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Original Article

ORIGINAL ARTICLE

Anti-nephrin antibody: a potential biomarker of minimal change disease

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ABSTRACT

Background. Minimal change disease (MCD) is a common pathological type of nephrotic syndrome in children and adults, and the mechanisms remain obscure. The diagnosis of MCD still relies on renal biopsy, lacking effective biological markers. This study explores the diagnostic value of circulating anti-nephrin antibody in MCD patients and evaluates the correlation with disease activity indicators such as proteinuria.

Methods. The study included 108 adult patients with glomerular disease, including 36 with MCD, 16 with primary focal segmental glomerulosclerosis (FSGS), 20 with primary membranous nephropathy (MN), 17 with diabetic nephropathy (DN) and 19 with immunoglobulin A nephropathy (IgAN). Twenty healthy volunteers were included. Circulating anti-nephrin antibody was detected by indirect immunofluorescence method of cell-based assay. The receiver-operating characteristic (ROC) curve was used to evaluate the role of circulating anti-nephrin antibody in the diagnosis of MCD. The correlations between anti-nephrin antibody and clinical parameters were analyzed.

Results. The prevalence of circulating anti-nephrin antibody was 19.44% (7 of 36) in MCD and 26.92% (7 of 26) in MCD patients with nephrotic proteinuria, which was higher than in FSGS, PMN, DN, IgAN and healthy volunteers. The ROC curve showed that the sensitivity of anti-nephrin antibody used in the diagnosis of MCD was 19.4% and the specificity was 97.8%. The MCD patients with positive anti-nephrin antibody had heavier proteinuria and higher serum lipid levels, while having lower serum albumin and blood IgG levels. Anti-nephrin antibody might turn to negative when the MCD patient had a response to therapy.

Conclusions. Circulating anti-nephrin antibody may be a potential biomarker of MCD and may play a role in the MCD diagnosis.

Keywords: anti-nephrin antibody, biomarker, minimal change disease, nephrin, podocyte damage

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KEY LEARNING POINTS

What was known:

- Minimal change disease (MCD) is a type of podocytopathy, which is caused by podocyte damage and leads to proteinuria.
- Previous studies have suggested that T-cell dysfunction plays a major role in the pathogenesis of MCD, but the details are still unclear.
- The current diagnosis of MCD is still dependent on renal biopsy, lacking effective biological markers.

This study adds:

- This was the first study explored the diagnostic value of circulating anti-nephrin antibody in MCD patients in China.
- This study adopted a new method—cell-based assay—to detect the circulating anti-nephrin antibody.

Potential impact:

- This study provides further evidence to support the changed paradigm of MCD etiology, suggesting that B-cell dysfunction also participates in the pathogenesis of MCD.
- Anti-nephrin antibody is worthy of further research as a blood-based biomarker for the diagnosis of MCD.

INTRODUCTION

Minimal change disease (MCD) is the most common pathological type of nephrotic syndrome in children [1] and accounts for approximately 15% of adult nephrotic syndrome [2]. The typical pathological characteristic of MCD is no obvious abnormalities besides podocyte foot process effacement observed by electron microscopy [2, 3]. To date, the pathogenesis of MCD is still unclear. It is widely accepted that MCD is a type of podocytopathy, which is caused by podocyte damage and leads to proteinuria [3–5]. Previous studies have suggested that T-cell dysfunction plays a major role in the pathogenesis of MCD, and some circulating factors such as Angiopoietin like-4 and CD80 may be involved [4, 6–8]. However, the recent discovery that B cell–targeted drugs such as rituximab can also effectively induce remission of MCD [9, 10] suggests that antibodies may also be involved in MCD.

Podocytes are one of the glomerular filtration barriers, wrapping around the glomerular capillaries via foot processes. The foot processes are connected through a special cell junction called the slit diaphragm (SD). The SD is considered as the final barrier of glomerular filtration [11] and is composed of multiprotein complexes including nephrin [12]. Nephrin was first discovered in an autosomal recessive disease called Congenital nephrotic syndrome of the Finnish type, with massive proteinuria caused by the mutation in the *NPHS1* gene encoding nephrin protein [13]. Nephrin is a 180-kDa transmembrane protein which has been not detected only in podocytes, but also in lymphoid tissues, brain, spine cord and pancreas [14]. Nephrin is regarded as the core component of SD and several studies focusing on the role of nephrin in glomerular disease have been performed. Kandasamy *et al*. found that urinary nephrin concentration in patients with glomerular disease was positively correlated with proteinuria and the severity of podocyte damage [15]. Giannou *et al*. had a similar finding in MCD patients [16], but controversies exist [17–19]. With the successful application of rituximab in MCD, researchers woke up to the role of anti-nephrin antibody in MCD. Hengel *et al*. conducted a multicenter study to analyze anti-nephrin antibodies in patients with glomerular diseases including MCD, focal segmental glomerulosclerosis (FSGS), primary membranous nephropathy (MN), lupus nephritis, immunoglobulin A nephropathy (IgAN) and ANCA-associated glomerulonephritis, and found that circulating anti-nephrin antibody was common in the MCD patients and appeared to be a marker of disease activity [20]. The details of anti-nephrin antibody in MCD have not been revealed yet. Further prospective studies and fundamental research are needed to evaluate the therapeutic significance of anti-nephrin antibody in MCD and to explore the pathogenicity of anti-nephrin antibody in MCD.

The current diagnosis of MCD is still dependent on renal biopsy, which is an invasive procedure with potential complications, such as bleeding and arteriovenous fistulas [21]. Biomarker-based assay is a promising method for diagnosing MCD. Thus, here, we would detect the circulating anti-nephrin antibody in patients with MCD to explore the value in diagnosis. Further, we would analyze the correlation between anti-nephrin antibody and clinical indices.

