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Clinical significance of PANoptosis-related proteins and their association with short-chain fatty acids in diabetic kidney disease

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Abstract

Background: Diabetic kidney disease (DKD) is one of the most common microvascular complications of diabetes and also a leading cause of end-stage renal disease (ESRD). PANoptosis is a form of programmed cell death that integrates the key characteristics of pyroptosis, apoptosis and necroptosis. Dysbiosis of the gut microbiota mediates the initiation and progression of DKD via the gut-kidney axis through a cascade mechanism involving metabolite imbalance, intestinal barrier disruption, and immune-inflammatory activation. Whether SCFAs synergistically promote renal inflammatory injury with Panoptosis in DKD is not yet known.

Purpose: This study aims to investigate the associations between PANoptosis-related proteins, SCFAs and the progression of DKD, and to explore their potential as diagnostic and therapeutic biomarkers for DKD.

Methods: Serum levels of core PANoptosis-related proteins in the three groups were measured by ELISA, while serum SCFAs concentrations were determined using GC-MS.

Results: ELISA revealed that PANoptosis-related proteins were significantly elevated in the DKD ($P < 0.0001$). PANoptosis-related proteins were positively correlated with HbA1c, BUN, duration of diabetes, and UACR ($r > 0.30$), and negatively correlated with eGFR ($r < -0.30$). GC-MS analysis demonstrated that serum acetate concentration was markedly higher in the DKD than in the pure T2DM ($P < 0.05$), whereas isovaleric acid showed an opposite trend ($P < 0.01$). Butyric acid, isobutyric acid and isovaleric acid were negatively correlated with necroptosis-associated proteins.

Conclusion: Panoptosis-related proteins were positively correlated with the progression of DKD; PANoptosis-related proteins were negatively correlated with isovaleric acid, isobutyric acid and butyric acid in DKD.

Keywords: Diabetic Kidney Disease, Pyroptosis, Apoptosis, Necroptosis, PANoptosis

Abbreviation: Gas chromatography-mass spectrometry (GC-MS); short-chain fatty acids (SCFAs); enzyme-linked immunosorbent assay (ELISA); blood urea nitrogen (BUN); estimated glomerular filtration rate (eGFR); glycated hemoglobin (HbA1c); triglycerides (TG); total cholesterol (TC); high-density lipoprotein cholesterol (HDL-C); low-density lipoprotein cholesterol (LDL-C)

1. Introduction

According to the 2025 report by the International Diabetes Federation, the global prevalence of diabetes among adults was 11.11% in 2024 and is projected to rise to 12.96% by 2050(1). Approximately 20% to 40% of patients with type 2 diabetes (T2DM) develop diabetic kidney disease (DKD) as a complication. In China, the overall prevalence of DKD among patients with type 2 diabetes is 21.8%. DKD is the primary microvascular complication of diabetes mellitus, characterized by persistent albuminuria, progressive decline in estimated glomerular filtration rate and ultimately leading to irreversible renal fibrosis, and has become the leading cause of end-stage renal disease (ESRD) worldwide(2). The therapeutic strategy for DKD based on strict glycemic and blood pressure management, the "four-pillar" therapy—combining renin-angiotensin system blockers, sodium-glucose cotransporter 2 inhibitors, glucagon-like peptide-1 receptor agonists, and non-steroidal mineralocorticoid receptor antagonists—has become the core strategy for delaying DKD progression(3,4). Although existing therapeutic drugs such as renin-angiotensin system blockers, sodium-glucose cotransporter 2 inhibitors, glucagon-like peptide-1 receptor agonists, and non-steroidal mineralocorticoid receptor antagonists have significantly improved the prognosis of patients with DKD, a considerable proportion of patients inevitably progress to ESRD. This suggests the existence of key molecular events in the pathogenesis of DKD that have not yet been fully elucidated.

Accumulating evidence indicates that multiple inflammatory cell death pathways, including pyroptosis, apoptosis, necroptosis, and ferroptosis, critically drive the

66 pathogenesis and progression of DKD(5). In 2019, Malireddi et al. first proposed the
67 concept of "PANoptosis," revealing a shared regulatory network among pyroptosis,
68 apoptosis, and necroptosis(6). PANoptosis is mediated by the PANoptosome complex
69 and represents a unique inflammatory programmed cell death modality that integrates
70 the core molecular features of the three classical death pathways, characterized by the
71 release of inflammatory cytokines (IL-1 β , IL-18) and damage-associated molecular
72 patterns (DAMPs), ultimately leading to cell death and tissue injury(7,8). The DKD
73 microenvironment, characterized by hyperglycemia and accumulated advanced
74 glycation end-products (such as free CML), activates the RAGE/NF- κ B signaling axis
75 to upregulate NLRP3 inflammasome, thereby promoting the assembly of the RIPK1-
76 PANoptosome complex and triggering classical PANoptosis in podocytes (9).
77 Concurrently, single-cell RNA sequencing has revealed significantly elevated
78 expression of TNFSF10 (TRAIL) and TNFRSF10B (DR5) in podocytes from DKD
79 patients; TNF- α stimulates autocrine TRAIL production in podocytes, which induces
80 PANoptosis via the death receptor DR5, and podocyte-specific ablation of Tnfsf10 or
81 Tnfrsf10b substantially attenuates kidney injury in diabetic mice (10). In renal tubular
82 epithelial cells and interstitial cells, co-activation of apoptotic, pyroptotic, and
83 necroptotic pathways has also been observed, suggesting that PANoptosis represents a
84 common pathway mediating the loss of resident renal cells in DKD (11). At the gene
85 expression level, multiple bioinformatics studies have systematically screened DKD
86 transcriptomic datasets and identified diagnostically relevant PANoptosis-related hub
87 genes, including CASP1, FAS, AKT3, FYN, and CD44. The expression levels of these

genes are significantly correlated with declining glomerular filtration rate and the degree of immune cell infiltration, and the risk scoring models constructed therefrom effectively distinguish DKD patients from healthy controls (11,12). From a therapeutic translation perspective, targeting key components of the PANoptosome—such as DR5-Fc fusion protein, the RAGE antagonist FPS-ZM1, or NLRP3 gene silencing—has demonstrated significant efficacy in reducing proteinuria and retarding renal function deterioration in animal models, while drug repurposing analyses further suggest that nodes including CASP1 and FAS possess clear intervention feasibility (13). Collectively, PANoptosis not only holds substantial significance in the pathogenesis and progression of DKD but also opens novel avenues for early biomarker screening and precision targeted therapy for this disease.

GSDMD is the terminal executor of pyroptosis. After cleavage by caspase-1, its N-terminal fragment is released and forms pores in the plasma membrane, leading to cell lysis and release of pro-inflammatory cytokines. Caspase-1 initiates the classical pyroptotic pathway, and its activation level directly reflects NLRP3 inflammasome assembly. Together, GSDMD and caspase-1 form the core "NLRP3–caspase-1–GSDMD" pyroptotic signalling axis, which has been shown to contribute substantially to tubular epithelial cell and podocyte injury in DKD(14,15). MLKL is a key effector of necroptosis. Following phosphorylation by the RIPK1–RIPK3 signalling axis, MLKL translocates to the plasma membrane and promotes the release of damage-associated molecular patterns (DAMPs), thereby amplifying inflammation. Fang et al. identified MLKL as a core mediator of resident renal cell death in DKD(15).

Caspase-3 is the executioner caspase of apoptosis, also acts as a molecular switch that modulates pyroptosis: in the presence of GSDME, caspase-3 cleaves GSDME to convert apoptotic signals into pyroptotic responses. Bioinformatics and machine-learning studies have identified CASP3 as a key predictive biomarker and therapeutic target in DKD, and these findings have been validated in animal models(11,16). Previous studies have mostly used high-glucose-stimulated renal cell lines or streptozotocin (STZ)-induced animal models. Although single-cell RNA sequencing has revealed co-activation of apoptotic, pyroptotic and necroptotic pathways in kidney tissues from patients with DKD, and bioinformatics analyses have identified PANoptosis-related hub genes including CASP1, FAS and AKT3, systematic evidence simultaneously assessing protein-level activation of these three pathways in DKD patients remains lacking(15,17). Accordingly, the current study proposes to systematically quantify serum levels of pyroptotic, apoptotic, and necroptotic effector proteins in patients with DKD, thereby furnishing direct clinical evidence for PANoptosis pathway activation during the course of DKD progression.

The protective role of gut microbial metabolites—short-chain fatty acids (SCFAs)—in diabetes and its complications has garnered increasing attention. SCFAs regulate immune-inflammatory responses through activation of GPR41/GPR43 receptors and inhibition of histone deacetylases(18). In the field of DKD, serum acetate and butyrate levels are negatively correlated with DKD risk, whereas isobutyrate shows a positive correlation (19–22). One study revealed that SCFAs (butyrate, propionate and acetate) can directly bind to ASC, a key adaptor protein of the inflammasome complex, thereby

promoting inflammasome activation and further inducing pyroptosis in macrophages to eliminate *Salmonella* infection(23). Another study demonstrated that SCFAs/butyrate regulate necroptosis (MLKL-mediated) and apoptosis through pathways including the NLRP3 inflammasome(24); Cooper KN, et al. reported that oral pathogens trigger necroptosis in THP-1 monocytes via the RIP1/RIP3/MLKL signaling pathway, and inhibition of RIP1 or MLKL markedly reduces cell death as well as the expression of TNF- α and IL-6(25). However direct evidence regarding whether SCFAs protect the DKD kidney by regulating the expression of PANoptosis-related proteins remains lacking.

In summary, PANoptosis plays an significant role in the pathogenesis of DKD, yet its interplay with gut microbial metabolite SCFAs remains unexplored. This study aimed to compare the serum levels of PANoptosis-related proteins (GSDMD, MLKL, caspase-1, caspase-3, IL-1 β , IL-18) and SCFAs in the serum of different study subjects and to analyze the correlations between them. The findings may provide novel clinical evidence indicating that SCFAs are involved in the regulation of DKD progression via PANoptosis.

2. Materials and methods

2.1 Reagents and antibodies

2.1.1 Reagents and antibodies of ELISA

The ELISA kit for GSDMD(Cat#: MK4219A)、MLKL(Cat#: MK4454A)、caspase-1(Cat#: MK3839A)、caspase-3(Cat#: MK3840A)、IL-1 β (Cat#: MK0181A)、IL-18

(Cat#: MK0139A) was purchased from Shenzhen Huitong Biotechnology Co., Ltd (Shenzhen, China)

2.1.2 Reagents and antibodies of GC-MS

Standard short-chain fatty acids, including acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid and caproic acid (purity $\geq$ 99%), were purchased from Sigma-Aldrich, USA. 2-ethylbutyric acid (purity $\geq$ 98%, Sigma-Aldrich, USA) or [$^{13}\text{C}_2$]-acetic acid-5 was used as the internal standard.

The derivatization reagents were N-(tert-butyldimethylsilyl)-N-methyltrifluoroacetamide (MTBSTFA) containing 1% tert-butyldimethylchlorosilane (Regis Technologies, USA) and isobutyl chloroformate (iBuCF, Aladdin, China). Organic solvents including methyl tert-butyl ether (MTBE), n-hexane, methanol, acetonitrile and isooctane were of chromatographic grade and obtained from Merck, Germany.

