

# Segmental Membranous Glomerulopathy in Adults

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## Keywords

Clinicopathological features · Segmental membranous glomerulopathy · M-type phospholipase A2 receptor 1 · Thrombospondin type 1 domain-containing 7A · Neural epidermal growth factor-like 1 protein

## Abstract

**Introduction:** The clinicopathological features of segmental membranous glomerulopathy (SMGN) have not been well characterized. The aim of this study was to investigate the prevalence and clinicopathological features of SMGN in adults. **Methods:** Adult patients with biopsy-confirmed SMGN in the native kidney at our center between January 2017 to September 2020 were identified. The clinicopathological features of SMGN were collected. The glomerular deposition of IgG subclasses, M-type phospholipase A2 receptor 1 (PLA2R), thrombospondin type 1 domain-containing 7A (THSD7A), and neural epidermal growth factor-like 1 protein (NELL1) were tested. Clinical and pathologic features were comparable between NELL1-positive and NELL1-negative SMGN. **Results:** A total of 167 patients with biopsy-proven SMGN were enrolled.

During the same period, 32,640 (33.0%) out of 98,939 renal biopsies were diagnosed with membranous nephropathy (MN) in adults. SMGN accounted for 0.17% of total kidney biopsies and 0.51% of MN in adults. One hundred and fifty (89.8%) cases were isolated SMGN, and 17 (10.2%) cases were complicated with other kidney disease. Clinically, the median age of isolated SMGN patients was 41.5 years, with female (74%) predominance, and 33.1% had full nephrotic syndrome. Pathologically, IgG1 was the dominant subclass (92.5%), followed by IgG4 (45.0%). PLA2R and THSD7A staining were done in 142 and 136 isolated SMGN cases, respectively, in which, all the cases showed negative. NELL1 staining was done in 135 isolated SMGN cases; 58 cases (43.0%) showed positive. Fifty-eight patients (41.1%) had diffuse ( $\geq 90\%$ ) foot process effacement, and 119 patients (83.8%) had either stage I (38.0%) or stage II (45.8%) membranous alterations in patients with SMGN. Most patients with NELL1-positive SMGN were female. Patients with NELL1-positive SMGN were more likely with lower prevalence of full nephrotic syndrome than NELL1-negative SMGN. **Conclusions:** SMGN is a relatively rare pathological type. Majority of patients with isolated SMGN were female, with a median age of 41.5 years, 33.1% had full

nephrotic syndrome, absence of PLA2R and THSD7A, 43.0% with NELL1-positive, and mainly stage I or II MN (83.8%). NELL1 is the major target antigen of SMGN in adults.

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## Introduction

Membranous nephropathy (MN), a common cause of the nephrotic syndrome in adults, is characterized by the accumulation of immune deposits along the glomerular capillary loops [1, 2]. Epidemiological data showed a remarkable rise of MN, and it has become the most common type of glomerulonephritis in patients aged 65 and above [3, 4]. The reported proportion of MN in primary glomerulonephritis range from 13% to 43% [4–7].

Segmental membranous glomerulopathy (SMGN), a variant of MN, is characterized by segmental staining of IgG in immunofluorescence (IF), segmental distribution of subepithelial deposits under light microscopy (LM), and electron microscopy (EM) [8–10]. Since the first report of the disease [11], so far only 200 cases have been reported [8–13]. Currently, there are few studies on the prevalence and clinicopathological characteristics of SMGN. M-type phospholipase A2 receptor 1 (PLA2R) and thrombospondin type 1 domain-containing 7A (THSD7A) antigen were the target antigens accounting for 70–80% and 1–5% of MN patients [14–17], respectively. Sethi et al. [18] recently detected neural epidermal growth factor-like 1 protein (NELL1) in the patients with PLA2R-negative MN. NELL1 is a cytoplasmic protein with a molecular weight of 90-kDa, which contains several highly conserved structural motifs including a secretory signal peptide, an N-terminal TSP-1-like (TSPN), a coiled-coil domain, 4 von Willebrand factor type C domains, and 6 epidermal growth factor-like domains [18]. PLA2R, THSD7A, and NELL1 were the target antigens of MN, and their expression in SMGN has been rarely studied. Therefore, in this report, we aimed to identify the clinicopathological characteristics of SMGN and analyze the presence of PLA2R, THSD7A, and NELL1 in SMGN patients.

## Materials and Methods

### Participants

In this study, SMGN was defined as subepithelial deposits less than 75% of the glomerular tuft [8] by LM, IF, and EM. SMGN secondary to hepatitis B virus, systemic lupus erythematosus, and Sjögren's syndrome were excluded. Adult patients with biopsy-

confirmed SMGN in the native kidney in Renal Pathology of King Medical Diagnostics Center between January 2017 to September 2020 were identified. The research was in compliance with the Declaration of Helsinki and approved by the Ethics Committee of King Medical Diagnostics Center, Guangzhou, China.