MATERIALS AND METHODS

Participants

A total of 108 adult patients with glomerular disease who were diagnosed by renal biopsy and 20 healthy volunteers (control group) were recruited from Fujian Medical University Union Hospital. Among these participants, 36 had MCD (MCD group), 16 primary FSGS (FSGS group), 20 PMN (MN group), 17 diabetic nephropathy (DN group) and 19 IgAN (IgAN group). Clinical data were collected, including gender, age, proteinuria, serum albumin, serum globulin, cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), blood urea nitrogen, creatinine, uric acid and IgG levels. Plasma samples were collected from the participants and stored in a 5 mL EDTA-K2 anticoagulant tube and centrifuged at 300*g*, 4°C for 10 min. Then the serum samples were extracted into 0.5-mL aliquots and stored at −80°C until the experiment. Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration formula. This study was conducted in accordance with the Declaration of Helsinki Principles and approved by the Ethics Committee of Fujian Medical University Union Hospital. All participants agreed to participate in this study and the informed consents were obtained.

Detection of circulating anti-nephrin antibody

Anti-nephrin antibody was analyzed using an indirect immunofluorescence cell-based assay. Full-length human nephrin and the reference gene that encodes the green fluorescent

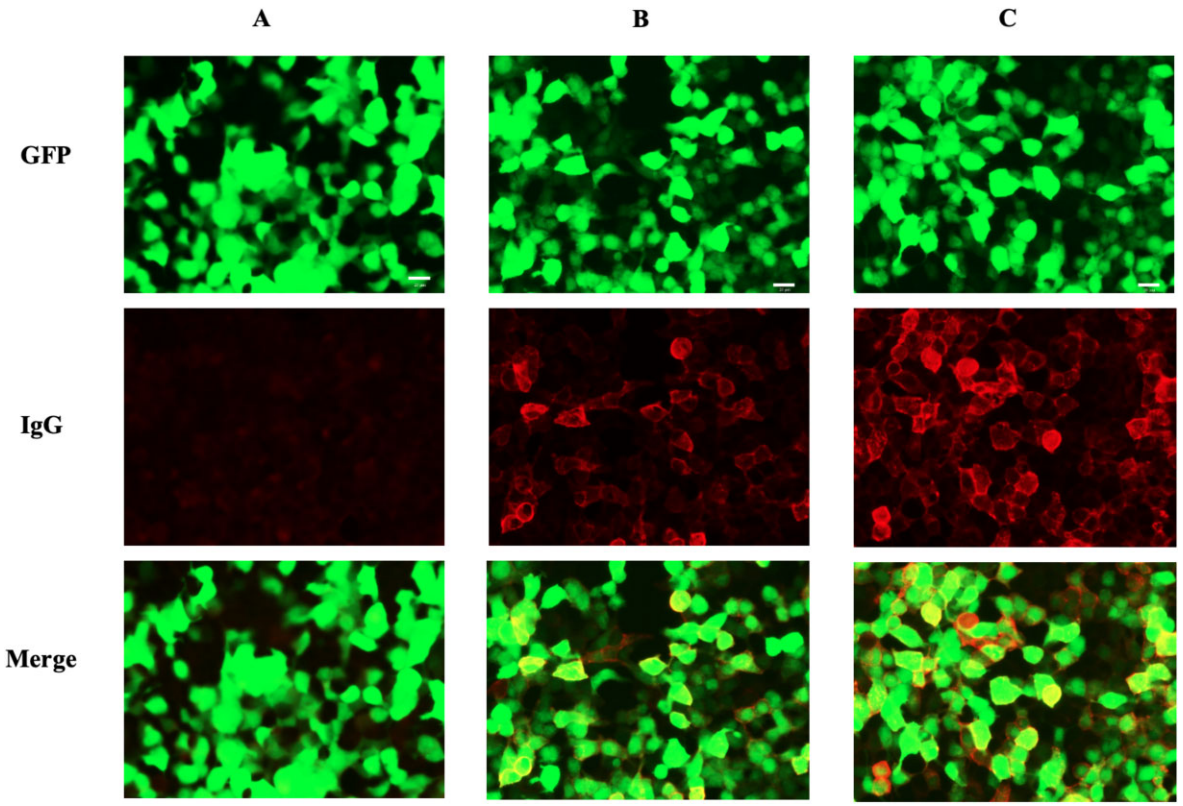

Figure 1: Detection of circulating anti-nephrin antibody by dual fluorescent cell transfection (cell based assay) method. (**A**) The green fluorescent is found, while the red fluorescent cannot be found, indicating a negative result of anti-nephrin antibody (MCD1–). (**B**, **C**) Both the green fluorescent and red fluorescent can be found, indicating a positive result of anti-nephrin antibody. Panel (B) shows anti-nephrin antibody 1:10 positive (MCD2+) and (C) shows antibody 1:32 positive (MCD3+). Scale bar: 20 μm.

protein (GFP) were inserted into the pcDNA3.1-EGFP plasmid. The plasmid was transiently transfected into HEK293 cells by Lipofectamine 2000 reagent. Then the cells were fixed on a 96-well plate to form the antigen substrate slide, which could be used to detect the circulating anti-nephrin antibody by indirect immunofluorescence method. Detection was performed according to the instructions of the reagent kit (membranous nephropathy autoantibody 10-item detection kit, Guangzhou ImmunoArt Biotechnology Company). The goat serum was diluted with phosphate-buffered saline (PBS) into Mixture A containing 10% goat serum, and then the fluorescent secondary antibody was diluted with Mixture A at 1:500 into Mixture B. The cells-coated plate was taken out and was equilibrated at room temperature (20–30°C) for 15–30 min. The reserved PBS in the well was discarded, and 100 μL PBS was added to wash once. Eighty microliters of 0.25% cell permeabilization agent was added to each well, then it was incubated at room temperature for 30 min, and the liquid in the well was discarded. Eighty microliters of Mixture A was added to the experimental well, and then 4 μL of serum sample was added into the well. The plate was shaken gently and was incubated at 37°C for 30 min. After incubation, the liquid was discarded, and the well was washed three times with 100 μL PBS. Eighty microliters of Mixture B was added to the sample well, and then the well was covered with tin foil and incubated in the dark at 37°C for 30 min. After this incubation, the liquid also was discarded, and the well was washed three times with 100 μL PBS. The liquid in the well was discarded, and 100 μL of PBS was added to the sample well to cover the cells. After that, the plate was observed by the fluorescence microscope. Firstly, the green light channel was used to observe, and green fluorescence would be found if the plasmid was successfully transfected into the cell. Secondly, the red-light channel was used to observe the anti-nephrin antibody. Two channels were merged to further confirm the results (Fig. 1). The detection was repeated twice. More images can be found in Supplementary data, Fig. S1.