2.2 Study design and Participants

This cross-sectional study was designed to assess the association of serum PANoptosis key protein and short-chain fatty acid levels with the extent of kidney damage in patients with T2DM. A total of 65 patients diagnosed with either type 2 diabetes mellitus (T2DM) alone or T2DM with diabetic kidney disease (DKD) were recruited from November 2024 to August 2025 at the Second People's Hospital of Yibin. After screening according to inclusion and exclusion criteria, a total of 60 patients were

eventually enrolled in the study and divided into two groups: type 2 diabetes mellitus alone group (DM, n=30) and type 2 diabetes mellitus with diabetic kidney disease group (DN, n=30). Meanwhile, 30 healthy individuals from the physical examination center of the same hospital were recruited as the control group, matched for age and gender. Demographic and clinical data including gender, age, dietary and exercise habits, smoking and drinking history, medical history, disease duration, body weight, BMI, blood pressure, and medication history were collected from all subjects. Serum laboratory indicators included HbA1c, TG, TC, HDL-C, LDL-C, urinary microalbumin, urinary creatinine, serum creatinine, BUN, and eGFR. The study design and baseline characteristics are described in Figure 1 and Table 1. All the participants provided written informed consent.

2.3 Quantification of serum PANoptosis proteins and SCFAs

2.3.1 PANoptosis proteins were measured by ELISA

Fasting venous blood samples (5 mL) were collected from all subjects after an overnight fast of more than 8 h. The supernatant was equally divided into 300 µL aliquots, transferred into two EP tubes, and stored at -80°C in an ultra-low temperature freezer. Serum PANoptosis-related protein levels were quantified via commercial enzyme-linked immunosorbent assay (ELISA) kits following manufacturer's instructions. Briefly, balanced serum samples and standards were incubated in microplate wells at 37 °C for specific antigen-antibody binding. After washing, horseradish peroxidase-conjugated secondary antibodies were added for further

incubation. Color development was performed with chromogenic reagents and terminated by stop solution. OD values at 450 nm were measured, and target protein concentrations were calculated via standard curves, with all samples tested in duplicate. The levels of GSDMD, MLKL, IL-1 β , IL-18, caspase-1 and caspase-3 in serum were subsequently measured.

2.3.2 SCFAs were detected by GC-MS

Serum SCFA levels in the DM group and DN group were determined using GC-MS. For standard preparation, short-chain fatty acid stock solutions were prepared in water or diethyl ether and serially diluted, with 4-methylvaleric acid as the internal standard. Ten standard working solutions were mixed at gradient concentrations, and stock solutions were stored at -20 °C while working solutions were freshly prepared. For metabolite extraction, samples were homogenized with phosphoric acid, internal standard and diethyl ether, centrifuged, and the supernatant was analyzed by GC-MS. MassHunter software was used for peak integration and quantification of SCFAs.

3. Results

3.1 PANoptosis-related proteins reflect the renal injury in T2DM

Serum levels of PANoptosis-related proteins include GSDMD, caspase-1, IL-1 β , IL-18, caspase-3 and MLKL, determined by ELISA in healthy subjects, T2DM and DKD. The results showed the all proteins were significantly higher in the DKD group (DN, n=30)

than in the T2DM group (DM,n=30), and higher in the DM than in the healthy control group (DN,n=30) (all $p < 0.0001$) (Fig. 2 A-F and Table 2). Subgroup analysis was performed based on eGFR levels, including the $\text{eGFR} \geq 60 \text{ mL/min/1.73 m}^2$ group (n=68) and the $\text{eGFR} < 60 \text{ mL/min/1.73 m}^2$ group (n=22). The serum levels of PANoptosis-related proteins were significantly higher in the $\text{eGFR} < 60 \text{ mL/min/1.73 m}^2$ group than in the $\text{eGFR} \geq 60 \text{ mL/min/1.73 m}^2$ group (all $p < 0.0001$) (Fig. 3 A-F and Table 2-1) . Subgroup analysis was conducted according to UACR (Albumin-to-Creatinine Ratio). Participants were divided into three groups: $\text{UACR} < 30 \text{ mg/g}$ (n=48), $30 \leq \text{UACR} \leq 300 \text{ mg/g}$ (n=25) and $\text{UACR} > 300 \text{ mg/g}$ (n=17). The results showed that the $\text{UACR} > 300 \text{ mg/g}$ group had the highest protein levels, followed by the $30 \leq \text{UACR} \leq 300 \text{ mg/g}$ group, while the $\text{UACR} < 30 \text{ mg/g}$ group had the lowest levels. No significant difference was observed between the $\text{UACR} > 300 \text{ mg/g}$ group and the $30 \leq \text{UACR} \leq 300 \text{ mg/g}$ group. However, significant differences were found when comparing the two higher UACR groups with the $\text{UACR} < 30 \text{ mg/g}$ group (all $p < 0.0001$) (Fig. 4 A-F and Table 2-2).

3.2 PANoptosis-Related Proteins Were Positively Correlated with UACR, BUN and Scr, and Negatively Correlated with eGFR

Correlations between serum levels of PANoptosis-related proteins and clinical characteristics were evaluated. The correlation matrix heatmap and detailed analyses (Fig.5A) revealed that all PANoptosis-related proteins were significantly positively correlated with HbA1c (all $p < 0.0001$), with correlation coefficients ranging from

0.484 to 0.541. Among them, IL-18 showed the strongest correlation with HbA1c ($r_s =$
0.541), followed by GSDMD ($r_s = 0.517$) and caspase-3 ($r_s = 0.514$). In addition, all
PANoptosis-related proteins were significantly positively correlated with urine
albumin-to-creatinine ratio (UACR) (all $p < 0.0001$), with correlation coefficients
ranging from 0.685 to 0.800. GSDMD showed the strongest correlation with UACR (r_s
 $= 0.800$), followed by caspase-1 ($r_s = 0.785$) and MLKL ($r_s = 0.752$). All PANoptosis-
related proteins were significantly negatively correlated with estimated glomerular
filtration rate (eGFR). Caspase-1 ($r_s = -0.370$, $p < 0.0001$) and GSDMD ($r_s = -0.360$, p
 < 0.0001) had the strongest correlations with eGFR, followed by caspase-3 ($r_s = -0.340$,
 $p < 0.01$). GSDMD was significantly positively correlated with BUN ($r_s = 0.350$, $p <$
0.0001) and Scr ($r_s = 0.349$, $p < 0.001$). Caspase-1 was also significantly positively
correlated with BUN ($r_s = 0.327$, $p < 0.01$) and Scr ($r_s = 0.363$, $p < 0.0001$). All
PANoptosis-related proteins were significantly positively correlated with the duration
of T2DM (all $p < 0.0001$), with correlation coefficients ranging from 0.683 to 0.783.
Caspase-1 ($r_s = 0.783$), MLKL ($r_s = 0.775$), and caspase-3 ($r_s = 0.763$) showed the
strongest correlations with diabetes duration.

Correlation matrix analysis further demonstrated that GSDMD, MLKL, caspase-1,
caspase-3, IL-1 β , and IL-18 were all highly positively correlated with each other, with
most correlation coefficients exceeding 0.9 (Fig.5B). This high collinearity suggests
that these molecules do not vary independently but are coordinately regulated by
common upstream signaling pathways, statistically supporting the biological concept
of PANoptosis as an integrated cell death network.

Correlations of PANoptosis-related proteins with age and BMI were weak or not statistically significant. Only MLKL ($r_s = 0.204$) and caspase-1 ($r_s = 0.218$) showed weak positive correlations with BMI ($p < 0.05$). No significant correlations were observed between any PANoptosis-related proteins and triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL), or low-density lipoprotein (LDL) (all $p > 0.05$).

In summary, Serum levels of key PANoptosis-related proteins were significantly elevated in DKD patients. These protein levels were negatively correlated with eGFR, while positively correlated with UACR, BUN, and Scr. Subgroup analysis further confirmed that lower eGFR or higher UACR levels were associated with increased levels of PANoptosis-related proteins, suggesting that these proteins may reflect the severity of renal injury.

3.3 Serum SCFA Profiles Differ Between DKD and T2DM Patients

PCA, OPLS-DA analysis, and the permutation test of the OPLS-DA model indicated that there were distinguishable overall differences in serum SCFA profiles between patients with T2DM and those with DKD (Fig. 6 A、B、C、E). Serum levels of seven short-chain fatty acids (SCFAs), including acetic acid, propionic acid, butyric acid, isobutyric acid, caproic acid, valeric acid and isovaleric acid, were determined by gas chromatography-mass spectrometry (GC-MS) in T2DM and DKD. Samples with over 50% of indicators below the Lower Limit of Quantification (LLOQ) or individual samples featuring multiple indicators below LLOQ were excluded. Finally, a total of

24 patients with T2DM (DM) and 27 patients with DKD (DN) were included in the analysis. The results showed that acetic acid exhibited the highest level of total SCFAs, followed by propionic acid, butyric acid and isovaleric acid, whereas isobutyric acid had the lowest level. There were significant differences among all SCFAs ($p < 0.0001$), except between isobutyric acid and isovaleric acid (Fig. 7 F、G). Comparisons of serum SCFAs between the two groups revealed that the level of acetic acid was significantly higher in the DN group than in the DM group ($p < 0.05$). The propionic acid level was slightly higher in the DN group without statistical significance ($p > 0.05$). No significant differences were found in propionic acid, isobutyric acid and butyric acid between the two groups ($p > 0.05$). The isovaleric acid level was markedly higher in the DM group than in the DN group ($p < 0.01$) (Fig. 7 A-E). Subgroup analysis was performed based on estimated glomerular filtration rate (eGFR). There were 35 participants in the eGFR ≥ 60 mL/min/1.73 m² group and 16 participants in the eGFR < 60 mL/min/1.73 m² group. Serum levels of acetic acid, propionic acid and butyric acid were comparable between the two groups. The levels of isobutyric acid and isovaleric acid were higher in the eGFR ≥ 60 mL/min/1.73 m² group, with no significant differences observed (Fig. 8 A-E). Subgroup analysis was also conducted according to albumin/creatinine ratio (UACR), including 16 cases in the UACR < 30 mg/g group, 21 cases in the $30 \leq \text{UACR} \leq 300$ mg/g group and 14 cases in the UACR > 300 mg/g group. The $30 \leq \text{UACR} \leq 300$ mg/g group presented higher serum acetic acid and propionic acid levels than the other two groups, and the UACR > 300 mg/g group had higher levels than the UACR < 30 mg/g group, yet all differences were not statistically significant. Serum isobutyric acid

and butyric acid levels were higher in the UACR < 30 mg/g group relative to the other two groups, with no significant differences. For isovaleric acid, levels in the UACR < 30 mg/g and $30 \leq \text{UACR} \leq 300$ mg/g groups were significantly higher than those in the UACR > 300 mg/g group ($p < 0.01$), while no difference was found between the former two groups(Fig. 8 F-J).

