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A renal biopsy–anchored multi-marker signature involving AOPEP SNP-driven splicing, miR-27b-3p and glycated albumin for stratifying renal damage in type 2 diabetes

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ABSTRACT

Aims: Stratifying renal damage in type 2 diabetes is challenging due to overlapping features between diabetic nephropathy (DN) and non-diabetic renal disease (NDRD). While miR-27b-3p modulates kidney fibrosis in DN through the lysine63 ubiquitination pathway, its upstream regulation and diagnostic relevance remain unclear.
Methods: In a biopsy-verified cohort of 231 chronic kidney disease (CKD) patients with and without type 2 diabetes, we investigated whether AOPEP promoter SNPs drive alternative splicing affecting intronic miR-27b-3p expression. We also assessed the diagnostic utility of combining these biomarkers with clinical parameters, such as glycated albumin (GA), to distinguish DN from NDRD.
Results: The rs10761364 minor allele was associated with reduced urinary miR-27b-3p and AOPEP splicing that excluded exons hosting the miR-23b/27b/24 cluster. This pattern, observed in vivo and under hyperglycemia in vitro, led to elevated AOPEP protein isoforms. A model combining rs10761364, GA, and urinary miR-27b-3p showed high diagnostic accuracy (AUC up to 0.93) in discriminating DN from NDRD. miR-27b-3p inversely correlated with GA, and GA-based models outperformed those using HbA1c.
Conclusion: We identify a genotype- and glucose-dependent mechanism regulating miR-27b-3p via AOPEP splicing and propose a biopsy-anchored, non-invasive biomarker panel (rs10761364, GA, miR-27b-3p) to differentiate DN from NDRD, supporting personalized nephrology care.

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1. Introduction

Diabetic Kidney Disease (DKD) is a major complication of type 2 diabetes and remains a leading cause of chronic kidney disease and end-stage renal disease. Clinically, DKD encompasses distinct histopathological entities and has been traditionally categorized in: i) diabetic nephropathy (DN), showing Kimmelstiel-Wilson lesions, thickened glomerular basal membranes, and mesangial matrix proliferation; ii) non-diabetic renal diseases (NDRD), encompassing vascular changes (arterioarteriolosclerotic) and glomerulonephritides without diabetic glomerulosclerosis; iii) mixed forms combining features of both (DN + NDRD) [1]. Distinguishing diabetic nephropathy (DN) from non-diabetic renal disease (NDRD) in patients with type 2 diabetes is critical since the conditions differ in underlying mechanisms, therapeutic strategies, and prognosis. Yet, despite their divergent management implications, diagnosis still relies on invasive kidney biopsy, as overlapping clinical features limit the accuracy of non-invasive assessment [2,3]. This diagnostic uncertainty underscores an unmet need for reliable biomarkers, linked to specific molecular mechanisms, capable of guiding patient stratification and prognosis in routine practice.

A central challenge lies in the incomplete understanding of the molecular mechanisms that drive renal fibrosis, a shared hallmark of both DN and NDRD. In DN, fibrosis is often linked to hyperglycemia-induced damage leading to both glomerulosclerosis with Kimmelstiel and Wilson lesions and tubulointerstitial fibrosis. In patients with NDRD instead, several different mechanisms, including hypertension, immune-mediated injury, or genetic syndromes, can activate fibrotic pathways through different molecular routes [4–10]. Recent studies have highlighted the role of non-coding RNAs in these processes, particularly microRNA-27b-3p (miR-27b-3p) has emerged as a post-transcriptional regulator of lysine 63 (K63)-linked polyubiquitination, a pathway implicated only in DN-associated fibrosis [11–13]. Reduced urinary levels of miR-27b-3p have been observed in DN patients [10–14], but the upstream mechanisms controlling its expression and their clinical relevance remain poorly understood.

Since miR-27b-3p is encoded within an intron of the AOPEP (C9orf3) gene, we hypothesized that genetic variations in AOPEP, especially promoter polymorphisms, might influence alternative splicing thereby modulating miR-27b-3p expression, kidney fibrosis and ultimately DN endophenotypes.

To test this hypothesis, we conducted a multi-tiered investigation in a biopsy-proven cohort of 231 diabetic patients with CKD in the presence or absence of type 2 diabetes. Our aims were to:

Identify functionally relevant SNPs in the AOPEP promoter region and evaluate their impact on miR-27b-3p expression.
Determine whether AOPEP alternative splicing acts as a mechanistic link between genetic variation and miR-27b-3p regulation.
Assess whether integrating genetic, transcriptomic (miR-27b-3p), and clinical (glycated albumin, GA) biomarkers can improve DN versus NDRD discrimination. In particular, we focused on glycated albumin (GA), recently recognized by ADA/AACC guidelines as a valuable glycaemic biomarker in CKD when HbA1c is less reliable [15].

Through this integrated approach, our study seeks to bridge clinical and molecular gaps, advancing biomarker-driven approaches for non-invasive stratification of renal disease in type 2 diabetes.

2. Materials and methods

2.1. Study design and population

This study was conducted on a cohort of 260 subjects enrolled at the Policlinico Hospital in Bari, Italy. A total of 231 patients with and without type 2 diabetes and clinical evidence of chronic kidney disease (CKD) (GFR < 60 ml/min or ACR higher than 30 mg/g) underwent diagnostic renal biopsy for histological classification. Control groups included patients with type 2 diabetes and preserved renal function (n = 14) and healthy subjects (n = 15). Exclusion criteria were the following: evidence of acute and chronic inflammatory diseases; ongoing viral or bacterial infectious diseases; decompensated chronic liver disease; pregnancy or puerperium. All participants provided informed consent, and the study was approved by the Independent Ethics Committee of Bari (Prot. N.4104/2013).

2.2. Renal histopathology

Renal damage in diabetes, referred to as DKD, was classified according to Mazzucco et al. [1] from two independent renal pathologists as:

i) DN with or without nephroangiosclerosis lesions (n = 58);
ii) NDRD (n = 95), mainly represented by nephroangiosclerosis lesions and including patients with membranous nephropathy (MN), IgA nephropathy (IgAN) and focal segmental glomerulosclerosis (FSGS);
iii) Mixed DN + NDRD (n = 17) including patients with MN, IgAN, FSGS superimposed on true DN;
iv) Non-diabetic CKD patients (n = 61) with renal impairment without diabetes, such as patients with MN, IgA and FSGS.

The main demographic and clinical characteristics of subjects enrolled in the study are summarised in Table 1.

2.3. SNP selection and genotyping

Using the UCSC Genome Browser (GRCh37/hg19) and GeneHancer database, common HapMap SNPs in the AOPEP promoter (chr9: 97764552–97773351) were identified and filtered by MAF > 10 % in CEU populations. SNPs rs10761364, rs4744422, and rs3802456 were genotyped from peripheral blood using TaqMan® assays (ThermoFisher Scientific) and confirmed by Sanger sequencing (Applied Biosystems). Sanger sequencing raw data are available in the open data publishing platform Dryad (https://doi.org/10.5061/dryad.6t1g1jx8p). Linkage disequilibrium (LD) and FORGEdB scores [16] were calculated using LDmatrix Tool (https://ldlink.nih.gov/?tab=ldmatrix).

2.4. RNA isolation and miRNA quantification

Urinary miRNA was isolated from cell-free urinary samples using the miRNeasy Serum/Plasma kit (Qiagen). Exogenous cel-miR-39 spike-in (Qiagen, Milan, Italy) diluted in Qiazol was added in equal amounts to all samples to monitor for the efficiency of RNA extraction. Expression of miR-27b-3p was quantified using miRCURY® LNA SYBR® Green PCR Kit (Qiagen) along with the pre-designed miRCURY LNA miR-27b-3p PCR Assay (Qiagen, Cat. No. / ID: 339306; GeneGlobe ID – YP00205915). Amplification was performed using the LightCycler® 96 Real-Time PCR System (Roche Diagnostics, Indianapolis, IN, USA).

2.5. AOPEP transcript and protein analysis

Total RNA, including small RNAs, was extracted from HK2 cells and PBMCs by the miRNeasy mini kit (Qiagen), according to the manufacturer instructions. Specific AOPEP isoforms were quantified by qPCR with primers targeting exons 1–2, 3–4, and 14–15 retrieved from the literature [17].

Immunoblotting and immunohistochemistry (IHC) were performed using AOPEP-specific antibodies on HK2 lysates and biopsy tissues. Briefly, HK2 Cells were lysed in RIPA buffer and 20 μg of proteins from each lysate were separated by SDS/PAGE (Bio-Rad) and then were electro-transferred onto 0.2 mm PVDF membrane. The filter was

Table 1
Main demographic and clinical characteristics of the 260 subjects enrolled in the study.