The diagnostic criteria for HBV-associated MN should meet the following conditions: (1) detection of the serologic manifestations of HBV infection and replicative virus in the blood; (2) detection of HBV-related protein antigens (HBsAg, HBeAg, and/or HBcAg) in the glomerular immune deposits; (3) excluding other secondary MN such as lupus nephritis [19, 20].

The diagnosis of Sjögren's syndrome was based on the revised version of the diagnostic criteria of American-European Consensus Group [21]. Patients who meet the diagnostic criteria for Sjögren's syndrome and were confirmed by renal biopsy as MN should be considered as MN associated with Sjögren's syndrome.

### Histopathologic Evaluation

The biopsy specimens were routinely analyzed by LM, IF, and EM. The number of glomeruli, glomerulosclerosis, segmental glomerulosclerosis, endocapillary hypercellularity, crescent formation, interstitial fibrosis and/or tubular atrophy (IF/TA)  $\geq 25\%$  was collected. Frozen sections were cut into 3–4  $\mu\text{m}$  and then used for direct IF staining by using fluorescein isothiocyanate (FITC)-labeled antibodies to human IgG, IgA, IgM, C3, C1q (DAKO, Denmark) and FITC-labeled antibodies to human IgG1, IgG2, IgG3, IgG4 (Gene, China). Indirect IF was used to detect PLA2R (Sigma, USA), THSD7A (Sigma, USA), NELL1 (Sigma, USA), light chain kappa (DAKO, Denmark), and lambda (DAKO, Denmark). For patients with positive serum HBsAg, HBV biomarkers including HBsAg, HBcAg, and HBeAg (Gene, China) were regularly detected in the kidney by indirect IF. Semi-quantitative scoring of IF staining intensity from 0 to 3+ (0 negative, 1+ weak staining, 2+ moderate staining, 3+ strong staining). For EM examination of thin sections, a JEM-1400 PLUS electron microscope (Jeol, Tokyo, Japan) was used. Ehrenreich and Churg classification was used for the ultrastructural staging of MN [22].

### Detection of Glomerular PLA2R, THSD7A, and NELL1 Expression By Indirect IF

Frozen kidney tissues were cut into 3–4  $\mu\text{m}$  thick slices and fixed with acetone. Rabbit anti-human PLA2R antibody (Sigma, HPA012657), mouse anti-human THSD7A antibody (Sigma, AMAB91233), and rabbit anti-human NELL1 antibody (Sigma, HPA051535) were used as primary antibodies. FITC-labeled swine anti-rabbit immunoglobulins (Dako, F0205), FITC-labeled rabbit anti-mouse immunoglobulins (Dako, F0261), and FITC-labeled swine anti-rabbit immunoglobulins (Dako, F0205) were used as secondary antibodies, respectively. Paraffin sections were roasted, dewaxed, and digested with protease K. The subsequent steps for the paraffin sections were the same as for the frozen samples. Kidney biopsies from other patients such as minimal change disease and IgA nephropathy were also stained as negative controls.

### Clinical and Laboratory Data

The data on age, gender, personal health history (hypertension, diabetes, tuberculosis, thyroid disease), edema, blood urea nitrogen (BUN), serum creatinine, hemoglobin, serum albumin, serum IgA, serum IgG, serum IgM, serum C3, microscopic hematuria (RBC  $>5/\text{HP}$ ), 24-h urinary protein, and secondary etiology

(autoantibodies, tumors, HBsAg, and medication usage) were obtained. Nephrotic range proteinuria was defined as proteinuria  $\geq 3.5$  g/d. Nephrotic syndrome was defined as nephrotic range proteinuria and hypoalbuminemia ( $<30$  g/L). The estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI equation [23]. Follow-up data included treatment, renal function, albumin, and urinalyses. Complete remission was defined as urine protein  $<0.3$  g/d (uPCR  $<300$  mg/g). Partial remission was defined as urine protein  $>0.3$  but  $<3.5$  g/d or at least 50% reduction at biopsy and  $<3.5$  g/d, along with improvement in serum albumin and stabilization of serum creatinine [19, 24].

#### Statistical Analysis

All statistics were performed using SPSS (version 26.0). The measurement data were shown as mean  $\pm$  standard deviation if they had a normal distribution. Median and interquartile range (25th to 75th percentiles) were used to show continuous variables having a skewed distribution. Student's *t* test or Mann-Whitney U test was used for continuous variables, and the  $\chi^2$  test was used for categorical variables. Two-tailed *p* values  $<0.05$  were considered statistically significant.

## Results

From January 2017 to September 2020, 32,640 (33.0%) out of 98,939 renal biopsies were diagnosed with MN in adults at our center. Among them, a total of 167 adult patients were identified as SMGN, from 127 hospitals across China. SMGN accounted for 0.17% of total kidney biopsies and 0.51% of MN in adults. One hundred and fifty (89.8%) of 167 patients with SMGN cases were isolated SMGN and 17 (10.2%) cases were complicated with other kidney disease, including 5 with hypertensive renal injury, 3 with IgA nephropathy, 3 with ANCA-related vasculitis, 3 with diabetic nephropathy, 1 with hyperuricemia nephropathy, 1 with ischemic kidney injury, and 1 with podocyte infolding glomerulopathy.

