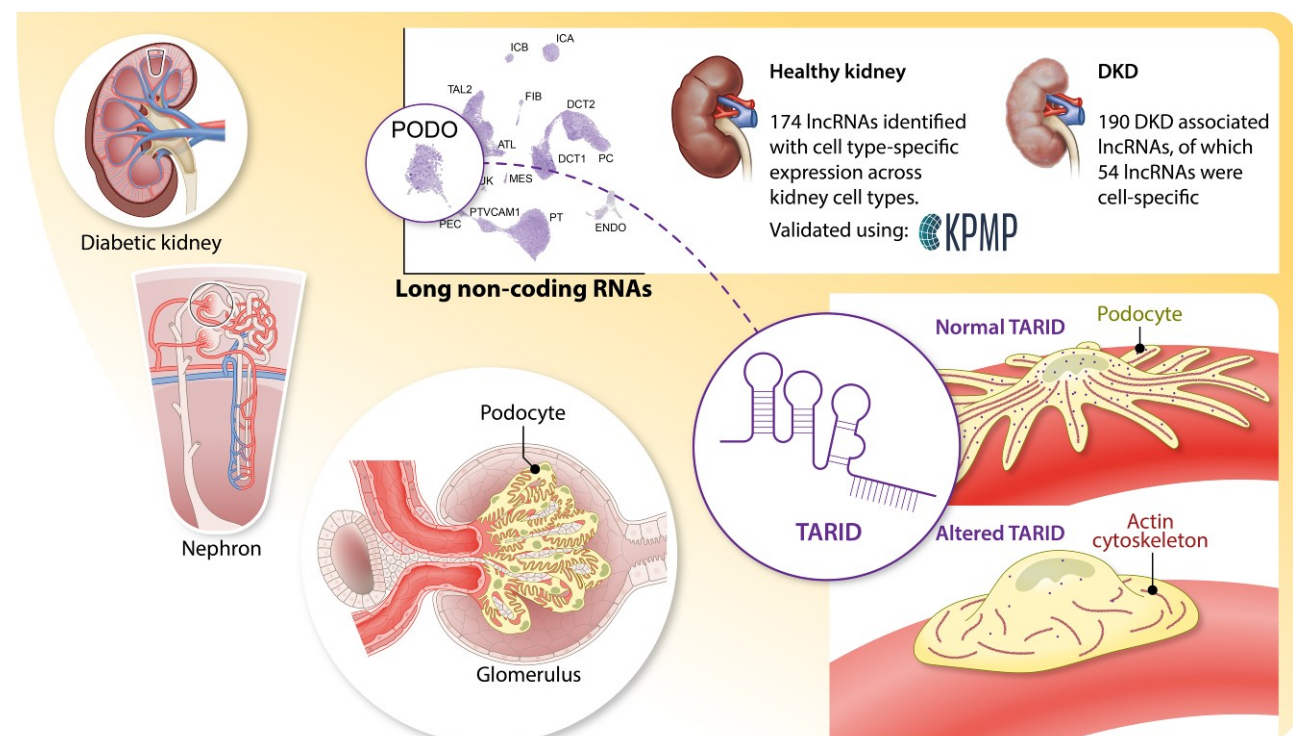
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ATL, ascending thin limb; DCT1, early distal convoluted tubule; DCT2, late distal convoluted tubule; DKD, diabetic kidney disease; ENDO, endothelial cells; FIB, fibroblasts; ICA, type A intercalated cells; ICB, type B intercalated cells; KPMP, Kidney Precision Medicine Project; LEUK, leukocytes; lncRNA, long noncoding RNA; MES, mesangial cells and vascular smooth muscle cells; PC, principal cells; PEC, parietal epithelial cells; PODO, podocytes; PT, proximal tubule; PT_VCAM1, VCAM1⁺ proximal tubule; TAL1, CLDN16⁺ thick ascending limb; TAL2, CLDN16⁺ thick ascending limb; TARID, *TCF21* antisense RNA inducing promoter demethylation.



Cell Type–Specific Expression of Long Noncoding RNAs in Human Diabetic Kidneys Identifies TARID as a Key Regulator of Podocyte Function

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Long noncoding RNAs (lncRNAs) play essential roles in cellular processes, often exhibiting cell type–specific expression and influencing kidney function. While single-cell RNA sequencing (scRNA-seq) has advanced our understanding of cellular specificity, past studies focus solely on protein-coding genes. We hypothesize that lncRNAs, due to their cell-specific nature, have crucial functions within particular renal cells and thereby play essential roles in renal cell function and disease. Using single-nucleus RNA-seq (snRNA-seq) data from kidney samples of five healthy individuals and six patients with diabetic kidney disease (DKD), we explored the noncoding transcriptome. Cell type–specific lncRNAs were identified, and their differential expression in DKD was assessed. Integrative analyses included expression quantitative trait loci (eQTL), genome-wide association studies (GWAS) for estimated glomerular filtration rate (eGFR), and gene regulatory networks. Functional studies focused on *TCF21* antisense RNA inducing promoter demethylation (*TARID*), a lncRNA with podocyte-specific expression, to elucidate its role in podocyte health. We identified 174 lncRNAs with cell type–specific expression across kidney cell types. Of these, 54 lncRNAs were differentially expressed in DKD. Integrative analyses, including eQTL data, GWAS results for eGFR, and gene regulatory networks,

ARTICLE HIGHLIGHTS

- This study provides a resource for kidney (cell type–specific) long noncoding (lnc)RNA expression and demonstrates the importance of lncRNAs in renal health.
- We identified 174 cell type–specific lncRNAs in the human kidney, with 54 showing altered expression in diabetic kidney disease.
- *TCF21* antisense RNA inducing promoter demethylation (*TARID*), a podocyte-specific lncRNA upregulated in diabetic kidney disease, is crucial for actin cytoskeleton reorganization in podocytes.

pinpointed *TARID*, a podocyte-specific lncRNA, as a key candidate upregulated in DKD. Functional studies confirmed *TARID*'s podocyte-specific expression and revealed its central role in actin cytoskeleton reorganization. Our study provides a comprehensive resource of single-cell lncRNA expression in the human kidney and highlights the importance of cell type–specific lncRNAs in kidney function and disease. Specifically, we demonstrate the functional relevance of *TARID* in podocyte health.

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Kidney disease remains a major health challenge with significant morbidity and mortality worldwide (1). Genome-wide association studies (GWAS) have provided valuable insights into the genetic underpinnings of kidney disease, highlighting key loci and potential biomarkers for disease susceptibility (2). Recent research has further expanded our understanding of the human genome, revealing that ~76–97% of it consists of noncoding sequences (3). Among these, long noncoding RNAs (lncRNAs) have emerged as key regulatory molecules involved in a wide range of cellular processes, including gene expression, epigenetic modulation, and disease pathogenesis (4). Notably, lncRNAs are increasingly recognized for their cell type-specific functions (5). Numerous lncRNAs have been implicated in kidney function regulation. Among them, metastasis-associated lung adenocarcinoma transcript 1 (*MALAT1*) stands out as a significant regulator in diabetic kidney disease (DKD) (6). Silencing *MALAT1* with siRNA has been shown to partially restore podocyte function, highlighting its role in kidney health (7). Similarly, nuclear paraspeckle assembly transcript 1 (*NEAT1*) is associated with renal inflammation and fibrosis, especially in DKD and chronic kidney disease, where it exacerbates inflammation by modulating immune-related gene expression (8). Additionally, the kidney-specific transforming growth factor- β /SMAD3-interacting lnc-RNA (*lnc-TSI*), inhibits renal fibrogenesis by impeding the transforming growth factor- β /SMAD3 pathway (9). Despite the growing interest in kidney-related lncRNAs, the specific functions and mechanisms many remain poorly understood, in part due to limited knowledge of their kidney cell type-specific expression and complex regulatory roles. Moreover, only a fraction of kidney-associated lncRNAs has been studied, leaving much of the lncRNA landscape in renal biology unexplored.

Single-cell RNA sequencing (scRNA-seq) as well as single nucleus RNA-sequencing (snRNA-seq) offer unprecedented resolution for exploring the transcriptomic landscape of the kidney and other tissues, enabling the dissection of gene expression profiles at the level of individual cell types. While previous studies have primarily focused on protein-coding genes, the rich lncRNA content of these data sets remains underexplored (10). Given the tissue specificity and functional significance of lncRNAs in kidney physiology, a focus on lncRNAs in scRNA-seq data sets is warranted to uncover their roles in both normal kidney function and disease states, including DKD.