3.4 Serum SCFAs Exhibit Generally Weak Correlations with Clinical Parameters

The correlations between SCFAs and clinical characteristics as a correlation matrix heatmap (Fig.6 D). For renal function parameters, weak positive correlations were observed between some SCFAs and UACR, with isobutyric acid showing the highest correlation coefficient ($r_s=0.3$). Butyric acid was weakly positively correlated with eGFR ($r_s=0.3$), while other SCFAs showed no strong correlations with eGFR, BUN or Scr. Regarding glucose metabolism, weak positive correlations were found between several SCFAs and HbA1c, with isovaleric acid showing the highest correlation ($r_s=0.3$). Propionic acid and isovaleric acid were also weakly positively correlated with the duration of T2DM (both $r_s=0.2$). No strong correlations were observed between SCFAs and lipid parameters including TC,TG,HDL-C,LDL-C. Correlations of SCFAs with age, BMI and HBP were generally weak. Moderate positive correlations were found between different SCFAs, such as acetic acid and propionic acid ($r_s=0.5$), propionic acid and butyric acid ($r_s=0.4$), suggesting coordinated changes in gut microbiota-derived SCFA metabolism.

The correlation analysis between PANoptosis-related proteins and SCFAs revealed that isovaleric acid showed significant negative correlations with caspase-3($r = -0.43$, $P = 0.001$); with MLKL($r = -0.38$, $P = 0.005$). Butyric acid and isobutyric acid also exhibited significant negative correlations with some PANoptosis markers (e.g., butyric acid with caspase-1: $r = -0.36$, $P = 0.010$; isobutyric acid with MLKL($r = -0.30$, $P = 0.030$). In contrast, acetic acid and propionic acid showed no statistically significant correlations with PANoptosis/inflammatory markers (Fig.4 B).

4. Discussion

In this study, we observed markedly elevated serum levels of PANoptosis-related proteins in patients with DKD. These proteins were closely correlated with renal injury biomarkers (UACR, BUN, Scr and eGFR), and their concentrations progressively increased alongside the aggravation of renal damage. Notably, coordinated alterations were identified between serum PANoptosis-related proteins and serum SCFA metabolism in our cohort. Collectively, this study indicates that PANoptosis-related proteins may serve as complementary biomarkers for inflammatory renal injury in diabetic nephropathy, with promising potential for early identification and risk stratification.

Consistent with previous animal experiments and clinical studies, the present study demonstrated that serum levels of GSDMD, caspase-1, IL-1 β , and IL-18 were significantly increased in patients with DKD(26,27). GSDMD、IL-18 were significantly positively correlated with HbA1c, indicating that chronic hyperglycemia is an important driving factor for the activation of the pyroptosis pathway. Mechanistic

studies have shown that high glucose can trigger NLRP3 inflammasome activation and GSDMD-mediated pyroptosis in renal tubular epithelial cells via the IRE1 α /sXBP1 pathway(28). GSDMD, IL-1 β , IL-18 and caspase-1 showed very strong positive correlations with UACR and significant negative correlations with eGFR . As UACR is a key indicator of renal tubular injury and proteinuria, and eGFR is a core marker of renal function, these strong correlations directly indicate that the activation level of the pyroptosis pathway is closely associated with the severity of renal injury in DKD. Pyroptosis not only induces the lysis and death of renal cells but also releases pro-inflammatory factors such as IL-1 β and IL-18, which recruit immune cell infiltration and form a vicious cycle of “inflammation and cell death”(29,30). In this study, the correlation coefficient between serum GSDMD levels and the UACR in patients with DKD was as high as 0.80, suggesting that this protein may serve as a potential biomarker for the diagnosis and disease severity assessment of DKD.

In the study, we found that serum levels of the apoptotic executioner protein caspase-3 and the necroptosis key protein MLKL were simultaneously elevated in DKD patients, suggesting a synergistic pathogenic effect between these two pathways and pyroptosis. Correlation analysis revealed that MLKL was strongly positively correlated with HbA1c and UACR , and negatively correlated with eGFR . Caspase-3 showed a correlation coefficient with UACR and HbA1c.Necroptosis is mediated by the RIPK1/RIPK3/MLKL axis and is characterized by plasma membrane rupture and the release of damage-associated molecular patterns (DAMPs, such as HMGB1), which subsequently activate the NLRP3 inflammasome in neighboring cells and indirectly

promote pyroptosis(31,32). In the present study, MLKL exhibited strong positive correlations with both GSDMD and caspase-1. Such inter-pathway correlations suggest extensive crosstalk among these cell death pathways and provide a rationale for why simultaneously targeting multiple death pathways may achieve better therapeutic efficacy than single-pathway intervention.

Traditionally, pyroptosis, apoptosis, and necroptosis have been regarded as parallel and independent cell death modalities. However, recent studies have proposed the integrated concept of “PANoptosis”(33,34). PANoptosis refers to a programmed cell death process triggered by specific stimuli, in which the above three cell death pathways are simultaneously regulated through a multifunctional molecular platform(35,36). The correlation matrix in the present study showed that the pairwise correlation coefficients among GSDMD, MLKL, caspase-1, caspase-3, IL-1 β , and IL-18 were mostly above 0.9 (Fig. 5B). Such high collinearity strongly suggests from a statistical perspective that these molecules do not change independently but are coordinately regulated by common upstream signaling pathways, which is consistent with the molecular network characteristics of PANoptosis. Additionally, all PANoptosis-related proteins were negatively correlated with eGFR, this support the driving role of cell death pathways in the loss of renal function. However, none of the PANoptosis-related proteins showed significant correlations with lipid profiles, including TG, TC, HDL-C, LDL-C (all $P > 0.05$). This may suggest that the activation of these cell death pathways is relatively independent of lipid metabolism disorders and may be more specifically associated with glucose metabolism and renal injury.

387

388 Serum acetic acid levels were significantly higher in the DKD group than in the T2DM
389 group, while isovaleric acid levels were significantly lower. No significant differences
390 were observed in propionic acid, isobutyric acid, or butyric acid between the two groups.
391 Acetic acid is one of the major metabolites produced by gut microbiota. To date, there
392 have been limited studies on serum acetic acid levels in DKD patients. In a multicenter
393 study that included a DKD control group, Koh et al. (2026) found no significant
394 difference in serum acetic acid levels between DKD patients and healthy controls, and
395 no studies have yet compared acetic acid levels between DKD and T2DM patients(37).
396 In contrast, serum isovaleric acid was markedly decreased in patients with DKD.
397 Subgroup analysis stratified by albumin-to-creatinine ratio (UACR) further showed that
398 the level of isovaleric acid was lower in the mUACRoalbuminuria group ($\text{UACR} > 300$
399 mg/g) than in the microalbuminuria group ($30 \leq \text{UACR} \leq 300 \text{ mg/g}$), indicating that
400 reduced isovaleric acid is closely associated with the severity of renal tubular injury. As
401 a branched-chain fatty acid, decreased isovaleric acid may reflect impaired metabolic
402 function of gut microbiota. Such metabolic disturbance can exacerbate renal damage
403 via the gut-kidney axis, which is consistent with findings from recent studies(38).
404 Subgroup analysis based on renal function ($\text{eGFR} \geq 60$ vs $\text{eGFR} < 60$) revealed no
405 significant differences in the levels of all SCFAs. This indicates that alterations in the
406 SCFA profile are more closely correlated with UACR than with a simple decline in
407 renal function, suggesting a specific association between SCFA dysregulation and renal
408 tubular injury. Although SCFAs showed weak positive correlations with renal function

indicators (eGFR, BUN, Scr) and weak negative correlations with glucose metabolism markers (HbA1c, duration of T2DM). Previous meta-analyses and dietary fiber intervention studies have also reported a positive correlation between SCFAs and eGFR(39,40); Animal experiments have demonstrated that SCFAs can reduce UACR, serum urea and creatinine, which are markers of renal dysfunction, and thereby alleviate renal damage(41). The overall weak correlations may be attributed to subject selection and the relatively low concentration of serum SCFAs.

The regulatory effects of gut microbiota metabolites on renal cell death pathways have attracted extensive attention in the context of DKD. Studies have shown that SCFAs can improve renal function, reduce blood glucose, alleviate fibrosis and collagen deposition, and inhibit cell apoptosis in DKD models by suppressing HDAC activity and the NF- κ B signaling pathway(42). Wang et al. further demonstrated that butyric acid could enhance autophagy and attenuate renal tubular cell apoptosis in diabetic mice via the HDAC2/ULK1 axis (43). At the cellular level, Andrade-Oliveira et al. reported that SCFAs treatment reduced ROS production, NF- κ B activation and nitric oxide generation in renal tubular epithelial cells, while improving mitochondrial function(44). These collective findings suggest that SCFAs may regulate the balance between cell death and survival in renal cells through multiple mechanisms. Current studies exploring the association between SCFAs and PANoptosis in the context of DKD remain limited, while a study in diabetic mouse model with periodontitis demonstrated have demonstrated that butyrate inhibits histone deacetylase 3 (HDAC3), thereby attenuating the transduction of the STAT1 signaling pathway and exerting

immunomodulatory effects, it also indicated that exogenous butyrate supplementation could effectively inhibit PANoptosis, Wenying Yang et al. provide theoretical evidence for the correlation between PANoptosis and SCFAs(45). Consistently, our study confirmed significant negative correlations: isovaleric acid with caspase-1 and MLKL, butyric acid with caspase-1, and isobutyric acid with MLKL.

This study has several limitations. On the one hand, intestinal microbiota composition was not directly detected, so the microbial sources responsible for altered SCFA profiles could not be clarified, on the other hand, as a cross-sectional study, the present work cannot establish a causal relationship between SCFA dysregulation and PANoptosis pathway activation. Finally, in vitro and animal experiments were not performed to verify the regulatory effects of SCFAs on the PANoptosis pathway, and the correlation between SCFAs and PANoptosis-related proteins remains to be further validated.

Future studies will adopt 16S rRNA sequencing to characterize gut microbiota composition. Combined with in vitro cellular assays, the regulatory roles of specific SCFAs in the PANoptosis pathway will be explored. In addition, animal models will be used to evaluate the therapeutic efficacy of interventions targeting gut microbiota or SCFAs for DKD.

5. Conclusion

This study demonstrated that serum levels of PANoptosis-related proteins were markedly elevated in DKD patients and strongly associated with renal function parameters, while being negatively correlated with isovaleric acid, isobutyric acid, and

butyric acid. In contrast, the absolute values of correlation coefficients between short-chain fatty acids (SCFAs) and indicators of renal function and glucose metabolism were all less than 0.4, indicating weak to moderate correlations. These weak associations suggest that , in addition to directly acting on the kidney, SCFAs may also participate in the progression of DKD through indirect pathways. Given the complexity and diverse regulatory patterns of PANoptosis, its specific mechanisms remain poorly understood. Further systematic experimental studies are therefore urgently needed to clarify this issue.

Author contributions

Conceptualization and methodology: Junling Gu; Investigation, data curation, and data collection: Yan Liu, Xiaolan Li, Hongfang Zhou, Cuiping Liu, Xin Liu, Yanyan Guo, Chengshan You; Formal analysis and visualization: Yan Liu, Linlin Huang; Laboratory testing and technical support: Bin Nie, Xiang Liu; Writing – original draft: Yan Liu, Xiaolan Li, Linlin Huang; Writing – review and editing, project administration, supervision, and funding acquisition: Junling Gu

Consent for publication

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473

474 **Declaration of competing interest**

475 Not applicable. The authors declare that they have no known competing financial
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488 **Ethics approval and consent to participate**

489 This study was approved by the Medical Ethics Committee of the Second People's
490 Hospital of Yibin (2025-177-01). All participants provided written informed consent.
491 All serum samples were analyzed anonymously in compliance with the Declaration of
492 Helsinki.