Immunofluorescence double-label staining analysis

Paraffin sections (1.5 μm) of human kidney biopsies were deparaffinized, followed by antigen repair for 25 min and circling. The sections were blocked at room temperature with 3% hydrogen peroxide solution and 3% bovine serum albumin. Then the sections were incubated with primary mouse anti-human IGG (Abcam; ab200699) (dilution ratio 1:2500), followed by incubation with the horseradish peroxidase (HRP)-labeled secondary goat anti-mouse IgG (Servicebio; GB23301) (dilution ratio 1:500). The corresponding tissue-specific antigen (TSA) dye was added, followed by antigen repair again and microwave treatment. After that, the second type of primary monoclonal mouse anti-human nephrin (Proteintech; 66970–1-lg) (dilution ratio 1:2500) were added, followed by HRP-labeled secondary goat anti-mouse IgG (Servicebio; GB23301) (dilution ratio 1:500), TSA dye and DAPI. Fluorescence quenching, sealing and image visualization were performed. IgG was red and nephrin was green.

Table 1: Clinical characteristics of the participants.

	MCD (n = 36)	FSGS (n = 16)	MN (n = 20)	DN (n = 17)	IgAN (n = 19)	Overall P
Age (years)	43.50 (23.75, 61.00)	47.00 (38.00, 66.75)	51.00 (37.75, 64.75)	51.00 (40.00, 58.50)	35.00 (28.00, 49.00)	.071
M/F (No.)	25/11	9/7	14/6	11/6	8/11	.307
Proteinuria (g/24 h)	7.76 (1.62, 14.38)	7.09 (3.00, 14.52)	4.00 (3.23, 10.37)	2.54 (1.32, 6.12)	1.23 (0.54, 2.39)	.000
Serum albumin (g/L)	20.40 (16.53, 32.90)	18.80 (15.95, 28.30)	22.00 (18.55, 25.68)	33.40 (26.80, 37.35)	37.40 (35.10, 39.70)	.000
Serum globulin (g/L)	21.40 (18.48, 23.75)	21.40 (19.03, 25.43)	20.45 (18.45, 21.85)	26.60 (24.25, 32.10)	27.40 (24.10, 29.20)	.000
Cholesterol (mmol/L)	8.21 (5.56, 11.11)	8.98 (5.72, 9.87)	7.10 (5.85, 8.35)	5.37 (4.40, 7.38)	5.03 (3.95, 5.61)	.000
Triglycerides (mmol/L)	2.03 (1.34, 3.13)	2.29 (1.66, 2.97)	1.69 (1.34, 2.39)	2.27 (1.25, 3.50)	1.97 (0.89, 2.85)	.700
HDL-C (mmol/L)	1.42 (1.02, 1.94)	1.10 (0.92, 1.75)	1.12 (0.92, 1.48)	0.98 (0.82, 1.68)	1.10 (0.88, 1.61)	.156
LDL-C (mmol/L)	5.84 (3.58, 8.71)	6.77 (3.02, 8.30)	4.93 (4.15, 6.09)	3.73 (2.68, 4.81)	2.79 (2.18, 3.37)	.000
Blood urea nitrogen (mmol/L)	5.35 (4.45, 8.25)	7.85 (4.80, 12.28)	5.40 (4.38, 6.40)	10.20 (6.6, 16.20)	4.80 (3.80, 11.10)	.006
Serum creatinine (μmol/L)	62.50 (53.25, 85.75)	84.50 (71.75, 117.00)	65.00 (42.00, 74.00)	130.00 (77.50, 242.50)	64.00 (57.00, 224.00)	.001
eGFR (mL/min/1.73 m^2)	108.66 (85.63, 129.85)	83.10 (52.19, 102.06)	103.36 (89.23, 120.81)	48.17 (27.70, 94.54)	106.11 (29.41, 115.38)	.001
Uric acid (μmol/L)	379.50 (282.00, 508.25)	400.50 (329.00, 494.25)	364.50 (284.50, 433.00)	407.00 (328.50, 474.00)	360.00 (267.00, 443.00)	.518

Data are presented as median (25–75%).
M: male; F: female.

Statistical analysis

Data were analyzed using SPSS (version 25.0; SPSS Inc., Chicago, IL, USA). For quantitative data, difference between two groups was compared using t-test or non-parametric test (Wilcoxon W test), and differences among multiple groups were analyzed using one-way analysis of variance or non-parametric test (Kruskal–Wallis test), and then differences within groups were analyzed using Dunnett's t-test. For qualitative data, group difference was compared using the chi-square test or Fisher's exact test, differences among multiple groups were analyzed using non- parametric test (Kruskal–Wallis test), and then differences within groups were analyzed using Bonferroni adjustment. Receiver-operating characteristic (ROC) curve analysis was performed to determine the diagnostic performance of anti-nephrin antibody by measuring the area under the ROC curve and calculating sensitivity and specificity. Spearman correlation analysis was conducted to evaluate the association between anti-nephrin antibodies and clinical indicators. $P < .05$ was considered significant.