This study aims to reassess a snRNA-seq data set of the human kidney (10), placing particular emphasis on the identification and characterization of lncRNAs. By leveraging the high-resolution data from snRNA-seq, we seek to determine cell type-specific expression of lncRNAs, investigate their potential associations with DKD, and integrate this information with genetic and regulatory network data to identify key lncRNAs involved in kidney function and pathology. Specifically, we focus on cell type-specific lncRNAs associated with DKD, a major complication of diabetes, which leads to progressive kidney dysfunction and is a leading cause of end-stage

kidney disease worldwide (11). Through this reanalysis, we aim to shed light on the underexplored landscape of lncRNAs in the kidney, providing novel insights into their potential roles in disease progression and offering new avenues for therapeutic targeting in DKD and other kidney-related disorders.

RESEARCH DESIGN AND METHODS

snRNA-seq

We reanalyzed our publicly available snRNA-seq data set (Gene Expression Omnibus [GEO] accession numbers GSE195460, GSE151302 and GSE131882) to focus on lncRNAs (10). Eleven kidney cortex samples were obtained from control participants ($n = 5$) and patients with DKD ($n = 6$). Tissue samples were collected either after nephrectomy for renal mass ($n = 10$) or from deceased organ donors ($n = 1$). For comprehensive details on data processing, cutoff thresholds, and cell quality control, please refer to the original publication by Wilson et al. (10). Briefly, snRNA-seq was conducted on 11 libraries using 10x Genomics, sequenced on an Illumina NovaSeq platform, and processed with Cell Ranger, Seurat (remove low-quality nuclei [features >500, features <5,000, RNA count <16,000, %Mitochondrial genes <0.5, %Ribosomal protein large or small subunits <0.3]), DoubletFinder (remove heterotypic doublets, assuming 6% of barcodes represent doublets), and Harmony for quality control, normalization, batch correction, clustering, and differential expression analysis. This workflow resulted in 39,176 high-quality nuclei from the kidney cortex. We focused on lncRNAs annotated in GENCODE version 32 (GRCh38, Ensembl release 98), the same version used in the previous study, to capture the non-coding transcriptome. For consistency, we used the Uniform Manifold Approximation and Projection (UMAP) clustering from the original study (10). Details on patient characteristics are also available in the original publication (10). Cell type-specific lncRNAs, defined by significantly higher expression in a single-cell type compared with others, and DKD-associated (per cell type) lncRNAs, were identified using the Seurat *FindMarkers()* function, focusing on transcripts detected in at least 25% of cells and with an effect size of $\geq 0.25 \log_2$ fold change ($\log_2$ FC). Statistical significance was determined using Bonferroni-adjusted P values, with a threshold of false discovery rate of <0.05. We used publicly available Kidney Precision Medicine Project (KPMP) data to validate our cell type-specific lncRNAs. This data set included single-cell RNA-seq data from 28 healthy individuals (12). Gene regulatory networks were constructed by calculating Pearson correlation coefficients to identify associations between gene expression profiles. Pathway enrichment was conducted with Reactome Pathway Analysis (PA) and Kyoto Encyclopedia of Genes and Genomes (KEGG) using clusterProfiler (v4.12.0) and ReactomePA (v1.48.0). All snRNA-seq analyses were performed using R (v4.4.0) and the R package Seurat (v5.1.0).

Expression Quantitative Trait Loci/GWAS Estimated Glomerular Filtration Rate Signals

To identify lncRNAs that may be causally related to kidney function, we used a publicly available GWAS (cat. no. GCST90019506) of estimated glomerular filtration rate (eGFR) traits. The GWAS included 349 significantly ($P < 5 \times 10^{-8}$) associated single nucleotide polymorphisms (SNPs) with eGFR. The Genotype-Tissue Expression (GTEx) database was used to screen for *cis* expression quantitative trait loci (*cis*-eQTLs). In addition, we validated key findings using an expanded GWAS meta-analysis of eGFR based on serum creatinine (eGFR_{crea}) and the Human Kidney eQTL Atlas from the same study (13).

In Situ Hybridization

Human formalin-fixed paraffin-embedded (FFPE) healthy kidney tissue of 4 μ m was stained with in situ hybridization, which was performed following the manufacturer's protocol (RNAscope Multiplex Fluorescent Reagent Kit v2). A custom probe for *TCF21* antisense RNA inducing promoter demethylation (*TARID*) on channel C3 was used (Hs-TARID-C3). To show podocyte specificity, we used a probe for *WT1* on channel 2 (Hs-WT1-C2).

Podocyte-Specific Expression of *TARID* in Kidney Organoids and Other scRNA-Seq Data Sets

TARID expression was analyzed in two independent scRNA-seq data sets. These data sets included untransplanted and vascularized transplanted kidney organoids from Koning et al. (14) and are available in the ArrayExpress repository under accession number E-MTAB-11429. Human-induced pluripotent stem cells were differentiated into kidney organoids following a previously described protocol (14). Briefly, human induced pluripotent stem cells were cultured as a monolayer for 7 days, then dissociated and transferred as 3-dimensional clumps onto a Transwell (0.4- μ m pore polyester membranes). APEL2 medium with recombinant human fibroblast growth factor-9 and heparin was added until 7 + 5 days. From day 7 + 5 onward, growth factors were removed, and the APEL2 medium was refreshed every other day for the remainder of the culture period. Organoids were cultured until transplantation in chicken embryos (day 7 + 12) or until day 7 + 20, coinciding with the harvest of transplanted organoids. scRNA-seq data and metadata from kidney organoids—both untransplanted and transplanted—were processed using R (v4.2.1) with the Seurat R package (v4.2.0). Specifically, the data were grouped according to the “Final_Clusters” and “transplantation” metadata information. Scaled expression values and percentage of cells expressing *TARID* were obtained for each cell identity and transplantation condition using the *DotPlot()* function. The final dotplot was prepared with the *ggplot2* package (v3.4.2) using the *geom_point()* function. Additional scRNA-seq data sets from the Kidney Interactive Transcriptome database were examined (15). These included publicly available data sets from human kidneys and kidney organoids. *TARID* expression was also examined in other kidney diseases using the CZ CELLxGENE portal (16).

Additional Glomeruli Samples

Microdissected glomeruli from a cohort of DKD patients with frozen biopsy specimens, described by Baelde et al. (17), were used to perform a real-time quantitative (q)PCR to determine *TARID* levels. These biopsy specimens were obtained from the pathology archives of the Leiden University Medical Center and the Institute for Clinical Pathology, Heidelberg. All patients with DKD ($n = 22$) were diagnosed with type 2 diabetes. The control group ($n = 10$) consisted of the nonaffected part of tumor nephrectomy samples or cadaver donor kidneys unsuitable for transplantation for technical reasons. The PALM Laser-MicroBeam System was used for microdissection of glomeruli from renal biopsy specimens (three 4- μ m cryosections). After selection of the glomeruli (20 ± 5 glomeruli from each specimen), they were dissected with a laser beam, followed by RNA isolation with TRIzol. cDNA was synthesized from the glomerular RNA using reverse transcription. The *TARID* RNA level was determined and corrected to the housekeeping genes *GAPDH*, and hypoxanthine phosphoribosyl transferase (*HPRT*). The number of podocytes was quantified by calculating the mean of the gene expression levels of podocyte-specific genes *NPHS1*, *NPHS2*, and *WT1*. Sirius Red staining was used to quantify the amount of interstitial fibrosis and served as a histological marker for the progression of renal disease.

In Vitro Experiments lncRNA *TARID*

TARID expression was downregulated with three antisense locked nucleic acid (LNA) oligonucleotides (Gapmers) targeting *TARID* (QIAGEN). We provided the sequence of *TARID* for which three LNA Gapmers were designed (Supplementary Figs. 1 and 2). Conditionally immortalized human podocytes (CIHP-1) (Ximbio) were cultured in RPMI 1640 medium (Gibco) containing 10% FBS (Gibco), 1% insulin-transferrin-selenium (ITS-G, 100X; Gibco) and 1% penicillin-streptomycin at 33°C with 5% CO₂. When cell density reached ~60%, CIHP-1 cells were exposed to Gapmers (QIAGEN) (three designs targeting *TARID*, *MALAT1* as positive control, and a scrambled negative control) for 24 h. Transfection of podocytes was performed using Lipofectamine LTX (Thermo Fisher Scientific). After 24 h, the podocytes were thermo-switched to 37°C with 5% CO₂ for 14 days to fully differentiate. *TARID* expression was also upregulated using a lentiviral vector (VectorBuilder) (Supplementary Fig. 3). Podocytes were transduced at 30% confluency with concentrated *TARID* lentiviral vector (5.43×10^8 transduction units/mL) or control vector (2.65×10^8 transduction units/mL) at multiplicity of infection of 5.4. Antibiotic selection was initiated using puromycin at a concentration of 1 μ g/mL for 4 days, followed by a second pulse of 2 μ g/mL puromycin. After successful transduction, podocytes were thermo-switched to 37°C with 5% CO₂ for 14 days to fully differentiate.

