

# The Efficacy of Cyclophosphamide Combined with Prednisone in Membranous Nephropathy Patients with Different Cytochrome P450 2B6 Gene Polymorphisms and Analysis of Factors Influencing the Efficacy

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**Objective:** To investigate the efficacy of cyclophosphamide combined with prednisone in membranous nephropathy (MN) patients with different cytochrome P450 (CYP) 2B6 gene polymorphisms and explore the factors influencing the efficacy.

**Methods:** We performed a prospective and interventional study including 153 outpatients diagnosed with membranous nephropathy from April 2020 to June 2020 in the Bayannur Hospital. Based on the results of CYP2B6 gene polymorphisms, patients were divided into CYP2B6\*4 group (785A>G) with 93 cases and CYP2B6\*5 group (1459C>T) with 60 cases, and all patients were treated with cyclophosphamide and prednisone. The efficacy of the two groups was compared, and the serum levels of antibody against thrombospondin type-1 domain-containing 7A (THSD7A-Ab), 8-hydroxy-2'-deoxyguanosine (8-OHdG) and antibody against the M-type phospholipase A2 receptor (PLA2R-Ab) determined by the enzyme-linked immunosorbent method were analyzed in the two groups before and after treatment.

**Results:** After the treatment with cyclophosphamide and prednisone, serum levels of THSD7A-Ab, 8-OHdG, and PLA2R-Ab were higher in the CYP2B6\*4 group than those in the CYP2B6\*5 group (All three  $P<0.001$ ). The univariate Logistic regression analysis showed that CYP2B6\*4 (OR = 1.727, 95% CI: 1.028–2.654.  $P=0.012$ ), CYP2B6\*5 (OR = 1.802, 95% CI: 1.179–2.752.  $P=0.031$ ), THSD7A-Ab (OR = 1.832, 95% CI: 0.871–2.348.  $P=0.023$ ), 8-OHdG (OR = 1.661, 95% CI: 1.123–2.231.  $P=0.009$ ), PLA2R-Ab (OR = 1.649, 95% CI: 1.083–2.761.  $P=0.017$ ) were independent influencing factors on the efficacy of MN. The multivariate Logistic regression analysis showed that CYP2B6\*4 (OR = 2.009, 95% CI: 1.327–2.703.  $P<0.001$ ), CYP2B6\*5 (OR = 3.009, 95% CI: 1.467–5.231.  $P=0.005$ ), THSD7A-Ab (OR = 1.396, 95% CI: 1.002–1.897.  $P=0.019$ ), 8-OHdG (OR = 0.704, 95% CI: 0.591–0.742.  $P<0.001$ ), PLA2R-Ab (OR = 2.761, 95% CI: 1.231–3.918.  $P=0.017$ ) were independent factors influencing the efficacy of cyclophosphamide and prednisone on MN.

**Conclusion:** Cyclophosphamide combined with prednisone was effective in treating MN with different CYP2B6 gene polymorphisms. CYP2B6\*4, CYP2B6\*5, and serum levels of THSD7A-Ab, 8-OHdG, and PLA2R-Ab were independent influencing factors on the outcome of MN.

**Keywords:** cyclophosphamide, prednisone, gene polymorphisms, membranous nephropathy, influencing factor

## Introduction

As a common cause of nephrotic syndrome in adults, membranous nephropathy (MN) accounts for about 20% of cases of primary nephrotic syndrome.<sup>1</sup> MN can occur at any age, and its incidence increases with age, with an average age of onset of

40 to 50 years.<sup>2,3</sup> 40–50% of renal biopsy-proven MN present with nephrotic syndrome, and is also one of the main types of hormone-resistant nephrotic syndrome.<sup>4</sup> According to the etiology, MN can be divided into secondary MN and idiopathic MN. The clinical symptoms of MN after the onset of the disease are predominantly persistent massive proteinuria, etc., but the severity of clinical symptoms is not the same, so the prognosis of patients is also different.<sup>5</sup> Chronic renal failure can occur when patients are not treated with timely and effective interventions, posing a serious threat to their health. According to clinical guidelines, an immunosuppressive therapy of cyclophosphamide combined with glucocorticoids is currently preferred for the treatment of MN, and has been proved be effective in treating patients with MN in recent years.<sup>6</sup>