#### Baseline Characteristics of Patients with SMGN and Isolated SMGN

The median age of SMGN patients was 44 years, 31.7% were male, 17.1% had hypertension, 7.5% had diabetes, 45.6% had microscopic hematuria, 34.0% had full nephrotic syndrome, 74.2% had edema, 24.0% were with positive ANA, and 6.2% were with positive HBsAg.

As shown in Table 1, the median age of isolated SMGN patients was 41.5 years, and 26.0% were male. The median 24-h urine protein was 3.3 g/d, 48.2% had nephrotic range proteinuria, 58.0% had hypoalbuminemia, 44.0% had microscopic hematuria, and 33.1% had full nephrotic syndrome. Median serum creatinine and eGFR were 61.0  $\mu\text{mol/L}$  and 107.1 mL/min/1.73 m<sup>2</sup>, respectively. Furthermore, 12.0% of them had eGFR less than 60 mL/min/1.73 m<sup>2</sup>. Serologic evaluation demonstrated positive ANA in 22.9%

patients. HBsAg serologies were positive in 5.4% patients. Of the 150 patients with isolated SMGN, 101 did not have an identifiable secondary etiology of MN, 27 had abnormal autoimmune findings, but the patients did not satisfy the criteria for diagnosis of lupus and Sjögren's syndrome (such as positive antinuclear antibody, anti-double stranded DNA, anti-SM, anti-SSA, 1 had undifferentiated connective tissue disease, 1 had rheumatoid arthritis, and 1 had ankylosing spondylitis), 7 had a history of HBV without deposition of HBV antigen in renal tissue, 5 had a history of tuberculosis, 9 had a history of hypothyroidism, 2 had plasma cell neoplasm, and one patient each had a history of esophageal cancer, colorectal carcinoma, thymic tumor, syphilis, and NSAID usage.

#### LM Results in Patients with Isolated SMGN

The GBMs of isolated SMGN had segmental "spikes" in 64 of 150 patients (shown in Fig. 1), with mild glomerular mesangial proliferation, 1 (0.7%) exhibited endocapillary hypercellularity, 1 (0.7%) exhibited crescent formation, 4 (2.7%) were with IF/TA  $\geq 25\%$ , and 13 (8.7%) were with segmental glomerulosclerosis. The median number of glomeruli and glomerulosclerosis in SMGN were 18 (13–23) and 0 (0–1), respectively.

#### IF Results in Patients with Isolated SMGN

IF revealed IgG segmental deposition (shown in Fig. 2a) in 92.7% cases, frequently accompanied by IgM (69.3%). Fifteen patients (10.0%) showed segmental stain of IgG along Bowman capsule (shown in Fig. 3a). None of the cases had full-house IF and tubular basement membrane deposit. Light chain kappa and lambda were tested in 59 cases, and no light chain restriction was found.

Forty cases had IgG subclass staining in isolated SMGN due to adequacy of samples. There were IgG1 staining in 37 cases (92.5%, shown in Fig. 2b), IgG4 in 18 biopsies (45.0%), IgG2 in 11 biopsies (27.5%), and IgG3 in 4 biopsies (10.0%).

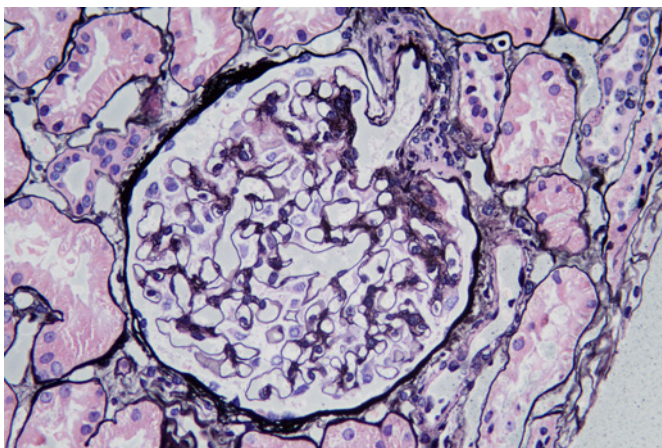
PLA2R and THSD7A staining were done in 142 and 136 isolated SMGN cases, respectively, in which, all the cases showed negative. NELL1 staining was done in 135 isolated SMGN cases; 58 cases (43.0%) showed positive.