Characteristic	CKD (61) CKD	CTRL (29) HS	T2D	DKD (170) DN	DN+ NDRD	NDRD	p-value[2]
	N = 61[1]	N = 15[1]	N = 14[1]	N = 58[1]	N = 17[1]	N = 95[1]	
Sex (m/f)	52/9	7/8	9/5	44/14	14/3	73/22	0.05[3]
Age (years)	58 (10)	56 (10)	59 (12)	63 (10)	65 (10)	64 (9)	0.4
Diabetes duration (years)	–	–	13.04 (10.59)	15.28 (10.83)	13.13 (10.12)	10.44 (8.19)	0.049
Basal glycemia (mg/dl)	86.50 (12.02)	–	150.38 (63.63)	120.19 (39.06)	129.47 (38.72)	113.91 (43.56)	0.010
HbA1c (mmol/mol)	41.00 (10.12)	–	55.23 (17.01)	51.64 (13.48)	58.62 (13.78)	47.27 (11.33)	0.017
sCr (mg/dl)	1.10 (0.26)	–	0.73 (0.19)	2.24 (1.36)	2.56 (1.71)	1.83 (1.62)	<0.001
Blood Urea Nitrogen (mg/dl)	45.00 (7.00)	–	41.64 (10.32)	91.85 (46.72)	97.06 (52.18)	68.69 (44.88)	<0.001
Albumin (g/dl)	2.80 (0.14)	–	–	3.34 (0.50)	3.58 (0.46)	3.49 (0.67)	0.047
Glycated albumin (%)	8.55 (4.17)	12.50 (1.27)	17.51 (5.07)	16.73 (4.42)	14.51 (3.43)	14.79 (3.73)	0.018
eGFR (ml/min)	71.50 (26.16)	–	–	41.94 (26.64)	36.76 (24.95)	55.66 (30.78)	0.010
Systolic BP (mmHg)	140.00 (0.00)	–	125.00 (16.64)	141.33 (22.60)	148.40 (23.18)	136.86 (18.46)	0.023
Diastolic BP (mmHg)	75.00 (7.07)	–	76.07 (9.84)	75.80 (11.26)	78.80 (13.88)	78.68 (11.49)	0.7
Proteinuria (mg/die)	6,247.50 (4,882.57)	–	–	2,641.23 (2,375.28)	3,664.41 (3,707.34)	2,223.90 (3,367.35)	0.002

[1]Mean (SD), [2]Kruskal-Wallis rank sum test, [3]Fisher's Exact Test for Count DataCKD: Chronic Kidney Disease; CTRL: Controls; DKD: Diabetic Kidney Disease; HS: Healthy Subjects; T2D: Type 2 Diabetes; DN: Diabetic Nephropathy; NDRD: Non-diabetic Renal Disease.

incubated with the anti-AOPEP antibody (Merck) and with anti-βactin antibody (Merck). The ECL enhanced chemiluminescence system was used for detection (GE Healthcare Life Sciences).

Immunohistochemistry against AOPEP was performed on 4 μm-thick paraffin-embedded human kidney biopsies. The antibody used in the IHC analysis against AOPEP (Sigma-Aldrich) is a rabbit polyclonal antibody with affinity for the N-terminal region of the protein target. Images were acquired using the Aperio ScanScope CS2 device (Aperio Technologies) and signals were analysed with ImageScope V12.1.0.5029 (Aperio Technologies). AOPEP staining was quantified using the Aperio Positive Pixel Count Algorithm tuned by the renal pathologist. Peri-vascular regions, the Bowman's capsule, and the limits of each biopsy section were excluded from the analysis. AOPEP staining was calculated as the ratio of the total number of positives (NP) on the area analysed (NP/Area).

Sirius Red/Fast Green staining was used to evaluate fibrosis. Digitalized imaging were quantified using a customised algorithm specifically generated by the renal pathologist (Image Analysis algorithm, Aperio).

2.6. In vitro hyperglycemia assays

HK2 cells are derived from a normal, human adult male kidney and were obtained from the American Type Culture Collection (Rockville). HK2 cells were cultured under normoglycemic (5.5 mM) and hyperglycemic (30 mM) conditions for up to 48 h in DMEM medium (Sigma-Aldrich). Splicing dynamics of AOPEP and miR-27b-3p levels were also monitored post-treatment by western blotting and real time PCR respectively.

2.7. Biochemical measurements

Glycated haemoglobin (HbA1c) was measured through high performance liquid chromatography (HPLC) using the single-cartridge Variant II assay (Bio-Rad). Glycated albumin (GA) was measured enzymatically (QuantILab Glycated Albumin assay, Instrumentation Laboratory SpA – A Werfen® Company) [18,19] and expressed as a percentage of total albumin.

2.8. Single-cell RNA-seq analysis

To examine AOPEP expression in specific nephron segments, publicly available scRNA-seq data from the Kidney Precision Medicine Project (KPMP) was first processed using SoupX (v1.5.0) [20] to correct the ambient mRNA expression and analysed by Seurat R package (version 4) for data processing [21]. In each dataset, we filtered cells out using cutoff of > 500 and < 5000 genes per cell, cell read counts were normalized and scaled; principal component analysis was performed using variable features. Reciprocal principal component analysis ('RPCA') method was used to integrate and generate a combined single cell dataset. The combined dataset was further processed which includes dimensionality reduction (Principal Component Analysis and Uniform Manifold Approximation and Projection), k.param nearest neighbors computation and unsupervised clustering.

2.9. Statistical analysis

Associations between genotypes and phenotype were assessed via chi-square, ANOVA, or Kruskal-Wallis tests as appropriate. Multivariate logistic regression with ROC analysis was used to assess diagnostic accuracy (AUC) of biomarkers for DN vs. NDRD classification. Analyses were conducted using R 4.2.2.

3. Results

3.1. Identification of AOPEP promoter SNPs and genotype distribution

To investigate if genetic variations in the promoter region of *AOPEP* could affect miR-27b-3p expression, we initially performed an *in silico* analysis. Using the UCSC Genome Browser, we identified nine HapMap SNPs within the *AOPEP* gene locus (Fig. 1A). Further refinement focusing on a 3566-nucleotide region of the promoter, and filtering for SNPs with a Minor Allele Frequency (MAF) > 10 % in the CEU population, yielded four candidate SNPs: rs4744422, rs4744423, rs3802456, and rs10761364. rs4744423 was excluded from further experimental investigation due to its proximity (155 nucleotides) to rs4744422 (Fig. 1B; Supplementary Table 1). The remaining three SNPs (rs3802456, rs4744422, and rs10761364) were predicted to be functionally relevant based on a FORGEdb score of 7 and exhibited strong linkage disequilibrium (LD; R^2 values ranging from 0.869 to 1). Across all analyzed SNPs, the minor allele was generally observed at its highest frequency in patients with DN lesions compared to those with NDRD, CKD, or CTRLs. While overall genotype frequencies did not show statistically significant differences across all patient classes for each SNP (potentially due to sample sizes within subgroups), a trend was observed for rs3802456 across all patient classes ($p = 0.049$, Fig. 1C) and for rs10761364 genotype frequencies when comparing DN versus NDRD ($p = 0.06$) and DN versus CTRLs ($p = 0.07$).