References

1. Genitsaridi I, Salpea P, Salim A, Sajjadi SF, Tomic D, James S, et al. 11th edition of the IDF Diabetes Atlas: global, regional, and national diabetes prevalence estimates for 2024 and projections for 2050. *Lancet Diabetes Endocrinol.* 2026 Feb;14(2):149–56. doi:10.1016/S2213-8587(25)00299-2 PubMed PMID: 41412135.
2. Thomas MC, Brownlee M, Susztak K, Sharma K, Jandeleit-Dahm KAM, Zoungas S, et al. Diabetic kidney disease. *Nat Rev Dis Primer.* 2015 Jul 30;1:15018. doi:10.1038/nrdp.2015.18 PubMed PMID: 27188921; PubMed Central PMCID: PMC7724636.
3. Ovid [Internet]. [cited 2026 May 24]. The Paradigm Shift in the Management of Diabetic... : APIK Journal of Internal Medicine. Available from: https://www.ovid.com/jnls/joim/fulltext/10.4103/ajim.ajim_3_26~the-paradigm-shift-in-the-management-of-diabetic-kidney doi:10.4103/ajim.ajim_3_26
4. Martinez Leon V, Hilburg R, Susztak K. Mechanisms of diabetic kidney disease and established and emerging treatments. *Nat Rev Endocrinol.* 2026 Jan;22(1):21–35. doi:10.1038/s41574-025-01171-3 PubMed PMID: 40935879.
5. Lv Z, Hu J, Su H, Yu Q, Lang Y, Yang M, et al. TRAIL induces podocyte PANoptosis via death receptor 5 in diabetic kidney disease. *Kidney Int.* 2025 Feb;107(2):317–31. doi:10.1016/j.kint.2024.10.026 PubMed PMID: 39571905.
6. Liu Y, Lu W, Xiang Z, Shen Y, Ni B, Zou H, et al. Integrated bioinformatics and machine learning reveal pan-apoptosis and immune infiltration signatures in diabetic nephropathy. *Front Immunol.* 2025;16:1659065. doi:10.3389/fimmu.2025.1659065

515 PubMed PMID: 41479901; PubMed Central PMCID: PMC12753429.

516 7. B F, W H, S D, Y H, F H, C Z. The Inflammatory Cell Death in Diabetic Kidney
517 Disease: Integrating Multifactorial Mechanisms into Novel Therapeutics. *Int J Mol*
518 *Sci.* 2025 Nov 14;26(22). doi:10.3390/ijms262211033 PubMed PMID: 41303516.

519 8. Ma L, Li Z, Liu Q, Wu N, Wang Q. PANoptosis in diabetes: immunometabolic
520 insights and treatments. *Apoptosis Int J Program Cell Death.* 2026 Jan 9;31(1):2.
521 doi:10.1007/s10495-025-02208-8 PubMed PMID: 41511602.

522 9. Zhang L, Chen L, Guo W, Cai L, Zeng X, Liu Y, et al. Free N ϵ -(carboxymethyl)-
523 lysine promotes diabetic kidney disease progression via RAGE/NF- κ B/NLRP3
524 pathway-mediated podocyte PANoptosis. *Free Radic Biol Med.* 2026 Aug
525 16;252:112–31. doi:10.1016/j.freeradbiomed.2026.04.159 PubMed PMID: 42066820.

526 10. Lv Z, Hu J, Su H, Yu Q, Lang Y, Yang M, et al. TRAIL induces podocyte
527 PANoptosis via death receptor 5 in diabetic kidney disease. *Kidney Int.* 2025
528 Feb;107(2):317–31. doi:10.1016/j.kint.2024.10.026 PubMed PMID: 39571905.

529 11. Chen Y, Yuan F, Li S, Liu L, Peng X, Zeng X, et al. Identification of PANoptosis
530 hub genes driving immune activation and tubulointerstitial injury in diabetic kidney
531 disease by integrative bioinformatics and machine learning. *Front Immunol.*
532 2026;17:1759781. doi:10.3389/fimmu.2026.1759781 PubMed PMID: 41878449;
533 PubMed Central PMCID: PMC13006297.

534 12. Zhang S, Zhang Y, Hu W, Huang C, Zhang Y, Chen X. Bioinformatics-based
535 analysis and experimental validation of PANoptosis-related biomarkers and immune
536 infiltration in diabetic nephropathy. *Front Endocrinol.* 2025;16:1610882.

doi:10.3389/fendo.2025.1610882 PubMed PMID: 40958910; PubMed Central
 PMCID: PMC12434962.

13. Guo Y, Zhao Y, Qiao Y, Xing Y, Fang Y, Zhao Y, et al. Targeting panoptosis: a
 narrative review of its therapeutic potential in kidney disease. *BMC Nephrol.* 2025
 Sep 29;26(1):545. doi:10.1186/s12882-025-04339-1 PubMed PMID: 41023669;
 PubMed Central PMCID: PMC12481750.

14. Li S, Feng L, Li G, Liu R, Ma C, Wang L, et al. GSDME-dependent pyroptosis
 signaling pathway in diabetic nephropathy. *Cell Death Discov.* 2023 May 11;9(1):156.
 doi:10.1038/s41420-023-01452-8 PubMed PMID: 37169767; PubMed Central
 PMCID: PMC10175547.

15. Fang B, Huang W, Du S, Hao Y, He F, Zhang C. The Inflammatory Cell Death in
 Diabetic Kidney Disease: Integrating Multifactorial Mechanisms into Novel
 Therapeutics. *Int J Mol Sci.* 2025 Nov 14;26(22):11033. doi:10.3390/ijms262211033
 PubMed PMID: 41303516; PubMed Central PMCID: PMC12652949.

16. Yu X, Hu Y, Jiang W. Integrative analysis of mitochondrial and immune pathways
 in diabetic kidney disease: identification of AASS and CASP3 as key predictors and
 therapeutic targets. *Ren Fail.* 2025 Dec;47(1):2465811.
 doi:10.1080/0886022X.2025.2465811 PubMed PMID: 39988817; PubMed Central
 PMCID: PMC11852243.

17. Zhang S, Zhang Y, Hu W, Huang C, Zhang Y, Chen X. Bioinformatics-based
 analysis and experimental validation of PANoptosis-related biomarkers and immune
 infiltration in diabetic nephropathy. *Front Endocrinol.* 2025 Sep 1;16:1610882.

doi:10.3389/fendo.2025.1610882 PubMed PMID: 40958910; PubMed Central
 PMCID: PMC12434962.

18. Geng L, Liu Y, Sun Y, Chen Y. Construction of a PANoptosis-Related Gene
 Signature for Diabetic Nephropathy. *Kidney Blood Press Res.* 2025;50(1):496–512.
 doi:10.1159/000546764 PubMed PMID: 40505639; PubMed Central PMCID:
 PMC12263135.

19. S Z, Y Z, W H, C H, Y Z, X C. Bioinformatics-based analysis and experimental
 validation of PANoptosis-related biomarkers and immune infiltration in diabetic
 nephropathy. *Front Endocrinol.* 2025 Jan 9;16. doi:10.3389/fendo.2025.1610882
 PubMed PMID: 40958910.

20. Vinolo MAR, Rodrigues HG, Nachbar RT, Curi R. Regulation of Inflammation
 by Short Chain Fatty Acids. *Nutrients.* 2011 Oct;3(10):858–76.
 doi:10.3390/nu3100858

21. Szrejder M, Piwkowska A. Gut Microbiome-Derived Short-Chain Fatty Acids in
 Glomerular Protection and Modulation of Chronic Kidney Disease Progression.
Nutrients. 2025 Jan;17(17):2904. doi:10.3390/nu17172904

22. Tsai HJ, Yu PS, Hung WW, Wu PH, Hung WC, Hwang SJ, et al. Trajectories of
 short-chain fatty acids and risk of adverse kidney outcomes in type 2 diabetes: a
 prospective cohort study. *Postgrad Med.* 2026 Mar;138(2):231–42.
 doi:10.1080/00325481.2026.2641270 PubMed PMID: 41789569.

23. Tsugawa H, Kabe Y, Kanai A, Sugiura Y, Hida S, Taniguchi S, et al. Short-chain
 fatty acids bind to apoptosis-associated speck-like protein to activate inflammasome

581 complex to prevent Salmonella infection. PLoS Biol. 2020 Sep;18(9):e3000813.
582 doi:10.1371/journal.pbio.3000813 PubMed PMID: 32991574; PubMed Central
583 PMCID: PMC7524008.

584 24. J W, J C. Alveolar Epithelial Cell Dysfunction in Acute Respiratory Distress
585 Syndrome: Mechanistic Insights and Targeted Interventions. Biomedicines. 2025 Sep
586 19;13(9). doi:10.3390/biomedicines13092299 PubMed PMID: 41007859.

587 25. Cooper KN, Potempa J, Bagaitkar J. Dying for a cause: The pathogenic
588 manipulation of cell death and efferocytic pathways. Mol Oral Microbiol. 2024
589 Aug;39(4):165–79. doi:10.1111/omi.12436 PubMed PMID: 37786286; PubMed
590 Central PMCID: PMC10985052.

591 26. Ding N, Wei C, Liu Q, Liu X, Huo L, Yu F, et al. Pyroptosis-mediator Gasdermin
592 D in serum: a potential biomarker in diabetic kidney disease. Front Endocrinol.
593 2026;17:1734036. doi:10.3389/fendo.2026.1734036 PubMed PMID: 41788766;
594 PubMed Central PMCID: PMC12957256.

595 27. Q C, L W, X W, M C, X Z, R M, et al. Puerarin alleviates diabetic nephropathy by
596 inhibiting Caspase-1-mediated pyroptosis. J Pharm Pharmacol. 2024 Apr 3;76(3).
597 doi:10.1093/jpp/rgad113 PubMed PMID: 38215026.

598 28. Liu X, Zhou D, Su Y, Liu H, Su Q, Shen T, et al. PDIA4 targets IRE1 α /sXBP1 to
599 alleviate NLRP3 inflammasome activation and renal tubular injury in diabetic kidney
600 disease. Biochim Biophys Acta Mol Basis Dis. 2025 Mar;1871(3):167645.
601 doi:10.1016/j.bbadis.2024.167645 PubMed PMID: 39743023.