#### EM Results in Patients with Isolated SMGN

Ultrastructural examination was available for 142 (including 1 case was unable to distinguish foot processes) of 150 isolated SMGN cases. As shown in Table 2, 58 patients (41.1%) had diffuse ( $\geq 90\%$ ) foot process effacement (shown in Fig. 4a), and 119 patients (83.8%) had either stage I (38.0%) or stage II (45.8%, shown in Fig. 4b) membranous alterations in patients with SMGN. Seventeen patients

**Table 1.** Clinical features of patients with isolated SMGN

	SMGN (N = 150)
Age, years	41.5 (32.8–51.3)
Male, n (%)	39/150 (26.0)
BUN, mmol/L	4.1 (3.3–6.0)
Serum creatinine, $\mu\text{mol/L}$	61.0 (50.0–77.1)
Serum albumin, g/L	26.0 (19.5–36.3)
eGFR, mL/min/1.73 m <sup>2</sup>	107.1 (87.3–120)
Hemoglobin, g/L	135.0 $\pm$ 20.8
Serum IgA, g/L	2.2 (1.6–2.8)
Serum IgG, g/L	7.2 (5.1–10.7)
Serum IgM, g/L	1.4 (0.9–2.0)
Serum C3, g/L	1.2 $\pm$ 0.3
Urine protein, g/day	3.3 (1.6–5.9)
No. of patients showing microscopic hematuria, %	62/141 (44.0)
No. of patients showing full nephrotic syndrome, %	42/127 (33.1)
No. of patients showing nephrotic range proteinuria, %	54/112 (48.2)
No. of patients showing hypoalbuminemia, %	83/143 (58.0)
Positive ANA, %	25/109 (22.9)
Positive HBsAg, %	7/130 (5.4)
No. of patients showing edema, %	103/139 (74.1)
No. of patients with eGFR<60 mL/min/1.73 m <sup>2</sup> , %	17/142 (12.0)



**Fig. 1.** Segmental “spikes” can be seen in SMGN on Jones methenamine silver ( $\times 200$ ).

(12.0%) with SMGN exhibited mesangial electron dense deposits (EDD), and 2 patients (1.4%) exhibited sub-endothelial EDD. None of the biopsies had endothelial tubuloreticular inclusions and substructure in the deposits.

#### *Clinical and Pathologic Characteristics of SMGN Patients with and without NELL1 Expression in Glomerular Immune Deposits*

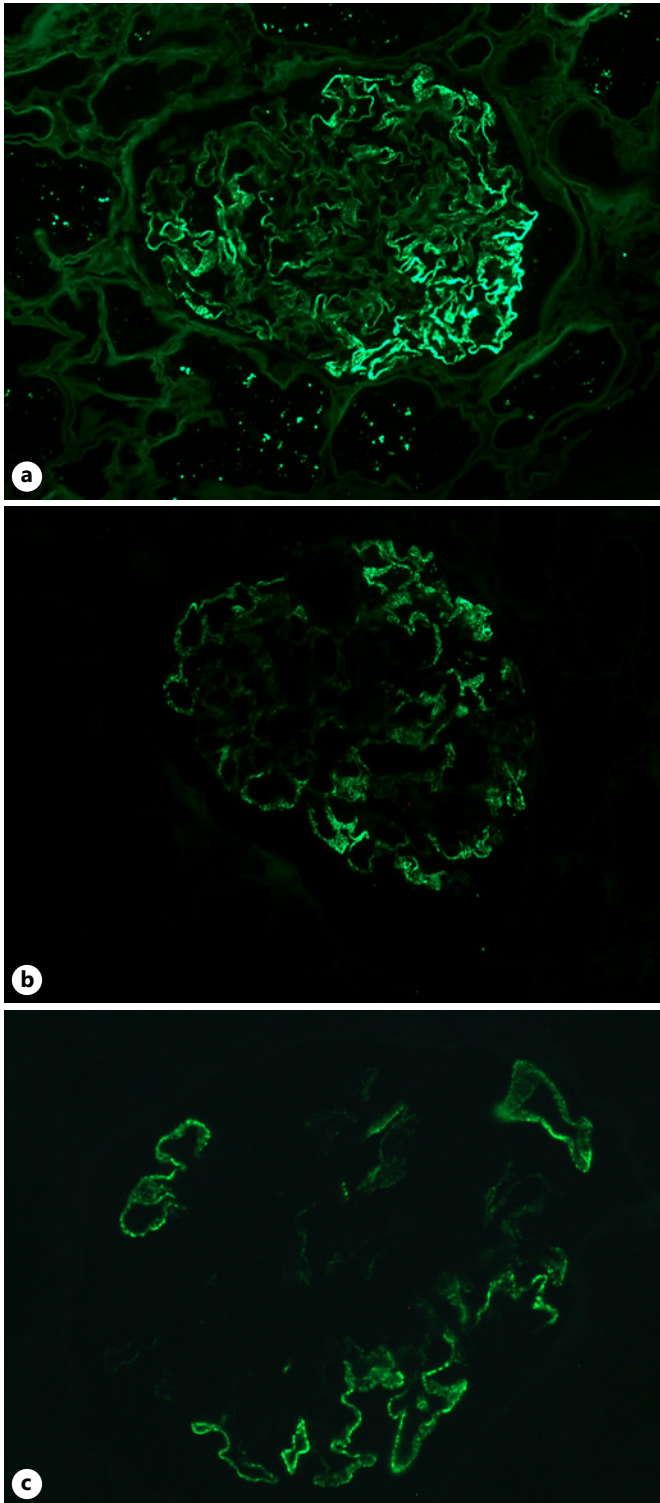
As shown in Table 3, among 58 patients with NELL1-positive SMGN, 13 men and 45 women (women-men ratio of 3.5), with a median age of 40 years, were analyzed.

