

SLC30A8 Promoter Hypomethylation is Associated with Impaired Renal Function in Type 2 Diabetes: A Cross-Sectional Study

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Background: Diabetic kidney disease (DKD) remains a major challenge in type 2 diabetes (T2DM) management. The solute carrier family 30 member 8 (SLC30A8) gene is a known T2DM susceptibility locus. However, the relationship between SLC30A8 promoter methylation and renal function in DKD remains unclear. This study aimed to investigate this association.

Methods: In this cross-sectional study, 351 hospitalized patients with T2DM (181 with DKD, 170 without) were enrolled. Peripheral blood DNA methylation at six CpG sites in the SLC30A8 promoter was quantified by pyrosequencing. Multivariate linear/logistic regression, smooth curve fitting, and subgroup analyses were used to evaluate the independent association between SLC30A8 methylation and estimated glomerular filtration rate (eGFR).

Results: SLC30A8 methylation was significantly lower in the DKD group ($73.6\% \pm 6.4\%$) than in the T2DM-only group ($88.7\% \pm 7.1\%$, $P < 0.001$), and progressively decreased across categories of declining eGFR and increasing albuminuria (all P for trend < 0.05). In a multivariate linear regression model adjusted for age, sex, BMI, systolic blood pressure, UACR, uric acid, lipids, fasting glucose, and diabetes duration, each 1% increase in methylation was independently associated with a $2.336 \text{ mL/min/1.73 m}^2$ higher eGFR (95% CI: $1.780\text{--}2.891$, $P < 0.001$). A nonlinear threshold was identified at 70.9% methylation. Below this, the association with eGFR was nonsignificant; above it, each 1% increase correlated with a $4.471 \text{ mL/min/1.73 m}^2$ increase in eGFR (95% CI: $3.961\text{--}4.981$, $P < 0.001$). This stable association was consistent across all clinical subgroups (P for interaction > 0.05).

Conclusion: SLC30A8 promoter hypomethylation is independently associated with impaired renal function in T2DM, exhibiting a dose-response relationship where lower methylation levels correlated with more severe kidney damage, and a distinct threshold effect at approximately 70.9% methylation, below which the association with eGFR was significantly stronger. These exploratory findings suggest that SLC30A8 promoter methylation may represent a promising epigenetic biomarker for DKD risk stratification, warranting further validation in prospective studies.

Keywords: type 2 diabetes mellitus, diabetic kidney disease, DNA methylation, SLC30A8, estimated glomerular filtration rate, epigenetics, biomarker

Introduction

Diabetic kidney disease (DKD) represents a prevalent and severe microvascular complication of type 2 diabetes mellitus (T2DM), standing as the leading cause of end-stage renal disease (ESRD) worldwide.¹ Its clinical hallmarks include a progressive decline in estimated glomerular filtration rate (eGFR) and the emergence of persistent albuminuria.² Despite advancements in therapeutic strategies, including renin-angiotensin system blockade and newer agents such as SGLT2 inhibitors, early and accurate identification of patients at high risk for DKD progression remains a significant clinical challenge. This is partly due to the inherent limitations of conventional biomarkers; urinary albumin-to-creatinine ratio (UACR) exhibits considerable variability, and eGFR often reflects established renal damage rather than incipient

risk.^{3,4} Consequently, there is a pressing need to elucidate novel molecular mechanisms and identify accessible biomarkers for improved early risk stratification.

Epigenetic regulation, particularly DNA methylation, has emerged as a critical interface between genetic predisposition, environmental exposures, and the pathophysiology of complex metabolic diseases.^{5,6} DNA methylation, involving the covalent addition of a methyl group to cytosine within CpG dinucleotides, serves as a key regulator of gene expression stability. It has been implicated in the phenomenon of “metabolic memory,” wherein prior hyperglycemic exposure can induce lasting epigenetic changes that drive diabetic complications, even after subsequent glycemic control is achieved.^{7,8} Genome-wide methylation studies have identified differentially methylated regions associated with both T2DM onset and DKD progression, suggesting that epigenetic alterations are active contributors to disease pathogenesis.^{9,10} These modifications, detectable in accessible tissues like peripheral blood, hold promise as potential biomarkers and may reveal novel therapeutic targets.¹¹

The solute carrier family 30 member 8 (*SLC30A8*) gene, encoding the zinc transporter 8 (ZnT8), is crucial for zinc transport into insulin secretory granules of pancreatic β -cells, a process essential for insulin processing and secretion.^{12,13} Large-scale genome-wide association studies (GWAS) have consistently identified *SLC30A8* as a susceptibility locus for T2DM across diverse populations.^{14,15} However, GWAS-identified variants explain only a fraction of disease heritability, shifting research focus toward the role of epigenetic regulation, such as promoter DNA methylation, in modulating this genetic risk.¹⁶ Emerging evidence indicates altered *SLC30A8* promoter methylation in patients with T2DM.^{17,18} Intriguingly, while some studies in specific populations have associated *SLC30A8* promoter hypermethylation with increased T2DM risk,¹⁸ the role of its epigenetic regulation in diabetic complications, particularly DKD, remains largely unexplored. Emerging evidence suggests that zinc dysregulation contributes to the pathogenesis of diabetic complications, including nephropathy, through pathways involving oxidative stress, inflammation, and fibrosis.¹⁹ As a key regulator of zinc homeostasis in insulin-producing cells and potentially other tissues, dysfunction of *SLC30A8* could have systemic metabolic consequences. Promoter DNA methylation is a key epigenetic mechanism that typically silences gene expression. Therefore, we postulate that altered methylation patterns in the *SLC30A8* promoter may modulate ZnT8 expression, leading to disturbances in intra- and extracellular zinc balance. This dysregulation could, in turn, exacerbate hyperglycemia-induced oxidative stress and inflammatory responses within the renal microenvironment, ultimately contributing to the decline in renal function observed in DKD. This biological plausibility provides a strong rationale for investigating the association between *SLC30A8* promoter methylation and kidney function in T2DM patients.