602 29. Q C, J P, Zl Z, F Y, Hy X, Pp C, et al. Caspase-11/4 and gasdermin D-mediated

603 pyroptosis contributes to podocyte injury in mouse diabetic nephropathy. *Acta*
 604 *Pharmacol Sin.* 2021 Jun;42(6). doi:10.1038/s41401-020-00525-z PubMed PMID:
 605 32968210.
 606 30. J G, K G, M G, W H, T Z, X L, et al. Targeting Pyroptosis: New Insights into the
 607 Treatment of Diabetic Microvascular Complications. *Evid-Based Complement Altern*
 608 *Med ECAM.* 2022 Sep 27;2022. doi:10.1155/2022/5277673 PubMed PMID:
 609 36204129.
 610 31. Zhang G, Wang J, Zhao Z, Xin T, Fan X, Shen Q, et al. Regulated necrosis, a
 611 proinflammatory cell death, potentially counteracts pathogenic infections. *Cell Death*
 612 *Dis.* 2022 Jul 22;13(7):637. doi:10.1038/s41419-022-05066-3 PubMed PMID:
 613 35869043; PubMed Central PMCID: PMC9307826.
 614 32. D F, Je V. Pyroptosis versus necroptosis: similarities, differences, and crosstalk.
 615 *Cell Death Differ.* 2019 Jan;26(1). doi:10.1038/s41418-018-0212-6 PubMed PMID:
 616 30341423.
 617 33. Wu S, Tian B, Pang X, Sui B. A potential strategy to rebuild the tumor immune
 618 microenvironment: PANoptosis. *Front Immunol.* 2025;16:1626411.
 619 doi:10.3389/fimmu.2025.1626411 PubMed PMID: 40831559; PubMed Central
 620 PMCID: PMC12360039.
 621 34. Christgen S, Zheng M, Kesavardhana S, Karki R, Malireddi RKS, Banoth B, et
 622 al. Identification of the PANoptosome: A Molecular Platform Triggering Pyroptosis,
 623 Apoptosis, and Necroptosis (PANoptosis). *Front Cell Infect Microbiol.* 2020;10:237.
 624 doi:10.3389/fcimb.2020.00237 PubMed PMID: 32547960; PubMed Central PMCID:

625 PMC7274033.

626 35. Wu S, Tian B, Pang X, Sui B. A potential strategy to rebuild the tumor immune
627 microenvironment: PANoptosis. *Front Immunol.* 2025;16:1626411.
628 doi:10.3389/fimmu.2025.1626411 PubMed PMID: 40831559; PubMed Central
629 PMCID: PMC12360039.

630 36. Sun Q, Hu J, Wang R, Guo S, Zhang G, Lu A, et al. Bioinformatics-based
631 screening and validation of PANoptosis-related biomarkers in periodontitis. *Front Cell*
632 *Dev Biol.* 2025;13:1619002. doi:10.3389/fcell.2025.1619002 PubMed PMID:
633 40612110; PubMed Central PMCID: PMC12222123.

634 37. Jh K, S P, M K, Ji P, J L, H C, et al. Altered acetate metabolism and signaling in
635 IgA nephropathy: an integrated gut microbiome and glomerular spatial transcriptome
636 analysis. *Front Immunol.* 2026 Jan 14;16. doi:10.3389/fimmu.2025.1665585 PubMed
637 PMID: 41613128.

638 38. Zhong C, Dai Z, Chai L, Wu L, Li J, Guo W, et al. The change of gut microbiota-
639 derived short-chain fatty acids in diabetic kidney disease. *J Clin Lab Anal.* 2021
640 Dec;35(12):e24062. doi:10.1002/jcla.24062 PubMed PMID: 34689373; PubMed
641 Central PMCID: PMC8649351.

642 39. Thakur D, Harmer MJ. Circulating Short-Chain Fatty Acid Levels in Chronic
643 Kidney Disease: A Systematic Review and Meta-Analysis. *Nutrients.* 2026 Apr
644 30;18(9):1440. doi:10.3390/nu18091440 PubMed PMID: 42124048; PubMed Central
645 PMCID: PMC13164688.

646 40. L M, L Z, J L, X Z, Y X, X L, et al. The potential mechanism of gut microbiota-

647 microbial metabolites-mitochondrial axis in progression of diabetic kidney disease.
648 Mol Med Camb Mass. 2023 Oct 31;29(1). doi:10.1186/s10020-023-00745-z PubMed
649 PMID: 37907885.

650 41. L Z, F Z, X D, G W, Yy L, X W, et al. Gut bacteria selectively promoted by
651 dietary fibers alleviate type 2 diabetes. Science. 2018 Sep 3;359(6380).
652 doi:10.1126/science.aao5774 PubMed PMID: 29590046.

653 42. Li L, Ma L, Fu P. Gut microbiota-derived short-chain fatty acids and kidney
654 diseases. Drug Des Devel Ther. 2017;11:3531–42. doi:10.2147/DDDT.S150825
655 PubMed PMID: 29270002; PubMed Central PMCID: PMC5729884.

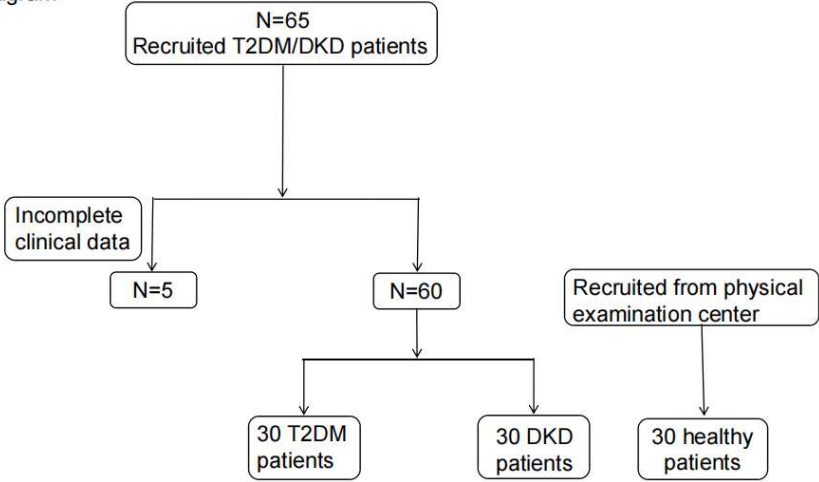
656 43. Ma X, Wang Q. Short-Chain Fatty Acids Attenuate Renal Fibrosis and Enhance
657 Autophagy of Renal Tubular Cells in Diabetic Mice Through the HDAC2/ULK1
658 Axis. Endocrinol Metab. 2022 Jun;37(3):432–43. doi:10.3803/EnM.2021.1336
659 PubMed PMID: 35574586; PubMed Central PMCID: PMC9262686.

660 44. Andrade-Oliveira V, Amano MT, Correa-Costa M, Castoldi A, Felizardo RJF, de
661 Almeida DC, et al. Gut Bacteria Products Prevent AKI Induced by Ischemia-
662 Reperfusion. J Am Soc Nephrol JASN. 2015 Aug;26(8):1877–88.
663 doi:10.1681/ASN.2014030288 PubMed PMID: 25589612; PubMed Central PMCID:
664 PMC4520159.

665 45. Yang W, Diao J, Zheng S, Xu Y, Liu Y, Xu S, et al. Butyrate Alleviates Diabetic
666 Periodontitis by Suppressing Macrophage M1 Polarization and PANoptosis-Like Cell
667 Death. FASEB J Off Publ Fed Am Soc Exp Biol. 2026 Jan 31;40(2):e71428.
668 doi:10.1096/fj.202504201R PubMed PMID: 41546529.

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Fig.1. Trial flow giagram



670

671 **Figure 1. Study flow diagram.** A total of 65 patients diagnosed with isolated type 2
672 diabetes mellitus (T2DM) or diabetic kidney disease (DKD) were recruited from The
673 Second People’s Hospital of Yibin between November 2024 and August 2025. After
674 screening according to inclusion and exclusion criteria, 5 patients were excluded and
675 60 diabetic subjects were finally enrolled, consisting of 30 patients with isolated T2DM
676 (DM group) and 30 patients with DKD (DN group). Meanwhile, 30 age- and
677 sex-matched healthy controls were recruited from the hospital physical examination
678 center.

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Table 1 Baseline characteristics of the study subjects

CHARACTERI STIC	NC (N=30)	DM(N=30)	DN(N=30)	P
AGE ,YEARS‡	53(51,59)	56(50,60.25)	57.50(52.75,61.25)	0.179

SEX, MALE	16 (53.3%)	16 (53.3%)	17 (56.7%)	0.956
(N, %)				
DURATION OF DIABETES , YEARS‡	-	0.00(0.00,0.02)	10.00(8.75,20.00)	0.000
BMI , KG/M²‡	23.85(23, 24.95)	24.60(23.25,29.22)	25.50(22.80,27.55)	0.205
HBA1C, % †	5.56±0.28 ^{2, 3}	10.82±2.97 ^{1, 3}	8.75±2.72 ^{1, 2}	0.000
TG, MMOL/L‡	1.68 (1.17, 2.28)	2.09(1.21,4.87)	1.63(1.01,2.87)	0.183
TC, MMOL/L‡	5.15 (4.41, 5.83)	5.45(4.71,6.18)	4.85(3.99,5.88)	0.269
HDL-C, MMOL/L‡	1.2 (1.07, 1.37)	1.15(0.99,1.40)	1.22(0.96,1.57)	0.616
LDL-C, MMOL/L‡	3.19 (2.81, 4.05)	3.65(3.21,4.18)	3.14(2.33,4.13)	0.259
BUN, MMOL/L‡	5.56 (4.79, 6.65) ³	5.22(4.47,6.28) ³	8.70(5.35,12.39) ^{1, 2}	0.000
SCR,MMOL/L‡	69.55 (57.85, 81.30) ³	60.65(51.82,74.57) ³	97.30(69.97,187.07) ^{1, 2}	0.000
EGFR, ML/MIN/1.73 M⁻²) †	86.36±18.93 ³	91.12±16.13 ³	58.39±38.45 ²	0.000
URINARY	7.79(6.42,13.68) ³	27.74(6.72,74.23	253.93(87.46,1898.	0.000

ALBUMIN, MG‡		) ³	96) ^{1, 2}	
URINARY CREATININE, MMOL/L‡	15.77(9.25,23.10) ³	12.67(8.84,21.58	6.91(4.40,11.64) ¹	0.001
UACR, MG/G‡	5.57(3.78,8.00) ³	17.76(6.42,52.70	321.29(90.93,1871.	0.000
		) ³	34) ^{1, 2}	

682 **Notes:** (1) ¹ $P < 0.05$ versus NC group; ² $P < 0.05$ versus DM group; ³ $P < 0.05$ versus
683 DN group. (2) †: mean $\pm$ standard deviation; ‡: median (interquartile range).