Median 24-h urine protein was 2.9 g/d, and 17.6% had full nephrotic syndrome. Serologic evaluation demonstrated positive ANA in 5 of 49 patients (10.2%) and low C3 in 2 of 39 patients (5.1%). Five patients had a history of tuberculosis. One patient had esophageal cancer, 1 patient had multiple myeloma, while the remaining 56 patients had no evidence of malignancy. NELL1 staining showed segmental bright granular GBM staining in all 58 patients. Patients with NELL1-positive SMGN were more likely with a higher serum albumin, lower urine protein, lower prevalence of full nephrotic syndrome, lower prevalence of ANA than NELL1-negative SMGN ( $p < 0.05$ ). While age, sex, BUN, serum creatinine, eGFR, and the incidence of cancer did not differ between the two groups ( $p > 0.05$ ).

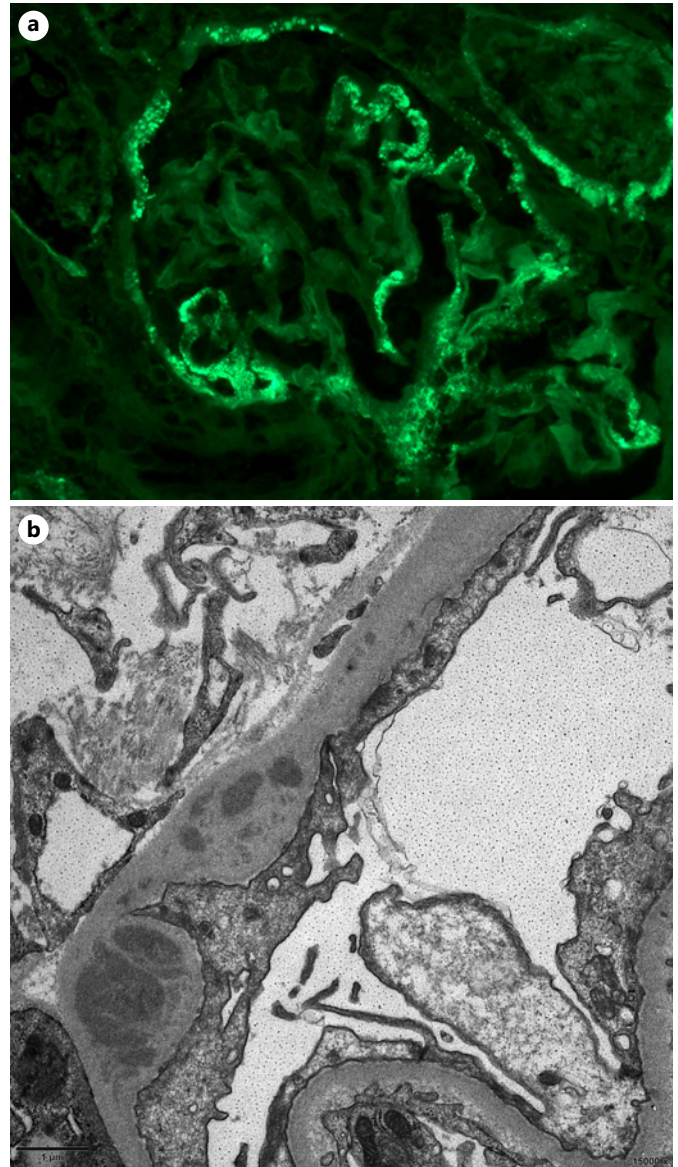
Biopsies from NELL1-positive SMGN patients showed no significant difference in IgG positivity (98.3 vs. 89.6%,  $p = 0.077$ ), IgA positivity (0.0 vs. 5.2%,  $p = 0.212$ ), IgM positivity (65.5 vs. 71.4%,  $p = 0.463$ ), C3 positivity (22.4 vs. 13.0%,  $p = 0.149$ ), C1q positivity (0.0 vs. 1.3%,  $p = 1.000$ ), compared to NELL1-negative SMGN. Twenty cases had IgG subclass staining in NELL1-positive SMGN due to adequacy of samples. There were IgG1 staining in 19 cases (95.0%), IgG4 in 12 biopsies (60.0%), IgG2 in 7 biopsies (35.0%), and IgG3 in 2 biopsies (10.0%).