It is important to acknowledge several methodological considerations inherent to the study design. First, DNA methylation patterns are often tissue-specific, and methylation measured in peripheral blood leukocytes may not directly reflect the epigenetic status of renal parenchymal cells.¹¹ However, accumulating evidence suggests that blood-derived DNA methylation can serve as a surrogate for systemic epigenetic changes and has been associated with various disease phenotypes, including kidney function decline.^{10,11} Second, the cross-sectional design precludes determination of the temporal direction of the observed association; thus, the possibility of reverse causality—whereby declining renal function itself induces epigenetic modifications—cannot be excluded. Third, while peripheral blood is an accessible and clinically practical tissue for biomarker discovery, extrapolating findings from blood to kidney pathology requires caution, as methylation changes in blood may reflect systemic metabolic or inflammatory states rather than direct renal mechanisms. Despite these inherent limitations, investigating *SLC30A8* methylation in peripheral blood represents a pragmatic first step toward identifying accessible epigenetic markers for DKD risk stratification. If validated, such markers could complement existing clinical tools and provide insights into systemic epigenetic dysregulation in diabetic complications.

Therefore, this study aimed to investigate the relationship between peripheral blood *SLC30A8* promoter DNA methylation levels and renal function in a well-characterized Chinese cohort of patients with T2DM, with and without DKD. We hypothesized that *SLC30A8* promoter hypomethylation is associated with worsening renal function and may serve as an independent epigenetic marker for DKD risk.

Methods

Study Design and Participants

This cross-sectional study was conducted at the First Affiliated Hospital of Shenzhen University. We consecutively enrolled 351 hospitalized patients diagnosed with type 2 diabetes mellitus (T2DM) between June 2019 and December 2023. The cohort included 181 patients diagnosed with diabetic kidney disease (DKD) and 170 T2DM patients without DKD. The diagnosis of T2DM was established according to the 2024 American Diabetes Association (ADA) guidelines,²⁰ based on at least one of the following criteria: fasting plasma glucose ≥ 7.0 mmol/L, random plasma glucose ≥ 11.1 mmol/L, 2-hour plasma glucose ≥ 11.1 mmol/L during a 75-g oral glucose tolerance test, or hemoglobin A1c (HbA1c) $\geq 6.5\%$. DKD was diagnosed according to the 2024 Kidney Disease: Improving Global Outcomes (KDIGO) guidelines,²¹ defined as the presence of persistent albuminuria (urinary albumin-to-creatinine ratio, UACR ≥ 30 mg/g) and/or a persistent reduction in estimated glomerular filtration rate (eGFR < 60 mL/min/1.73 m²) for more than 3 months in patients with diabetes, after excluding other primary renal diseases. Exclusion criteria were: history of dialysis, acute infection, viral hepatitis, inflammatory bowel disease, autoimmune diseases, malignancy, organ transplantation, pregnancy, non-diabetic renal disease, neurodegenerative diseases, or the use of corticosteroids or immunosuppressants within the preceding three months. We confirm that the study was conducted in accordance with the Declaration of Helsinki, approved by the Clinical Research Ethics Committee of the First Affiliated Hospital of Shenzhen University (Approval No: 2024-199-01PJ), and written informed consent was obtained from all participants.

Definitions and Data Collection

Demographic characteristics (age and sex), anthropometric measurements, medical history, and laboratory data were extracted from the hospital's electronic medical record system. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Renal function was assessed by eGFR, calculated using the simplified four-variable Modification of Diet in Renal Disease (MDRD) study equation, which is validated for Chinese populations.²²

Measurement of SLC30A8 Promoter DNA Methylation

Genomic DNA was extracted from peripheral blood leukocytes using a magnetic bead-based automated nucleic acid extraction system. DNA concentration and purity were assessed by spectrophotometry (OD_{260/280} ratio 1.8–2.0), and samples with concentrations > 50 ng/ μ L were used for subsequent analysis.

Bisulfite conversion was performed using the EZ DNA Methylation-Gold Kit (Zymo Research) according to the manufacturer's protocol. A region encompassing six adjacent CpG sites within the *SLC30A8* promoter (GRCh38 chr8:118,188,200–118,188,250) was amplified via PCR using the following primers: forward, 5'-GGGAGTTGGTTT TAGTATTGGTTAGTTT-3'; reverse, 5'-[Biotin]ACCTTCCTTCAATATACATTAACTAATC-3'. PCR reactions were conducted under the following conditions: initial denaturation at 95°C for 3 min; 40 cycles of denaturation at 94°C for 30s, annealing at 50°C for 30s, and extension at 72°C for 1 min; final extension at 72°C for 7 min.

Pyrosequencing was performed on a PyroMark Q48 system (Qiagen) using the sequencing primer 5'-GTTAGTTT TAGAGAGGGG-3'. Methylation percentages at each of the six CpG sites were quantified using PyroMark Q48 software (version 2.5.8), and the average methylation level across all six sites was used for statistical analysis. The intra-assay coefficient of variation for replicate samples was $< 5\%$.