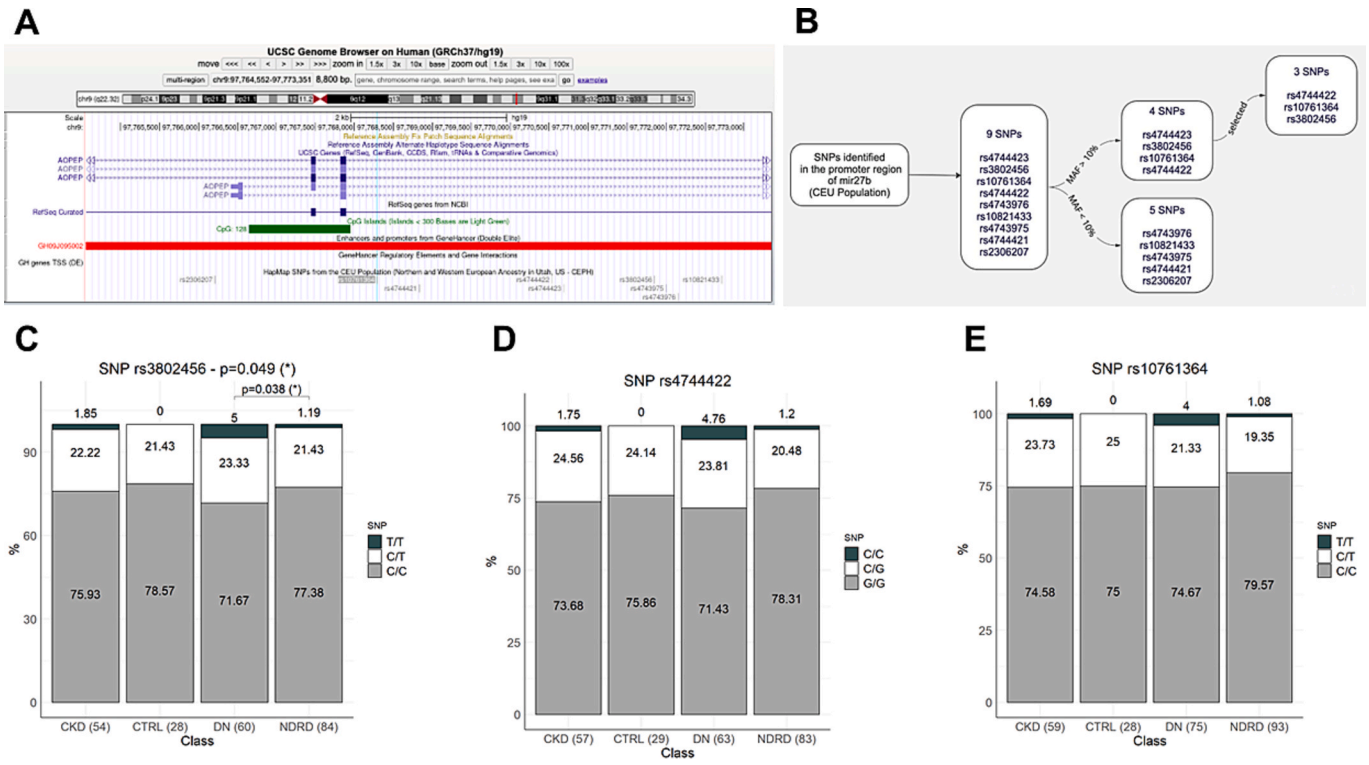

Fig. 1. **A:** UCSC Genome browser showing the genomic position of the 9 identified hapmap SNPs in the promoter region of *AOPEP (C9orf3)* gene. **B:** Workflow of the final selection of the 3 HapMap SNPs (rs4744422, rs3802456, rs10761364) within the promoter region of *AOPEP (C9orf3)* investigated in the present study. **C-E:** different distribution of genotype frequency of the SNP rs3802456 (C), rs4744422 (D) and SNP rs10761364 among control subjects (CTRL) and different classes of renal damage in diabetic patients (DN: diabetic nephropathy; NDRD: non-diabetic renal disease) and non-diabetic (CKD: chronic kidney disease) patients.

3.2. Urinary miR-27b-3p levels are reduced in DN and associated with AOPEP genotype

Urinary miR-27b-3p levels were significantly lower in DN compared to NDRD and control groups ($p < 0.01$; Fig. 2A). Across all three SNPs, individuals homozygous for the minor allele had the lowest miR-27b-3p levels (e.g., for rs10761364 TT vs CC $p < 0.00016$; rs3802456 TT vs CC $p < 0.00019$; rs4744422 GG vs CC $p < 0.000179$, Fig. 2B-D), implicating AOPEP genotype as a key determinant of miRNA expression.

3.3. Association of SNP genotypes with clinical parameters

The rs10761364 and rs3802456 SNPs genotypes were significantly associated with differences in systolic blood pressure ($p = 0.046$ and $p = 0.045$, respectively), tubular fibrosis ($p = 0.01$ and $p = 0.02$, respectively), glomerular fibrosis (both $p = 0.04$), and proteinuria ($p = 0.03$ and $p = 0.026$, respectively). These findings support the functional relevance of the AOPEP locus in modulating renal injury severity.

3.4. Glycated albumin outperforms HbA1c and correlates with fibrosis and miR-27b-3p

GA levels were significantly higher in DN compared to NDRD (Kruskal-Wallis $p = 0.009$; Fig. 3A). GA levels demonstrated statistically significant positive correlations with diabetes duration ($R = 0.251$, $p = 5.24e-03$, Fig. 3B), glycemia ($R = 0.375$, $p = 8.63e-06$, Fig. 3C), and the extent of tubular fibrosis (Sirius Red staining, $R = 0.365$, $p = 0.00415$, Fig. 3D). Conversely, GA was negatively correlated with eGFR ($R = -0.251$, $p = 5.06e-03$, Fig. 3E). Notably, we observed an inverse correlation between GA levels and urinary miR-27b-3p levels ($R = -0.253$, $p = 0.053$, Fig. 3F). HbA1c levels also varied across histological classes (Kruskal-Wallis $p = 1.8e-02$, Fig. 3G) and showed a strong positive correlation with GA levels ($R = 0.608$, $p = 1.19e-12$, Fig. 3H). HbA1c showed a similar inverse correlation with miR-27b-3p ($R = -0.29$, $p = 0.03$; Fig. 3I).

3.5. Multivariate models identify predictive biomarker combinations

A logistic regression model combining urinary miR-27b-3p with the rs10761364 genotype (minor allele as risk) yielded an AUC of 0.867 (95 % CI: 0.7983–0.9463), with 82 % specificity and 79 % sensitivity in distinguishing DN from NDRD (Table 2, Fig. 3J).

A model combining GA and urinary miR-27b-3p further improved performance (AUC = 0.932; Fig. 3K; Table 3a), significantly outperforming the HbA1c-based model (AUC = 0.774; Fig. 3L; Table 3b).

In addition, we estimated post-test probabilities across different DN prevalences for each two-marker model. Using the study's biopsy prevalence of 44.1 %, the miR-27b-3p + rs10761364 model (Se = 0.79, Sp = 0.82) gave PPV = 77.6 % / NPV = 83.2 %, while GA + miR-27b-3p (AUC = 0.932; Youden-optimal Se = 0.86, Sp = 0.88) gave PPV = 85.0 %/NPV = 88.8 %. As context, the HbA1c + miR-27b-3p model (AUC = 0.774) yielded PPV ≈ 67.6 % and NPV ≈ 77.8 % when evaluated at the Youden-optimal operating point on its ROC (Se ≈ 0.74, Sp ≈ 0.72) (Supplementary Table II). These data reinforce the superior translational performance of the GA-based combination relative to HbA1c-based pairing, consistent with the main AUC comparisons.

3.6. AOPEP alternative splicing driven by SNP and glucose exposure

AOPEP protein expression was markedly increased in glomerular (DN vs CTRL, $p = 0.02$), tubular (DN vs CTRL, $p = 0.01$; DN vs NDRD, $p = 0.004$), and vascular compartments (DN vs CTRL, $p = 0.02$) of DN patients compared to NDRD patients or control kidneys (Fig. 4 A-L). Despite increased AOPEP protein levels, expression of different AOPEP exonic regions in PBMCs from DKD patients stratified by their rs10761364 genotype, demonstrated a marked reduction in the

