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Original Article

Expression of SIRT6 and VNN1 in children with primary nephrotic syndrome and their correlation with acute kidney injury

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Abstract: Objective: To explore the diagnostic and prognostic values of Sirtuin 6 (SIRT6) and Vanin-1 (VNN1) in peripheral blood monocytes of children with primary nephrotic syndrome (PNS) and their correlation with acute kidney injury (AKI). Methods: A retrospective analysis was conducted on 101 children (observation group) diagnosed with PNS and treated at the Shanxi University of Traditional Chinese Medicine Affiliated Hospital from December 2021 to December 2023. These children were categorized into two groups: the AKI group (n=35) and the non-AKI group (n=66), based on the presence of AKI. Additionally, 101 healthy children who underwent physical examinations during the same period served as the control group. Western blotting and RT-PCR were employed to measure the protein and mRNA levels of SIRT6 and VNN1 in monocytes across the three groups. The correlation between SIRT6 and VNN1 mRNA levels and clinical data, as well as kidney function indicators, was analyzed. The diagnostic value of SIRT6 and VNN1 mRNA levels for AKI in PNS was assessed using ROC curves. Multivariate logistic regression identified independent factors influencing AKI in PNS. The mRNA levels of SIRT6 and VNN1 were also compared before and after treatment in children with PNS. Results: The AKI group exhibited lower SIRT6 protein and mRNA levels, and higher VNN1 protein and mRNA levels in monocytes compared to the other groups (all $P<0.05$). Correlation analysis revealed that SIRT6 mRNA levels were positively correlated with serum creatinine (Scr), uric acid (UA), blood urea nitrogen (BUN), 24-hour urine protein (24h UP), cystatin C (Cys-C), and β2-microglobulin (β2-MG), but negatively correlated with albumin (ALB) and estimated glomerular filtration rate (eGFR) (all $P<0.05$). In contrast, VNN1 levels showed the opposite correlations ($P<0.05$). ROC curve analysis showed that the AUC for SIRT6 or VNN1 mRNA alone in diagnosing AKI was above 0.8, with a combined diagnostic AUC exceeding 0.9. Logistic regression indicated that eGFR, β2-MG, Cys-C, and the mRNA levels of SIRT6 and VNN1 were independent risk factors for AKI in PNS (all $P<0.05$). After treatment, SIRT6 mRNA levels significantly decreased, while VNN1 mRNA levels increased in children with PNS (both $P<0.05$). Conclusion: SIRT6 and VNN1 are closely associated with AKI in children with PNS and may serve as valuable biomarkers for the diagnosis of AKI.

Keywords: Primary nephrotic syndrome, SIRT6, VNN1, acute kidney injury

Introduction

Primary nephrotic syndrome (PNS) is a common condition in children [1, 2]. Currently, there is no specific treatment, and glucocorticoids or cyclophosphamide are the main clinical options. However, some children with PNS do not achieve symptom remission, and others may experience relapse. Additionally, some children may develop resistance or dependence on glucocorticoids, significantly affecting their prognosis [3, 4]. Acute kidney injury (AKI) is a frequent complication of PNS, leading to a rapid decline in renal function [5-7]. Clinically, AKI diagnosis is primarily based on renal biopsy or serum creatinine (Scr) testing. However, biopsy is invasive, and Scr testing can be delayed in reflecting kidney damage. Therefore, it is crucial to identify non-invasive, highly sensitive biomarkers for early diagnosis of AKI [8].

Recent studies have shown that aberrant Sirtuin 6 (SIRT6) can exacerbate renal insufficiency, tubular injury, and renal fibrosis, which are closely linked to kidney disease [9-11].

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Vanin-1 (VNN1), a glycosylated phospholipid adenoinositol-anchored hydrolase in epithelial cells, has been reported to inhibit platelet antioxidant capacity, exacerbate oxidative stress, and induce inflammatory responses, thereby affecting renal function [12]. To date, no studies have explored the expression levels of SIRT6 and VNN1 in PNS complicated by AKI. The existing research provides a basis for investigating VNN1 and SIRT6 levels in PNS with AKI. This study aims to assess the clinical significance of SIRT6 and VNN1 in peripheral blood monocytes of children with PNS and AKI, potentially offering valuable insights for clinical diagnosis and prognosis.