As shown in Table 4, ultrastructural examination was available for 54 of 58 NELL1-positive SMGN and 75 of 77 NELL1-negative SMGN. Eleven of NELL1-positive SMGN cases showed diffuse ( $\geq 90\%$ ) foot process effacement, 4 exhibited mesangial EDD, and none of the





**Fig. 2.** IF findings in SMGN. **a** Segmental granular staining of IgG ( $\times 200$ ) along the capillary loops. **b** Segmental granular staining of IgG1 ( $\times 200$ ) along the capillary loops. **c** Segmental granular staining of neural epidermal growth factor-like 1 protein (NELL1) ( $\times 400$ ) along the capillary loops.



**Fig. 3.** Deposits in glomerular Bowman capsule in SMGN patients. **a** Segmental deposition of IgG ( $\times 400$ ) in glomerular Bowman capsule. **b** EDD can be seen in glomerular Bowman capsule by EM.

biopsies had subendothelial EDD. Patients with NELL1-positive SMGN were more likely with a lower prevalence of diffuse ( $\geq 90\%$ ) foot process effacement. There was a significant difference in the composition ratio of stage II and III MN between NELL1-positive and negative patients ( $p < 0.05$ ).

#### *Follow-Up*

Follow-up data were obtained for 35 of 150 patients with isolated SMGN. Of the 35 patients, 20 received immunosuppression (6 with glucocorticoids and calcineurin

**Table 2.** Pathologic features of patients with isolated SMGN

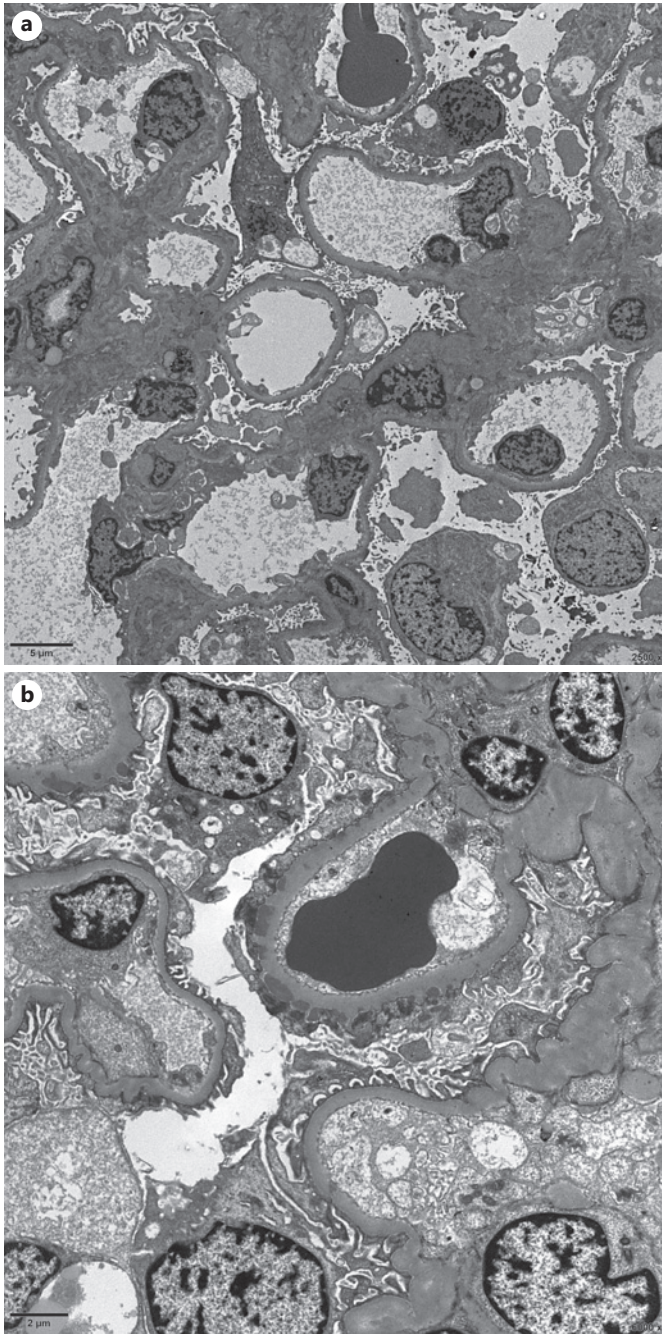
	SMGN (N = 150)
<i>Immunofluorescence</i>	
IgG positivity, %	139/150 (92.7)
IgA positivity, %	6/150 (4.0)
IgM positivity, %	104/150 (69.3)
C3 positivity, %	25/150 (16.7)
C1q positivity, %	2/150 (1.3)
Full-house IF, %	0/150 (0.0)
IgG Bowman capsule deposits, %	15/150 (10.0)
PLA2R, %	0/142 (0.0)
THSD7A, %	0/136 (0.0)
NELL1, %	58/135 (43.0)
<i>IgG subclass (N = 40)</i>	
IgG1, %	37/40 (92.5)
IgG2, %	11/40 (27.5)
IgG3, %	4/40 (10.0)
IgG4, %	18/40 (45.0)
<i>Electron microscopy (N = 142)</i>	
No. diffuse ( $\geq 90\%$ ) foot process effacement, %	58/141 (41.1)
<i>Stage of MN</i>	
Stage I, %	54/142 (38.0)
Stage II, %	65/142 (45.8)
Stage III, %	20/142 (14.1)
Stage IV, %	3/142 (2.1)
Mesangial EDD, %	17/142 (12.0)
Subendothelial EDD, %	2/142 (1.4)