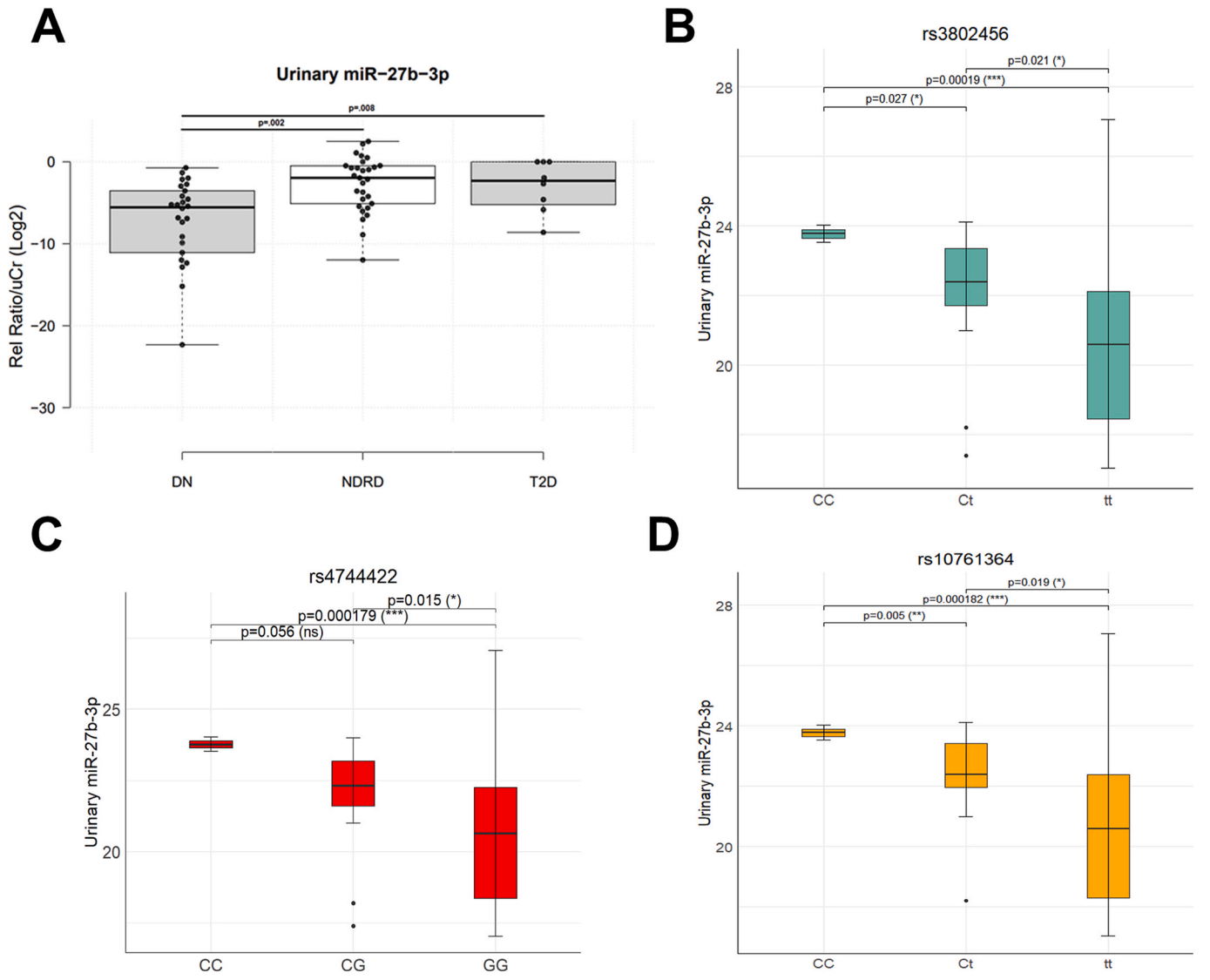

Fig. 2. **A:** miR-27b-3p urinary expression levels in patients with diabetic nephropathy (DN, n = 26), non-diabetic renal disease (NDRD, n = 29) or patients without renal damage (CTRLs, n = 8). Center lines show the medians; box limits indicate the 25th and 75th percentiles as determined by R software; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles; outliers are represented by dots. **B:** distribution of miR-27b-3p levels across genotypes of the SNPs rs3802456. **C:** distribution of miR-27b-3p levels across genotypes of the rs4744422. **D:** distribution of miR-27b-3p levels across genotypes of the rs10761364. **E:** ROC curve describing the sensitivity and specificity of miRNA 27b-3p urinary expression levels and SNP rs10761364 to discriminate DN vs NDRD.

expression of the transcript segment containing exons 14–15 (which hosts miR-27b-3p) in rs10761364 TT carriers ($p < 0.01$; Fig. 4M). In contrast, individuals with CC or CT genotypes showed comparable expression levels across these exonic regions.

Furthermore, in vitro experiments using HK2 human kidney tubular cells (rs10761364 GG genotype) demonstrated that exposure to hyperglycemic conditions led to a marked downregulation of the *AOPEP* transcript containing exons 14–15 (Fig. 4N), while transcripts for exons 1–2 and 3–4 remained relatively unchanged. Consistent with this, high glucose stimulation of HK2 cells resulted in an overall increase in AOPEP protein expression, particularly of isoforms smaller than 50 kDa, potentially representing products of alternative splicing (Fig. 4O).

These results support a glucose- and genotype-driven splicing mechanism of AOPEP gene.

3.7. *AOPEP expression in kidney cell types*

Publicly available scRNA-seq data from the Kidney Precision Medicine Project (KPMP) confirmed AOPEP expression enrichment in kidney tissue from DKD patients compared to healthy controls, with increased expression in proximal tubule and TAL segments in DKD, consistent with tissue compartment findings in IHC analysis (Fig. 5 A-B).

4. Discussion

Accurate stratification of renal damage in type 2 diabetic patients remains a cornerstone of personalized nephrology but continues to be challenged by the overlap of DN and NDRD phenotypes. Renal biopsy remains the gold standard, but its invasiveness and associated risks necessitate the development of robust, non-invasive biomarkers [22]. In this study, we present a novel, biopsy-anchored framework for renal damage classification in diabetes, integrating AOPEP promoter SNP rs10761364, urinary miR-27b-3p expression, and GA as a composite signature. This multi-marker approach achieved high discriminative performance in distinguishing DN from NDRD and offers translational promise.

Mechanistically, our findings reveal a regulatory mechanism where rs10761364, a SNP in the AOPEP promoter region, modulates

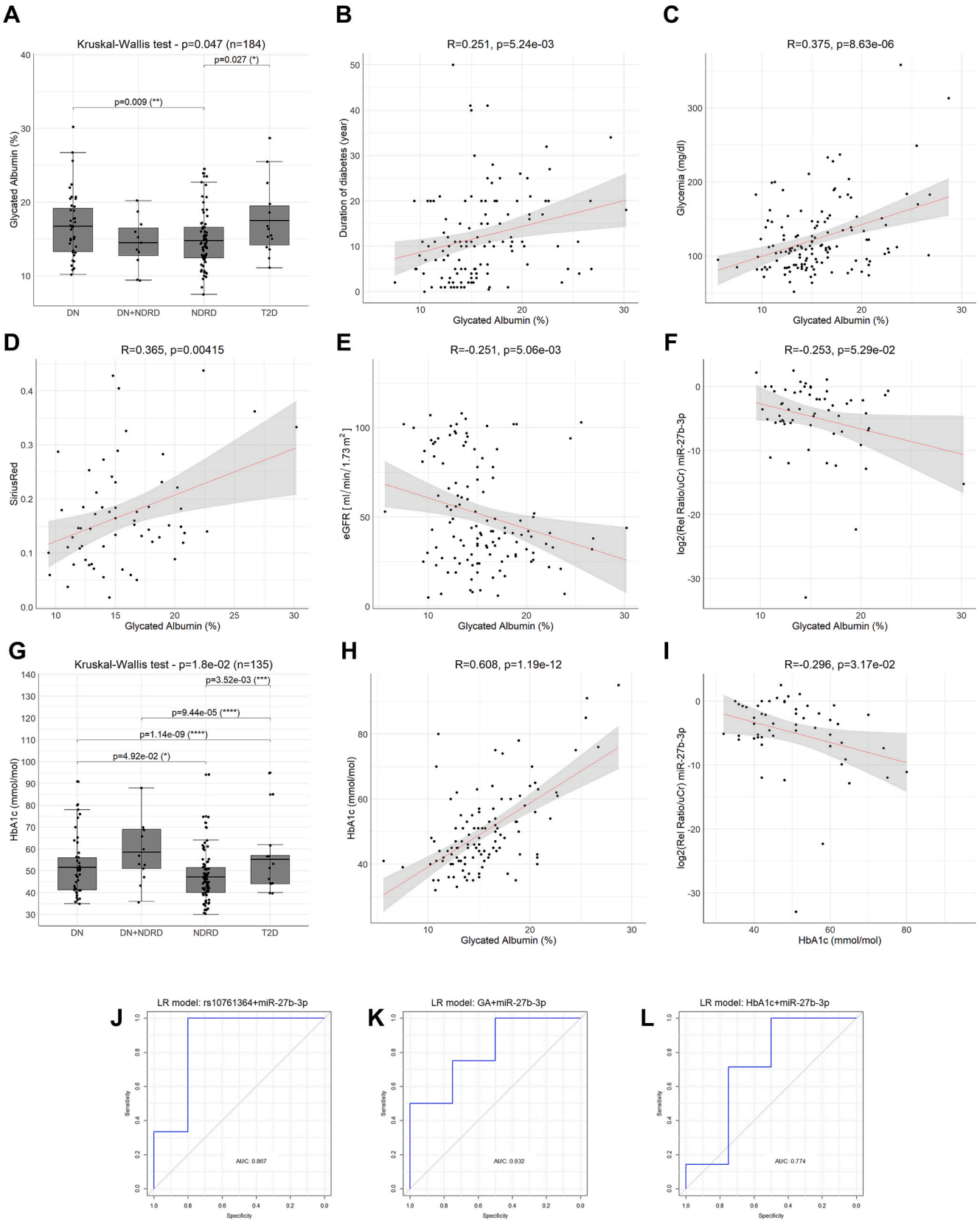

Fig. 3. A: Glycated albumin distribution in DN, DN + NDRD, NDRD and T2D. **B-F:** Correlation analysis between glycated albumin and: diabetes duration (B), glycemia (C), renal fibrosis evaluated through Sirius Red staining (D), eGFR (E) and miR-27b-3p urinary expression (F). **G:** Glycated haemoglobin distribution in DN, DN + NDRD, NDRD and T2D. **H-I:** Correlation analysis between glycated haemoglobin and: glycated albumin (H), and urinary miR-27b-3p expression (I). **J:** ROC curve describing the sensitivity and specificity of rs10761374 and miR-27b-3p. **K:** ROC curve describing the sensitivity and specificity of glycated albumin and miR-27b-3p to discriminate DN vs NDRD. **L:** ROC curve describing the sensitivity and specificity of glycated haemoglobin and miR-27b-3p to discriminate DN vs NDRD. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
Logistic regression model to discriminate diabetic nephropathy (DN) vs non diabetic renal disease (NDRD).

Characteristic	OR[1]	95 % CI[1]	p-value*
rs10761364 (2)	1.24	1.14, 1.67	0.01
miR-27b-3p	0.83	0.72, 0.97	0.03

[1]OR = Odds Ratio, CI = Confidence Interval, Bootstrap based on 1000 samples, 2 = minor allele

Table 3
Logistic regression model to discriminate diabetic nephropathy (DN) vs non diabetic renal disease (NDRD) based on a) GA (glycated albumin) and miR-27b-3p levels or b) HbA1C (glycated hemoglobin) and miR-27b-3p levels.