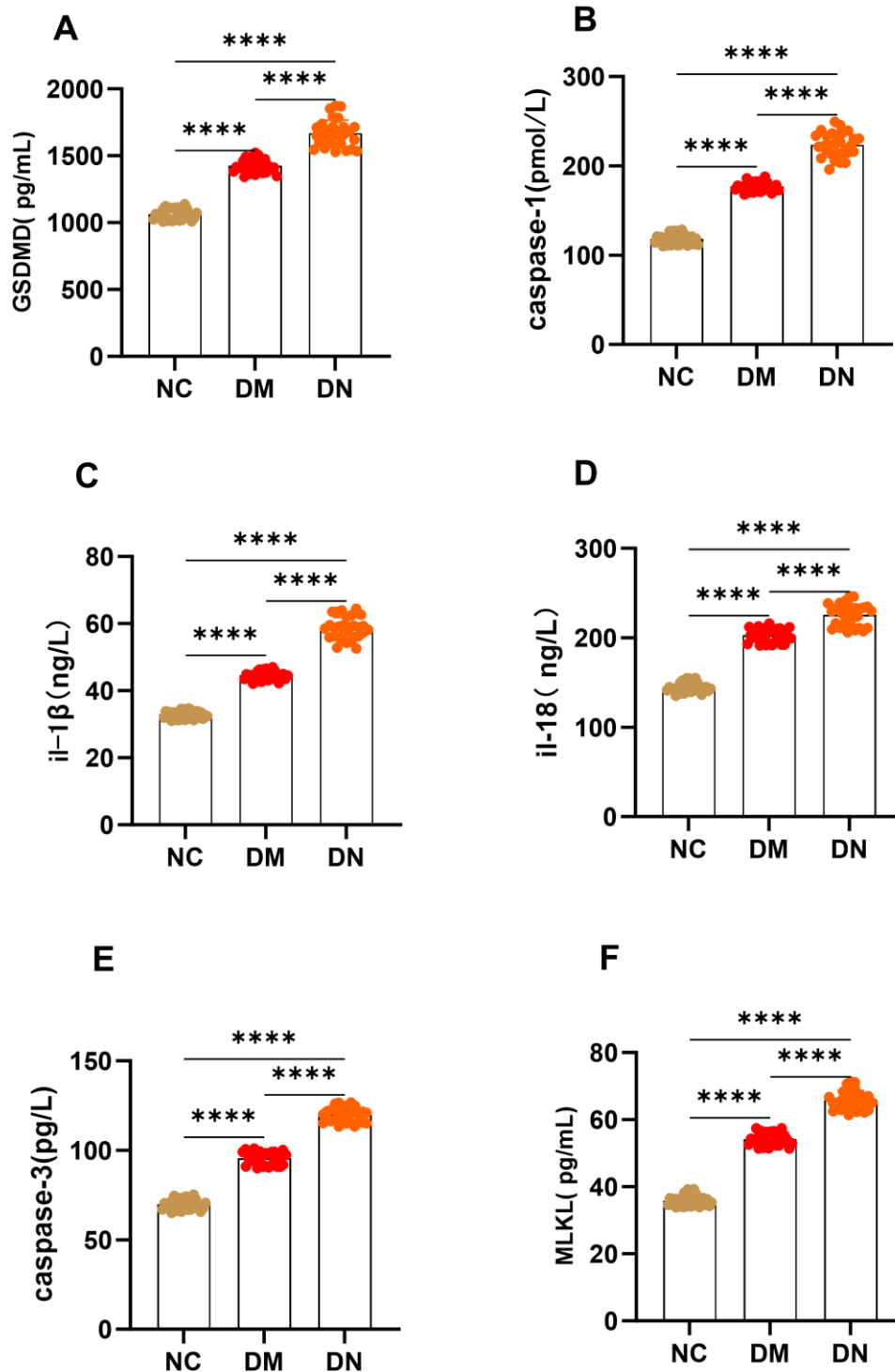


Figure 2. Serum levels of PANoptosis-related proteins in three groups detected by ELISA. NC (healthy control group, n=30), T2DM (DM group, n=30), DKD (DN group, n=30) Statistical significance was set at $P < 0.05$. Significant differences between groups are marked with * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $p < 0.0001$.

Table 2 Serum levels of key PANoptosis proteins in the three groups				
CHARACTERISTIC	NC (N=30)	DM(N=30)	DN(N=30)	P
GSDMD,pg/mL‡	1052.11 (1024.52, 1104.39)	1411.78 (1382.93, 1467.70)	1665.71 (1591.55, 1716.05)	0.00
MLKL,pg/ml‡	35.60 (34.50, 36.73)	54.07 (52.83, 55.62)	65.38 (63.20, 67.83)	0.00
Caspase-1,pmol/L†	118.298±5.768	176.908±5.078	223.553±13.315	0.00
Caspase-3,pmol/L‡	69.86 (67.30, 72.03)	96.19 (91.56, 98.56)	120.23 (115.64, 124.28)	0.00
IL-1β ,ng/L†	32.90±1.16	44.51±1.30	58.51±3.28	0.00
IL-18 ,ng/L†	144.45±5.45	202.83±7.50	225.36±12.32	0.00

Notes: †, mean ± SD; ‡, median (IQR)

**TABLE 2-1 KEY SERUM PANOPTOSIS-RELATED PROTEINS ACROSS
EGFR SUBGROUPS**

CHARACTERISTIC	eGFR$\geq$60 (ml/min/1.73m²) (n=68)	eGFR<60 (ml/min/1.73m²) (n=22)	Total(n=90)	P
GSDMD,pg/mL	1320.89 $\pm$ 233.96	1584.84 $\pm$ 234.59	1385.41 $\pm$ 259.24	0.000
MLKL,pg/mL	49.06 $\pm$ 11.59	60.90 $\pm$ 11.31	51.95 $\pm$ 12.55	0.000
Caspase-1,pmol/L	161.52 $\pm$ 39.44	208.15 $\pm$ 39.87	172.92 $\pm$ 44.18	0.000
Caspase-3,pmol/L	89.62 $\pm$ 18.64	112.17 $\pm$ 18.66	95.13 $\pm$ 20.95	0.000
IL-1 β ,ng/L	42.25 $\pm$ 9.07	54.74 $\pm$ 10.14	45.31 $\pm$ 10.74	0.000
IL-18,ng/L	182.72 $\pm$ 32.77	216.12 $\pm$ 31.61	190.88 $\pm$ 35.39	0.000

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700 Table 2-2 Key serum PANoptosis-related proteins across UACR subgroups

Characteristic	UACR<30	30$\leq$UACR$\leq$3	UACR>300	Total	P
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	(n=48)	00 (n=25)	(n=17)	(n=90)	
GSDMD,pg/m	1192±173.50	1559.01±123	1676.17±116	1385.41±259	0.00
L		.00	.60	.24	0
MLKL,pg/mL	42.84±9.19	60.74±6.95	64.77±3.50	51.95±12.55	0.03
					3
Caspase-	139.70±28.4	204.38±25.5	220.44±15.8	172.92±44.1	0.04
1,pmol/L	4	5	8	8	5
Caspase-	79.63±13.25	108.46±12.5	119.29±8.51	95.13±20.95	0.04
3,pmol/L		1			5
IL-1β,ng/L	37.42±6.01	51.57±7.42	58.37±4.85	45.31±10.74	0.04
					0
IL-18,ng/L	167.10±30.2	212.13±14.0	210.00±13.8	190.88±35.3	0.04
	7	5	5	9	0

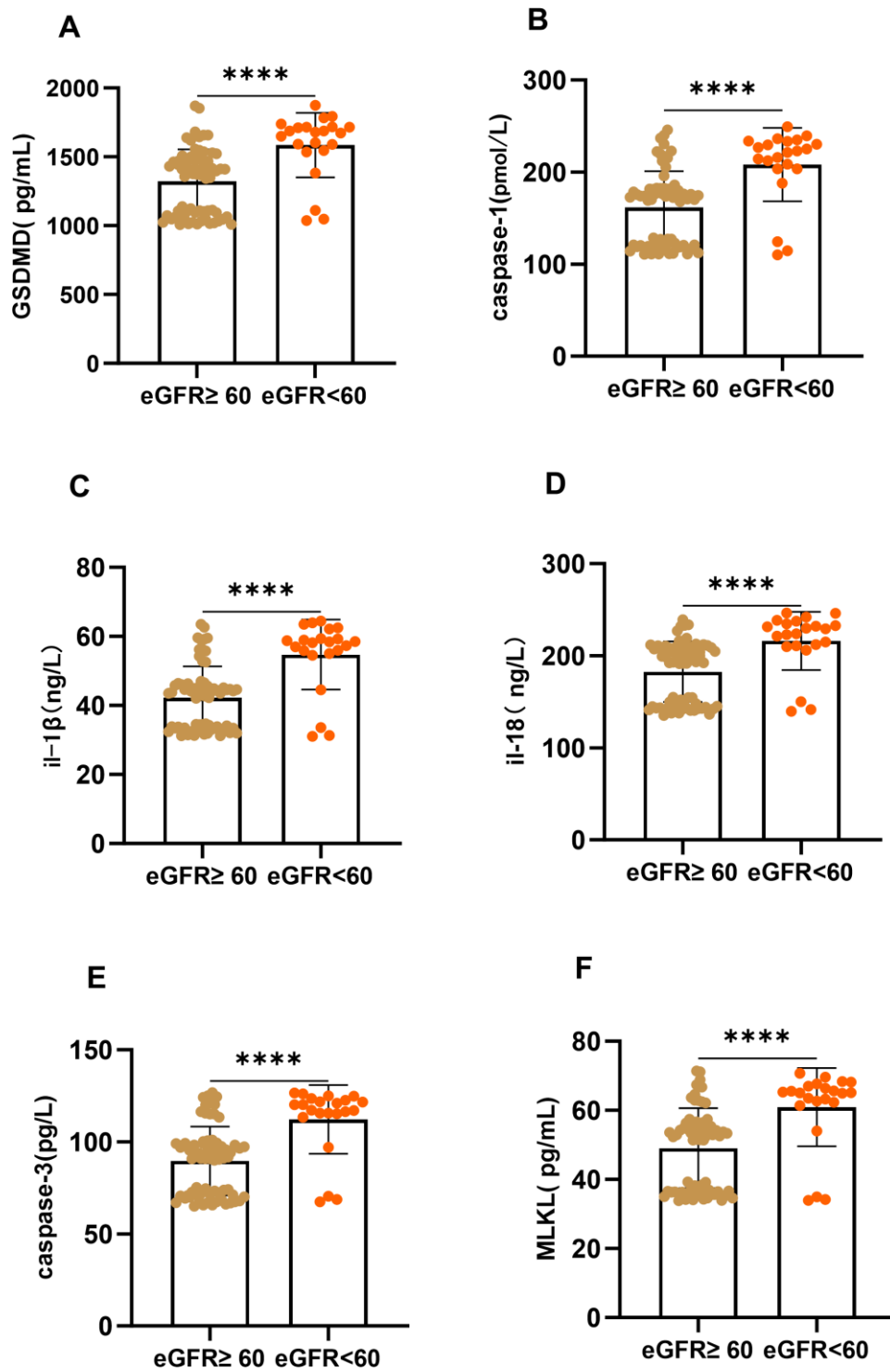
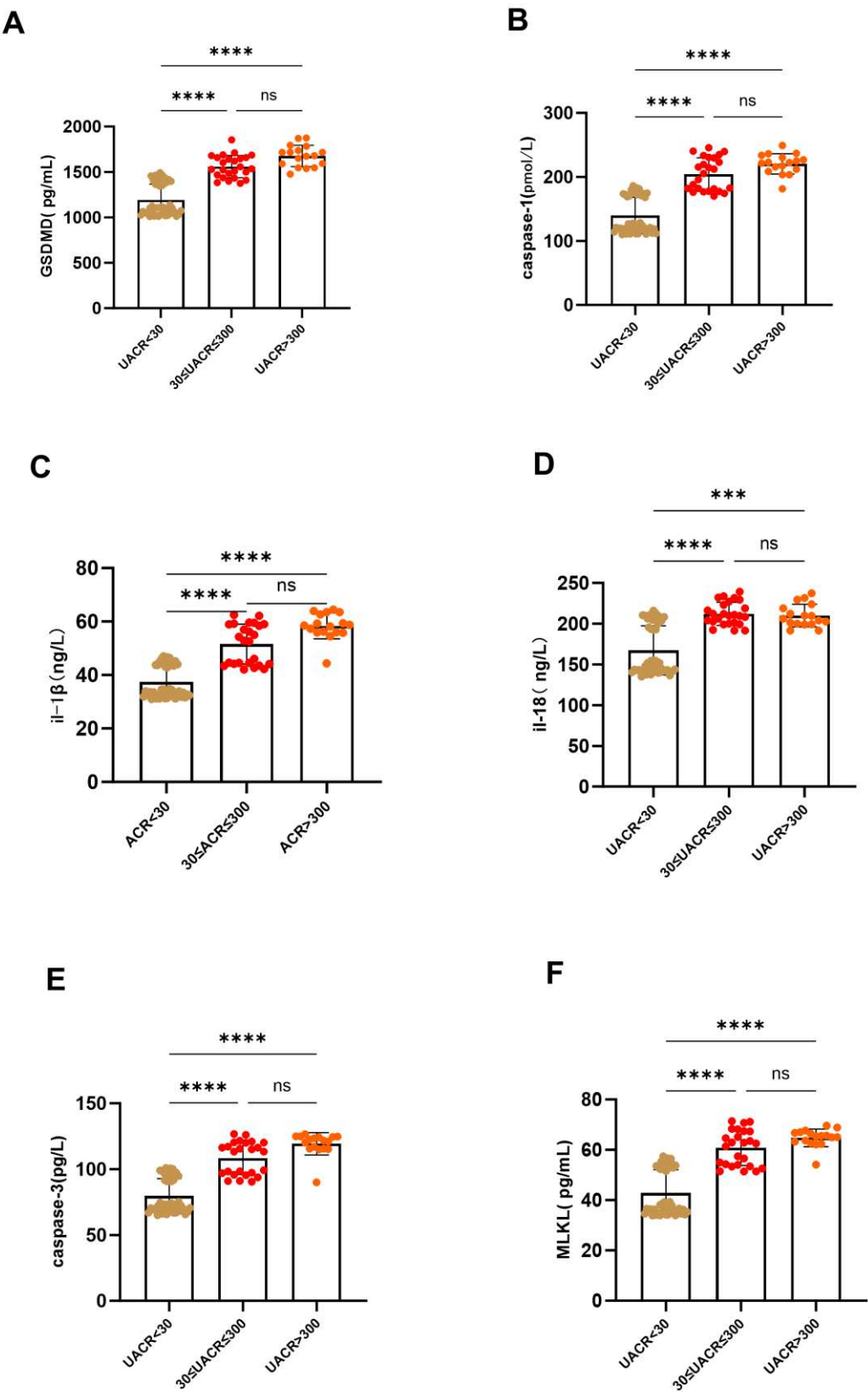