RESULTS

Clinical characteristics of participants

As shown in Table 1, there was no statistical difference for sex, age, triglycerides, HDL-C and uric acid across the five groups ($P > .05$). However, there were statistical differences between the five groups in baseline proteinuria, serum albumin, serum globulin, cholesterol, LDL-C, blood urea nitrogen, serum creatinine and baseline eGFR ($P < .05$). Further comparation within groups showed that there was no statistical difference between MCD group and FSGS group in baseline proteinuria, serum albumin, serum globulin, cholesterol, LDL-C, blood urea nitrogen, serum creatinine and eGFR, and the same applied to the MCD group and MN group (Supplementary data, Table S1 and Fig. S2).

Prevalence of anti-nephrin antibody in MCD

This study showed that 7 of 36 patients (19.44%) with MCD, and 2 of 16 patients (12.5%) with FSGS detected positive for circulating anti-nephrin antibody, whereas all 20 patients with MN, 17 patients with DN, 19 patients with IgAN and all 20 healthy controls tested negative. The prevalence of anti-nephrin antibody had significant difference between groups ($P = .011$) (Fig. 2). However, within-groups analysis showed that there was no significant difference between the MCD group and any other group (Supplementary data, Table S2). Further analysis found that 7 of 26 patients (26.92%) had positive anti-nephrin antibody in MCD patients with nephrotic-range proteinuria (urine protein excretion >3.5 g/day).

ROC curve analysis was performed to determine the diagnostic efficacy of anti-nephrin antibody in MCD. The ROC curve revealed the area under the curve was 0.586, the sensitivity was 19.4% and the specificity was 97.8% (Fig. 3).

Association between anti-nephrin antibody and disease activity

According to the anti-nephrin antibody, 36 MCD patients were divided into two groups: anti-nephrin antibody–positive ($n = 7$) and –negative ($n = 29$). The clinical data of the two groups of patients were compared. There were statistical differences in proteinuria, serum albumin, cholesterol, triglycerides, LDL-C

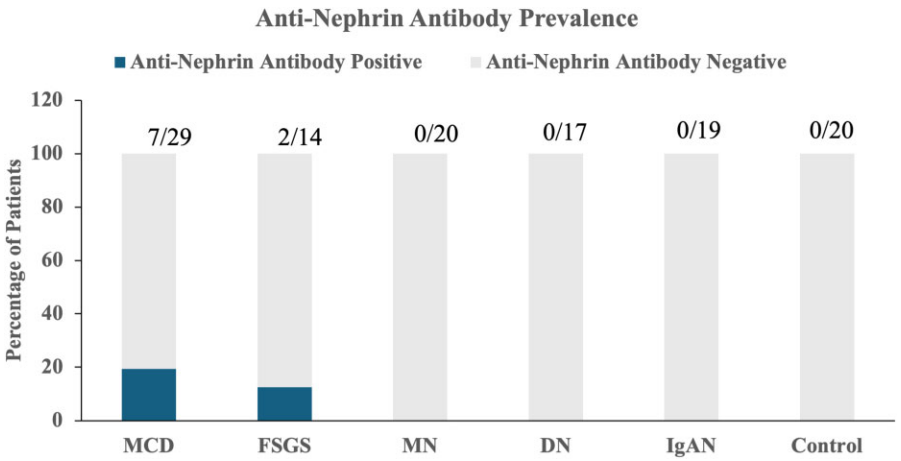

Figure 2: Prevalence of circulating anti-nephrin antibody in patients with glomerular diseases and in controls. Panel shows the prevalence of anti-nephrin antibody in the MCD, FSGS, MN, DN, IgAN and controls.

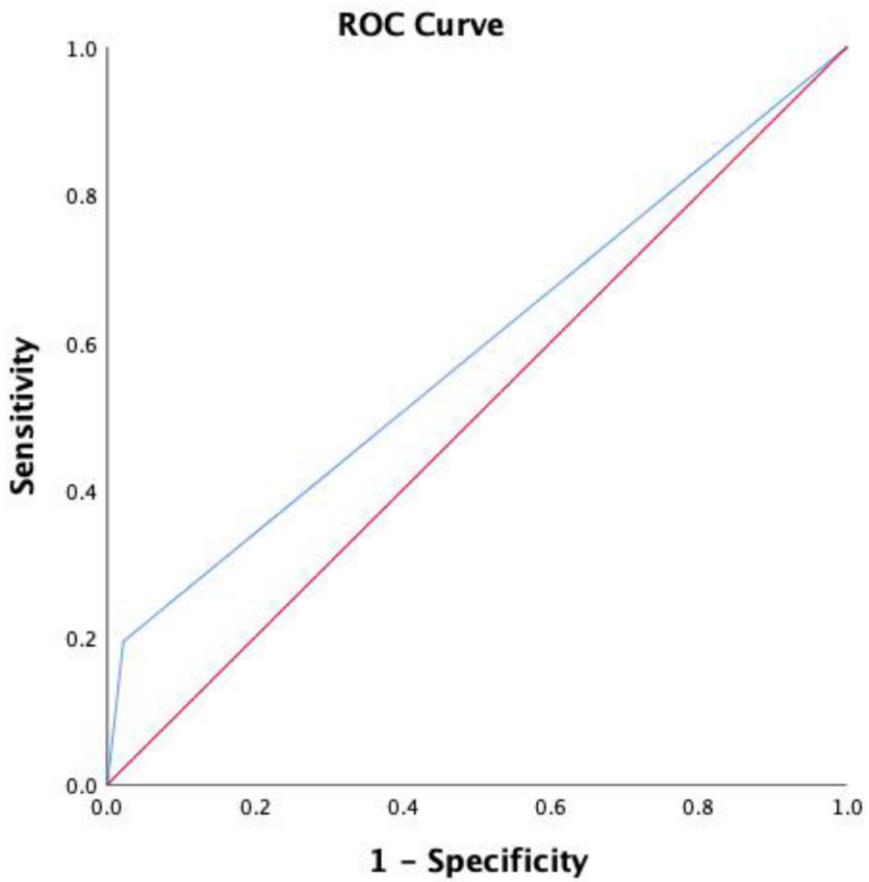

Figure 3: Analysis of the discriminatory power of circulating anti-nephrin antibody in MCD. The graph shows the ROC curve analysis of circulating anti-nephrin antibody in discriminating patients with MCD from other type glomerular diseases and healthy controls.