Characteristic	OR[1]	95 % CI[1]	p-value
a)			
Glycated Albumin	1.08	1.02, 1.12	0.03
miR-27b-3p	0.83	0.73, 0.98	0.03
b)			
HbA1c	1.02	0.98, 1.09	0.06
miR-27b-3p	0.82	0.70, 0.97	0.03

[1]OR = Odds Ratio, CI = Confidence Interval

alternative splicing of AOPEP transcripts. Notably, the presence of the TT genotype was associated with reduced transcription of exons harboring the miR-23b/27b/24 cluster, suggesting a mechanism of miRNA silencing through exon exclusion. This is consistent with rs10761364′s annotation as an sQTL in GTEx project data [23] and aligns with previous large-scale miRNA-eQTL mapping by Sonehara et al., who identified rs10761364 as a significant modulator of miR-27b-3p expression in PBMCs [24]. Importantly, these effects were reproduced under hyperglycemic conditions in vitro, indicating that the SNP-mediated splicing mechanism operates synergistically with metabolic stress.

The host gene AOPEP, also known as C9orf3, encodes aminopeptidase O, previously implicated in angiotensin processing and diabetes-related hypertension [25] and our observation of associations between rs10761364/rs3802456 genotypes and systolic blood pressure, as well as external database support for associations with T2D, blood pressure, and serum creatinine from resources like the T2D Knowledge Portal [26], further underscores the potential clinical relevance of genetic variation in this locus. The intronic location of the miR-27b cluster within AOPEP implies that splicing decisions directly impact miRNA maturation. Hyperglycemia also downregulates the exon 14–15-containing transcript in HK2 cells, highlighting a convergence between genetic and metabolic stress in modulating AOPEP splicing. The observed downregulation of miR-27b-3p in DN patients thus reflects a convergence of genetic and environmental pressures, highlighting the underexplored influence of alternative splicing in the regulation of non-coding RNAs [27,28].

The role of epigenetic regulation and of non-coding RNAs such as long ncRNAs, small interfering RNAs, or miRNAs in kidney diseases and diabetic kidney disease is increasing over time.

Epigenetic regulation has emerged as a key contributor to the progression of kidney disease in diabetic patients. Beyond genetic predisposition, DNA methylation, histone modifications, and non-coding RNAs alter gene expression in response to hyperglycaemia [29,30]. Hypomethylation at promoters such as EDN1 and hyperacetylation of histones at inflammatory loci like NF-κB enhance pro-inflammatory and profibrotic signalling [31]. Importantly, such alterations contribute to the phenomenon of metabolic memory, where persistent epigenetic changes sustain pathogenic gene expression despite normoglycemia [32,33]. In addition, several miRNAs (e.g., miR-21, miR-29, miR-200, miR-192) can act as pro-fibrotic drivers thus amplifying fibrotic and inflammatory pathways modulating the TGFβ signaling pathway, collagen expression, PTEN-dependent pathways or other pathways such as TIMP3 or MMP2 [34,35].

Among these miRNAs, miR-27b-3p has previously been identified as a suppressor of lysine 63-linked polyubiquitination, a pathway critical to profibrotic signalling in renal epithelial cells [11]. Loss of this miRNA has been associated with tubular injury and interstitial fibrosis in DN, as demonstrated in both human and animal models [12,13]. In our cohort, miR-27b-3p levels were inversely correlated with histological fibrosis scores, reinforcing its relevance as a non-invasive marker of fibrotic burden [14]. The mechanistic link to AOPEP splicing provides new insights into its regulation. Additionally, miR-27b-3p is part of a highly conserved triad (miR-23b/27b/24–1) implicated in epithelial integrity, oxidative stress, and inflammation [36–38]. The broader implications of altered cluster expression go beyond fibrosis and may influence tubular cell survival and repair mechanisms. It is plausible that SNP-driven dysregulation of this cluster contributes to a global shift toward a pro-fibrotic, pro-inflammatory microenvironment in diabetic kidneys [39].

Beyond mechanistic novelty, our multivariate logistic models underscore the clinical utility of combining genotype (rs10761364), urinary miR-27b-3p, and GA, a clinically validated marker of glycemic control reflecting glycemic variability in diabetic complications [40,41]. The pairing of urinary miR-27b-3p with either rs10761364 genotype or GA yielded AUCs up to 0.93 for DN vs. NDRD classification. These results outperform HbA1c-based models (AUC = 0.77), consistent with evidence that GA is a superior glycaemic index in CKD due to reduced red cell turnover and less susceptibility to renal anaemia [42]. Serum GA reflects the average glucose concentration over 2–3 weeks [43], emerged as a potentially superior marker in diabetic patients with all CKD stages [44]. GA is also an early precursor of advanced glycation end products (AGEs), molecules that play an important role in the induction of deleterious down-stream signals in various cell types and can also promote several processes involved in DN such as ROS generation, inflammation and fibrosis [45].

Importantly, this multi-marker model offers a practical roadmap for risk stratification. It could be deployed as a pre-biopsy triage tool, helping nephrologists prioritize patients with ambiguous presentations. For example, patients with preserved eGFR but elevated proteinuria and diagnostic uncertainty could benefit from an algorithm incorporating genotype and urinary miRNA. In high-risk or elderly patients, it could help avoid biopsy altogether. Integration into clinical decision support tools is feasible through laboratory automation of GA and TaqMan-based miRNA quantification [46]. This study also opens the door to combining miR-27b-3p with other emerging biomarkers such as NGAL, KIM-1, or miR-21, creating multiplex panels tailored to DKD sub-phenotypes. While some markers, like miR-21, broadly indicate fibrosis or tubular stress, miR-27b-3p may add a layer of specificity linked to genetic susceptibility and AOPEP-related pathways [47,48].

Single-cell RNA-seq data from the Kidney Precision Medicine Project (KPMP) [49] demonstrated AOPEP expression enrichment in proximal tubules and thick ascending limbs, nephron segments commonly affected in DN. Immunohistochemistry in biopsy samples confirmed these expression patterns. Interestingly, total AOPEP protein levels were elevated in DN tissues, while transcript variants including exons 14–15 were suppressed, especially in TT homozygotes. This indicates a decoupling between transcription and translation that may reflect compensatory or isoform-specific responses to injury. Such complexity highlights the importance of isoform-resolved analyses in transcriptomic profiling.

Our findings support a model in which upregulation of non-miRNA-hosting isoforms compensates for transcript loss through alternative splicing. Future studies may explore whether specific isoforms of AOPEP possess catalytic or regulatory properties that influence fibrosis, inflammation, or renin-angiotensin axis dynamics.

Key strengths of this study include the use of a renal biopsy–validated cohort [50], rigorous histological classification, and integration of multi-omics datasets (genomic, transcriptomic, proteomic, and clinical). The use of external datasets, such as GTEx and KPMP,