The cytochrome P450 superfamily (CYP) is an important key enzyme for drug metabolism in vivo and includes multiple isoforms. The C64T, G516T, C777A, A785G and C1459T mutations of CYP2B6 were found at frequencies of 5.3%, 28.6%, 0.5%, 32.6% and 14.0%, respectively.<sup>7</sup> Among them, CYP2B6 is an important enzyme that metabolizes about 3.0% of therapeutic drugs, including cyclophosphamide and antiepileptic drugs.<sup>8</sup> However, there are few studies on the efficacy of intravenous cyclophosphamide therapy combined with prednisone in MN patients with different CYP2B6 gene polymorphisms. Based on this, this study was designed to investigate the efficacy of cyclophosphamide combined with prednisone in MN patients with CYP2B6 gene polymorphisms and analyze the related influencing factors.

## Materials and Methods

### Patients Data

This prospective and interventional study was conducted following the ethical principles of the World Medical Association Declaration of Helsinki, approved by the ethics committee of Bayannur Hospital. All patients were fully informed of all information of this treatment and signed an informed consent. The study included 153 outpatients with renal biopsy-diagnosed MN from April 2020 to June 2020 in the Department of Nephrology, Bayannur Hospital. Based on the results of CYP2B6 gene polymorphisms, patients were divided into CYP2B6\*4 group (785A > G) with 93 cases and CYP2B6\*5 group (1459C > T) with 60 cases.

### Inclusion Criteria and Exclusion Criteria

Inclusion criteria were patients diagnosed with renal biopsy-proven MN and histologically graded according to the previous diagnostic criteria,<sup>9</sup> and with normal aminotransferase levels and serum creatinine, and without immune, hormonal or renal transplantation treatment prior to treatment.

Exclusion criteria were patients with unstable renal function or severe liver and kidney disease, those with pre-treatment estimated glomerular filtration rate (eGFR) <30 mL/min/1.73m<sup>2</sup>, those receiving conservative treatment, pregnant or lactating women, those with hepatitis or autoimmune disease, those taking Chinese medicine at the same time, and with other factors interfering with drug concentration, and those not taking drugs regularly.

### Gene Polymorphisms Detection

The gene polymorphisms detection of CYP2B6 was performed according to previous studies.<sup>10</sup> 4mL of fasting peripheral venous blood was drawn from all subjects, placed in Ethylene Diamine Tetraacetic Acid (EDTA) anticoagulation test tubes. DNA was extracted within 48 hours. Firstly, 200 µL blood was added to the tube, as well as 20 µL proteinase K solution. Then, 200 µL of buffer was added, fully inverted and mixed, and placed at 56°C for 10 minutes. 200 µL of absolute ethanol was added to the adsorption column and be put into the waste liquid collection tube, followed by centrifugation at 8000 rpm for 1 min at room temperature, and the waste liquid was discard. Then the adsorption column was added with 500 µL of buffer, and centrifugated at 14,000 rpm for 3 min at room temperature, and the waste solution discarded. The adsorption column was transferred into a 1.5mL tube, added with 50–200 µL of elution buffer AE, placed at room temperature for 2–5 minutes, and centrifugated at 8000rpm for 1min at room temperature. Finally, the solution was collected, which was the obtained DNA solution.