Real-Time qPCR

Real-time qPCR was used to quantify the transcript expression of *TARID*, *TCF21*, *MALAT1*, *WT1*, *CDC42BPG*,

ASB2, and *NEDD9*. *GAPDH* was used as a housekeeping gene. The RNA from CIHP-1 cells was extracted with the RNeasy Micro Kit (QIAGEN). Levels of RNA were quantified using a NanoDrop One Spectrophotometer (Thermo Fisher Scientific). cDNA was synthesized with ~200 ng RNA using the SuperScript II Reverse Transcriptase kit (Invitrogen). Individual gene expression levels were detected in triplicate using the SYBR Green Master Mix kit (Applied Biosystems). Primers are provided in Supplementary Table 1.

Bulk RNA-Seq

RNA samples extracted from CIHP-1 podocytes treated with *TARID* Gapmer or control were sent for bulk RNA-seq to Novogene. In short, mRNA was purified from total RNA using poly-T oligo-attached magnetic beads. After fragmentation, the first-strand cDNA was synthesized using random hexamer primers, followed by the second-strand cDNA synthesis. The library was ready after end repair, A-tailing, adapter ligation, size selection, amplification, and purification. The library was checked with Qubit and real-time PCR for quantification and Bioanalyzer for size distribution detection. Quantified libraries were pooled and sequenced on Illumina platforms, according to effective library concentration and data amount. Sequencing reads were aligned to the human genome (GRCh38 release 112) using Spliced Transcripts Alignment to a Reference (STAR; v2.7.7a). Mapped reads were quantified for genomic features with featureCounts. Low-expressed RNAs were filtered out (threshold of mean five or more copies), and read counts were normalized using the Trimmed Mean of M-values (TMM) method. For differential expression analysis, a quasi-likelihood negative binomial generalized log-linear model was applied using the edgeR package (v3.18) in R. Genes were considered differentially expressed if the contrast between *TARID* knockdown and control reached statistical significance, defined by a false discovery rate-adjusted *P* value of <0.05. Pathway enrichment was conducted with Gene Ontology (GO) using clusterProfiler (v4.12.0) and STRING (18). All statistical analyses were performed in R (v4.4.0), and figures were generated with ggplot2 (v3.5.1) and enrichplot (v1.24.0).

Immunofluorescent Staining and Imaging

After full differentiation of podocytes and treatment with *TARID* and scrambled Gapmers (knockdown), and *TARID* and control vectors (overexpression), the cells were fixed with 4% paraformaldehyde and stained with Rhodamine Phalloidin (1:500; Invitrogen R415) to visualize F-actin and Hoechst 33258 (1:1000; Molecular Probes) for nuclear visualization. Blocking buffer contained 2% albumin from BSA in PBS. Imaging was performed using a high-content confocal microscope (ImageXpress Micro Confocal, Molecular Devices) equipped with a 20× objective (Nikon Plan Apo Λ; numerical aperture = 0.75). Detailed protocols for staining and imaging are described by Postma et al. (19). Images were quantified with ImageJ (v1.54 m) Ridge

Detection Plugin (v1.4.0) by measuring the total length and the number of the actin filaments. A Student *t* test was used to evaluate significance, and a *P* value <0.05 was considered significant.

Statistical Analyses

The correlation between *TARID* expression and Sirius Red staining was assessed using the Pearson correlation coefficient. Statistical significance of *TARID* upregulation in additional glomerular RNA samples and *TARID* knockdown in podocytes was evaluated using a Student *t* test. A *P* value <0.05 was considered significant.

Data and Resource Availability

Data sets have been deposited in the GEO and are available under accession numbers GSE195460, GSE151302, and GSE131882. The study also provides the processed count matrices for 11 snRNA-seq libraries, available under GEO GSE195460 (10). All other data are included in the manuscript and/or Supplementary Data Appendix.

RESULTS

snRNA-seq Identifies Cell Type-Specific lncRNAs

SnRNA-seq data of five control individuals and six DKD individuals were reanalyzed. The snRNA-seq data set included 39,176 cells (n_{cells} control = 27,667, n_{cells} DKD = 11,509), encompassing all key cell types present in the kidney cortex: proximal tubule (PT) VCAM1⁺ proximal tubule (PT_VCAM1), parietal epithelial cells (PEC), ascending thin limb (ATL), CLDN16[−] thick ascending limb (TAL1), CLDN16⁺ thick ascending limb (TAL2), early distal convoluted tubule (DCT1), late distal convoluted tubule (DCT2), principal cells (PC), type A intercalated cells (ICA), type B intercalated cells (ICB), podocytes (PODO), endothelial cells (ENDO), mesangial cells and vascular smooth muscle cells (MES), fibroblasts (FIB), and leukocytes (LEUK) (Fig. 1A). Original UMAP clustering was preserved (10). Notably, clustering based solely on lncRNA annotations produced a similar structure (Supplementary Fig. 4). A total of 17,910 lncRNAs are available in GENCODE version 32 (Supplementary Table 2), of which 10,327 lncRNAs were present in the snRNA-seq. Most cell types have ~15% of the total transcripts mapped to lncRNAs (Fig. 1B and C). We found that 349 lncRNAs exhibited cell type enriched or specific expression in 15 cell types (Fig. 1D and Supplementary Table 3). Most cell type-specific lncRNAs were specific for the PT cluster, followed by TAL1 and PODO. We observed a correlation between the number of cells in a cluster and the number of cell type-specific lncRNAs in a cluster ($R^2 = 0.78$, $P < 0.001$) (Supplementary Fig. 5). However, some cell types have more cell-specific lncRNAs than expected based on the number of cells in that cluster, for example, in podocytes. Fibroblasts were the only cell type without (statistically significant) cell type-specific expression. The expression of individual cell type-specific lncRNAs indicated a high correlation with