Materials and methods

Clinical data

This retrospective analysis was conducted on 101 children (observation group) diagnosed with PNS and treated at the Shanxi University of Traditional Chinese Medicine Affiliated Hospital from December 2021 to December 2023. These children were categorized into two groups: the AKI group (n=35) and the non-AKI group (n=66), based on the presence of AKI. Additionally, 101 healthy children who underwent physical examinations during the same period served as the control group. The study received approval from the Ethics Committee of the Shanxi University of Traditional Chinese Medicine Affiliated Hospital.

Inclusion and exclusion criteria for the observation group

Inclusion criteria: (1) Diagnosis in accordance with the "2016 Evidence-Based Guidelines for the Diagnosis and Treatment of Children with Hormone-Sensitive, Relapsing/Dependent Nephrotic Syndrome" by the Pediatrics Branch of the Chinese Medical Association [13]; (2) First-time diagnosis; (3) Age ≤16 years; (4) No history of hormone use or other medications that could affect experimental outcomes within the past 3 months; (5) No history of infection, chronic kidney disease, or other related diseases within the past 3 months; (6) Complete clinical data.

Exclusion criteria: (1) Presence of malignant tumors; (2) Incomplete clinical data; (3) Intolerance to the medications used in this study; (4) Presence of other allergic diseases; (5) Severe diseases affecting the heart, liver, or other organs and tissues; (6) Secondary nephrotic syndrome caused by drugs or systemic diseases.

Treatment methods

Children with PNS received supportive care upon admission, including electrolyte regulation, acid-base balance management, fluid supplementation, and diuretics. Additionally, they were treated with prednisone acetate tablets (Guangdong Huanan Pharmaceutical Group Co., Ltd., National Medicine Standard: H44 020682) at an initial dose of 2 mg/kg once daily, not exceeding a maximum dose of 40 mg, administered orally in the morning. The prednisone dosage was gradually tapered based on the patient's condition, decreasing by 2.5 mg every 2 weeks until reaching a maintenance dose of 10 mg/day. Cyclophosphamide (Shanxi Pude Pharmaceutical Co., Ltd., National Medicine Standard: H14023686) was administered intravenously at 10 mg/kg once a month, accompanied by vitamin B6 injections to prevent vomiting. Symptomatic treatments, including lipid regulation and liver protection, were provided according to the children's specific needs.

Observation indicators

The flowchart is shown in **Figure 1**.

Clinical data collection

Clinical data were collected from all study participants, including age, gender, body mass index (BMI), blood lipid levels [total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C)], serum creatinine (Scr), albumin (ALB), uric acid (UA), blood urea nitrogen (BUN), glomerular filtration rate (eGFR), 24-hour urinary protein (24h UP), cystatin C (Cys-C), and β2-microglobulin (β2-MG).

SIRT6 and VNN1 mRNA detection

In the control group, 5 mL of peripheral blood was collected at the time of enrollment. In the observation group, 5 mL of peripheral blood was collected at enrollment (before treatment) and after 4 weeks of treatment. Blood cells were separated by centrifugation at 4,000 r/min, and peripheral mononuclear cells were