inhibitors, 6 with glucocorticoids only, 3 with glucocorticoids and cyclophosphamide, 2 with glucocorticoids and *Tripterygium wilfordii*, 1 with glucocorticoids and hydroxychloroquine, 1 with *Tripterygium wilfordii* only, and 1 with calcineurin inhibitors only), 13 received non-immunosuppressive therapy (12 received renin angiotensin aldosterone system inhibition, and 1 received traditional Chinese medicine), 1 died of tumor, and 1 underwent maintenance hemodialysis. Among the 33 patients, the mean follow-up was  $12.1 \pm 10.5$  months. The median proteinuria at follow-up was 0.28 (0.08–1.76) g/d. The average eGFR at follow-up was  $107.8 \pm 15.2$  mL/min/1.73 m<sup>2</sup>, and serum albumin was  $40.6 \pm 7.5$  g/L. Among the 35 patients mentioned above, 31 underwent NELL1 staining, of which 14 were NELL1-positive and 17 were NELL1-negative (including 1 died of tumor). At the end of the follow-up period, 7 patients (50.0%) had complete remission of proteinuria, 3 (21.4%) had partial remission, 4 (28.6%) had no remission in patients with NELL1-positive. One patient with NELL1-positive was diagnosed with ovarian cancer. Ten patients (62.5%) had complete remission of proteinuria, 3 (18.8%) had partial remission, 3 (18.8%) had no remission in patients with NELL1-negative. There were no significant differences observed in the treatment response between two groups ( $p > 0.05$ ).

## Discussion

To the best of our knowledge, our current study is the first and the largest multicenter cohort of SMGN. We found that SMGN is a relatively rare pathological type, accounting for 0.17% of total kidney biopsies and 0.51% of MN in adults. The frequency of SMGN in patients with MN is unclear and varies greatly in the reported literature. It represents 2.5% of MN in adults [8], however, up to 29% in children [10]. The data in the above articles were all higher than ours. The demographics and diagnostic criteria may affect the frequency of SMGN. There are no definite diagnostic criteria for SMGN. Obana et al. [10] defined SMGN as partial glomerular tuft involvement but did not give the percentage of glomerular clusters. Segawa et al. [25] identified SMGN as lesions comprising <50% of glomerular clusters. Kudose et al. [8] proposed a definition that requires the involvement of glomerular tuft at least 25% and no more than 75%. However, in our data, we have 28 cases (18.7%) with isolated SMGN less than 25% of the glomerular tuft by IF or EM. These cases do not meet the diagnostic criteria of minimal change disease. Thus, we included these patients in the study and defined SMGN as no more than 75% of the total glomerular capillaries.





**Fig. 4.** EM findings. **a** Electron micrograph reveals extensive effacement of the podocyte foot processes. **b** Normal capillary loop and capillary loop with segmental subepithelial EDD.

So far, the clinicopathological features of SMGN have not been well characterized [8–10, 12, 13]. In pediatric patients, Obana et al. [10] found that SMGN had a higher frequency of C1q staining and mesangial EDD, but their clinical manifestations and staging of MN were similar to

those of MN. Segawa et al. [25] showed that IgG1 and IgG3 were the dominant subclasses. In adult patients, Kudose et al. [8] showed that SMGN had an elderly age, about one-third had nephrotic syndrome, IgG1 predominance, and predominantly early-stage MN. In our current study, the median age of isolated SMGN patients was 41.5 years, with female predominance, 12.0% with mesangial EDD, and the majority of patients absent of C1q staining. Our data confirmed that IgG1 was the dominant subclass, followed by IgG4, and rarely accompanied IgG3. The above results suggest that the clinicopathological and pathogenesis of SMGN in childhood may be different from that in adults. In addition, we also found IgG deposition in the Bowman capsule in 10% of SMGN patients, which we currently cannot explain.