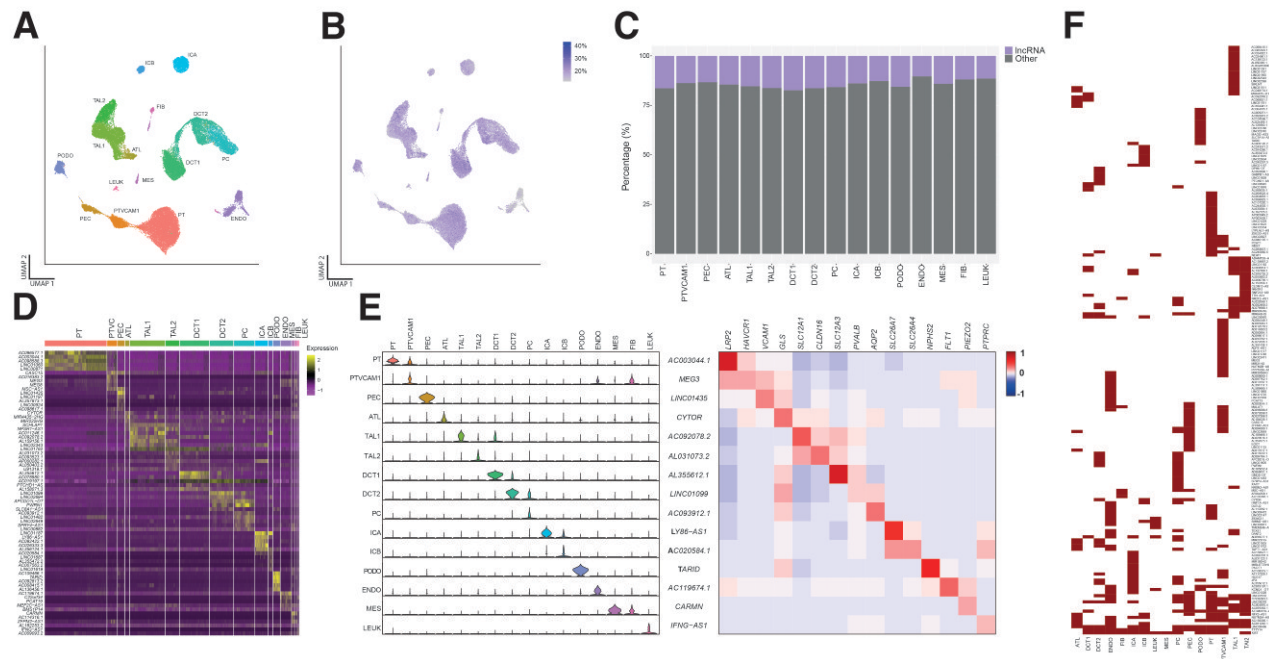


Figure 1—snRNA-seq of human kidney reveals cell type-specific lncRNAs. **A:** UMAP visualization of snRNA-seq data, highlighting overall cellular clustering. Cell types: proximal tubule (PT), VCAM1⁺ proximal tubule (PT_VCAM1), parietal epithelial cells (PEC), ascending thin limb (ATL), CLDN16⁺ thick ascending limb (TAL1), CLDN16⁺ thick ascending limb (TAL2), early distal convoluted tubule (DCT1), late distal convoluted tubule (DCT2), principal cells (PC), type A intercalated cells (ICA), type B intercalated cells (ICB), podocytes (PODO), endothelial cells (ENDO), mesangial cells and vascular smooth muscle cells (MES), fibroblasts (FIB), and leukocytes (LEUK). **B:** UMAP projection illustrating the distribution of lncRNAs within the snRNA-seq data set. **C:** Distribution of lncRNAs across different cell types. **D:** Identification of 349 cell type-specific lncRNAs, with the top 5 lncRNAs for each cell type displayed. **E:** Analysis of cell type-specific lncRNAs and their correlation with known protein-coding marker genes. Left: Violin plots showing expression patterns of representative lncRNAs in specific cell types. Right: Heat map depicting the correlation between these lncRNAs and cell type-specific protein-coding marker genes, with color intensity indicating the degree of correlation. **F:** Heat map of 190 lncRNAs uniquely associated with DKD in specific kidney cell types, identified from a total of 405 DKD-associated lncRNAs. Each red mark indicates a significant association between a lncRNA and DKD in that particular cell type. Several lncRNAs show significant associations in multiple cell types.

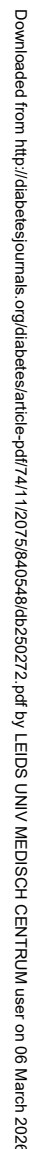
canonical protein-coding marker genes (Fig. 1E). For example, *TARID*, a lncRNA specifically expressed in podocytes, showed a strong correlation with *NPHS2* (podocin), a key podocyte marker gene, exhibiting similar expression patterns.

Using the KPMP data to validate our cell type-specific lncRNAs, we found that of the 349 cell type-specific lncRNAs identified in our analyses, we were able to validate 174 that showed cell type-specific expression in a corresponding cell type within the KPMP data set (Supplementary Figs. 6 and 7, Supplementary Table 4). We therefore proceeded with these validated cell type-specific lncRNAs in our downstream analyses. We identified 405 lncRNAs associated with DKD within a specific cell type (Supplementary Fig. 8 and Supplementary Table 5). Of these, 190 lncRNAs were unique, with several exhibiting differential expression across multiple cell types (Fig. 1F and Supplementary Fig. 9). Notably, this set also includes X-inactive specific transcript (*XIST*), a well-characterized lncRNA involved in X-chromosome inactivation (20). Of these DKD-associated lncRNAs, 54 demonstrated cell type-specific expression (Fig. 2A and Supplementary Table 6). We observed that the endothelial-specific lncRNA *PCAT19* is upregulated in DKD, consistent with its previously reported role in vascular injury (21). Additionally, we identified several novel lncRNAs

with specific expression patterns. For example, lncRNA *AL355612.1* is primarily expressed in early distal convoluted tubule cells and to a lesser extent in late distal convoluted tubule cells and is also downregulated in DKD. Furthermore, lncRNA *TARID* and *AC092813.2* show podocyte-specific expression and are upregulated in DKD (Fig. 2B). lncRNA *AC003044.1* is uniquely expressed in both VCAM1⁺ and VCAM1[−] proximal tubule cells and is significantly upregulated in DKD, is specifically expressed in parietal epithelial cells and downregulated in DKD, and is specifically expressed in CLDN16[−] thick ascending limb cells, with lower expression in CLDN16⁺ cells, and is upregulated in DKD. Another lncRNA, *NEAT1*, is expressed in all cell types but exhibits the highest expression in proximal tubule cells, with even greater expression in DKD. Notably, *NEAT1* has been previously implicated in DKD pathogenesis (22) (Supplementary Figs. 10 and 11).

eQTL/GWAS Analysis Identifies Podocyte-Specific lncRNA *TARID*

To identify lncRNAs in our data set that may be causally related to kidney function, we next analyzed a GWAS data set that investigated SNPs in relation to kidney function.



Among 349 independent SNPs that are significantly associated with eGFR ($P < 5 \times 10^{-8}$) from GWAS (GWAS cat. no. GCST90019506), only 2 SNPs fell in the 1-megabase centered region of the 54 lncRNAs that were cell type-specific and associated with DKD: *TARID* rs74379084-C and rs17643040-C (Supplementary Table 7). Both SNPs were also significantly associated with eGFR_{crea} in a larger meta-analysis by Liu et al. (Science, 2025). Of these 2 SNPs, rs17643040-C was a *cis*-eQTL in multiple tissues, including the kidney cortex for

TARID. This *cis*-eQTL is associated with lower *TARID* expression in the kidney cortex ($P = 4.1 \times 10^{-9}$, normalized effect size = -0.77) and higher eGFR ($P = 3 \times 10^{-9}$, $\beta = 0.0199$, 95% CI 0.013–0.027) (Fig. 2C). This eQTL association with *TARID* was independently confirmed using the kidney-specific eQTL data set from Liu et al. (Science, 2025). Therefore, suggesting that altered *TARID* expression has an effect on eGFR. Interestingly, *TARID* was exclusively expressed in almost all podocytes and not in other cell

types (Fig. 3A) and significantly upregulated in DKD (adjusted $P = 1.3 \times 10^{-9}$) (Fig. 3B).

Confirmation of Increased *TARID* Levels in the Validation Cohort of Glomerular RNA Samples

To confirm the upregulation of *TARID* in DKD, RNA samples from glomerular kidney biopsy specimens of patients with DKD were analyzed for *TARID* expression. We observed a significant upregulation of the lncRNA *TARID* in patients with DKD compared with control participants ($P = 0.01$), which is in line with the snRNA-seq analysis (Fig. 3C). Furthermore, *TARID* expression showed correlation with Sirius Red staining in DKD samples ($R^2 = 0.44$,

$P = 0.045$) and not in control samples ($R^2 = -0.18$, $P = 0.65$) (Fig. 3D). Sirius Red is a well-established marker of interstitial fibrosis and a crucial indicator of disease progression. In addition, we also observed that *TARID* is upregulated in acute kidney injury (logFC = 0.71, $P = 2.6 \times 10^{-81}$), chronic kidney disease (logFC = 0.55, $P = 1.1 \times 10^{-91}$), and type 2 diabetes (logFC = 1.75, $P = 7.3 \times 10^{-123}$) (Supplementary Fig. 12).

Validation of Podocyte-Specific *TARID* Expression in Human Kidneys and Kidney Organoids

To validate the podocyte-specific expression of *TARID* indicated by snRNA-seq data, we performed RNAscope analysis.