Statistical Analysis

Statistical analyses were performed using R software (version 4.3.0, R Foundation for Statistical Computing) and EmpowerStats (version 4.1, X&Y Solutions). For participant characteristics: Continuous variables were tested for normality using the Shapiro–Wilk test. Normally distributed continuous variables (eg., age, BMI, systolic blood pressure, eGFR, and SLC30A8 methylation levels) are presented as mean $\pm$ standard deviation (SD) and were compared between the T2DM and DKD groups using Student's *t*-test. Categorical variables (eg., sex) are presented as numbers (percentages) and were compared using the Chi-square test. For trend analyses across multiple groups: When comparing methylation levels across CKD stages (Figure 1A) and UACR categories (Figure 1B), one-way ANOVA (with post-hoc Bonferroni correction) was used for

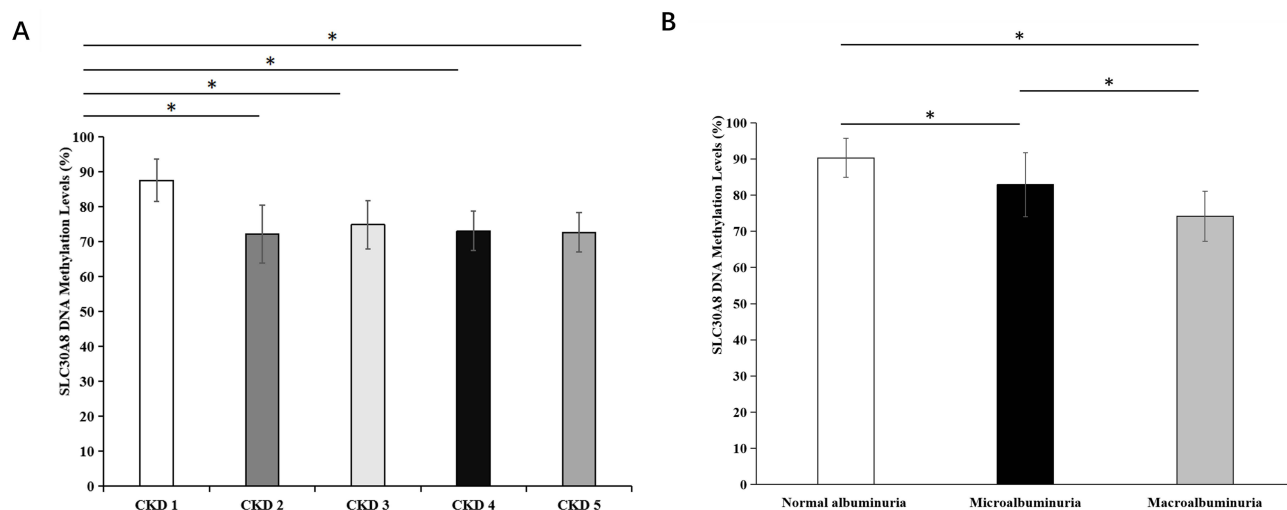


Figure 1 SLC30A8 promoter methylation levels in patients with diabetic kidney disease and type 2 diabetes mellitus. **(A)** SLC30A8 promoter methylation levels across chronic kidney disease (CKD) stages in patients with diabetic kidney disease (DKD, $n=181$). CKD stages are defined according to the KDIGO 2024 guidelines based on estimated glomerular filtration rate (eGFR): Stage 1 (≥ 90 mL/min/1.73 m^2), Stage 2 (60–89), Stage 3 (30–59), Stage 4 (15–29), and Stage 5 (<15). Data are presented as mean $\pm$ SD. Comparisons among stages were performed using one-way ANOVA with post-hoc Bonferroni correction. * $P < 0.05$ for indicated pairwise comparisons. **(B)** SLC30A8 promoter methylation levels across urinary albumin-to-creatinine ratio (UACR) categories in the total type 2 diabetes mellitus (T2DM) cohort ($n=351$). UACR categories are defined as: normoalbuminuria (<30 mg/g), microalbuminuria (30–300 mg/g), and macroalbuminuria (≥ 300 mg/g). Data are presented as mean $\pm$ SD. Comparisons among groups were performed using one-way ANOVA with post-hoc Bonferroni correction. * $P < 0.05$ for all pairwise comparisons.

Abbreviations: CKD, chronic kidney disease; DKD, diabetic kidney disease; eGFR, estimated glomerular filtration rate; SD, standard deviation; T2DM, type 2 diabetes mellitus; UACR, urinary albumin-to-creatinine ratio.

normally distributed data, and the Kruskal–Wallis test was applied where appropriate. The linear trends were assessed using the Cochran–Armitage trend test or linear regression contrast, as indicated. For the primary analysis: The independent association between SLC30A8 methylation (independent variable) and eGFR (continuous dependent variable) was assessed using multiple linear regression, reported as β coefficient with 95% confidence interval (CI). Covariates were selected based on their known or potential associations ($P < 0.05$) with both methylation and eGFR, as identified in prior literature and univariate analyses. Age, sex, and BMI were included as basic demographic and anthropometric confounders. Three models were constructed: Model 1 (crude, unadjusted); Model 2 adjusted for age, sex, and BMI; and Model 3 further adjusted for systolic blood pressure, UACR, serum uric acid, total cholesterol, LDL-C, fasting glucose, and diabetes duration. Additionally, to assess the robustness of the association using an alternative clinically relevant outcome, we performed logistic regression analysis with impaired renal function (defined as $eGFR < 60$ mL/min/1.73 m^2) as the dependent variable. The same covariate adjustment strategy (Models I and II) was applied. For non-linearity exploration: Potential non-linearity in the methylation-eGFR relationship was explored using generalized additive models (GAM). If a threshold was suggested, a two-piecewise linear regression model was applied, with the inflection point determined by a recursive algorithm and compared to a simple linear model using the log-likelihood ratio test. For subgroup analyses: The consistency of the association was examined across prespecified strata using interaction tests within the multiple linear regression framework. P for interaction < 0.05 was considered indicative of significant effect modification. All statistical tests were two-tailed, and $P < 0.05$ was considered statistically significant.