and IgG levels ($P < .05$) (Table 2). Anti-nephrin antibody–positive group patients had heavier proteinuria, higher cholesterol, triglyceride and LDL-C levels, while they had lower serum albumin and IgG levels (Fig. 4). Spearman analysis also showed significant correlations between the anti-nephrin antibody and certain clinical indicators: proteinuria ($P = .022$), serum albumin ($P = .015$), cholesterol ($P = .001$), triglycerides ($P = .038$), LDL-C ($P = .001$) and IgG levels ($P = .044$) (Table 3).

Table 2: Comparison of the clinical data of MCD patients based on anti-nephrin antibody status.

	Anti-nephrin antibody–positive group ($n = 7$)	Anti-nephrin antibody–negative group ($n = 29$)	P-value
Age (years)	44.57 ± 20.32	41.62 ± 18.59	.713
M/F (No.)	5/2	20/9	.898
Proteinuria (g/24 h)	14.49 (8.80, 18.11)	6.42 (0.52, 12.84)	.024
Serum albumin (g/L)	16.20 (11.80, 20.20)	21.10 (17.90, 33.45)	.017
Serum globulin (g/L)	19.89 ± 1.80	21.75 ± 3.37	.169
Cholesterol (mmol/L)	12.90 (9.30, 15.79)	6.76 (5.26, 9.90)	.000
Triglycerides (mmol/L)	2.55 (2.43, 3.25)	1.70 (1.16, 2.90)	.040
HDL-C (mmol/L)	1.62 (0.95, 2.38)	1.35 (1.04, 1.92)	.826
LDL-C (mmol/L)	10.99 (7.04, 13.53)	4.84 (3.33, 7.35)	.002
Blood urea nitrogen (mmol/L)	6.80 (5.00, 15.50)	5.10 (4.30, 8.10)	.101
Serum creatinine (μmol/L)	67.00 (58.00, 124.00)	62.00 (52.00, 85.50)	.549
eGFR (mL/min/1.73 m^2)	95.05 (51.86, 140.50)	109.23 (89.37, 129.75)	.536
Uric acid (μmol/L)	427.00 (311.00, 539.00)	379.00 (273.00, 487.00)	.576
Serum IgG (g/L)	2.89 (1.81, 4.00)	6.49 (3.43, 8.42)	.047

The normal distribution data are present as mean ± standard deviation. The non-normal distribution data are present as median (25%–75%).
M: male; F: female.

Correlation between anti-nephrin antibody and therapeutic response

Four of seven MCD patients with positive anti-nephrin antibody had available follow-up serum samples. Among these four patients, two used rituximab and low-dose glucocorticoid, one used rituximab alone and one was treated with glucocorticoid. During the follow-up, two patients experienced complete remission, and the other two patients did not have remission, which might be due to the short follow-up time (<6 months) (Table 4). Although no remission occurred for the two patients, but their serum albumin increased and proteinuria decreased compared with the baseline, indicating that the condition has improved. The anti-nephrin antibody in these four MCD patients all turned negative during follow-up (Fig. 5).

IgG colocalizes with nephrin in anti-nephrin antibody–positive MCD biopsies

To further explore the potential role of anti-nephrin antibody, we analyzed the expression of IgG and nephrin using double immunofluorescence staining. In the anti-nephrin antibody–postive MCD (MCD+) biopsies, we observed colocalization of IgG with nephrin (Fig. 6).

DISCUSSION

The mechanism of MCD remains obscure. The diagnosis still relies on renal biopsy and lacks effective biological markers. A total of 108 patients with glomerular disease and 20 healthy volunteers were included in the study, and the circulating anti-nephrin antibody was detected. The study found that the prevalence of circulating anti-neprhin antibody in MCD patients was higher than that of healthy volunteers, and FSGS, MN, DN and IgAN patients. Further analysis found that circulating anti-nephrin antibody levels in MCD patients are related to the disease severity, and anti-nephrin antibody might turn to negative when the MCD patient had a response to therapy. IgG were found colocalized with nephrin in anti-nephrin antibody–positive MCD biopsies.

In this study, the positive rate of anti-nephrin antibody was 19.44% (7/36) in MCD patients and 26.92% (7/26) in MCD patients with nephrotic-range proteinuria. Watts *et al*. collected serum samples of MCD patients from the Nephrotic Syndrome Study Network (NEPTUNE) cohort and four medical institutions, and then found that the positive rate of circulating anti-nephrin antibody in 62 MCD patients whose urine protein creatinine ratio (UPCR) >3 g/g was 29.03% (18/62) [22], which was consistent with our study. Hengel *et al*. included 105 MCD patients from the Hamburg Glomerulonephritis Registry and the University of Bari Aldo Moro, and found that the positive rate of circulating anti-nephrin antibody was 43.81% (46/105) [20]. Different detection methods of antibody may be one of the reasons for the different positive rate. The study by Hengel *et al*. used recombinant human nephrin ectodomain for immunoprecipitation reaction, while our study used indirect immunofluorescence method, resulting in a decrease in sensitivity. Although ROC curve analysis showed that the sensitivity of using circulating anti-nephrin antibody for MCD diagnosis was only 19.4%, the specificity was as high as 97.8%. All the anti-nephrin antibody tests in healthy people, MN patients, DN patients and IgAN patients were negative, which were consistent with studies from other centers. For patients with contraindications for renal biopsy, non-invasive detection of circulating anti-nephrin antibody may be able to have a role in the diagnosis of MCD. Of course, these require more large-sample studies to confirm.