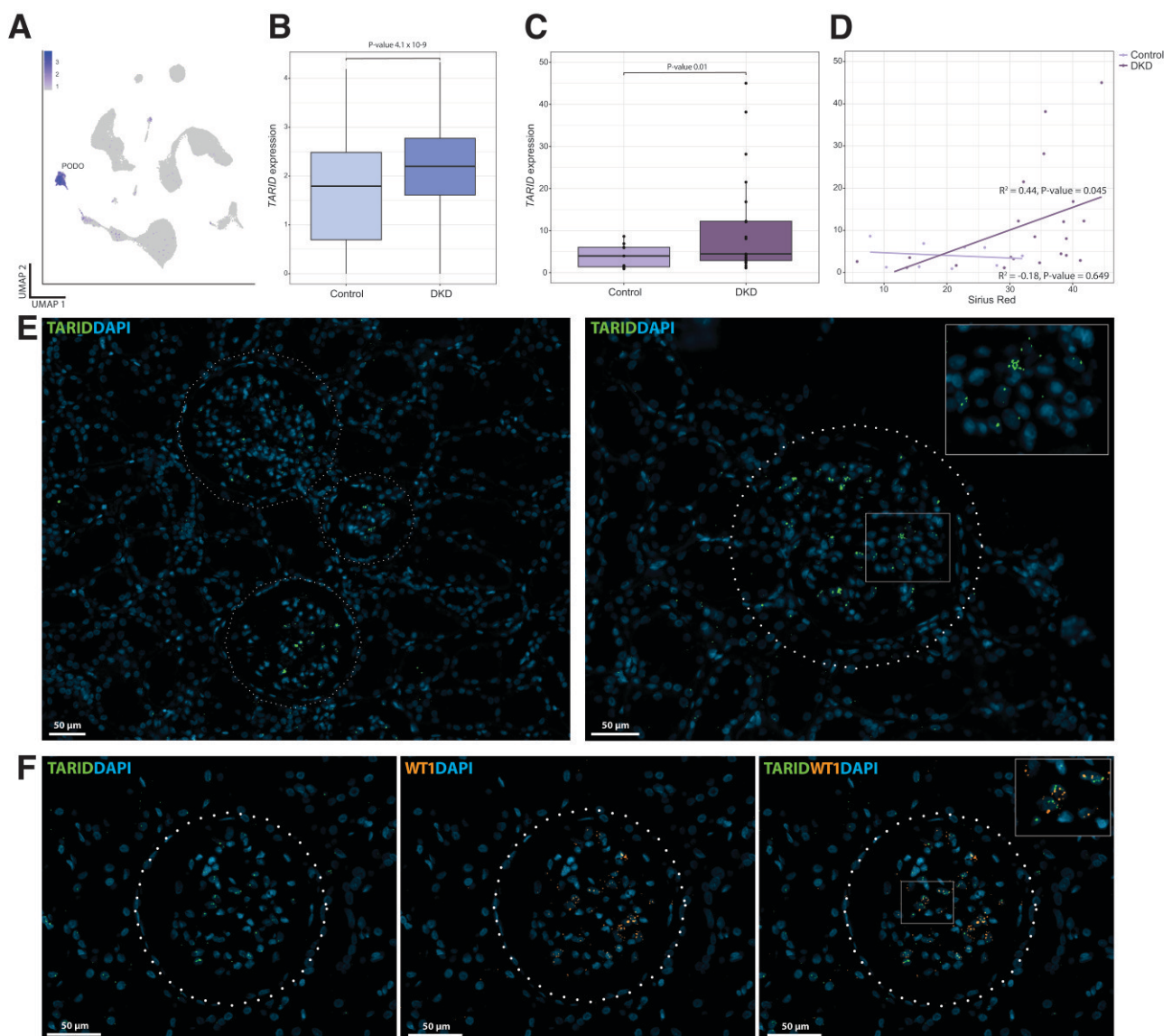


Figure 3—*TARID* is a podocyte-specific lncRNA. A: UMAP projection showing *TARID* expression across kidney cell types. Expression of *TARID* in healthy kidneys and in kidneys of DKD patients analyzed by snRNA-seq (B), by qPCR analysis of additional RNA samples ($n = 32$) from glomerular kidney biopsy specimens from patients with DKD and healthy control participants (C) and correlation with Sirius red (D). E: In situ hybridization of *TARID* in human FFPE kidney tissue. The glomeruli is displayed within the dotted line, bar = 50 μm . Left and right display different glomeruli on different slides. DAPI was used to stain for nucleus. F: In situ hybridization of *TARID* and *WT1* and their colocalization in human FFPE kidney tissue. The glomeruli is displayed within the dotted line, bar = 50 μm . DAPI was used to stain for nucleus.

TARID showed specific staining in glomerular cells, that based on their location most likely represent podocytes, within healthy human kidney sections (Fig. 3E). Additionally, costaining with the podocyte marker Wilms tumor 1 (*WT1*) demonstrated partial colocalization of *TARID* and *WT1* in glomerular podocytes, further confirming the podocyte-specific expression of *TARID* (Fig. 3F). Furthermore, in two independent scRNA-seq data sets of kidney organoids—comprising both untransplanted and transplanted kidney organoids, we assessed the specific expression of *TARID* (14). In both data sets, *TARID* displayed podocyte-specific expression, which was consistently observed across all developmental stages of these cells (Supplementary Fig. 13). In addition, podocyte-specific expression of *TARID* was also confirmed in other scRNA-seq data sets of the human kidney/kidney organoid in the Kidney Interactive Transcriptome database (Supplementary Fig. 14 and Supplementary Table 8).

Gene Regulatory Networks Indicate *TARID* Associates With Podocyte Morphology

We set up a gene regulatory network to explore the functional role of *TARID* in podocytes. This was done by correlating *TARID* with all other genes in the snRNA-seq specifically in podocytes using the Pearson correlation coefficient (Supplementary Table 9). Eight of the 12 most highly correlated genes with *TARID* expression (Pearson correlation coefficient ≥ 0.5): *PARD3B* (Fig. 4A), *ST6GALNAC3*, *COL4A3*, *PLCE1*, *MAGI2*, *CCDC91*, *NEBL*, and *IQGAP2*, have been linked to podocyte dysfunction in literature (Fig. 4B). The genomic locus of *TARID* lies adjacent to the *TCF21* gene, transcribed in the opposite direction (Supplementary Fig. 15). Previous studies have demonstrated that *TARID* plays a critical role in activating *TCF21* expression by promoting promoter demethylation, suggesting its involvement in regulating gene expression through epigenetic modifications (23). We found that 101

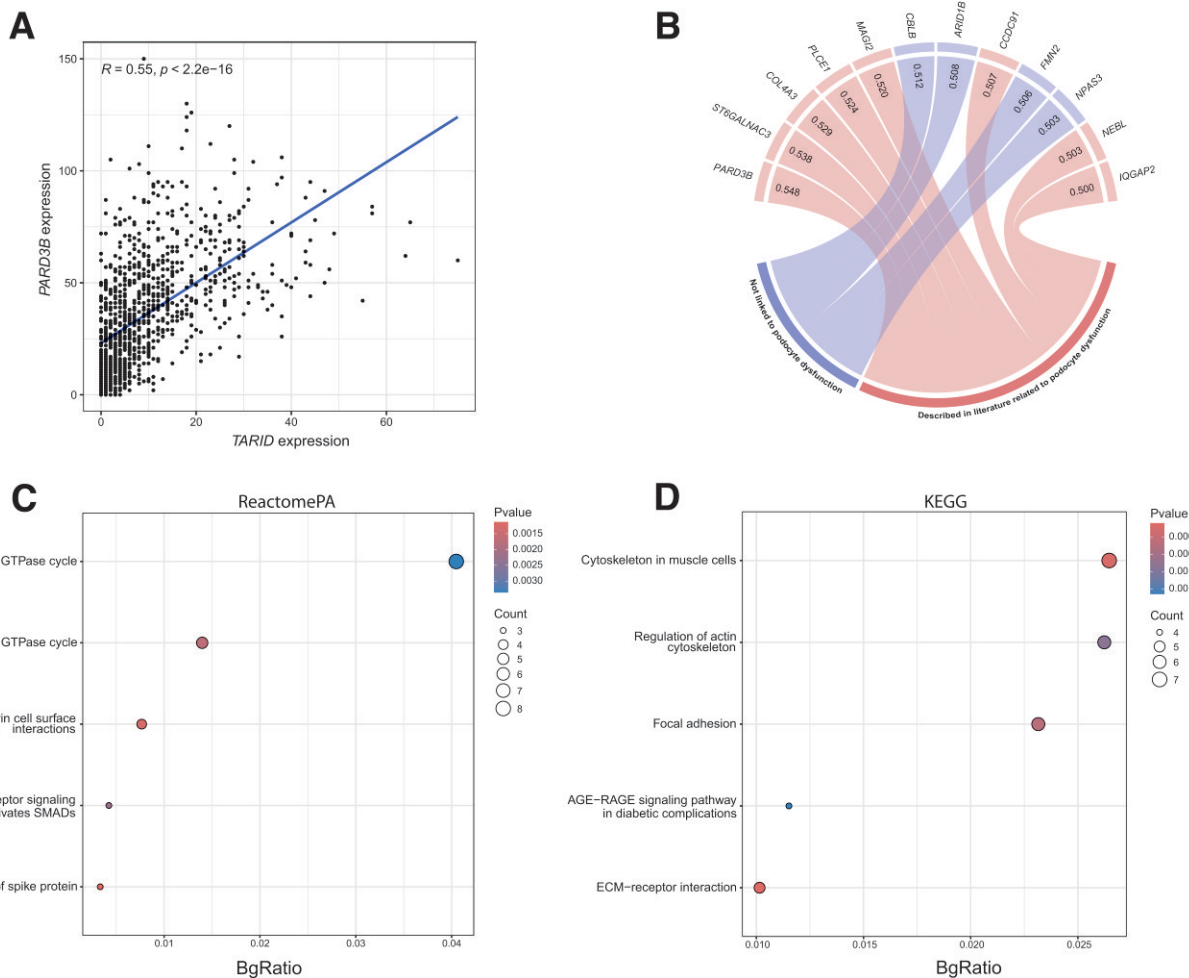


Figure 4—Gene regulatory network of *TARID*. A: Correlation analysis of *PARD3B* and *TARID* in podocytes. B: Circos plot showing correlation coefficient (>0.5) for *TARID* with genes that are linked to podocyte dysfunction (red) and not previously linked to podocyte dysfunction (blue). Pathway enrichment analysis (genes = 101, Pearson correlation coefficient >0.4) with Reactome Pathway Analysis (PA) (C) or KEGG (D). Color of dots indicates *P* value, and size of dots indicates gene count. The x-axis shows the BgRatio (ratio of the number of genes within that distribution that are annotated to the total number of genes in the background distribution).

genes had a Pearson correlation coefficient ≥ 0.4 . However, it is notable that *TCF21* itself was not included in this correlation, despite its close proximity to *TARID*. Pathway enrichment with ReactomePA and KEGG on the correlated genes pointed out enrichment for Rho GTPase cycle (Fig. 4C and Supplementary Table 10) and regulation of the actin cytoskeleton (Fig. 4D and Supplementary Table 11). Additionally, using EnrichR, we found enrichment for abnormal podocyte foot process morphology, podocyte foot process effacement (MGI Mammalian Phenotype Level 4 2024), and primary focal segmental glomerulosclerosis, nephrotic syndrome (WikiPathways 2024 Human) (Supplementary Table 12) (24–26). Similarly, we performed pathway analysis on the genes correlated with the 54 lncRNAs identified in this study (Supplementary Table 13). This revealed several diabetes- and DKD-relevant pathway enrichments, including primary focal segmental glomerulosclerosis associated with lncRNA *AC092813.2*, insulin signaling with *LINC00472*, and epidermal growth factor/epidermal growth factor receptor signaling with *NEAT1* (Supplementary Table 14).