Primers for polymerase chain reaction (PCR) were designed and synthesized by Sangon Biotech Co. Ltd. (Shanghai, China). CYP2B6\*4: forward 5'-AGAAGCACCGTGAAACCC-3', reverse 5'-TCTCCATCTTGCCCTCCC-3'. CYP2B6\*5: forward 5'-ACCACCATCCTCCAGAAC-3', reverse 5'-TTGCGGGGAGTCAGAGCC -3'. The PCR amplification was conducted according to the previous study.<sup>10</sup>

## Treatment Protocol

According to previous studies,<sup>11</sup> all study participants were given 0.8–1.0 g of cyclophosphamide (Shanxi PUDE Pharmaceutical Co., Ltd., China. LOT.NO, 20,170,102) intravenously each month for 6 consecutive months, with extended dosing intervals for every 3 months depending on the patient's condition, and a cumulative dose of 8–10 g. The starting dose of prednisone (Zhejiang Xianju Pharmaceutical Co., Ltd., China. LOT.NO, 201,811,281) was 0.8–1.0 mg/kg per day and the drug dose was gradually reduced to less than 0.5mg/kg after 8–12 weeks of administration. Depending on the patient's condition, anti-platelet adhesion drugs, lipid-lowering drugs, osteoporosis prevention and blood pressure control therapies are applied. Five patients in CYP2B6\*4 group had hemorrhagic cystitis, but not in the CYP2B6\*5 group. Total incidence of adverse reactions in the CYP2B6\*4 group was 11.83% and 1.67% for CYP2B6\*5 group, including hemorrhagic cystitis, nausea and vomiting.

## Primary Observation Schedule

The primary observation indicators of the study included total remission rate, complete remission rate, partial remission rate, and no remission rate. Complete remission was defined as serum albumin >35 g/L, 24-h urine protein <0.3 g/24 h, and stable renal function. Partial remission was defined as 24-h urine protein reduction >50%, 24-h urine protein ≤3.5 g/24 h and basically stable renal function. No remission was defined as the patient's clinical symptoms did not improve significantly and might even worsen. Total remission rate was defined as the sum of complete and partial remission rates.

## Measurement of Serum Indicators

3–8 mL fasting venous blood was collected, centrifuged at 10,000 r/min for 10 min, and the serum was taken for measurement. The serum levels of the antibody against thrombospondin type-1 domain-containing 7A (THSD7A-Ab), 8-hydroxy-2'-deoxyguanosine (8-OHdG) and antibody against the M-type phospholipase A2 receptor (PLA2R-Ab) were determined using the enzyme-linked immunosorbent assay kits purchased from the Enzyme Link Biotechnology Co. (Shanghai, China) according to the manufacturer's instructions (THSD7A-Ab, E-EL-R2856c; 8-OHdG, bs-0782R; PLA2R, ab66579).

## Statistical Analysis

SPSS 23.0 software (SPSS Inc., Armonk, NY, USA) was applied for statistical analysis of the data. The measurement data conforming to normal distribution were expressed by mean ± standard deviation (SD), and analyzed by *t*-test. While non-normally distributed measurement data were expressed as median (interquartile range), and the comparisons were examined by Mann–Whitney test. The categorical data were expressed as n (%) and compared using the  $\chi^2$  test. Univariate/multivariate Logistic regression analysis was used to analyze the influencing factors related to the efficacy of gene polymorphisms in MN. The difference was considered statistically significant when  $P \leq 0.05$ .

## Results

### Baseline Characteristics of MN Patients

Patients with CYP2B6\*4 gene polymorphisms accounted for 60.78% (93/153) of MN patients, while CYP2B6\*5 gene polymorphisms accounted for 39.22% (60/153). As shown in Table 1, there was no significant difference in gender, body mass index (BMI), systolic blood pressure, diastolic blood pressure, histology grading, and blood lipid levels between the CYP2B6\*4 group and CYP2B6\*5 group ( $P > 0.05$ ).

### Comparison of Clinical Outcomes Between Groups of CYP2B6\*4 and CYP2B6\*5

Total remission rate was defined as the sum of complete remission rate and partial remission rate. As shown in Table 2, the difference between the total remission rates of the two groups was not statistically significant (CYP2B6\*4 group VS CYP2B6\*5 group: 93.55% VS 95.00%,  $P > 0.05$ ).