MCD and FSGS have significant differences in the treatment and prognosis, although both are podocytopathies. Sometimes it is difficult to differentiate them even after renal biopsy due to the limitations of pathological specimens. Chebotareva *et al*. used the enzyme immunoassay method to quantitatively detect anti-nephrin antibody and found that the anti-nephrin antibody titer in MCD patients was higher than that in FSGS patients, although there was no statistical difference between the two groups [23]. In our study, the positive rate of anti-nephrin antibody in FSGS patients was 12.50% (2/16), which was close to the Hengel's (9.46%) [20], was also lower than that in MCD patients. Although there was no statistical difference about the positive rate between MCD and FSGS group which might be due to small sample, it still suggested that anti-nephrin antibody perhaps can be used to distinguish MCD from FSGS, warranting further large-sample investigation.

Scientists had established animal models of podocytopathies by administering with anti-nephrin antibody. Kawashima *et al*. injected anti-nephrin antibody into C57BL/6 mice via the tail vein and found that podocyte foot process effacement was

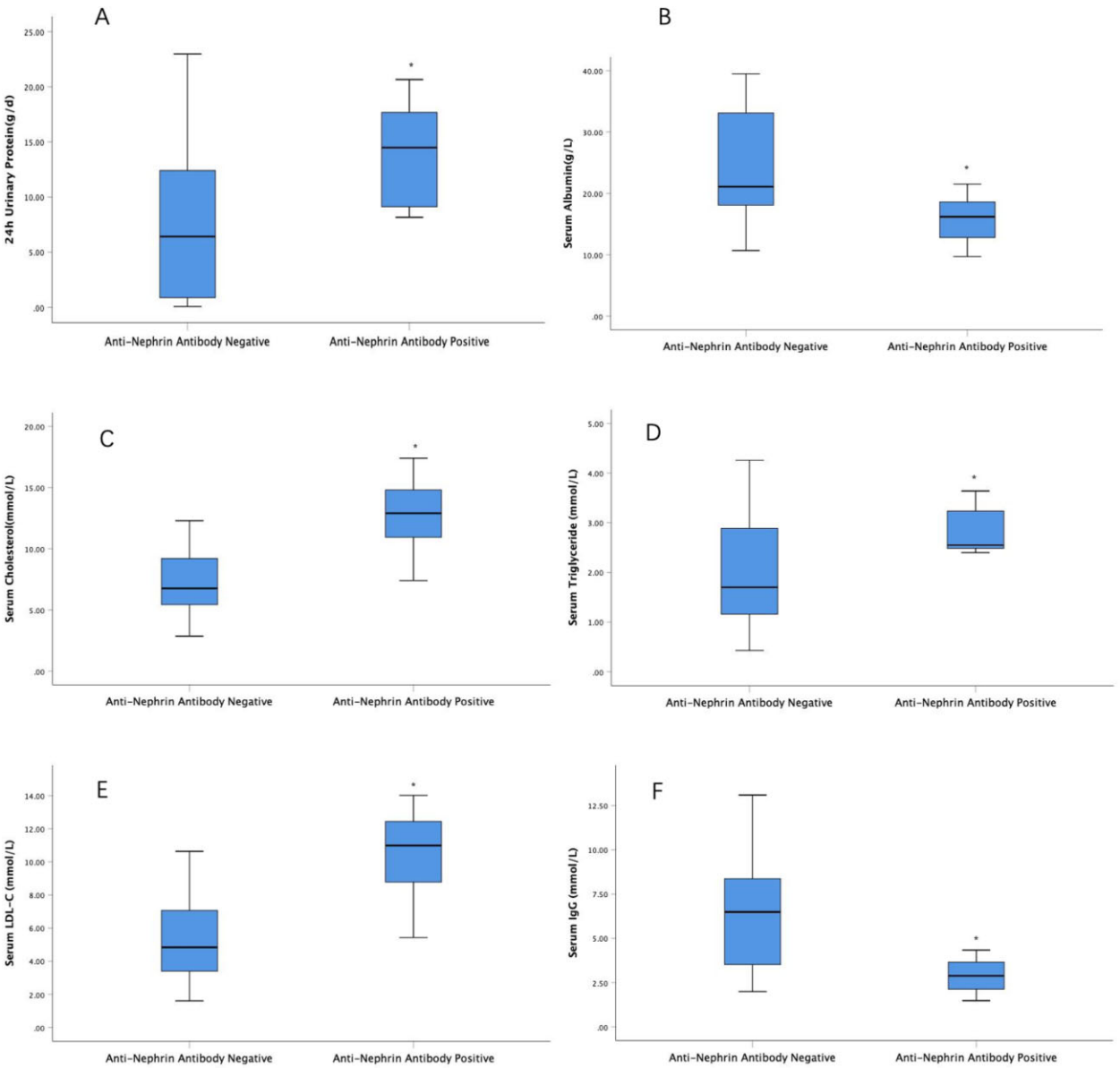

Figure 4: Comparison of the clinical data in anti-nephrin antibody–negative MCD patients and positive MCD patients. (**A**) Anti-nephrin antibody–positive group patients had significantly heavier proteinuria. (**B**) Anti-nephrin antibody–positive group patients had significantly lower serum albumin level. (**C–E**) Anti-nephrin antibody–positive group patients had significantly higher cholesterol, triglyceride and LDL-C levels. (**F**) Anti-nephrin antibody–positive group patients had significantly lower serum IgG levels. Data are presented as median (25%–75%). $^{*}P < .05$.