Figure 3. Serum levels of PANoptosis-related proteins in different eGFR.

Statistical significance was set at $P < 0.05$. Significant differences between groups are marked with * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $p < 0.0001$.



708 **Figure 4. Serum levels of PANoptosis-related proteins in different UACR.**

Statistical significance was set at $P < 0.05$. Significant differences between groups are marked with * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $p < 0.0001$.

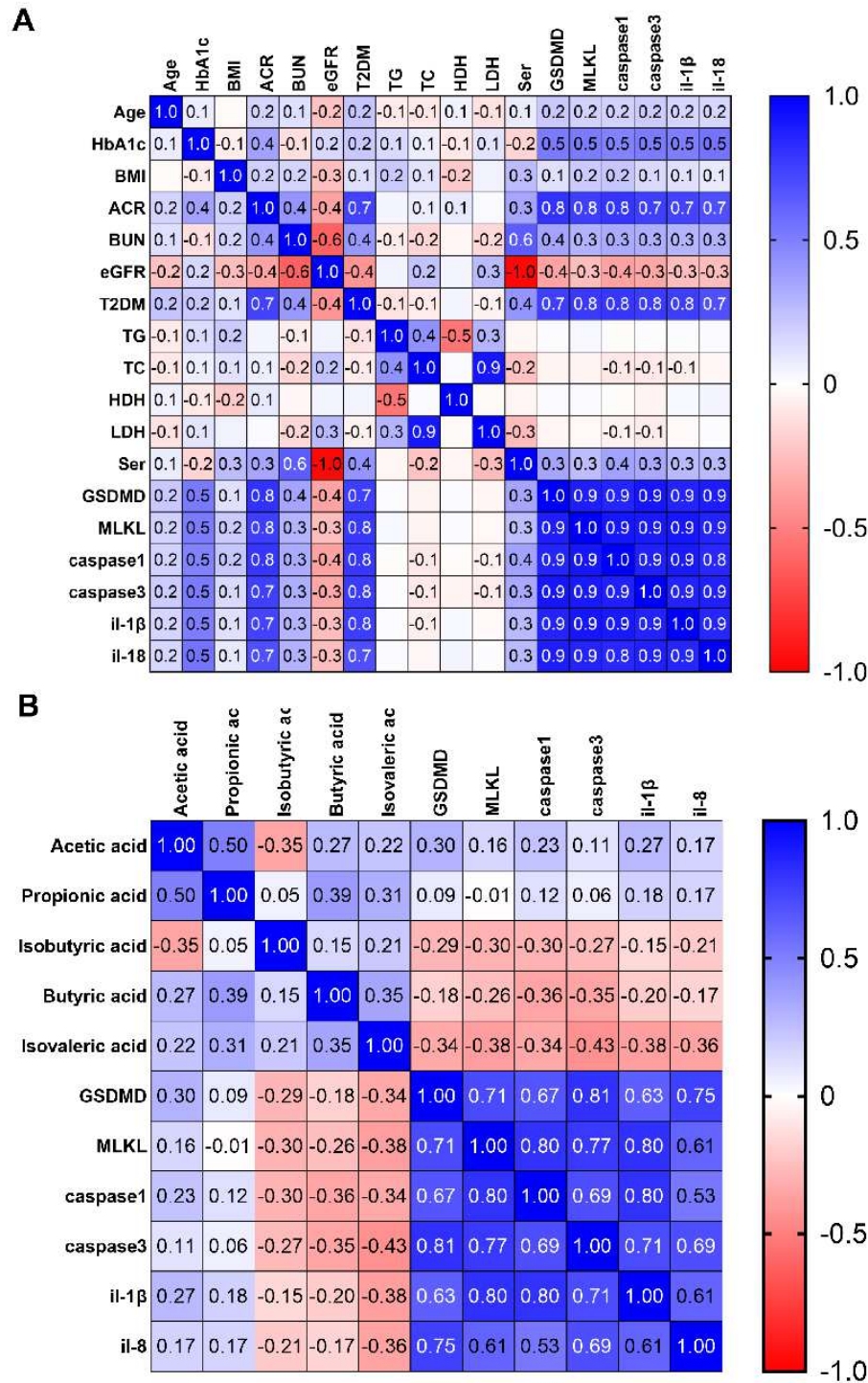
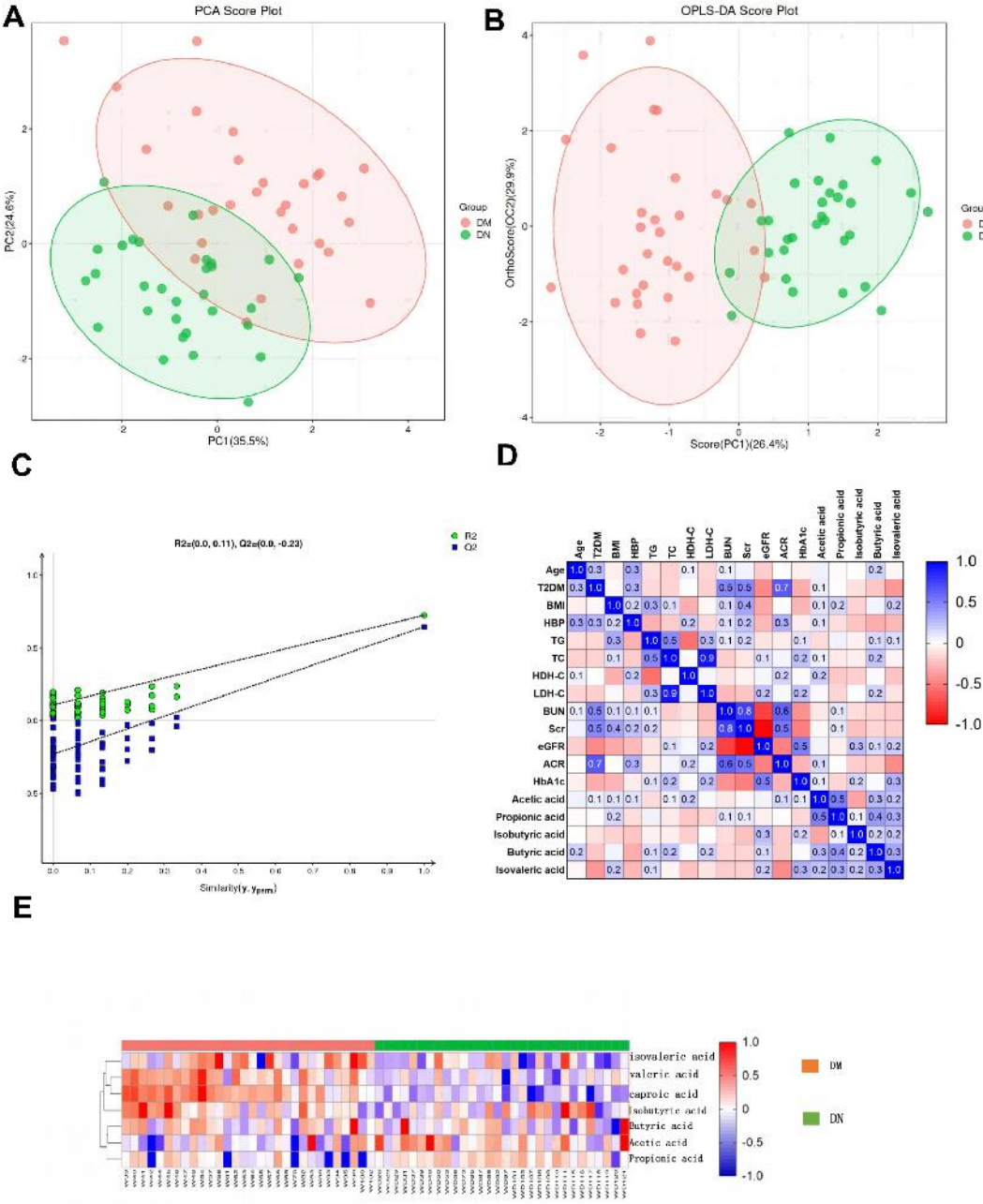


Figure5. Correlation of PANoptosis with clinical parameters and SCFAs

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719 **Figure 6. Overall differences in serum SCFA levels between T2DM and DKD**