### Comparison of Serum Levels of THSD7A-Ab, 8-OHdG and PLA2R-Ab in Each Group

Before the treatment with cyclophosphamide and prednisone, there was no statistically significant difference in the serum levels of THSD7A-Ab, 8-OHdG and PLA2R-Ab between the two groups ( $P > 0.05$ ). After the treatment, serum levels of THSD7A-Ab

**Table 1** Baseline Characteristics of MN Patients

Baseline Characteristics	CYP2B6*4 Group (n=93)	CYP2B6*5 Group (n=60)	t/ $\chi^2$	P value
Age (years)	48.87±4.03	48.82±4.02	0.075	0.937
Gender (Male/Female)	27/66	18/42	0.016	0.898
BMI (kg/m <sup>2</sup> )	24.91±2.38	24.89±2.41	0.051	0.958
Blood pressure (mm Hg)				
Systolic	139.51±8.53	138.28±8.48	0.866	0.388
Diastolic	87.31±5.28	88.09±5.17	-0.889	0.375
Blood glucose (mmol/L)	4.42±0.38	4.44±0.41	-0.308	0.759
Leukocyte (×10 <sup>9</sup> /L)	10.89±2.19	10.81±2.23	0.219	0.827
Erythrocytes (×10 <sup>9</sup> /L)	4.81±0.61	4.79±0.59	0.201	0.841
Hemoglobin (g/L)	132.87±12.18	130.29±12.23	1.277	0.204
Platelets (×10 <sup>9</sup> /L)	302.29±25.29	310.37±28.28	-1.841	0.068
Serum albumin (g/L)	24.42±3.89	24.58±3.91	0.248	0.804
Histology grading (n)			3.513	0.061
Stage I	65 (69.89%)	33 (55.00%)		
Stage II	23 (24.73%)	23 (38.33%)		
Stage III	5 (5.38%)	4 (6.67%)		
TG (mmol/L)	1.93±0.23	1.91±0.25	0.507	0.613
HDL (mmol/L)	2.19±0.28	2.15±0.31	0.827	0.408
LDL (mmol/L)	3.59±0.34	3.51±0.39	1.341	0.182
Total cholesterol (mmol/L)	5.09±1.02	5.12±1.04	-0.176	0.861
Urinary protein (g/24 h)	4.34±1.02	4.31±1.07	0.174	0.862

**Notes:** Data were expressed as mean ± standard deviation or number. *P* value was calculated by analysis of variance,  $\chi^2$  test, or independent *t*-test, as appropriate.

**Abbreviations:** MN, membranous nephropathy; BMI, Body mass index; TG, Triglyceride; HDL, High-density lipoprotein; LDL, Low-density lipoprotein;

**Table 2** Comparison of Clinical Outcomes Between Groups of CYP2B6\*4 and CYP2B6\*5

Outcomes	CYP2B6*4 Group (n=93)	CYP2B6*5 Group (n=60)	$\chi^2$	P value
Complete remission	35 (37.63%)	32 (53.33%)		
Partial remission	52 (56.99%)	25 (41.67%)		
No remission	6 (6.45%)	3 (5.00%)		
Total remission rate	87 (93.55%)	57 (95.00%)	0.139	0.709

**Notes:** Data were expressed as number (%). *P* value was calculated by analysis of variance,  $\chi^2$  test.

(CYP2B6\*4 VS CYP2B6\*5: 209.17 ± 29.86 VS 122.23 ± 28.76 µg/L), 8-OHdG (CYP2B6\*4 VS CYP2B6\*5: 218.76 ± 29.65 VS 193.23 ± 24.39 µg/L), and PLA2R-Ab (CYP2B6\*4 VS CYP2B6\*5: 517.28 ± 34.38 VS 421.76 ± 25.35 µg/L) were higher in the CYP2B6\*4 group than those in the CYP2B6\*5 group, with statistically significant differences (All three *P* < 0.001), as shown in Table 3.