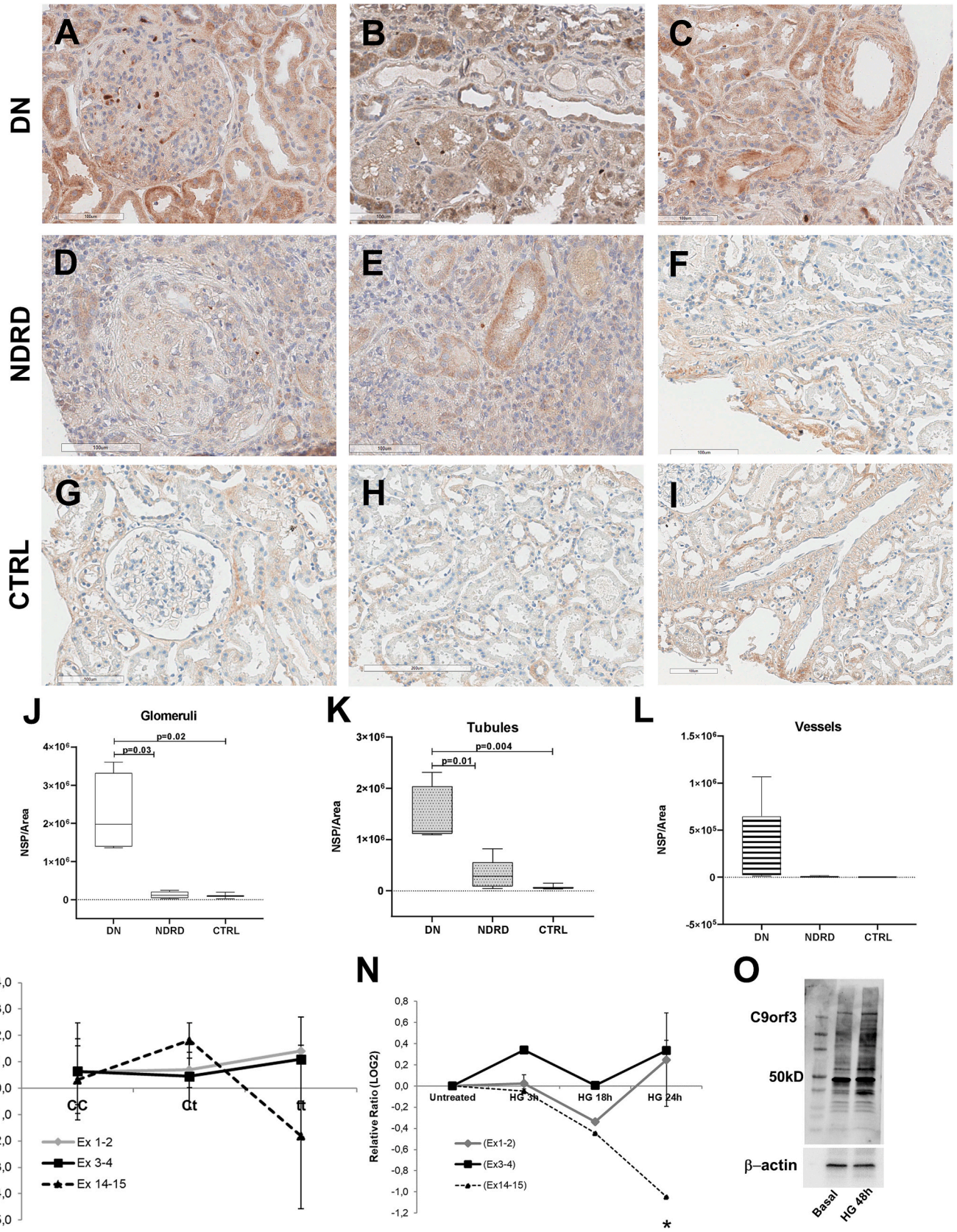

Fig. 4. A-L: AOPEP (C9orf3) protein expression levels in the glomerular (A, D, G, J), tubular (B, E, H, K) and vascular (C, F, I, L) compartments of patients with DN (A-C), NDRD (D-F) and controls (G-I). **M–N**: Expression levels of *AOPEP (C9orf3)* exons 1 to 2, 3 to 4 and 14 to 15 in: HK2 tubular cells under hyperglycaemic conditions for 3, 18 or 24 h (M); PBMC from diabetic patients with CKD and different allelic forms of the SNP rs10761364. **O:** Expression levels of AOPEP (C9orf3) protein isoforms in HK2 cells under hyperglycaemic conditions. *$p < 0.05$.

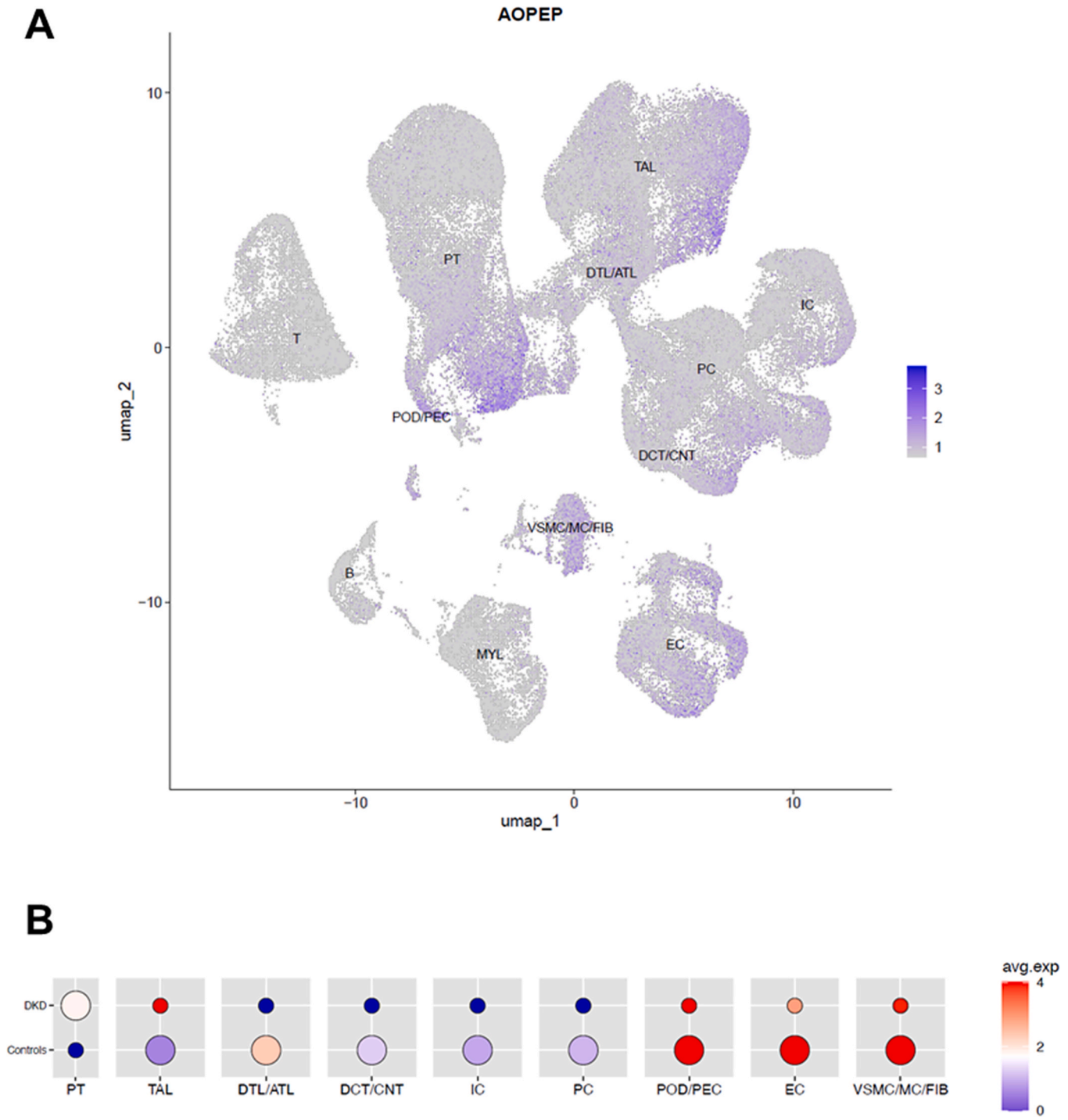

Fig. 5. *AOPEP (C9orf3)* kidney single cell expression by the Kidney Precision Medicine Project (KPMP- https://atlas.kpmp.org/), in healthy subjects and DKD patients. **A**: UMAP embedding of the single cell profiles from the DKD and healthy control samples showing the expression of AOPEP mapped to the nephron segments. Purple color indicates cells expressing AOPEP. **B:** Single cell RNAseq expression of AOPEP in both healthy controls and DKD samples. Dot sizes indicate the percentage of cells in which AOPEP expression was detected (larger dots indicate higher levels of AOPEP detected). Dot color indicates the average expression of AOPEP in the cell type. Abbreviations.ATL-ascending thin loop of Henle, CNT-connecting tubule, DCT-distal convoluted tubule, DTL-descending loop of Henle, EC-endothelial cell, FIB-fibroblast, IC-intercalated cell, MC-mesangial cell, PC- principal cell, PEC-parietal epithelial cell, POD-podocyte, PT-proximal tubular epithelial cell, TAL-thick ascending loop of Henle, VSMC-vascular smooth muscle cell. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

further validates the biological relevance of our findings. Additionally, the replication of splicing effects in vitro under hyperglycemic conditions lends mechanistic support and open the way to the possibility of novel class of drugs–splicing-modifying therapeutics in CKD and type 2 diabetes [51].

Nonetheless, limitations exist. The cohort was derived from a single center and may not capture global population heterogeneity. Although in vitro validation was performed, functional characterization of distinct AOPEP isoforms and their role in kidney injury was beyond the study's scope. It would be interesting in the future, to explore synergy with other

non-coding RNAs, including long non-coding RNAs (lncRNAs) that may act upstream or downstream of miR-27b-3p [52,53].

The lack of prospective validation limits immediate clinical translation. Moreover, while urinary miRNAs are stable and reproducible, pre-analytical variables such as storage, normalization methods, and sequencing depth require harmonization for clinical adoption. Thus, future investigations should aim to validate this biomarker panel in multicenter, multi-ethnic cohorts with longitudinal follow-up and assess the predictive utility of the panel for DKD progression and response to interventions (e.g., SGLT2 inhibitors, GLP-1 receptor agonists).