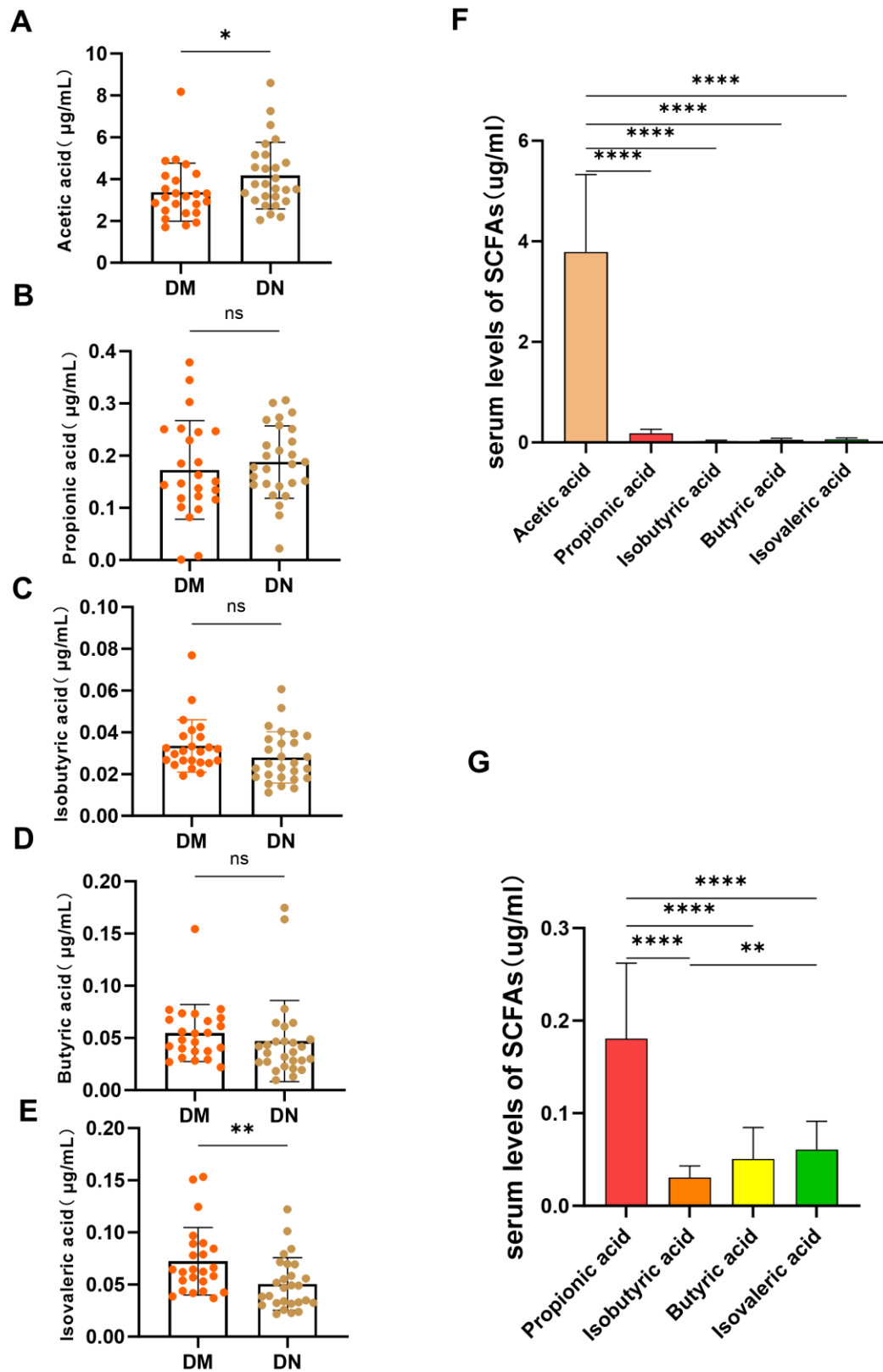


Figure 7. Comparison of SCFAs levels among subjects with T2DM and DKD.

Statistical significance was set at $P < 0.05$. Significant differences between groups are

marked with * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $p < 0.0001$.

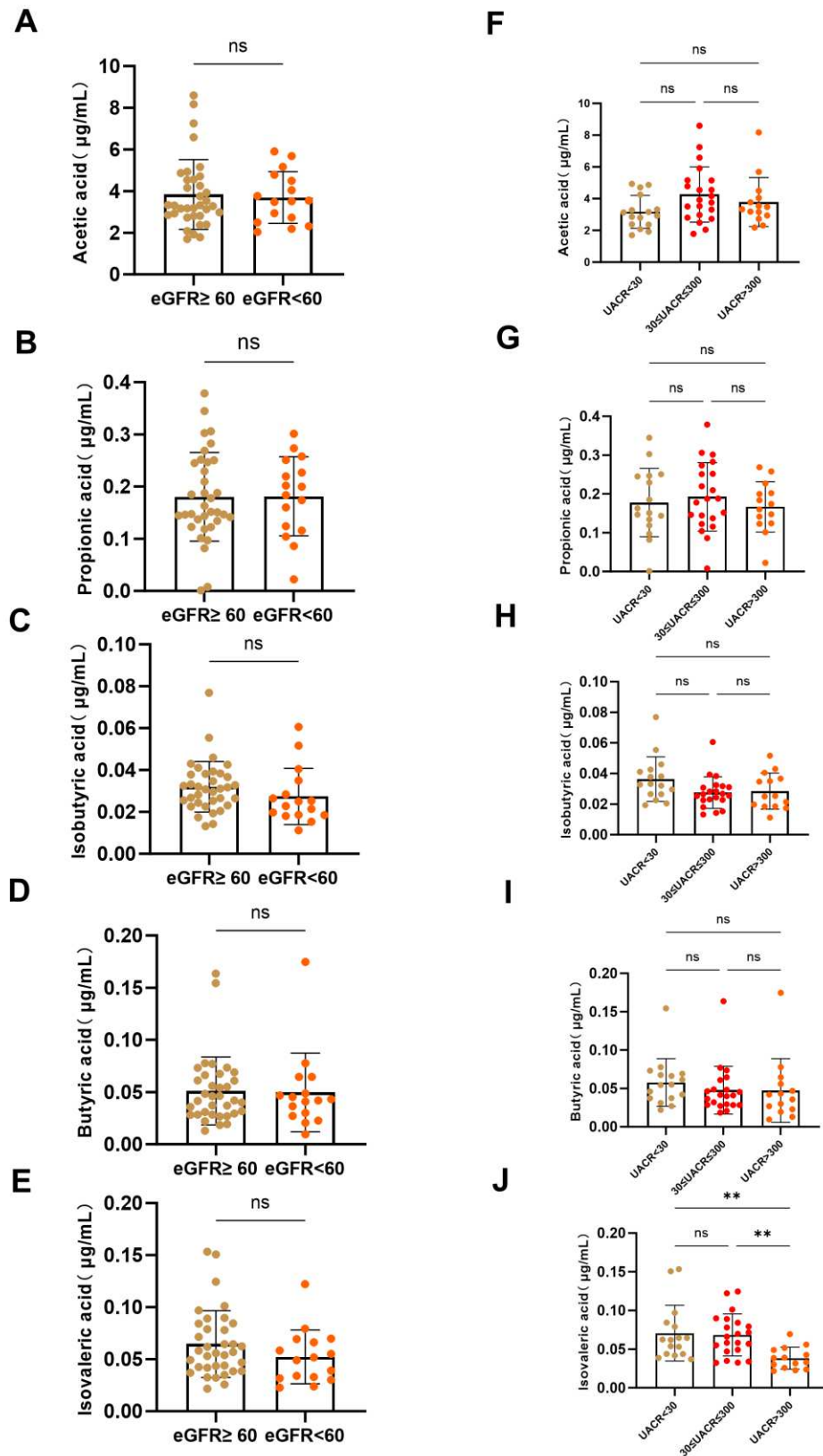


Figure 8. Comparison of SCFAs levels among different eGFR、UACR. Statistical

significance was set at $P < 0.05$. Significant differences between groups are marked with * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Supplementary Materials / Supplementary data

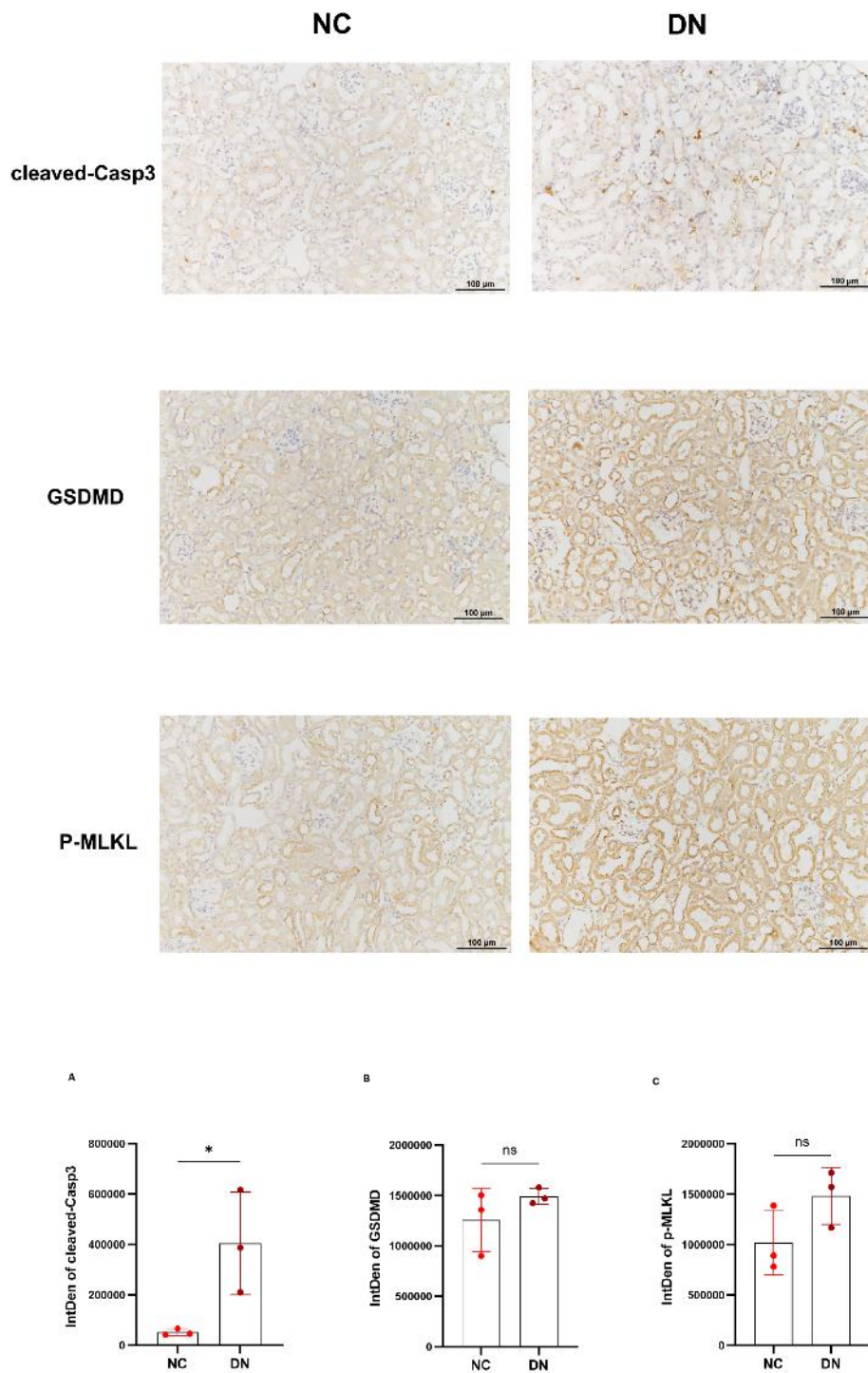


Figure 9. Immunohistochemistry for PANoptosis proteins in mouse kidneys. NC (healthy control group, n=3), DKD (DN group, n=3). Statistical significance was set at

$P < 0.05$. Significant differences between groups are marked with * $P < 0.05$, ** $P < 0.01$,
*** $P < 0.001$, **** $p < 0.0001$.