evident only 3 h after injection [24]. Takeuchi *et al.* successfully made a nephrotic syndrome mice model induced by polyclonal rabbit anti-mouse nephrin antibody, in which podocyte numbers were clearly decreased after antibody injection [25]. However, the details of the mechanism have not yet been elucidated. In our study, IgG deposition colocalizing with nephrin were found under double-immunofluorescence in the renal tissue of patients with anti-nephrin antibody positivity, but the sample size was limit. Some experts believed that it was different from the traditional immune complex deposition leading renal damage [22]. New *et al.* proposed that the regulatory effect of nephrin was determined by the level of phosphorylation at the tyrosine site of its intracellular domain [26], and the nephrin phosphorylation was required for the stabilization and repair of podocyte foot process structure after injury [27]. Loss of nephrin tyrosine phosphorylation in podocytes was considered as a driver of renal disease progression [28]. Hengel *et al.* conducted proteomics research on the experimental mice and found that anti-nephrin antibody participated in nephrin-related signaling pathways, which could change the phosphorylation of nephrin and lead to the redistribution of nephrin, and then destroy the integrity of SD, resulting in proteinuria [20]. There is a lack of systemic research on renal tissue of MCD patients.

Our study also found that the MCD patient with positive anti-nephrin antibody had significantly higher proteinuria and lower serum albumin level, which suggested severe conditions. Watts *et al.* found that the UPCR of the

Table 3: Correlation between anti-nephrin antibody and clinical data in MCD patients.

	Correlation coefficient	P-value
Age (years)	0.047	.784
Proteinuria (g/24 h)	0.382	.022
Serum albumin (g/L)	−0.402	.015
serum globulin (g/L)	−0.237	.165
Cholesterol (mmol/L)	0.544	.001
Triglycerides (mmol/L)	0.348	.038
HDL-C (mmol/L)	0.037	.830
LDL-C (mmol/L)	0.530	.001
Blood urea nitrogen (mmol/L)	0.277	.102
Serum creatinine (μmol/L)	0.101	.556
eGFR (mL/min/1.73 m^2)	−0.105	.543
Uric acid (μmol/L)	0.095	.583
Serum IgG (g/L)	−0.398	.044

anti-nephrin antibody–positive group was higher than that of the –negative group, although there was no statistical difference [22]. Only four patients out of the anti-nephrin antibody–positive group had follow-up data in our study, and all of them improved during the follow-up period, with anti-nephrin antibodies becoming negative. Although two patients still had heavy proteinuria with negative anti-nephrin antibody during follow-up, the proteinuria had alleviated compared with baseline. Further long-term, large-sample research is needed to explore whether the observed phenomenon was due to insufficient follow-up duration or whether antibody conversion preceded proteinuria remission. In the study by Hengel *et al.*, 18 MCD patients had follow-up data, and linear repeated-measures models revealed a strong association between anti-nephrin antibody positivity and protein levels in urine [20]. Research by Watts *et al.* also found that MCD patients with circulating anti-nephrin antibody were more prone to relapse [22]. To date, there few studies suggest that there exists correlation between circulating anti-nephrin antibody and MCD disease activity, but whether it has a similar predictive value like anti-PLA2R antibody in MN still needs to be confirmed by more large-sample clinical studies.

Although we attempt to explore the value of circulating anti-nephrin antibody in the diagnosis of MCD, the sample size is too small. The types of glomerular diseases also need to be expanded, such as lupus nephritis. More prospective studies are needed to gather more follow-up data to evaluate its predictive value for MCD treatment effect and prognosis. In this study, the positive rate of circulating anti-nephrin antibody is low respectively, which may be related to the detection method. Different detection methods need to be compared find a suitable diagnostic way and attain quantification. Further experiments

Table 4: Follow-up of four MCD patients with positive anti-nephrin antibody.

		Baseline			Last visit			
	Therapy	Serum albumin (g/L)	Proteinuria (g/day)	Anti-nephrin antibody	Follow-up time (months)	Serum albumin (g/L)	Proteinuria (g/day)	Anti-nephrin antibody
1	RTX + low-dose Pred	16.2	11.35	1:10(+)	2	34.2	0.15	(–)
2	RTX	9.7	14.49	1:10(+)	1	13	12.39	(–)
3	Pred	17	9.46	1:10(+)	4	16	5.06	(–)
4	RTX + low-dose Pred	11.8	20.66	1:32(+)	9	37.1	0.06	(–)

RTX: rituximab; Pred: prednisone.

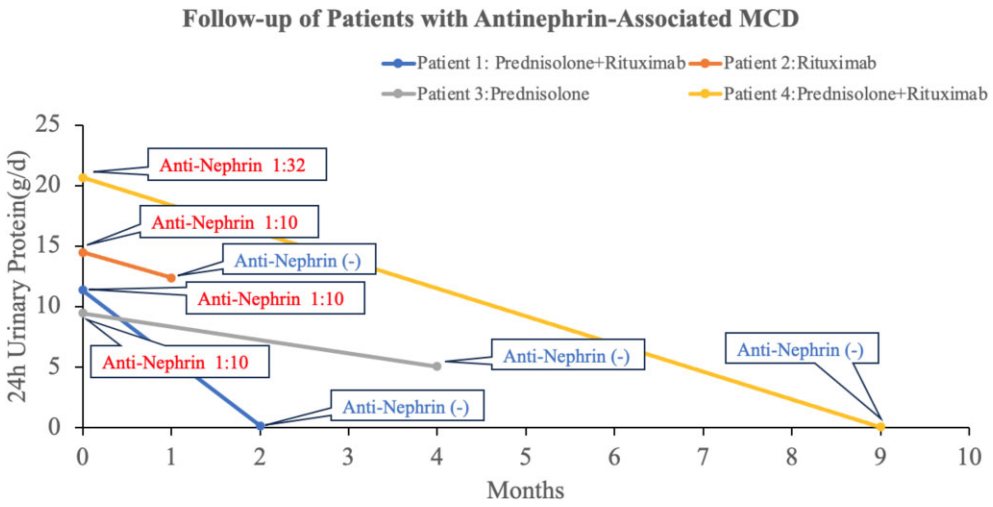

Figure 5: Follow-up of MCD patients with positive anti-nephrin antibody. Panel shows the disease course, treatment therapy, the 24-h urinary protein levels and the anti-nephrin antibody status of the four MCD patients with positive circulating anti-nephrin antibody at study inclusion.