## Univariate Logistic Regression Analysis of Factors Influencing MN Outcomes

The data with significant differences in the above comparisons, including CYP2B6\*4, CYP2B6\*5, THSD7A-Ab, 8-OHdG, and PLA2R-Ab were used as independent variables, and the outcomes of MN was used as the dependent variable for the univariate Logistic regression analysis. The results showed that CYP2B6\*4 with an odd ratio (OR) = 1.727, and 95% confidence interval (CI) = 1.028–2.654 (*P* = 0.012), CYP2B6\*5 (OR = 1.802, 95% CI: 1.179–2.752. *P* = 0.031), THSD7A-Ab (OR = 1.832, 95% CI: 0.871–2.348. *P* = 0.023), 8-OHdG (OR = 1.661, 95% CI: 1.123–2.231. *P* =

**Table 3** Comparison of Serum Levels of THSD7A-Ab, 8-OHdG and PLA2R-Ab in Each Group

Indicators	Time	CYP2B6*4 Group (n=93)	CYP2B6*5 Group (n=60)	t	P value
THSD7A-Ab (μg/L)	Pre-treatment	320.28±31.18	321.22±30.87	-0.183	0.855
	Post-treatment	209.17±29.86	122.23±28.76	17.837	<0.001
8-OHdG (μg/L)	Pre-treatment	251.87±30.37	250.76±21.19	0.247	0.805
	Post-treatment	218.76±29.65	193.23±24.39	5.561	<0.001
PLA2R-Ab (μg/L)	Pre-treatment	727.39±35.29	723.19±28.76	0.771	0.442
	Post-treatment	517.28±34.38	421.76±25.35	18.509	<0.001

**Notes:** Data were expressed as mean ± standard deviation. P value was calculated by analysis of variance, independent t-test.

**Abbreviations:** THSD7A-Ab, antibody against thrombospondin type-I domain-containing 7A; 8-OHdG, 8-hydroxy-2'-deoxyguanosine; PLA2R-Ab, antibody against phospholipase A2 receptor.

0.009), PLA2R-Ab (OR = 1.649, 95% CI: 1.083–2.761.  $P = 0.017$ ) were independent factors influencing the efficacy of cyclophosphamide and prednisone on MN, as shown in Table 4.

## Multivariate Logistic Regression Analysis of Factors Influencing MN Outcomes

The above-mentioned data with differences in CYP2B6\*4, CYP2B6\*5, THSD7A-Ab, 8-OHdG, and PLA2R-Ab were further applied to multivariate Logistic regression analysis, the results showed that CYP2B6\*4 (OR = 2.009, 95% CI: 1.327–2.703.  $P < 0.001$ ), CYP2B6\*5 (OR = 3.009, 95% CI: 1.467–5.231.  $P = 0.005$ ), THSD7A-Ab (OR = 1.396, 95% CI: 1.002–1.897.  $P = 0.019$ ), 8-OHdG (OR = 0.704, 95% CI: 0.591–0.742.  $P < 0.001$ ), PLA2R-Ab (OR = 2.761, 95% CI: 1.231–3.918.  $P = 0.017$ ) were independent influencing factors on the efficacy of MN, as shown in Table 5.

**Table 4** Univariate Logistic Regression Analysis of Factors Influencing MN Outcomes

Influencing Factors	B	$\chi^2$	P value	OR (95% CI)
CYP2B6*4 (n=93)	0.549	5.876	0.012	1.727 (1.028–2.654)
CYP2B6*5 (n=60)	0.991	7.091	0.031	1.802 (1.179–2.752)
THSD7A-Ab (n=153)	0.578	4.987	0.023	1.832 (0.871–2.348)
8-OHdG (n=153)	0.511	6.653	0.009	1.661 (1.123–2.231)
PLA2R-Ab (n= 153)	0.498	5.591	0.017	1.649 (1.083–2.761)

**Abbreviations:** MN, membranous nephropathy; THSD7A-Ab, antibody against thrombospondin type-I domain-containing 7A; 8-OHdG, 8-hydroxy-2'-deoxyguanosine; PLA2R-Ab, antibody against phospholipase A2 receptor.