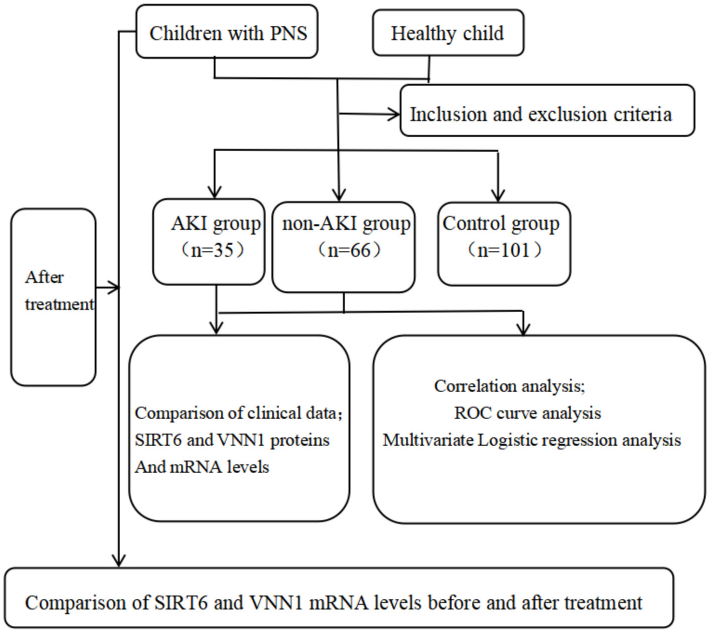

Figure 1. Flowchart. AKI: acute kidney injury, PNS: primary nephrotic syndrome, SIRT6: Sirtuin 6, VNN1: Vanin-1.

Table 1. Primer sequences

Primers	Sequence
SIRT6 upstream	5'-CCCACGGAGTCTGGACCAT-3'
SIRT6 downstream	5'-CTCTGCCAGTTTGTCCCTG-3'
VNN1 upstream	5'-GCGCTCTCATCAGGAAACAA-3'
VNN1 downstream	5'-CAGTGTGCAGTCCGTCAAATG-3'
GADPH upstream	5'-AAGATCTGCCGAGTAAACCG-3'
GADPH downstream	5'-TCCCGTGAAATACACCTCAA-3'

isolated using a cell isolation solution. RNA was extracted and reverse transcribed using the ReverTra Ace qPCR RT kit (Takara, Japan, Item No. FSQ-101). SIRT6 and VNN1 mRNA expression was detected using C1000 RT-PCR (Bio-Rad, USA). The reaction conditions were as follows: 95°C pre-denaturation for 30 s, 95°C for 5 s, and 62°C for 30 s, with 35 cycles. The $2^{-\Delta\Delta Ct}$ method was used to determine the relative expression levels of SIRT6 and VNN1 mRNA in each group, with GADPH serving as the internal reference. Each sample was tested in triplicate, and the average value was recorded. Primers were designed and synthesized by Beijing Dingguo Biotechnology Co., with sequences provided in **Table 1**.

Total protein from the peripheral blood was extracted according to the protocol of the protein extraction kit. Western blotting was performed following the instructions of the Western blot kit, including membrane transfer and blocking. Rabbit antibodies against SIRT6, VNN1, and β-actin were obtained from Wuhan Three Eagles Bio-Technology Co., Ltd. (item no. 10144-1-AP, 27613-1-AP, 25687-AP), diluted 1:300, and incubated with the samples overnight. HRP-labeled goat anti-rabbit secondary antibody (Abcam, UK, item number: ab6872) was added, followed by a 2-hour incubation. Imaging and gray value calculations were performed, with the experiments repeated three times.

Statistical analysis

Data were analyzed using SPSS 22.0. Normally distributed measurement data were expressed as mean ± standard deviation ($\bar{x}\pm$sd). One-way ANOVA was used for comparisons among multiple groups, with LSD-t tests for pairwise comparisons. Categorical data were expressed as percentages (%), with comparisons made using the χ^2 test. Multivariate analysis was performed using logistic regression. Correlation analysis was conducted using Pearson's method. ROC curves were plotted, and a P value <0.05 was considered statistically significant.

Results

Comparison of general information among groups

The results indicated that the AKI group had significantly higher levels of UA, BUN, 24h UP, Cys-C, and β2-MG, and significantly lower levels of ALB and eGFR compared to the control group and the non-AKI group (all P<0.05), as shown in **Table 2**.