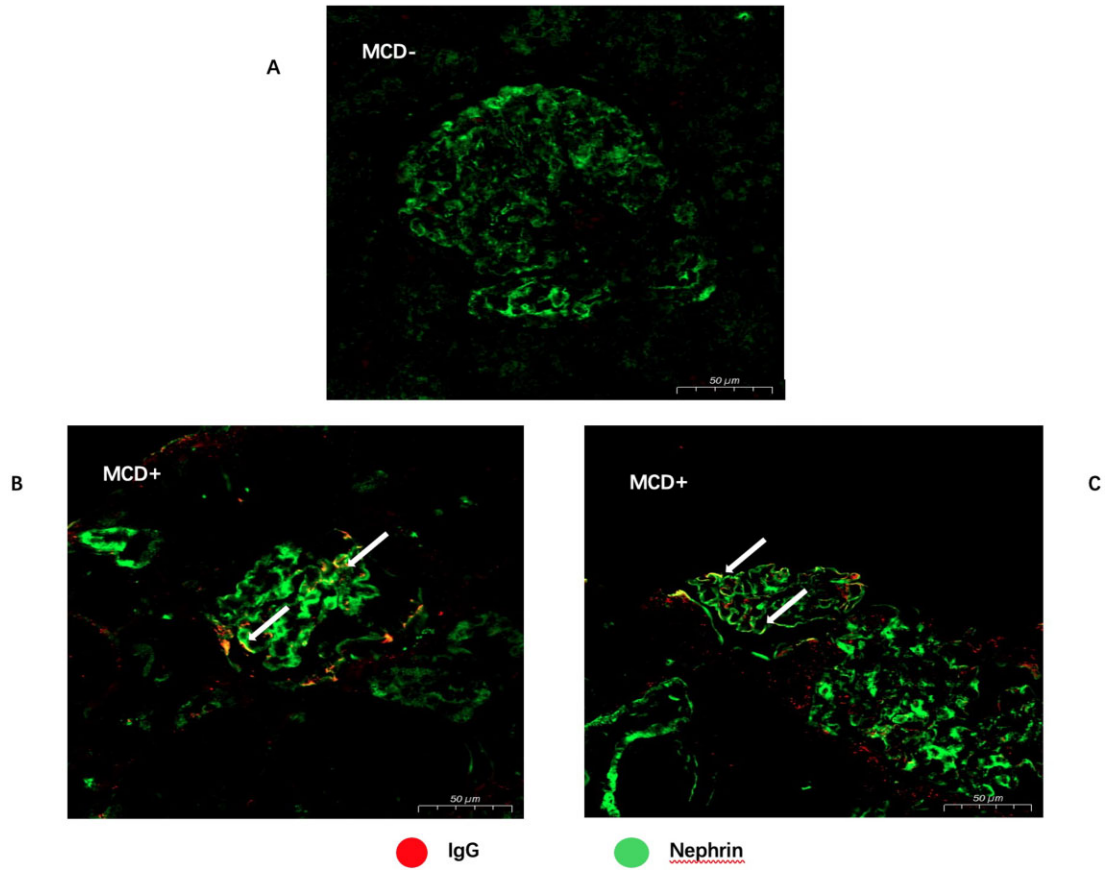

Figure 6: IgG deposition colocalized with nephrin in anti-nephrin antibody–positive MCD biopsies. (**A**) Immunofluorescence double-label staining image of glomerulus in anti-nephrin antibody–negative MCD (MCD–) biopsy. (**B**, **C**) Immunofluorescence double-label staining images of glomeruli in anti-nephrin antibody–positive MCD (MCD+) biopsies. There is an overlap (yellow) of IgG with nephrin (white arrows). Scale bar: 50 μm.

on animals and human are needed to explore the mechanisms of anti-nephrin antibody in the development and progression of MCD.

In conclusion, this study found that circulating anti-nephrin antibody has high specificity in the diagnosis of MCD patients and has a correlation with the disease activity of MCD. In the past, it was believed that MCD was mainly caused by T-cell dysfunction. Our study suggests that B-cell dysfunction may also play an important role in the pathogenesis of MCD. Although the positive rate of anti-nephrin antibody in this study is not high, which means that anti-nephrin antibody associated MCD may be just one type of MCD, it also provides clues for the further research. Taken together, this study demonstrates that circulating anti-nephrin antibody may be a potential biomarker of MCD and may play a role in the MCD diagnosis.

SUPPLEMENTARY DATA

Supplementary data are available at *Clinical Kidney Journal* online.

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AUTHORS' CONTRIBUTIONS

Conceptualization: L.W., Q.C., L.L. Methodology: S.C., R.G., Y.X., J.X. Investigation: Q.C., S.C., L.L., Q.Y., W.L., Y.L., B.L. Funding acquisition: Q.C. Project administration: L.W. Supervision: L.W., Q.C. Writing—original draft: Q.C., S.C. Writing—review & editing: L.W., L.L.

DATA AVAILABILITY STATEMENT

The data underlying our article will be shared on reasonable request to the corresponding author. Our supplementary data has been fully uploaded at the time of submission.

CONFLICT OF INTEREST STATEMENT

All authors have no conflicts of interest.

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