**Table 5** Multivariate Logistic Regression Analysis of Factors Influencing MN Outcomes

Influencing Factors	B	$\chi^2$	P value	OR (95% CI)
CYP2B6*4 (n=93)	1.619	0.503	<0.001	2.009 (1.327–2.703)
CYP2B6*5 (n=60)	1.592	3.381	0.005	3.009 (1.467–5.231)
THSD7A-Ab (n=153)	0.329	3.671	0.019	1.396 (1.002–1.897)
8-OHdG (n=153)	1.028	3.182	<0.001	0.704 (0.591–0.742)
PLA2R-Ab (n= 153)	1.241	2.902	0.017	2.761 (1.231–3.918)

**Abbreviations:** MN, membranous nephropathy; THSD7A-Ab, antibody against thrombospondin type-I domain-containing 7A; 8-OHdG, 8-hydroxy-2'-deoxyguanosine; PLA2R-Ab, antibody against phospholipase A2 receptor.

## Discussion

MN is a pathomorphological diagnostic term that is a common cause of adult nephrotic syndrome with typical clinical manifestations of massive proteinuria, hypoalbuminemia, edema, and hyperlipidemia. It is typically characterized by immune complex deposition under the glomerular basement membrane epithelium with diffuse thickening of the basement membrane visible on light microscopy, and with granular, diffuse IgG and complement C3 along the capillary wall seen by immunofluorescence, and with the deposition of subepithelial granular electron-dense material seen by electron microscopy.<sup>12</sup> Currently, clinical treatment of MN includes non-immunosuppressive symptomatic supportive therapy (control of blood pressure, reduction of urinary protein levels, application of angiotensin converting enzyme inhibitors/angiotensin II receptor blockers, etc.), glucocorticoids and immunosuppressive therapy.<sup>13</sup> Glucocorticoids can affect human glucose metabolism, and the glucocorticoids commonly used in clinical treatment are prednisone and hydrocortisone. Among which, prednisone can reduce cell membrane and capillary permeability, reduce the inflammatory response, inhibit the release of histamine, promote the decomposition of immune complexes, and reduce the utilization of glucose, thus reducing the inflammatory response and reducing the accumulation of immune complexes.<sup>14</sup> However, the administration of excessive doses of prednisone can affect serum creatinine and impair the renal function.<sup>15</sup>

As one of the immunosuppressants widely used in clinical practice for the treatment of MN, cyclophosphamide is a bifunctional agent and cell cycle non-specific drug. On the one hand, cyclophosphamide can effectively inhibit the division of proliferating lymphocytes and reduce the number of B lymphocytes after entering the human body. On the other hand, it can interfere with the synthesis and transcription of DNA in quiescent B lymphocytes, which in turn reduces the synthesis of immunoglobulins and decreases their content, and finally inhibits the immune response ability of the body.<sup>15</sup> Scolari et al proved that cyclophosphamide was a precursor drug, which could be efficiently metabolized by CYP450 enzymes in the liver to eventually produce active metabolites.<sup>16</sup> Under normal conditions, the metabolism of cyclophosphamide by CYP mainly involves deoxyethylation and oxidation of cyclophosphamide, and its active products may be further decomposed to produce acrolein and phosphoramidate mustard after entering the target cells.<sup>15</sup> CYP gene polymorphisms can affect the balance of Phase I and Phase II metabolizing enzymes of drugs in vivo, and regulate the metabolism of drugs entering the body.<sup>17</sup> As one of critical CYP enzymes in liver, CYP2B6 is also expressed in human brain, intestine and kidney.<sup>18</sup> CYP2B6 metabolizes about 3.0% of therapeutic drugs, including cyclophosphamide, bupropion, and methadone,<sup>8</sup> and participates in the oxidation of cyclophosphamide C-4. CYP2B6 has multiple polynucleotide polymorphisms with widely varying activities. Theoretically, reduced enzyme activity can lead to decreased production of active metabolites, finally inducing a poorer efficacy. CYP2B6\*4 mutations could enhance enzyme activity and thus affect cyclophosphamide efficacy and adverse effects.<sup>10</sup> In a study of proliferative lupus nephritis patients treated with cyclophosphamide, Takada et al noted that patients homozygous for CYP2B6\*5 had reduced enzyme activity and were more likely to reach end-stage renal disease, as well as a lower probability of complete renal response.<sup>19</sup> In this study, the results of Logistic regression analysis of factors influencing MN outcomes showed that CYP2B6\*4 and CYP2B6\*5 were independent factors influencing the efficacy of cyclophosphamide and prednisone on MN.