Antisense LNA Gapmer Knockdown of *TARID* in Podocytes

Next, we sought to further explore the function of *TARID* in podocytes. To that end, *TARID* expression was downregulated with three different antisense LNA Gapmer designs in CIHP-1. One Gapmer seemed most effective in knockdown of *TARID* expression, Gapmer design 2 (Supplementary Fig. 16). Using this *TARID* Gapmer for further studies, the next eight samples were selected for bulk RNA-seq (NC Gapmer $n = 4$, *TARID* Gapmer 2 $n = 4$). Both qPCR and bulk RNA-seq revealed efficient knockdown of *TARID* (Fig. 5A and Supplementary Fig. 17). Our results show that *TARID* knockdown leads to a modest reduction (16%, not significant) in *TCF21* gene expression (Supplementary Fig. 18). In total, we found 258 statistically significant differentially expressed genes upon *TARID* knockdown, including 171 downregulated and 87 upregulated genes (Fig. 5B and Supplementary Table 15), of which 44 are podocyte specific in our snRNA-seq data set (Supplementary Table 16). Among the differentially expressed genes were, *NOTCH3* and *WNT2*, which are two well-known genes associated with kidney and podocyte function. Of the 101 genes that correlated with *TARID* in the snRNA-seq (Pearson correlation coefficient ≥ 0.4), we found 3 genes that also significantly associated with *TARID* knockdown with similar direction of effect, *SPOCK1*, *SULF1*, and *NEDD9* (Supplementary Fig. 19). Of which, *SULF1* is associated with podocyte foot process effacement and *NEDD9* is an actin-related gene (27,28). Of the 12 most highly correlated genes within the snRNA-seq, 8 have a similar direction of effect in the *TARID* knockdown experiment: *PARD3B*, *ST6GALNAC3*, *MAGI2*, *CBLB*, *ARID1B*, *CCDC91*, *NPAS3*, and *NEBL* (Supplementary Fig. 20). In line with the snRNA-seq-based *TARID* gene regulatory network analysis, pathway enrichment analysis on differentially expressed genes upon

TARID knockdown revealed genes related to among others cytoskeleton reorganization (Fig. 5C and Supplementary Table 17). Further investigation of the genes associated with cytoskeleton reorganization revealed a specific involvement with the actin cytoskeleton (Supplementary Table 18 and Supplementary Fig. 21). As such, the F-actin cytoskeleton organization was measured after knockdown of *TARID* in CIHP-1 cells. Indeed, we confirmed that knockdown of *TARID* results in altered actin cytoskeleton structure (Fig. 6A). We observed an upregulation in the total length of the actin filaments ($P = 0.02$) (Fig. 6B). Furthermore, we also observed an upregulation in the total number of actin filaments upon *TARID* knockdown ($P = 0.03$) (Fig. 6C).

Lentiviral Overexpression of *TARID* in Podocytes

To further investigate the functional role of *TARID* in cytoskeletal regulation, we overexpressed *TARID* in CIHP-1 podocytes using a lentiviral vector. Successful overexpression was confirmed by qPCR, showing an $\sim 1,800$ -fold increase in *TARID* expression compared with cells transduced with a control vector ($P < 0.0001$). The expression of the podocyte marker *WT1* remained unchanged (Fig. 7A). We next assessed the expression of four genes potentially regulated by *TARID*: *TCF21*, *CDC42BPG*, *ASB2*, and *NEDD9*. Among these, *CDC42BPG* was significantly upregulated upon *TARID* overexpression ($P = 0.0007$), consistent with previous observations. *TCF21* expression showed a modest, nonsignificant increase. Similarly, *ASB2* and *NEDD9* levels were not significantly affected, although *ASB2* showed a slight, nonsignificant upward trend (Fig. 7A). Next, we assessed the effect of *TARID* overexpression on the actin cytoskeleton. In contrast to the knockdown results, overexpression of *TARID* led to a marked reduction in both the total length ($P = 0.04$) and the total number ($P = 0.04$) of actin filaments in podocytes (Fig. 7B). These findings confirm that *TARID* modulates actin cytoskeletal organization in a bidirectional manner, consistent with a regulatory role in cytoskeletal dynamics.

DISCUSSION

The human transcriptome is predominantly composed of noncoding RNAs, with lncRNAs forming a significant subset (29). lncRNAs are recognized for their varied roles in many biological processes such as cell differentiation and transcriptional regulation. However, the precise biological functions of most lncRNAs are still largely unclear, and a comprehensive understanding of lncRNAs in different kidney cell types in human kidney disease is lacking (4). To explore this, we assessed our (publicly available) snRNA-seq data set with a specific focus on lncRNAs in the human kidney (10) and demonstrated clear cell type- and DKD-specific expression of lncRNAs.

Most scRNA- and snRNA-seq studies have centered on the protein-coding genome, leaving a wealth of noncoding RNA biology largely unexplored. Interestingly, many lncRNAs exhibit tissue-specific expression patterns, often confined to a single cell type, which provides valuable insights into their