In conclusion, we identified a novel regulatory axis in diabetic nephropathy patients involving AOPEP SNP rs10761364 and glucose-driven alternative splicing that suppresses miR-27b-3p expression. This mechanism is associated with histologic fibrosis, systolic blood pressure and proteinuria and can be captured through a non-invasive biomarker panel incorporating urinary miRNA, genotype, and GA. Anchored in biopsy-confirmed diagnosis, our study bridges mechanistic insight and translational relevance, offering a step toward precision medicine in nephrology. This strategy not only improves diagnostic accuracy but paves the way for genotype-informed biomarker deployment that could refine DKD management across heterogeneous clinical settings.

Data availability statement

SNPs Sanger sequencing raw data are available in the open data publishing platform Dryad (https://doi.org/10.5061/dryad.6t1g1jx8p).

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CRediT authorship contribution statement

Francesca Conserva: Writing – original draft, Validation, Supervision, Project administration, Methodology, Data curation. **Francesco Pesce:** Writing – review & editing, Supervision, Software, Methodology, Investigation, Formal analysis, Data curation. **Claudia Cinefra:** Methodology, Formal analysis, Data curation. **Tommaso Maria Marvulli:** Software, Methodology, Formal analysis, Data curation. **Ighli Di Bari:** Software, Methodology, Formal analysis, Data curation. **Alessandra Stasi:** Writing – original draft, Supervision, Methodology, Formal analysis, Data curation. **Maria Felicia Faienza:** Visualization, Methodology, Investigation, Data curation. **Michele Rossini:** Supervision, Methodology, Investigation, Formal analysis, Data curation. **Adriano Montinaro:** Methodology, Formal analysis, Data curation. **Elena Squiccimarro:** Visualization, Methodology, Formal analysis, Data curation. **Giorgia Sclavo:** Writing – review & editing, Visualization, Investigation, Data curation. **Annalisa Schirinzi:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Francesca Di Serio:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Viji Nair:** Writing – review & editing, Writing – original draft, Validation, Software, Methodology, Formal analysis, Data curation. **Damian Fermin:** Writing – review & editing, Writing – original draft, Validation, Software, Methodology, Formal analysis, Data curation. **Rajasree Menon:** Writing – review & editing, Writing – original draft, Validation, Software, Methodology, Formal analysis, Data curation. **Edgar Otto:** Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Formal analysis, Data curation. **Fabio Sallustio:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Anna Gallone:** Writing – review & editing, Visualization, Data curation. **Giovanni Stallone:** Writing – review & editing, Visualization, Data curation. **Gianluigi Zaza:** Writing – review & editing, Visualization, Data curation. **Luigi Laviola:** Writing – review & editing, Visualization, Investigation, Data curation. **Marco Fiorentino:** Writing – review & editing, Visualization, Methodology, Data curation. **Francesco Giorgino:** Writing – review & editing, Supervision, Investigation, Data curation. **Maria F. Gomez:** Writing – review & editing, Validation, Funding acquisition, Formal analysis, Data curation. **Matthias Kretzler:** Writing – review & editing, Validation, Resources, Investigation, Funding acquisition, Formal analysis, Data curation. **Loreto Gesualdo:** Writing – review & editing, Supervision, Resources, Investigation, Funding acquisition, Data curation, Conceptualization. **Paola Pontrelli:** Writing – review & editing, Validation, Supervision, Project administration, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: LG received grant support from Abionyx, Sanofi and Astrazeneca to his University department (DIMEPRE-J); He has been a member of advisory boards for Astrazeneca, Baxter, Chinook, GSK, Mundipharma, Novartis, Pharmadoc, Roche, Sanofi, Travere, Vifor Pharma and an invited speaker at meetings supported by Astrazeneca, Astellas, Estor, Fresenius, Werfen, Medtronic, Travere, GSK. PP has been a member of advisory board for Sanofi and was an invited speaker at meetings supported by Werfen and Estor. FP was an invited speaker at meetings supported by Astrazeneca. MK receives grants and contracts through the University of Michigan with the National Institutes of Health, Chan Zuckerberg Initiative, JDRF, Alport Foundation, amfAR, AstraZeneca, NovoNordisk, Eli Lilly, Gilead, Janssen, Boehringer-Ingelheim, Moderna, European Union Innovative Medicine Initiative, Certa, Chinook, Angion, RenalytixAI, Travere, Regeneron, IONIS, Sanofi and Maze Therapeutics. He has received consulting fees through the University of Michigan from Janssen, NovoNordisk, Otsuka, Alexion, Variant Bio. Dr. Kretzler served on the NIH-NCATS council and is on the board of Nephcure Kidney International. Dr. Nair, and Dr. Kretzler hold a patent PCT/EP2014/073413 “Biomarkers and methods for progression prediction for chronic kidney disease” licensed. MFG has received financial and non-financial (in kind) support from Boehringer Ingelheim Pharma GmbH, JDRF International, Eli Lilly, AbbVie, Sanofi-Aventis, Astellas, Novo Nordisk A/S, Bayer AG, within EU grant H2020-JTI-lMl2-2015-05 (Grant agreement number 115974 - BEAt-DKD). She has also received financial and in-kind support from Novo Nordisk, Pfizer, Follicum, Coegin Pharma, Abcentra, Probi, Johnson & Johnson, within a project funded by the Swedish Foundation for Strategic Research on precision medicine in diabetes (LUDC-IRC #15-0067). Dr. Gomez has received personal consultancy fees from Lilly and Tribune Therapeutics AB. FC, CC, TMM, IdB, AS, MFF, MR, AM, ES, GS, AS, FdS, VN, DF, RM, EO, FS, AG, GS, GZ, LL, MF, FG have nothing to disclosure.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.diabres.2025.112460.