Results

Demographic and Clinical Characteristics of the Study Population

A total of 351 patients with T2DM were included in this study, comprising 170 patients without DKD (hereafter referred to as the T2DM-only group) and 181 patients with DKD (DKD group). The demographic and clinical characteristics of the two groups were summarized in Table 1. Compared to the T2DM group, patients in the DKD group were significantly older (61.5 ± 13.5 vs. 55.6 ± 14.9 years, $P < 0.001$), had a longer duration of diabetes (14.2 ± 9.2 vs. 10.0 ± 8.7 years, $P < 0.001$), higher systolic blood pressure, and worse renal function parameters, including significantly elevated UACR, serum creatinine, urea nitrogen, and uric acid (all $P < 0.001$). Consequently, eGFR was markedly lower in the DKD group (22.3 ± 22.6 vs. 116.9 ± 30.9 mL/min/1.73 m^2 ,

Table 1 Demographic and Clinical Characteristics of Type 2 Diabetes Patients with and without Diabetic Kidney Disease

Variable	T2DM-Only group (n =170)	DKD Group (n =181)	P value
Male, n (%)	101 (59.4)	121 (66.9)	0.149
Age (years)	55.6 ± 14.9	61.5 ± 13.5	<0.001
BMI (kg/m ²)	24.3 ± 3.8	23.8 ± 5.2	0.916
Systolic BP (mmHg)	122.9 ± 17.0	149.3 ± 23.2	< 0.001
Hemoglobin (g/L)	133.0 ± 15.6	97.9 ± 20.0	< 0.001
UACR (mg/g)	18.5 ± 14.8	3929.5 ± 2818.3	<0.001
Serum albumin (g/L)	41.5 ± 6.0	33.3 ± 7.1	< 0.001
ALT (U/L)	23.7 ± 21.9	20.4 ± 27.2	0.085
AST (U/L)	36.6 ± 20.2	27.1 ± 30.3	0.021
Blood urea nitrogen (mmol/L)	5.5 ± 1.7	18.0 ± 8.8	< 0.001
Serum creatinine (μmol/L)	63.3 ± 18.4	425.2 ± 273.4	< 0.001
Serum uric acid (μmol/L)	348.8 ± 88.4	430.3 ± 108.8	< 0.001
Serum potassium (mmol/L)	4.0 ± 0.3	4.4 ± 0.6	< 0.001
Fasting plasma glucose (mmol/L)	6.6 ± 2.3	6.9 ± 3.3	0.860
Triglycerides (mmol/L)	1.7 ± 1.4	2.1 ± 2.0	0.077
Total cholesterol (mmol/L)	4.5 ± 1.2	4.9 ± 1.9	0.463
HDL-C (mmol/L)	1.1 ± 0.3	1.1 ± 0.3	0.750
LDL-C (mmol/L)	2.8 ± 1.0	3.1 ± 1.8	0.268
Age at diabetes onset (years)	44.6 ± 13.0	47.4 ± 12.2	0.049
Diabetes duration (years)	10.0 ± 8.7	14.2 ± 9.2	< 0.001
SLC30A8 promoter DNA methylation (%)	88.7 ± 7.1	73.6 ± 6.4	< 0.001
eGFR (mL/min/1.73 m ²)	116.9 ± 30.9	22.3 ± 22.6	< 0.001

Notes: Data are presented as mean ± standard deviation (SD) or number (percentage). Comparisons between groups were performed using Student's t-test for normally distributed continuous variables and the χ^2 -test for categorical variables. Normality was assessed using the Shapiro-Wilk test.

Abbreviations: BMI, body mass index; BP, blood pressure; UACR, urinary albumin-to-creatinine ratio; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; eGFR, estimated glomerular filtration rate; T2DM, type 2 diabetes mellitus; DKD, diabetic kidney disease.

$P < 0.001$). Crucially, the average DNA methylation level of the *SLC30A8* promoter was significantly lower in the DKD group compared to the T2DM group ($73.6\% \pm 6.4\%$ vs. $88.7\% \pm 7.1\%$, $P < 0.001$).

SLC30A8 Methylation Levels Across Renal Function Categories

When patients were stratified according to KDIGO categories based on eGFR and UACR,²¹ a clear trend was observed. Among the 181 DKD patients, *SLC30A8* methylation levels showed a significant decreasing trend across advancing CKD stages (P for trend < 0.05 , Figure 1A). Furthermore, across the entire cohort of 351 T2DM patients, methylation levels progressively declined from the normoalbuminuria group ($90.3\% \pm 5.4\%$), to the microalbuminuria group ($82.9\% \pm 8.8\%$), and were lowest in the macroalbuminuria group ($74.2\% \pm 6.9\%$), with a significant decreasing trend across increasing albuminuria categories (P for trend < 0.05 , Figure 1B).

Association Between SLC30A8 Methylation and eGFR

Univariate linear regression analysis revealed that *SLC30A8* promoter methylation was strongly and positively associated with eGFR ($\beta = 4.070$, 95% CI: 3.702 to 4.438, $P < 0.001$), alongside other expected clinical correlates such as age, systolic blood pressure, and serum creatinine (full univariate results were shown in Supplementary Table S1). This association remained robust in multivariate linear regression models. After adjusting for age, sex, and BMI (Model 2), each 1% increase in methylation was associated with a 3.866 mL/min/1.73 m² increase in eGFR (95% CI: 3.474 to 4.257, $P < 0.001$). Further comprehensive adjustment for systolic blood pressure, UACR, uric acid, lipid profiles, fasting glucose, and diabetes duration (Model 3) attenuated the effect size, but the association remained highly significant ($\beta = 2.336$, 95% CI: 1.780 to 2.891, $P < 0.001$) (Table 2).

Table 2 Multivariate Linear Regression Analysis of SLC30A8 Methylation and eGFR

Variable	Model	β (95% CI)	P value
SLC30A8 methylation (%)	Model 1 (Crude)	4.070 (3.702, 4.438)	< 0.001
	Model 2	3.866 (3.474, 4.257)	< 0.001
	Model 3	2.336 (1.780, 2.891)	< 0.001

Notes: The β coefficient represents the change in eGFR (mL/min/1.73 m²) per 1% increase in SLC30A8 promoter methylation level. Model 1: Crude, unadjusted. Model 2: Adjusted for age, sex, and body mass index (BMI). Model 3: Further adjusted for systolic blood pressure, urinary albumin-to-creatinine ratio (UACR), serum uric acid, total cholesterol, low-density lipoprotein cholesterol (LDL-C), fasting plasma glucose, and diabetes duration.