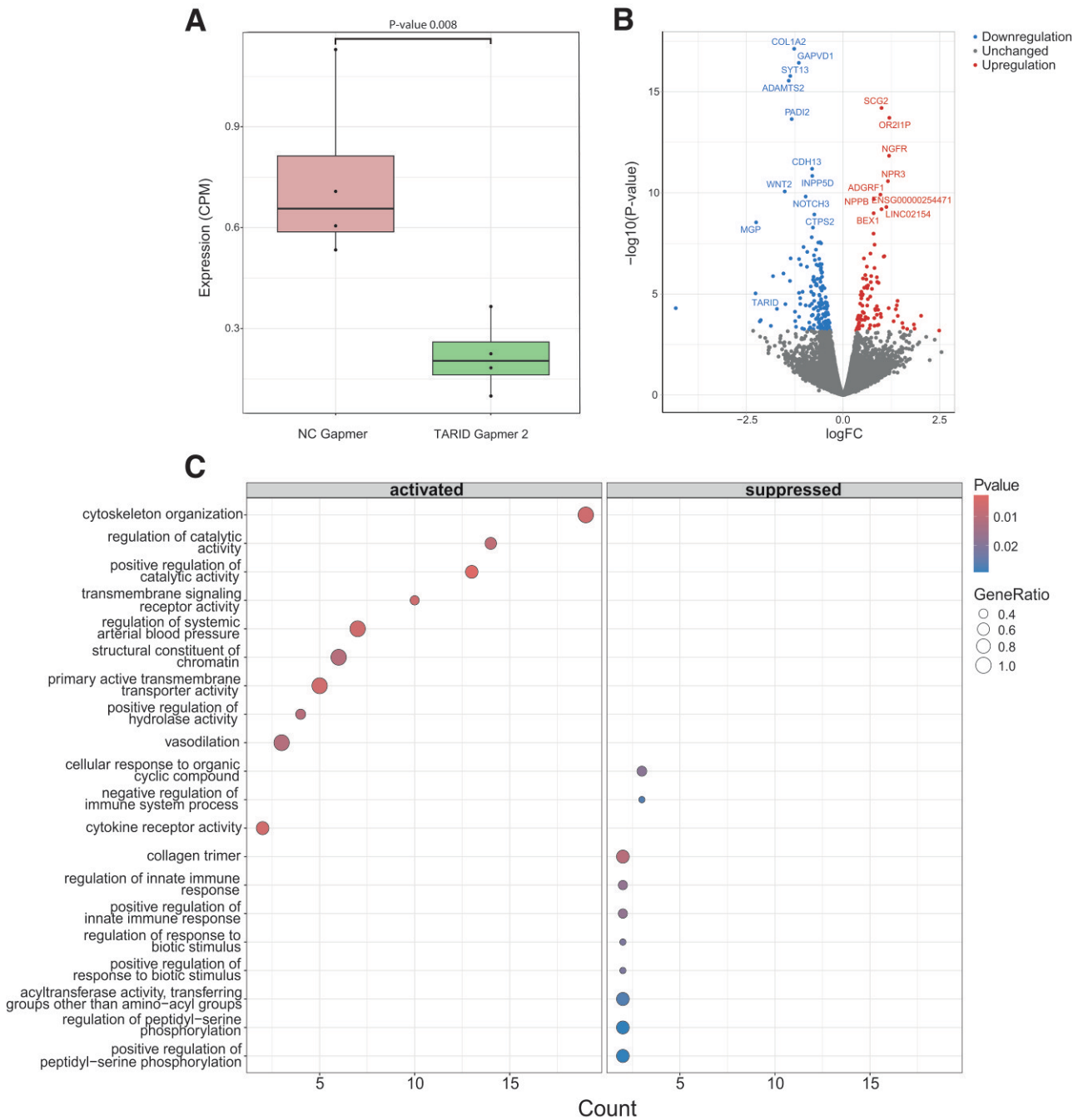


Figure 5—Bulk RNA-seq analysis of podocytes following *TARID* knockdown. **A**: RNA-seq analysis of *TARID* expression in CIHP-1 cells upon transfection with scrambled Gapmer and *TARID* Gapmer (design 2). CPM, counts per million. **B**: Volcano plot displaying differentially expressed genes in CIHP-1 cells following *TARID* knockdown. Blue dots indicate downregulated genes ($n = 171$), red dots indicate upregulated genes ($n = 87$), and gray dots indicate not significantly changed genes. Gene names of the top 20 associated genes and *TARID* are displayed in the volcano plot. **C**: Pathway enrichment analysis results of significantly differentially expressed genes. Left panel indicates activated pathways. Right panel indicates suppressed pathways. Color of dots indicates P value, and size of dots indicates GeneRatio. The x-axis shows the count.

specialized roles within cells (4). It is believed that lncRNAs are often tissue-specific due to regulation by tissue-specific promoters and epigenetic markers, as well as specific signaling pathways active in certain tissues (30,31). They help regulate genes crucial for the identity and function of particular

cell types (32). Additionally, their proximity to tissue-specific genes and alternative splicing contribute to their specialized expression and roles (33). This allows lncRNAs to participate in complex regulatory networks essential for cell specialization across different tissues (30).

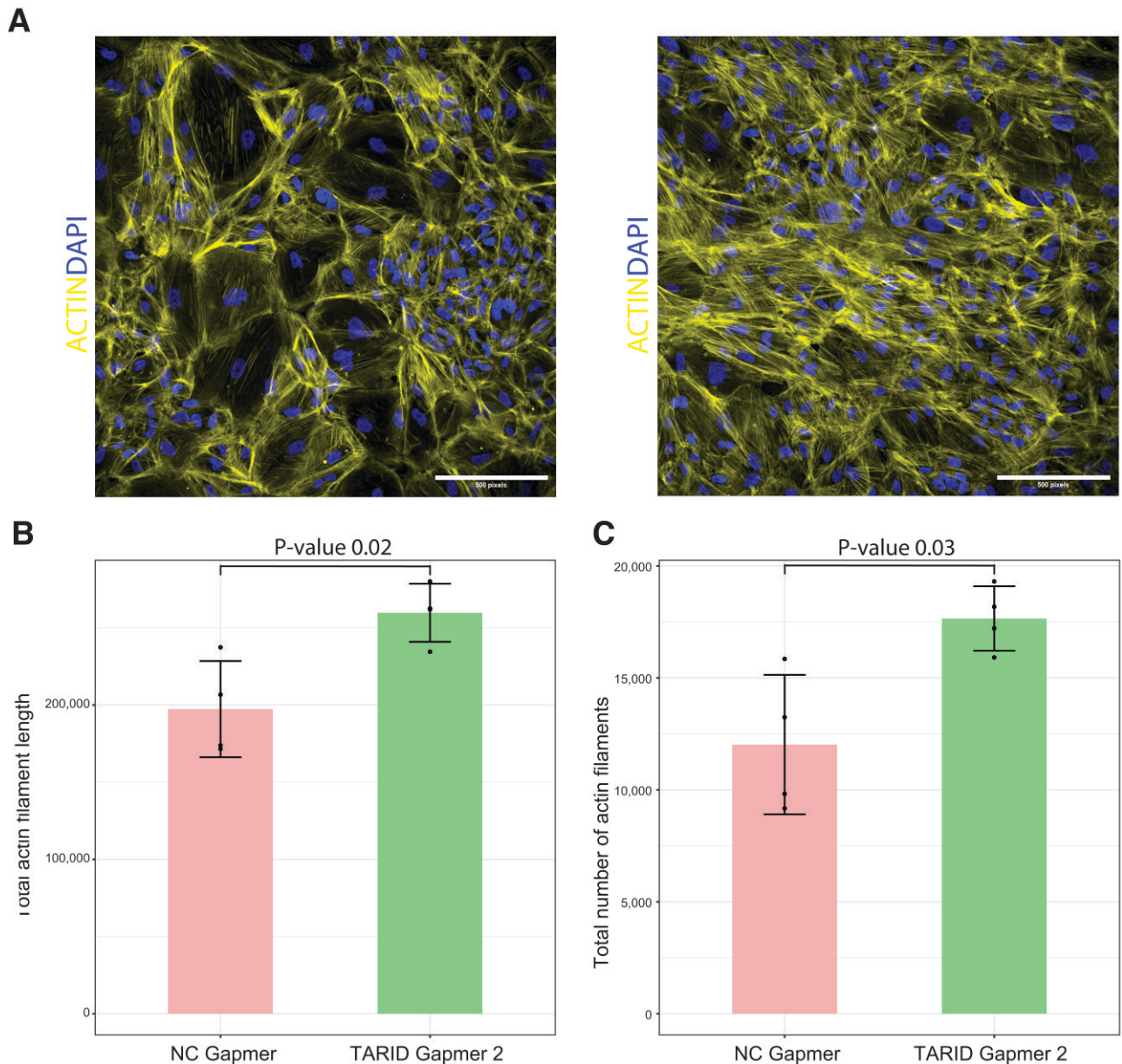


Figure 6—Actin staining analysis of podocytes following *TARID* knockdown. **A**: Microscopic images of podocytes (CIHP-1) transfected with scrambled Gapmer (left) and *TARID* Gapmer (right) stained for actin and DAPI, bar = 500 pixels = 180 μ m. **B**: Quantification of total length of the actin filaments in podocytes (CIHP-1) treated with scrambled Gapmer ($n = 4$) and *TARID* Gapmer ($n = 4$). **C**: Quantification of total number of actin filaments in podocytes (CIHP-1) treated with scrambled Gapmer (NC, normal control) ($n = 4$) and *TARID* Gapmer ($n = 4$).

A previous study that focused on lncRNAs in the mouse kidney found cell-type and age-specific expression of lncRNAs in the kidney (5). However, sequence conservation between human and mice for lncRNAs is poor, which makes the understanding of the noncoding genome an exciting new area to understand human renal disease. As such, this study is the first to characterize lncRNAs across cell types in the human kidney, offering a valuable resource for understanding lncRNA expression patterns within this organ in humans. In total, we found that 10,327 lncRNAs are present in the snRNA-seq, which was 37% of the total amount of transcripts. We identified 349 lncRNAs

with cell type-specific expression across all cell types in the kidney cortex, except for fibroblasts. These cell type-specific lncRNAs exhibited strong correlation with canonical protein-coding RNA markers, suggesting that lncRNAs may serve as reliable biomarkers for distinguishing cell clusters. Notably, proximal tubular cells, which represent the largest cell population, displayed the highest number of cell type-specific lncRNAs. A significant positive correlation was observed between cell count and the number of cell type-specific lncRNAs across most cell types. Interestingly, podocytes presented an exception to this trend, with an unexpectedly high number of cell type-specific lncRNAs (number of