Comparison of SIRT6 and VNN1 mRNA levels among groups

The AKI group exhibited significantly higher levels of VNN1 mRNA and significantly lower levels

Table 2. Comparison of the general data in each group

Indicators	AKI group (n=35)	Non-AKI group (n=66)	Control group (n=101)	F/χ^2	*P*
Age (years)	11.8±3.4	12.2±3.1	12.0±3.8	0.156	0.856
Gender					
Male	22	45	73	1.143	0.565
Female	13	21	28		
Body Mass Index (kg/m^2)	23.04±3.61	22.78±4.25	23.40±4.57	0.422	0.656
TC (mmol/L)	6.27±1.14	5.89±0.98	6.12±1.09	1.673	0.190
TG (mmol/L)	1.95±0.69	1.73±0.55	1.64±0.74	2.748	0.066
HDL-C (mmol/L)	2.85±0.51	2.74±0.46	2.61±0.65	2.615	0.076
LDL-C (mmol/L)	1.49±0.42	1.43±0.51	1.38±0.46	0.757	0.470
Scr (μmol/L)	184.73±27.15[a,b]	126.22±24.27[a]	75.98±22.41	288.598	<0.001
ALB (g/L)	19.22±8.47[a,b]	24.82±8.71[a]	37.65±6.87	96.578	<0.001
UA (μmol/L)	289.26±48.29[a,b]	264.05±51.17[a]	236.53±47.36	16.994	<0.001
BUN (mmol/L)	18.98±6.64[a,b]	10.85±3.37[a]	4.14±1.29	252.329	<0.001
eGFR (mL/min/1.73 m^2)	43.74±10.29[a,b]	76.29±13.38[a]	109.84±10.21	487.848	<0.001
24h Up (g/24 h)	0.19±0.03[a,b]	0.15±0.04[a]	0.10±0.03	107.451	<0.001
Cys-C (mg/L)	3.67±0.63[a,b]	2.31±0.64[a]	0.72±0.29	526.675	<0.001
β2-MG (mg/L)	7.91±1.04[a,b]	3.28±0.94[a]	1.09±0.27	1193.414	<0.001

Note: [a]Compared with the control group, P<0.05; [b]Compared with the non-AKI group, P<0.05. AKI: acute kidney injury, TC: total cholesterol, TG: triglyceride, LDL-C: low-density lipoprotein, HDL-C: high-density lipoprotein, Scr: serum creatinine, ALB: albumin, UA: uric acid, BUN: urea nitrogen, eGFR: glomerular filtration rate, 24h UP: 24-hour urinary protein, Cys-C: cystatin C, β2-MG: β2-microglobulin.

Table 3. Comparison of SIRT6 and VNN1 mRNA levels in each group

Indicators	AKI group (n=35)	Non-AKI group (n=66)	Control group (n=101)	*F*	*P*
SIRT6	0.59±0.21[a,b]	0.96±0.22[a]	1.44±0.31	152.247	<0.001
VNN1	1.73±0.30[a,b]	1.36±0.25[a]	0.89±0.37	102.401	<0.001

Note: [a]Compared with the control group, P<0.05; [b]Compared with the non-AKI group, P<0.05. AKI: acute kidney injury, SIRT6: Sirtuin 6, VNN1: Vanin-1.

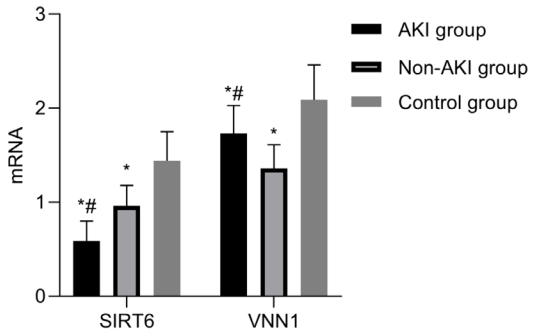

Figure 2. Relative mRNA expression levels of SIRT6 and VNN1 in each group. Note: *Compared with the control group, P<0.05; #Compared with the non-AKI group, P<0.05. AKI: acute kidney injury, SIRT6: Sirtuin 6, VNN1: Vanin-1.

of SIRT6 mRNA compared to the control and non-AKI groups (all P<0.05), as presented in **Table 3** and **Figure 2**.