Abbreviation: CI, confidence interval.

Non-Linear Relationship and Threshold Effect Analysis

Smooth curve fitting suggested a non-linear relationship between *SLC30A8* methylation and eGFR. A two-piecewise linear regression model identified a significant inflection point at a methylation level of 70.90% (log-likelihood ratio test $P=0.003$). When methylation was below this threshold, its association with eGFR was not significant ($\beta = 0.230$, $P=0.857$). However, above 70.90%, each 1% increase in methylation was strongly associated with a 4.471 mL/min/1.73 m² increase in eGFR (95% CI: 3.961 to 4.981, $P<0.001$). The piecewise model provided a significantly better fit than the simple linear model, confirming the presence of a threshold effect. (Figure 2 and Table 3).

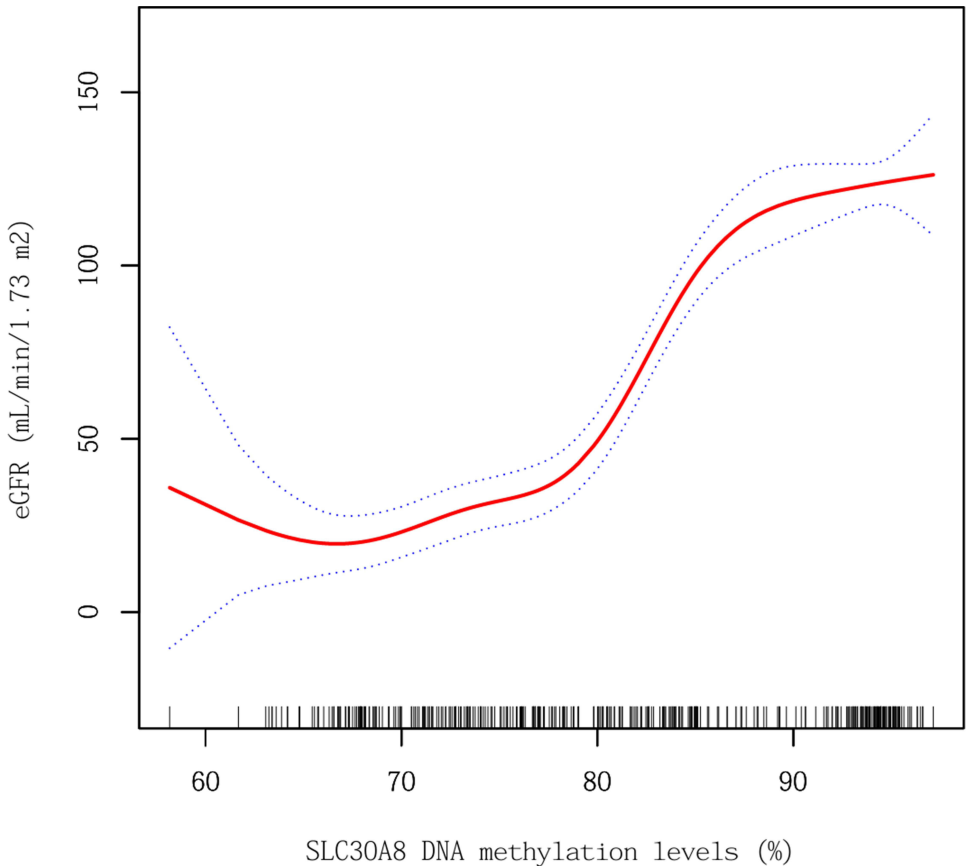


Figure 2 Non-linear association between SLC30A8 promoter methylation and estimated glomerular filtration rate (eGFR). The solid red line represents the fitted curve from the generalized additive model (GAM), and the shaded area indicates the 95% confidence band. A two-piecewise linear regression model identified a significant inflection point at 70.9% methylation. The log-likelihood ratio test confirmed that the piecewise model provided a significantly better fit than the simple linear model ($P = 0.003$). Below the threshold ($\leq 70.9\%$), the association with eGFR was non-significant ($\beta = 0.230$, $P = 0.857$); above the threshold ($> 70.9\%$), each 1% increase in methylation was associated with a 4.471 mL/min/1.73 m² increase in eGFR (95% CI: 3.961–4.981, $P < 0.001$).

Abbreviations: eGFR, estimated glomerular filtration rate; GAM, generalized additive model; CI, confidence interval.

Table 3 Threshold and Saturation Effect Analysis of SLC30A8 Promoter Methylation on eGFR (n = 351)

Model	β (95% CI)	P value
Linear model	3.995 (3.584, 4.405)	< 0.001
Two-piecewise model		
Inflection point (K)	70.902%	
Slope $\leq$ K	0.230 (−2.264, 2.723)	0.857
Slope $>$ K	4.471 (3.961, 4.981)	< 0.001
Difference in slopes ($>$ K vs $\leq$ K)	4.241 (1.469, 7.013)	0.003
Predicted eGFR at inflection point (mL/min/1.73 m ²)	16.201 (8.410, 23.992)	
Log-likelihood ratio test (piecewise vs linear)	—	0.003

Notes: The inflection point (K = 70.9%) was identified by a recursive algorithm that maximizes the model fit. The β coefficient indicates the change in eGFR (mL/min/1.73 m²) per 1% increase in SLC30A8 methylation within each segment. The log-likelihood ratio test was used to compare the two-piecewise linear model with the simple linear model; a significant P-value (P = 0.003) indicates that the piecewise model provides a significantly better fit, confirming a threshold effect.