THSD7A, the second podocyte autoantigen identified clinically, can be one of the key indicators to identify patients with primary or secondary MN.<sup>20</sup> THSD7A is involved in the stabilization of the slit diaphragm of mature podocytes, and its autoantibodies might structurally and functionally alter the slit diaphragm's permeability to protein, thus causing proteinuria or pathological changes in patients.<sup>21</sup> 8-OHdG is an end product of oxidative metabolism that can occur only through DNA oxidation, which is relatively stable in vivo and can be used as a sensitive indicator to assess the status of oxidative stress and oxidative damage.<sup>22</sup> PLA2R is a transmembrane protein on podocytes, and when it appears antigenic, it can induce IgG4-based immunoglobulin antibodies to bind to it and form in situ immune complexes, which then cause morphological changes in podocytes and trigger clinical symptoms such as proteinuria and hypoproteinemia so that PLA2R may be involved in the development of MN as a pathogenic antigen.<sup>23</sup> The inducement by exogenous antigen molecules or unknown factors may further lead to conformational changes in PLA2R in vivo, which accelerates the formation of in situ immune complexes and activates complement and generates cascade reactions.<sup>24</sup> PLA2R can be used as a specific target antigen in MN patients and PLA2R-Ab can effectively assess the severity of the disease and prognosis of MN.<sup>25</sup> In the present study, before the treatment with cyclophosphamide and prednisone, there



was no statistically significant difference in the serum levels of THSD7A-Ab, 8-OHdG and PLA2R-Ab between the two groups ( $P > 0.05$ ). After the treatment, THSD7A-Ab, 8-OHdG, and PLA2R-Ab were higher in the CYP2B6\*4 group than in the CYP2B6\*5 group (All three  $P < 0.001$ ), suggesting that possible association between CYP2B6 gene polymorphisms and the effects of the treatment with cyclophosphamide and prednisone on serum levels of THSD7A, 8-OHdG, and PLA2R in MN patients.

Furtherly, univariate/multivariate Logistic regression analyses were applied to evaluate the possible association between CYP2B6 gene polymorphisms and the treatment effects. The results showed that CYP2B6\*4, CYP2B6\*5, THSD7A-Ab, 8-OHdG, and PLA2R-Ab were independent factors influencing treatment efficacy on MN. This may be because the CYP450 superfamily has multiple polynucleotide polymorphisms, and CYP2B6 are located on human chromosome 19 and include nine exons.<sup>7</sup> The lysine 262-to-arginine mutant of CYP2B6\*4 had been shown to have differential effects on CYP2B6 catalytic activity,<sup>26</sup> causing a decrease in the metabolism of cyclophosphamide, which in turn induced the inability to effectively metabolize cyclophosphamide into active compounds after shock therapy treatment, and caused abnormalities in the antigenic and oxidative stress states of the body.

There were still some limitations in this study. Firstly, this study was a single-center study with a small sample size, which may lead to biased findings. Secondly, this study did not present adverse events during the treatment to clarify the influence of CYP2B6 gene polymorphisms on them. In addition, this study did not conduct a longer follow-up to evaluate the influence of CYP2B6 gene polymorphisms on the recurrence rate of MN. Finally, there were few clinical studies on CYP2B6\*4 and CYP2B6\*5 in MN, and it is expected that a multicenter study with a large sample size will confirm the results of this study in the future.

In conclusion, cyclophosphamide combined with prednisone was effective in treating MN patients with CYP2B6 gene polymorphisms. In addition, CYP2B6\*4, CYP2B6\*5, and serum levels of THSD7A-Ab, 8-OHdG, and PLA2R-Ab were independent factors influencing the efficacy of cyclophosphamide and prednisone on MN.

## Data Availability Statement

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

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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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