Comparison of SIRT6 and VNN1 protein levels among groups

In comparison to the control and non-AKI groups, the AKI group showed a significantly higher relative expression level of VNN1 protein and a significantly lower relative expression level of SIRT6 protein (both P<0.05), as illustrated in **Figure 3**.

Correlation analysis results

Correlation analysis revealed that SIRT6 mRNA levels were positively correlated with Scr, UA, BUN, 24h UP, Cys-C, and β2-MG, and negatively correlated with ALB and eGFR (P<0.05). In contrast, VNN1 mRNA levels were negatively correlated with Scr, UA, BUN, 24h UP, Cys-C, and β2-MG, and positively correlated with ALB and eGFR (all P<0.05), as detailed in **Table 4**.

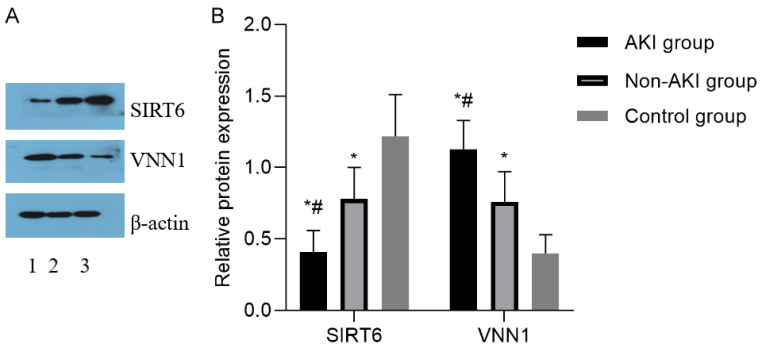

Figure 3. Western blot and expression levels of SIRT6 and VNN1 in each group. Note: (A) Western blot diagram, 1: control group, 2: non-AKI group, 3: AKI group; (B) Relative expression of SIRT6 and VNN1 proteins in each group. *Compared with the control group, P<0.05; #Compared with the non-AKI group, P<0.05. AKI: acute kidney injury, SIRT6: Sirtuin 6, VNN1: Vanin-1.

Table 4. Results of correlation analysis

Indicators	SIRT6 mRNA		VNN1 mRNA	
	r	*P*	*r*	*P*
Scr (μmol/L)	0.479	0.013	-0.452	0.024
ALB (g/L)	-0.344	<0.001	0.417	<0.001
UA (μmol/L)	0.362	<0.001	-0.396	<0.001
BUN (mmol/L)	0.396	<0.001	-0.441	<0.001
eGFR (mL/min/1.73 m^2)	-0.414	<0.001	0.392	0.008
24h Up (g/24 h)	0.428	0.025	-0.403	<0.001
Cys-C (mg/L)	0.441	<0.001	-0.377	<0.001
β2-MG (mg/L)	0.387	0.019	-0.419	<0.001

Note: Scr: serum creatinine, ALB: albumin, UA: uric acid, BUN: urea nitrogen, eGFR: glomerular filtration rate, 24h UP: 24-hour urinary protein, Cys-C: cystatin C, β2-MG: β2-microglobulin.

Table 5. Assignment table

Indicators	Assignment
Scr (μmol/L)	≥126.22=1; <126.22=0
ALB (g/L)	≤24.82=1; >24.82=0
UA (μmol/L)	≥264.05=1; <264.05=0
BUN (mmol/L)	≤10.85=1; >10.85=0
eGFR (mL/min/1.73 m^2)	≤76.29=1; >76.29=0
24h Up (g/24 h)	≥0.15=1; <0.15=0
Cys-C (mg/L)	≥2.31=1; <2.31=0
β2-MG (mg/L)	≥3.28=1; <3.28=0
SIRT6 mRNA	≤0.96=1; >0.96=0
VNN1 mRNA	≥1.36=1; <1.36=0

Note: Scr: serum creatinine, ALB: albumin, UA: uric acid, BUN: urea nitrogen, eGFR: glomerular filtration rate, 24h UP: 24-hour urinary protein, Cys-C: cystatin C, β2-MG: β2-microglobulin, SIRT6: Sirtuin 6, VNN1: Vanin-1.