Abbreviation: CI, confidence interval.

Sensitivity Analyses

To evaluate the robustness of the association between SLC30A8 promoter methylation and renal function, we conducted an additional analysis using an alternative outcome definition—impaired renal function defined as eGFR <60 mL/min/1.73 m²—with logistic regression models (Table 4). In the unadjusted model, each 1% increase in methylation was associated with a 23.8% lower odds of impaired renal function (OR = 0.762, 95% CI: 0.722–0.804, P < 0.001). After adjustment for demographic and clinical covariates (Model I: age, sex, BMI, systolic blood pressure, UACR, uric acid, total cholesterol, LDL-C), the association remained highly significant (OR = 0.715, 95% CI: 0.609–0.838, P < 0.001). Further adjustment for fasting glucose and diabetes duration (Model II) slightly attenuated the effect, but the association persisted (OR = 0.624, 95% CI: 0.470–0.828, P < 0.001). These results are consistent with the primary linear regression findings using continuous eGFR, confirming that higher SLC30A8 methylation is associated with better renal function regardless of whether eGFR is analyzed as a continuous or dichotomous outcome.

Stability of the Association in Subgroup Analyses

Subgroup analyses were performed to evaluate the consistency of the positive association between SLC30A8 methylation and eGFR. No significant effect modifications were observed across predefined subgroups stratified by sex, age (<60 vs. $\geq$ 60 years), BMI (<24 vs. $\geq$ 24 kg/m²), fasting glucose levels, or diabetes duration (all P for interaction >0.05). This indicated that the positive association between higher SLC30A8 methylation and better renal function (ie., higher eGFR) was stable and independent of the common clinical variables examined (sex, age, BMI, fasting glucose, and diabetes duration) (Figure 3).

Table 4 Logistic Regression Analysis of the Association Between SLC30A8 Promoter Methylation and Impaired Renal Function (eGFR <60 mL/min/1.73 M²)

Variable	Model	OR (95% CI)	P value
SLC30A8 methylation (%)	Unadjusted	0.762 (0.722, 0.804)	<0.001
	Model I	0.715 (0.609, 0.838)	<0.001
	Model II	0.624 (0.470, 0.828)	<0.001

Notes: The OR represents the change in the odds of having impaired renal function (eGFR <60 mL/min/1.73 m²) associated with each 1% increase in SLC30A8 promoter methylation level. Model I: Adjusted for age, sex, and body mass index (BMI). Model II: Further adjusted for systolic blood pressure, urinary albumin-to-creatinine ratio (UACR), serum uric acid, total cholesterol, low-density lipoprotein cholesterol (LDL-C), fasting plasma glucose, and diabetes duration.

Abbreviations: OR, odds ratio; CI, confidence interval.

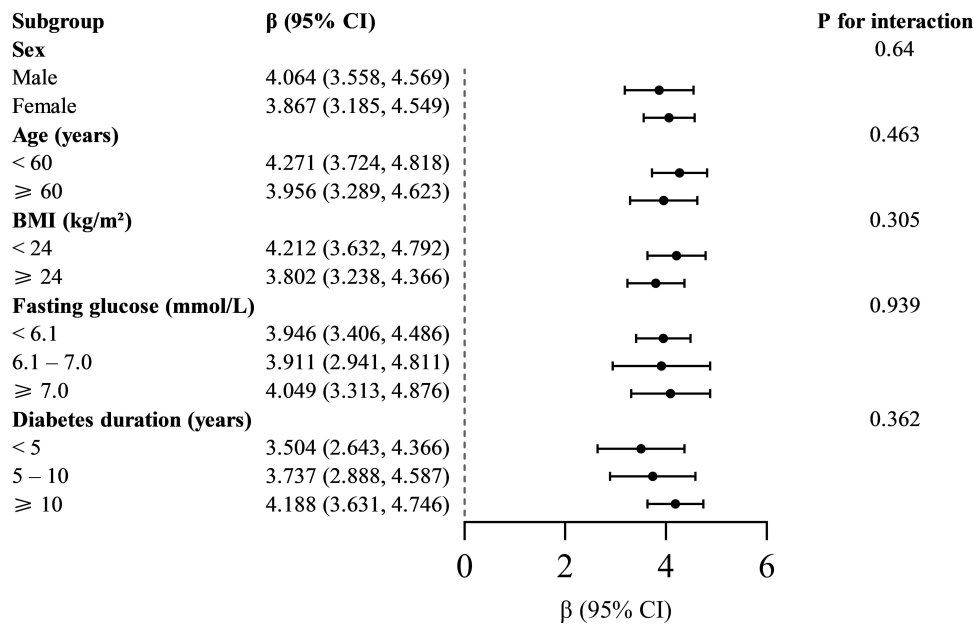


Figure 3 Subgroup analysis and interaction test of the association between DNA methylation level of *SLC30A8* promoter and estimated glomerular filtration rate (eGFR) in T2DM patients. Forest plot displays β -coefficients (solid squares) and 95% confidence intervals (horizontal lines) representing the change in eGFR (mL/min/1.73 m²) per 1% increase in *SLC30A8* methylation across prespecified subgroups. All analyses were adjusted for covariates in Model 3 (age, sex, BMI, systolic blood pressure, UACR, uric acid, total cholesterol, LDL-C, fasting glucose, and diabetes duration). The vertical dashed line at $\beta = 0$ indicates the null effect. P-values for interaction (P for interaction) are shown for each subgroup; all $P > 0.05$, indicating no significant effect modification and demonstrating the robustness of the association across different patient characteristics. **Abbreviations:** eGFR, estimated glomerular filtration rate; BMI, body mass index; UACR, urinary albumin-to-creatinine ratio; LDL-C, low-density lipoprotein cholesterol; CI, confidence interval.