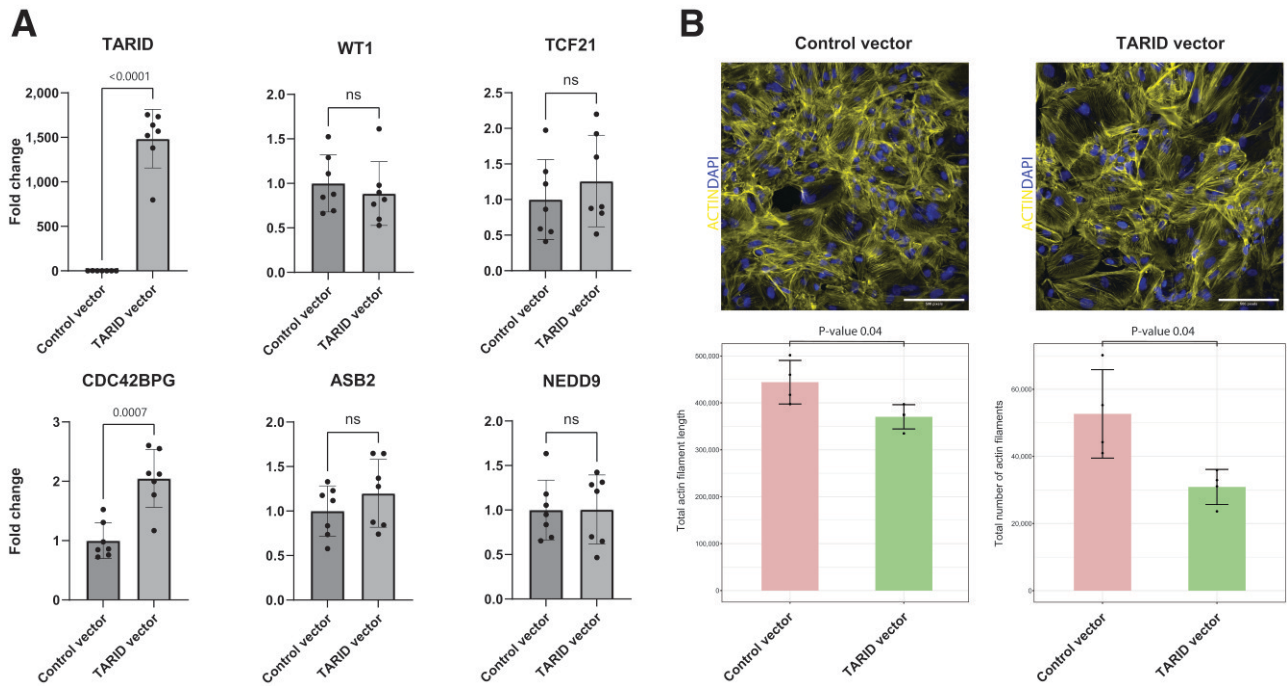


Figure 7—Actin cytoskeleton analysis of podocytes following *TARID* overexpression. **A:** Podocytes (CIHP-1) were transduced with a lentiviral vector overexpressing *TARID* or a control vector. Gene expression was assessed by qPCR. *TARID* expression was robustly increased in *TARID*-transduced cells compared with control ($P < 0.0001$). The expression of the podocyte marker *WT1* remained unchanged. Among the potentially downstream target genes analyzed (*TCF21*, *CDC42BPG*, *ASB2*, and *NEDD9*), only *CDC42BPG* showed a significant upregulation ($P = 0.0007$). Expression of *TCF21*, *ASB2*, and *NEDD9* was not significantly affected. Data are presented as mean $\pm$ SD ($n = 8$ per group). Statistical analysis was performed using unpaired two-tailed Student *t* tests. ns, not significant. **B:** Microscopic images of CIHP-1 podocytes transduced with control vector (left) or *TARID*-overexpressing vector (right), stained for actin and nuclei (DAPI); scale bar = 500 pixels = 180 μ m. Quantification of the total actin filament length (left) and total number of actin filaments (right), $n = 4$ per group.

podocyte specific lncRNAs = 42), indicating that these cells may depend on a unique lncRNA expression profile to support their specialized functions. After validation in KPMP data, this resulted in 174 cell type-specific lncRNAs.

Furthermore, we identified 405 lncRNAs associated with DKD within a specific cell type, including 190 unique lncRNAs. We observed noticeable bias toward cells originating from female donors. Notably, *XIST*, an essential lncRNA responsible for X chromosome inactivation in females, was associated with DKD across multiple clusters. Since *XIST* is typically not expressed in males (except during germline development), this association may be biased by our study design, in which three female control participants were compared with two women with DKD, potentially skewing results for lncRNAs located on the X chromosome (20). Of the 405 lncRNAs associated with DKD, 54 exhibited cell type-specific expression. To demonstrate the relevance of our lncRNA resource, we further investigated the function of *TARID* as a case study.

Our focus on *TARID* stemmed from its unique association with the eGFR-related SNP, rs17643040-C, which is linked to reduced *TARID* expression in, among others, the kidney cortex and an increase in eGFR. *TARID* is notably upregulated in DKD and is specifically expressed in podocytes. Through network analysis, we identified 12 genes with strong correlations to *TARID*, including 8 that are crucial for podocyte function, highlighting its potential

role in podocyte health and DKD. For instance, *PARD3B*, a key regulator of podocyte architecture, connects polarity signaling with actin regulation by modulating Rho-GTP levels, underscoring a functional pathway through which *TARID* may influence podocyte integrity (34). Additional key genes associated with *TARID* include *ST6GALNAC3*, a recognized marker of podocyte injury (35), and *COL4A3*, mutations in which are linked to Alport syndrome. Mutations in *PLCE1* contribute to recessive nephrotic syndrome by influencing GTPase activity and actin dynamics in podocytes (36,37). Furthermore, *MAGI2* plays a vital role in preventing podocyte injury, progressive kidney disease, and renal failure (38). These findings suggest that *TARID* and its network of associated genes are likely critical for maintaining podocyte function and kidney health, particularly in the context of DKD. Furthermore, pathway enrichment on genes correlated with *TARID* (Pearson correlation coefficient ≥ 0.4) showed enrichment for the *Rho GTPase* cycle. Additional pathway enrichment with KEGG showed enrichment for regulation of actin cytoskeleton. We thus hypothesized that *TARID* may be involved in the GTPase cycle to regulate actin cytoskeleton organization. This is a crucial component of podocytes as podocyte function is dependent on actin cytoskeleton regulation within the foot process (39).

This study is the first to establish a connection between *TARID* and podocyte function. *TARID* has been previously

characterized in cancer, where it promotes activation of the tumor suppressor *TCF21*. In cancer cells, *TARID* recruits *GADD45A* to facilitate DNA demethylation, thereby reactivating *TCF21* expression in otherwise silenced cells (23). *TARID* and *TCF21* are located adjacent to each other on the genome in opposite orientations. Our findings indicate that *TARID* knockdown slightly decreases (nonsignificant) *TCF21* expression in podocytes and that *TARID* overexpression slightly increases (nonsignificant) *TCF21* expression in podocytes. However, we found no correlation between *TARID* and *TCF21* expression in podocytes in the snRNA-seq. Further studies are needed to determine whether *TARID* also exerts its effects in podocytes through *TCF21*.

We validated *TARID* expression in podocytes through reanalysis of two scRNA-seq data sets from untransplanted and transplanted kidney organoids and confirmed its localization with in situ hybridization in human kidney tissue sections (14). Furthermore, knockdown of *TARID* in podocytes led to differential expression of 258 genes; among others, we observed reduced expression of *WNT2* and *NOTCH3*. Both genes have previously been implicated in podocyte injury. Evidence suggests that the WNT pathway plays a critical role in podocyte injury because WNT ligand expression is elevated in both murine experimental models for podocyte injury as in human biopsy samples from individuals with glomerular diseases (40). Similarly, *NOTCH3* is only expressed in injured podocytes, and *Notch3*-knockout mice, as well as wild-type mice treated with a *Notch3* inhibitor, showed protection against renal function decline (41). In vivo studies have also linked *Notch3* activation in podocytes to morphological changes (42). The overlap between genes correlated with *TARID* in the snRNA-seq data and the differentially expressed genes following *TARID* knockdown was minimal (3). This discrepancy may arise because correlation analyses and gene knockdown experiments measure distinct aspects of gene regulation and the effects of knockdown may not always manifest directly. Nevertheless, we demonstrate that *TARID* knockdown significantly influences genes involved in diverse cellular processes, particularly actin cytoskeleton organization, for example, *ASB2*, *S1PR1*, *NEDD9*, *KIT*, and *CDC42BPG*, aligning with the pathways associated with *TARID*-correlated genes identified in the snRNA-seq data. To further investigate this, we assessed the actin cytoskeleton following *TARID* knockdown and overexpression, confirming that *TARID* plays a critical role in actin cytoskeleton organization. We show that the organization, length, and total number of actin filaments is increased upon *TARID* knockdown and decreased upon *TARID* overexpression. Based on these findings, we hypothesize that *TARID* knockdown may influence podocyte health by remodeling the actin cytoskeleton specifically in podocytes.

Conclusion

This study offers a valuable resource for examining lncRNA expression across various cell types in the human kidney. Through the identification of the podocyte-specific lncRNA

TARID, we underscore the potential significance of cell type-specific lncRNAs in the pathogenesis of DKD.

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