Logistic regression analysis results

The indicators that showed statistical significance in univariate analysis were assigned values (**Table 5**) and analyzed by multivariate logistic regression using the stepwise method (Forward: conditional, with an entry criterion of α=0.05 and an exclusion criterion of α=0.1). Multivariate logistic regression analysis demonstrated that eGFR, β2-MG, Cys-C, SIRT6, and VNN1 were all independent risk factors for AKI in PNS (all P<0.05), as shown in **Table 6**.

ROC curve results

The ROC curve analysis revealed that the AUC for diagnosing AKI using SIRT6 or VNN1 mRNA individually was greater than 0.8, while the AUC for their combined diagnosis was greater than 0.9, as depicted in **Table 7** and **Figure 4**.

Comparison of SIRT6 and VNN1 mRNA levels before and after treatment

Following treatment, SIRT6 mRNA levels in children with PNS significantly decreased, while VNN1 mRNA levels significantly increased (P<0.05), as shown in **Table 8**.

Discussion

PNS is a common immune-related kidney disease, with over 70% of cases occurring in children. It is also the leading cause of mortality among children with kidney diseases in China [14]. Currently, clinical practice primarily involves supportive and symptomatic treatments, but symptoms in some patients remain difficult to alleviate even after treatment [15, 16]. AKI is one of the common complications of PNS, characterized by the rapid onset of oliguria or anuria, leading to renal artery obstruction within hours to days. In severe cases, patients may develop

Table 6. Logistic regression analysis results

Indicators	β	SE	Wald	OR	95% CI	P
eGFR (ml/min/1.73 m^2)	1.550	0.492	9.925	4.71	3.10-6.84	<0.001
Cys-C (mg/L)	2.115	0.617	11.750	8.29	3.98-25.26	<0.001
β2-MG (mg/L)	1.495	0.522	8.202	4.46	2.29-9.34	<0.001
SIRT6 mRNA	0.820	0.295	7.727	2.27	1.87-5.09	<0.001
VNN1 mRNA	1.175	0.419	7.864	3.24	1.97-7.33	<0.001

Note: eGFR: glomerular filtration rate, Cys-C: cystatin C, β2-MG: β2-microglobulin, SIRT6: Sirtuin 6, VNN1: Vanin-1.

Table 7. ROC curve results

Variable	Cut-off value	AUC	95% CI	Sensitivity	Specificity	P
SIRT6 mRNA	0.90	0.89	0.83, 0.95	0.73	0.91	<0.001
VNN1 mRNA	1.47	0.82	0.73, 0.91	0.80	0.79	<0.001
Combined factor	-	0.91	0.85, 0.97	0.80	0.92	<0.001

Note: SIRT6: Sirtuin 6, VNN1: Vanin-1.

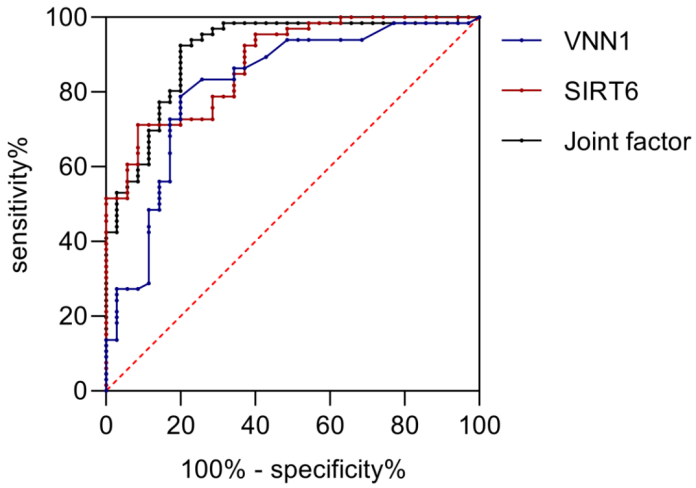

Figure 4. ROC curve. Note: SIRT6: Sirtuin 6, VNN1: Vanin-1.