Discussion

In this cross-sectional study of Chinese patients with T2DM, we identified a significant and independent association between peripheral blood *SLC30A8* promoter hypomethylation and impaired renal function, which in this study was primarily manifested as reduced eGFR. Our key findings are threefold: *SLC30A8* methylation levels were markedly lower in patients with DKD compared to those with T2DM alone and exhibited a progressive, graded relationship with worsening eGFR and higher UACR categories—ie., lower methylation levels consistently corresponded to more advanced stages of renal impairment, demonstrating a biological gradient; second, after comprehensive adjustment for conventional and diabetes-related risk factors, higher *SLC30A8* methylation remained a strong, independent predictor of better renal function; third, and most notably, this association demonstrated a clear non-linear threshold effect at approximately 70.9% methylation and was remarkably stable across all key demographic and clinical subgroups. The robustness of this association was further supported by sensitivity analysis using an alternative definition of renal impairment (eGFR <60 mL/min/1.73 m²), which yielded consistent results in logistic regression models. These results suggest that *SLC30A8* promoter hypomethylation may serve as a novel, accessible epigenetic biomarker associated with DKD risk and severity, and suggest a potential quantitative threshold for risk stratification that warrants further investigation.

Our observation of reduced *SLC30A8* methylation in DKD aligns with and extends the growing body of research linking epigenetic alterations to diabetic complications. While prior genetic studies have firmly established *SLC30A8* as a susceptibility locus for T2DM,^{14,15} and some epigenetic studies have reported promoter hypermethylation associated with T2DM risk in specific populations,^{17,18} the epigenetic landscape in DKD appears distinct. Our findings resonate with emerging evidence that differential DNA methylation patterns in peripheral blood are associated with kidney function decline and DKD progression.^{10,11} The present study shifts the focus from static genetic predisposition to dynamic epigenetic regulation, adding *SLC30A8* to the list of epigenetically regulated genes implicated in DKD pathophysiology. This moves beyond its established role in diabetes susceptibility, suggesting a potential contributory role in its microvascular complications, and warrants further investigation as a potential therapeutic target within the metabolic syndrome spectrum.

In epidemiological studies, the presence of a biological gradient—where increasing exposure levels (here, lower methylation) are associated with progressively greater severity of the outcome (lower eGFR)—is one of Bradford Hill’s criteria for supporting a causal relationship. Our findings demonstrated that methylation levels not only differed between the T2DM-only and DKD groups but also decreased incrementally across advancing CKD stages and across normoalbuminuria, microalbuminuria, and macroalbuminuria categories (both P for trend < 0.05). This consistent stepwise pattern strengthens the inference that the association is unlikely to be due to chance or confounding alone. Furthermore, the identification of a threshold effect at 70.9% methylation adds another layer of complexity to this dose-response relationship: below this threshold, the association with eGFR was non-significant, whereas above it, each 1% increase in methylation was associated with a substantial 4.47 mL/min/1.73 m² increase in eGFR. This suggests that the relationship is not simply linear but exhibits a non-linear dose-response pattern, with a critical threshold beyond which the protective effect of higher methylation becomes markedly pronounced. Such non-linear dose-response relationships are increasingly recognized in epigenetic epidemiology and may reflect biological switch mechanisms or saturation effects.¹¹ The independent and dose-dependent relationship between *SLC30A8* methylation and eGFR, which persisted after adjusting for diabetes duration and measures of glycemic control, is particularly noteworthy. This suggests that the observed epigenetic change may represent more than a mere consequence of chronic hyperglycemia; it could be a component of “metabolic memory”. The metabolic memory phenomenon posits that early hyperglycemic exposures can induce persistent epigenetic modifications that drive the progression of complications, even after subsequent glycemic improvement.^{6,7,23} The stability of the *SLC30A8* methylation–eGFR association across different strata of fasting glucose and diabetes duration in our subgroup analyses supports this notion. It implies that this epigenetic mark might reflect a lasting, programmed change induced by the diabetic milieu, and may be associated with sustained renal risk independently of current metabolic status—a key consideration for long-term disease management.

An important question arising from our findings is what factors might drive the observed *SLC30A8* hypomethylation in patients with DKD. DNA methylation patterns can be influenced by both genetic variants (methylation quantitative trait loci, meQTLs) and long-term environmental exposures.¹⁶ Chronic hyperglycemia itself can induce epigenetic changes through mechanisms involving oxidative stress. Furthermore, emerging evidence suggests that inflammatory cytokines, which are elevated in DKD, can modulate DNA methyltransferase activity, leading to locus-specific hypomethylation.²⁴ Thus, the hypomethylation observed in our DKD patients may represent an integrated epigenetic response to the cumulative burden of hyperglycemia, inflammation, and oxidative stress over time. Regarding how *SLC30A8* methylation status might contribute to DKD progression, our findings suggest a potential role extending beyond insulin regulation. ZnT8, the protein encoded by *SLC30A8*, is not exclusively expressed in pancreatic β -cells; low-level expression has been reported in kidney proximal tubular cells.²⁵ In the kidney, zinc homeostasis is critical for maintaining tubular integrity and protecting against oxidative injury. Zinc acts as a cofactor for superoxide dismutase (SOD), a key antioxidant enzyme, and can inhibit NF- κ B-mediated inflammatory pathways.²⁶ Therefore, we hypothesize that *SLC30A8* hypomethylation—which we postulate may lead to dysregulated ZnT8 expression—could disrupt local zinc handling within the kidney. This would impair antioxidant defenses and promote a pro-inflammatory and pro-fibrotic microenvironment, accelerating the glomerulosclerosis and tubulointerstitial fibrosis characteristic of DKD.^{4,27} This hypothesized mechanism aligns with our observation that the methylation–eGFR association persisted after adjusting for glycemic control, suggesting a direct renal effect rather than one mediated solely through systemic glucose metabolism.