References

[1] Mazzucco G, Bertani T, Fortunato M, Bernardi M, Leutner M, Boldorini R, et al. Different patterns of renal damage in type 2 diabetes mellitus: a multicentric study on 393 biopsies. Am J Kidney Dis 2002;39(4):713–20.
[2] Gesualdo L, Di Paolo S. Renal lesions in patients with type 2 diabetes: a puzzle waiting to be solved. Nephrol Dial Transplant 2015;30(2):155–7.
[3] Gesualdo L, Fiorentino M, Conserva F, Pontrelli P. Should we enlarge the indication for kidney biopsy in patients with diabetes? The pro part. Clin Kidney J 2023;17(1):sfad266.
[4] de Boer IH, Caramori ML, Chan JC, Heerspink HJ, Hurst C, Khunti K, Liew A, Michos ED, Navaneethan SD, Olowu WA, Sadusky T. KDIGO 2020 clinical practice guideline for diabetes management in chronic kidney disease. Kidney Int. 2020;98(4):S1–115.
[5] Oshima M, Shimizu M, Yamanouchi M, Toyama T, Hara A, Furuichi K, et al. Trajectories of kidney function in diabetes: a clinicopathological update. Nat Rev Nephrol 2021;17(11):740–50.
[6] Zhou D, Liu Y. Renal fibrosis in 2015: Understanding the mechanisms of kidney fibrosis. Nat Rev Nephrol 2016;12(2):68–70.
[7] Brosius 3rd FC. New insights into the mechanisms of fibrosis and sclerosis in diabetic nephropathy. Rev Endocr Metab Disord 2008;9(4):245–54.
[8] Limonte CP, Kretzler M, Pennathur S, Pop-Busui R, de Boer IH. Present and future directions in diabetic kidney disease. J Diabetes Complications 2022;36(12):108357.
[9] Sandholm N, Cole JB, Nair V, Sheng X, Liu H, Ahlqvist E, et al. GENIE Consortium. Genome-wide meta-analysis and omics integration identifies novel genes associated with diabetic kidney disease. Diabetologia 2022;65(9):1495–509.
[10] Salem RM, Todd JN, Sandholm N, Cole JB, Chen WM, Andrews D, et al. SUMMIT consortium, DCCT/EDIC research group, GENIE consortium. Genome-wide association study of diabetic kidney disease highlights biology involved in glomerular basement membrane collagen. J Am Soc Nephrol 2019;30(10):2000–16.
[11] Pontrelli P, Conserva F, Papale M, Oranger A, Barozzino M, Vocino G, et al. Lysine 63 ubiquitination is involved in the progression of tubular damage in diabetic nephropathy. FASEB J 2017;31(1):308–19.
[12] Pontrelli P, Oranger A, Barozzino M, Divella C, Conserva F, Fiore MG, et al. Deregulation of autophagy under hyperglycemic conditions is dependent on increased lysine 63 ubiquitination: a candidate mechanism in the progression of diabetic nephropathy. J Mol Med (Berl) 2018;96(7):645–59.
[13] Pontrelli P, Conserva F, Menghini R, Rossini M, Stasi A, Divella C, et al. Inhibition of lysine 63 ubiquitination prevents the progression of renal fibrosis in diabetic DBA/2J mice. Int J Mol Sci 2021;22(10):5194.
[14] Conserva F, Barozzino M, Pesce F, Divella C, Oranger A, Papale M, et al. Urinary miRNA-27b-3p and miRNA-1228-3p correlate with the progression of kidney fibrosis in diabetic nephropathy. Sci Rep 2019;9(1):11357.
[15] Sacks DB, Arnold M, Bakris GL, Bruns DE, Horvath AR, Lernmark Å, et al. Executive summary: guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. Diabetes Care 2023;46(10):1740–6.
[16] Breeze CE, Haugen E, Gutierrez-Arcelus M, Yao X, Teschendorff A, Beck S, et al. FORGEdb: a tool for identifying candidate functional variants and uncovering target genes and mechanisms for complex diseases. Genome Biol 2024;25(1):3.
[17] Nishida K, Kuwano Y, Rokutan K. The MicroRNA-23b/27b/24 cluster facilitates colon cancer cell migration by targeting FOXP2. Cancers (Basel) 2020 Jan 10;12(1):174. https://doi.org/10.3390/cancers12010174.
[18] Kouzuma T, Uemastu Y, Usami T, Imamura S. Study of glycated amino acid elimination reaction for an improved enzymatic glycated albumin measurement method. Clin Chim Acta 2004;346(2):135–43.
[19] Kohzuma T, Yamamoto T, Uematsu Y, Shihabi ZK, Freedman BI. Basic performance of an enzymatic method for glycated albumin and reference range determination. J Diabetes Sci Technol 2011;5(6):1455–62.
[20] Young MD, Behjati S. SoupX removes ambient RNA contamination from droplet-based single-cell RNA sequencing data. GigaScience 2020;9(12):giaa151.
[21] Hao Y, Hao S, Andersen-Nissen E, Mauck 3rd WM, Zheng S, Butler A, et al. Integrated analysis of multimodal single-cell data. Cell 2021;184(13):3573–3587.e29.
[22] Gooding KM, Lienczewski C, Papale M, Koivuviita N, Maziarz M, et al. BEAt-DKD consortium. Prognostic imaging biomarkers for diabetic kidney disease (iBEAt): study protocol. BMC Nephrol 2020;21(1):242.
[23] GTEx Consortium. The GTEx Consortium atlas of genetic regulatory effects across human tissues. Science 2020;369(6509):1318–30.
[24] Sonehara K, Sakaue S, Maeda Y, Hirata J, Kishikawa T, Yamamoto K, et al. Genetic architecture of microRNA expression and its link to complex diseases in the Japanese population. Hum Mol Genet 2022;31(11):1806–20.
[25] Ichikawa M, Konoshita T, Makino Y, Suzuki J, Ishizuka T, Nakamura H. An association study of C9orf3, a novel component of the renin-angiotensin system, and hypertension in diabetes. Sci Rep 2020;10(1):16111.
[26] Costanzo MC, von Grotthuss M, Massung J, Jang D, Caulkins L, Koesterer R, et al. The type 2 diabetes knowledge portal: an open access genetic resource dedicated to type 2 diabetes and related traits. Cell Metab 2023;35(4):695–710.e6.
[27] Park E, Pan Z, Zhang Z, Lin L, Xing Y. The expanding landscape of alternative splicing variation in human populations. Am J Hum Genet 2018;102(1):11–26.
[28] Scotti M, Swanson M. RNA mis-splicing in disease. Nat Rev Genet 2016;17:19–32.
[29] Mahtal N, Lenoir O, Tinel C, et al. MicroRNAs in kidney injury and disease. Nat Rev Nephrol 2022;18:643–62.
[30] Liebisch M, Wolf G. Role of epigenetic changes in the pathophysiology of diabetic kidney disease. Glomerular Dis 2024;4(1):211–26.
[31] Lu HC, Dai WN, He LY. Epigenetic histone modifications in the pathogenesis of diabetic kidney disease. Diabetes Metab Syndr Obes 2021;14:329–44.
[32] Kato M, Natarajan R. Epigenetics and epigenomics in diabetic kidney disease and metabolic memory. Nat Rev Nephrol 2019;15(6):327–45.
[33] Liu Z, Liu J, Wang W, An X, Luo L, Yu D, et al. Epigenetic modification in diabetic kidney disease. Front Endocrinol (Lausanne) 2023;14:1133970.
[34] Szostak J, Gorący A, Durys D, Dec P, Modrzejewski A, Pawlik A. The role of MicroRNA in the pathogenesis of diabetic nephropathy. Int J Mol Sci 2023;24(7):6214.
[35] Casagrande V, Federici M, Menghini R. TIMP3 involvement and potentiality in the diagnosis, prognosis and treatment of diabetic nephropathy. Acta Diabetol 2021;58(12):1587–94.
[36] Xu W, Li F, Liu Z, Xu Z, Sun B, Cao J, et al. MicroRNA-27b inhibition promotes Nrf2/ARE pathway activation and alleviates intracerebral hemorrhage-induced brain injury. Oncotarget 2017;8:70669–84.
[37] Zhao XR, Zhang Z, Gao M, Li L, Sun PY, Xu LN, et al. MicroRNA-27a-3p aggravates renal ischemia/reperfusion injury by promoting oxidative stress via targeting growth factor receptor-bound protein 2. Pharmacol Res 2020;155:104718.
[38] Silva-Llanes I, Shin CH, Jiménez-Villegas J, Gorospe M, Lastres-Becker I. The transcription factor NRF2 has epigenetic regulatory functions modulating HDACs, DNMTs, and miRNA biogenesis. Antioxidants (Basel) 2023;12(3):641.
[39] Lu Z, Liu N, Wang F. Epigenetic regulations in diabetic nephropathy. J Diabetes Res 2017;2017:7805058.
[40] Park SB, Kim SS, Kim IJ, Nam YJ, Ahn KH, Kim JH, et al. Variability in glycated albumin levels predicts the progression of diabetic nephropathy. J Diabetes Complications 2017;31(6):1041–6.
[41] Wang N, Xu Z, Han P, Li T. Glycated albumin and ratio of glycated albumin to glycated hemoglobin are good indicators of diabetic nephropathy in type 2 diabetes mellitus. Diabetes Metab Res Rev 2017;33(2).
[42] Kuo IC, Lin HY, Niu SW, Lee JJ, Chiu YW, Hung CC, et al. Anemia modifies the prognostic value of glycated hemoglobin in patients with diabetic chronic kidney disease. PLoS One 2018;13(6):e0199378.
[43] Gan T, Liu X, Xu G. Glycated albumin versus HbA1c in the evaluation of glycemic control in patients with diabetes and CKD. Kidney Int Rep 2017;3(3):542–54.
[44] Speeckaert M, Van Biesen W, Delanghe J, Slingerland R, Wiecek A, Heaf J, et al. European renal best practice guideline development group on diabetes in advanced CKD. Are there better alternatives than haemoglobin A1c to estimate glycaemic control in the chronic kidney disease population? Nephrol Dial Transplant 2014;29(12):2167–77.
[45] Rodiño-Janeiro BK, González-Peteiro M, Ucieda-Somoza R, González-Juanatey JR, Alvarez E. Glycated albumin, a precursor of advanced glycation end-products, up-regulates NADPH oxidase and enhances oxidative stress in human endothelial cells: molecular correlate of diabetic vasculopathy. Diabetes Metab Res Rev 2010;26(7):550–8.
[46] Sun IO, Lerman LO. Urinary microRNA in kidney disease: utility and roles. Am J Physiol Renal Physiol 2019;316(5):F785–93.
[47] Castillo-Rodriguez E, Fernandez-Prado R, Martin-Cleary C, Pizarro-Sánchez MS, Sanchez-Niño MD, Sanz AB, et al. Kidney injury marker 1 and neutrophil gelatinase-associated lipocalin in chronic kidney disease. Nephron 2017;136(4):263–7.
[48] Fiorentino L, Cavalera M, Mavilio M, Conserva F, Menghini R, Gesualdo L, et al. Regulation of TIMP3 in diabetic nephropathy: a role for microRNAs. Acta Diabetol 2013;50(6):965–9.
[49] de Boer IH, Alpers CE, Azeloglu EU, Balis UGJ, Barasch JM, Barisoni L, et al. Kidney precision medicine project. Rationale and design of the kidney precision medicine project. Kidney Int 2021;99(3):498–510.
[50] Fiorentino M, Bolignano D, Tesar V, Pisano A, Biesen WV, Tripepi G, et al. ERA-EDTA immunonephrology working group. Renal biopsy in patients with diabetes: a pooled meta-analysis of 48 studies. Nephrol Dial Transplant 2017;32(1):97–110.

[51] Stevens M, Oltean S. Alternative splicing in CKD. J Am Soc Nephrol 2016;27(6):1596–603.
[52] Zhang Q, Shao W, Li Y, Liu L, Chen W, Wang C, et al. Long non-coding RNA LINC01128 affects proliferation, migration, and invasion of glioma cells by regulating miR-27b-3p. Folia Neuropathol 2022;60(3):338–45.
[53] Li S, Zhao X, Cheng S, Li J, Bai X, Meng X. Downregulating long non-coding RNA PVT1 expression inhibited the viability, migration and phenotypic switch of PDGF-BB-treated human aortic smooth muscle cells via targeting miR-27b-3p. Hum Cell 2021;34(2):335–48.