Table 8. Comparison of SIRT6 and VNN1 mRNA levels before and after treatment

Time	SIRT6 mRNA	VNN1 mRNA
Before treatment (n=101)	0.83±0.28	1.49±0.33
4 weeks after treatment (n=101)	1.50±0.32	1.80±0.51
t	15.599	10.048
P	<0.001	<0.001

acute heart failure, significantly increasing the risk of death [17, 18]. Therefore, identifying potential biomarkers that may influence patient prognosis is crucial.

VNN1 is widely distributed in epithelial tissues and can indirectly reduce the synthesis of glutathione, thereby weakening the body's ability to counteract oxidative stress and reducing the inflammatory response [19]. Studies have confirmed that urinary VNN1 is significantly elevated in patients with kidney disease, suggesting it as a biomarker for tubular injury [20]. This study found that, compared to healthy children, children with PNS exhibited increased levels of VNN1 mRNA in mononuclear cells, with even higher levels in those with concurrent AKI. These findings align with related research [20]. Additionally, VNN1 mRNA levels were closely correlated with kidney function and disease severity indicators such as Scr, UA, BUN, 24h UP, Cys-C, β2-MG, and eGFR. The study also observed a decrease in VNN1 mRNA levels following treatment, suggesting that VNN1 may contribute to PNS development by modulating inflammatory and oxidative stress responses, and it is closely linked to disease severity and renal function impairment.

SIRT6 is a member of the Sir2 family, located in the cell nucleus [21]. It regulates glucose metabolism homeostasis by inhibiting glycolysis-related genes. When SIRT6 is inactivated, acetylation levels of glycolysis gene promoters and multiple metabolic genes increase, ultimately affecting glycolysis. The kidneys require

substantial adenosine triphosphate production via mitochondria to maintain physiological functions. Since kidney injury primarily affects renal tubules, which contain numerous mitochondria, SIRT6 is closely related to mitochondrial function [22]. SIRT6 plays a vital role in antioxidant stress, mitochondrial substrate metabolism, and cell survival, protecting mitochondrial integrity and reducing renal tubular injury severity [23]. This study demonstrated that, compared to healthy children or those with PNS alone, children with PNS and AKI had significantly lower SIRT6 mRNA levels and protein expression, with SIRT6 levels closely related to kidney function indicators. A possible explanation is that reduced SIRT6 increases apoptosis of renal tubular epithelial cells and mitochondrial damage, compromising kidney structural function and contributing to nephrotic syndrome. Additionally, reduced SIRT6 may exacerbate oxidative stress and inflammatory damage [24, 25]. After treatment, increased SIRT6 levels correlated with improved kidney function, further indicating the protective role of SIRT6.

Logistic regression analysis identified eGFR, β2-MG, Cys-C, SIRT6, and VNN1 as independent risk factors for AKI in PNS. Clinically, patients with lower eGFR and higher levels of β2-MG, Cys-C, SIRT6, and VNN1 should be closely monitored, and timely interventions should be implemented to prevent disease progression.

The study also explored the clinical value of SIRT6 and VNN1 in assessing AKI. The results showed that the AUC for diagnosing AKI using SIRT6 or VNN1 mRNA alone was greater than 0.8, with the combined diagnostic AUC exceeding 0.9. This suggests that combined detection offers higher clinical value in assessing AKI and is crucial for evaluating the prognosis of children with PNS.

Limitations of this study include: (1) the lack of exploration into the long-term prognostic value of SIRT6 and VNN1 levels in children; and (2) the study's limited sample size, sourced from a single hospital. Future multi-center studies with larger samples are necessary to confirm these findings.

In conclusion, SIRT6 levels in mononuclear cells of children with PNS significantly decrease, while VNN1 levels significantly increase. These changes are closely related to disease severity, kidney damage, and prognosis. Combined detection of SIRT6 and VNN1 offers valuable diagnostic and prognostic insights for children with PNS.

Disclosure of conflict of interest

None.

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