The biological plausibility of our findings is supported by the known function of *SLC30A8*. The gene encodes ZnT8, a zinc transporter critical for zinc influx into insulin secretory granules in pancreatic β -cells, a process essential for insulin crystallization, storage, and secretion.^{12,13} Promoter hypomethylation is typically associated with increased gene expression. We postulate that *SLC30A8* hypomethylation, potentially leading to its dysregulated expression, might disrupt systemic zinc homeostasis. Zinc acts not only as an insulin chaperone but also as a crucial cofactor for numerous enzymes and possesses significant antioxidant and anti-inflammatory properties.²⁶ Dysregulated zinc transport could therefore contribute to the oxidative stress and inflammatory pathways that are central to DKD pathogenesis.^{4,27} Furthermore, emerging evidence implicates *SLC30A8* in hepatic insulin clearance;²⁸ its dysregulation might exacerbate systemic insulin resistance and metabolic dysfunction, potentially contributing to a vicious cycle that may exacerbate kidney injury.

The discovery of a distinct threshold effect at approximately 71% methylation provides a novel and potentially transformative insight for risk stratification and personalized medicine. The strong linear association above this threshold suggests that higher methylation levels above this threshold were associated with better renal function, whereas levels below it may signify a state of heightened vulnerability. This non-linear relationship underscores the complexity of epigenetic regulation. Such threshold effects are increasingly recognized in epigenetic epidemiology and are key for defining actionable biomarker cut-offs with direct clinical utility.¹¹ From a translational perspective, the magnitude of eGFR difference associated with a 10% increase in methylation above the threshold (approximately 45 mL/min/1.73 m²) is substantial and compares favorably with the annual eGFR preservation achieved by current cornerstone therapies such as SGLT2 inhibitors or finerenone,^{29,30} highlighting its potential clinical relevance for risk stratification.

The primary strengths of our study include the well-phenotyped cohort, the use of quantitative pyrosequencing for precise methylation measurement, extensive adjustment for a wide array of confounders, and rigorous exploration of non-linearity and subgroup consistency, which collectively strengthen the inference of an independent and robust association. However, several limitations must be acknowledged. First, the cross-sectional design precludes any causal inference. We cannot determine whether *SLC30A8* hypomethylation is a cause, a consequence, or a parallel marker of declining renal function. Second, our findings are based on a Chinese hospital-based population, which may limit the generalizability to other ethnicities and community settings. Third, while we adjusted for major clinical confounders, unmeasured or residual confounding (eg., dietary zinc intake, detailed medication history) cannot be ruled out. Fourth, we measured methylation in peripheral blood leukocytes, which may not directly mirror the methylation status or transcriptional activity in renal or pancreatic tissues. Future studies should prioritize several key areas. First, longitudinal cohort studies are needed to establish the temporal relationship between *SLC30A8* methylation changes and eGFR decline, and to determine whether baseline methylation levels predict future DKD progression. Second, multi-ethnic and community-based studies are essential to validate the generalizability of our findings and to explore potential ethnic-specific differences in this epigenetic marker. Third, mechanistic studies—including in vitro models using renal tubular cells exposed to diabetic conditions and in vivo studies using *SLC30A8* knockout or overexpression models—are crucial to elucidate whether altered ZnT8 expression directly contributes to kidney injury and to identify the specific molecular pathways involved. Fourth, given that DNA methylation is potentially reversible, future research should investigate whether interventions that modulate zinc homeostasis or target epigenetic machinery (eg., demethylating agents) could influence renal outcomes in DKD, though such approaches would require careful evaluation of safety and specificity.

It is important to interpret our findings within the appropriate context. As an exploratory cross-sectional study, this work was designed to generate hypotheses and identify potential epigenetic signals associated with DKD, rather than to establish definitive clinical utility or causal relationships. The observed associations, while robust and consistent across multiple sensitivity analyses, require validation in independent cohorts and, ideally, in prospective longitudinal settings. Such validation studies should assess whether baseline *SLC30A8* methylation levels predict the rate of eGFR decline over time, and whether incorporating this epigenetic marker improves risk prediction beyond conventional clinical factors. Furthermore, given the exploratory nature of our threshold analysis, the specific cutoff of 70.9% methylation should be interpreted with caution and validated in external populations before considering any clinical application.

Conclusion

In summary, this exploratory cross-sectional study identifies that *SLC30A8* promoter hypomethylation is robustly and independently associated with worse renal function in Chinese patients with T2DM. It exhibits a clear dose-response relationship with kidney damage severity and a clinically informative threshold effect around 71%, though this cutoff requires validation in independent cohorts. The stability of this association across key patient subgroups supports the hypothesis that *SLC30A8* methylation may represent a promising epigenetic biomarker associated with renal function, which could inform risk stratification. These findings contribute to the understanding of epigenetic dysregulation in DKD and highlight a candidate for further investigation. However, given the exploratory nature of this study, prospective longitudinal studies and mechanistic experiments are essential to confirm the predictive value of this marker and to determine whether it represents a modifiable target for intervention.

Data Sharing Statement

The data used in this study are available from the corresponding author upon reasonable request. The datasets generated and/or analyzed during the current study are not publicly deposited but can be shared by the corresponding author, subject to appropriate scientific and ethical considerations.

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Author Contributions

Changxiu Guo: Conceptualization, Writing – original draft, Writing – review & editing, Visualization, Formal analysis. Weidong Yang: Conceptualization, Methodology, Formal analysis, Investigation, Writing – review & editing. Rui Xue: Writing – review & editing, Investigation. Qinghui Zhou: Investigation, Data curation, review & editing. Yukun Liang: Investigation, Data curation, Writing – review & editing. Xiuli Zhang: Writing – review & editing, Conceptualization, Resources, Supervision, Project administration, Funding acquisition. All authors took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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