The pathogenesis of SMGN is still incompletely elucidated [8–10]. One hypothesis is that SMGN is an early stage of idiopathic MN [10]. Repeated renal biopsy was performed on one child in our center. Two years later, after the 1st biopsy, the patient showed persistent SMGN same as the first biopsy. Similar cases were also found in the report of Kudose et al. [8] and Obana et al. [10]. These repeat biopsy results contradict the above hypothesis. Another hypothesis is that SMGN may be a secondary MN [25]. In our study, some patients with SMGN had autoimmune abnormalities, tumors, HBV, and thyroid diseases, indicating that some SMGN patients may be secondary MN. Last but not least, the target antigens are involved in the pathogenesis of SMGN. In this study, PLA2R and THSD7A staining were both negative, which is consistent with Kudose et al. [8]. The reported proportion of NELL1 staining in SMGN was 29.4% [8]. In this study, 43.0% of isolated SMGN patients were NELL1-positive. The prevalence of NELL1-positive SMGN was higher in our study than previously reported. NELL1, rather than PLA2R or THSD7A, is the target antigen of SMGN in adults [8, 18]. Therefore, the remaining nearly 50% of the target antigens of SMGN have remained elusive. With the application of mass spectrometry [26, 27], we believe that novel antigens of SMGN will be discovered soon.

NELL1, as a newly recognized target antigen for MN, has not yet been fully recognized. In this study, the majority of NELL1-positive SMGN were female, with a median age of 40 years, unlike in the study by Sethi et al. [18]. We compared the clinicopathological features between NELL1-positive and negative SMGN, and the prevalence of full nephrotic syndrome of NELL1-positive SMGN was significantly lower than that of NELL1-negative SMGN. However, during follow-up, we found

**Table 3.** Clinical characteristics of isolated SMGN patients with and without NELL1 expression in glomerular immune deposits

	NELL1-positive	NELL1-negative	<i>p</i> value
Patients, <i>n</i>	58	77	
Age, years	40.0 (32.8–50.3)	44.0 (33.0–52.0)	0.574
Male, <i>n</i> (%)	13/58 (22.4)	24/77 (31.2)	0.259
BUN, mmol/L	4.0 (3.2–5.4)	4.4 (3.5–6.2)	0.220
Serum creatinine, $\mu$ mol/L	56.0 (47.9–71.4)	62.5 (53.3–79.5)	0.068
Serum albumin, g/L	32.4 (24.1–36.7)	22.5 (17.9–35.6)	0.004
eGFR, mL/min/1.73 m <sup>2</sup>	110.5 (97.9–122.7)	105.4 (86.1–117.7)	0.161
Urine protein, g/day	2.9 (1.4–4.4)	3.9 (2.6–7.5)	0.003
No. of patients showing microscopic hematuria, %	23/56 (41.1)	30/71 (42.3)	0.893
No. of patients showing full nephrotic syndrome, %	9/51 (17.6)	30/63 (47.6)	0.001
No. of patients showing nephrotic range proteinuria, %	16/46 (34.8)	34/55 (61.8)	0.007
No. of patients showing hypoalbuminemia, %	23/55 (41.8)	52/74 (70.3)	0.001
Positive ANA (%)	5/49 (10.2)	15/53 (28.3)	0.021
Positive HBsAg (%)	3/51 (5.9)	3/68 (4.4)	1.000
No. of patients showing edema, %	37/52 (71.2)	56/73 (76.7)	0.483
No. of patients with eGFR<60 mL/min/1.73 m <sup>2</sup> , %	9/56 (16.1)	6/72 (8.3)	0.177
Cancer, %	2/58 (3.4)	2/77 (2.6)	1.000

**Table 4.** Pathologic features in isolated SMGN patients with and without NELL1 expression in glomerular immune deposits

	NELL1-positive	NELL1-negative	<i>p</i> value
<i>Immunofluorescence</i>			
IgG positivity, %	57/58 (98.3)	69/77 (89.6)	0.077
IgA positivity, %	0/58 (0.0)	4/77 (5.2)	0.212
IgM positivity, %	38/58 (65.5)	55/77 (71.4)	0.463
C3 positivity, %	13/58 (22.4)	10/77 (13.0)	0.149
C1q positivity, %	0/58 (0.0)	1/77 (1.3)	1.000
IgG Bowman capsule deposits, %	5/58 (8.6)	7/77 (9.1)	0.924
<i>IgG subclass</i>			
IgG1, %	19/20 (95.0)	16/17 (94.1)	1.000
IgG2, %	7/20 (35.0)	4/17 (23.5)	0.447
IgG3, %	2/20 (10.0)	0/17 (0.0)	0.489
IgG4, %	12/20 (60.0)	12/17 (70.6)	0.501
<i>Electron microscopy</i>			
No. diffuse ( $\geq$ 90%) foot process effacement, %	11/53 (20.8)	41/75 (54.7)	<0.001
<i>Stage of MN</i>			
Stage I, %	20/54 (37.0)	31/75 (41.3)	0.622
Stage II, %	30/54 (55.6)	26/75 (34.7)	0.018
Stage III, %	2/54 (3.7)	18/75 (24.0)	0.002
Stage IV, %	2/54 (3.7)	0/75 (0.0)	0.173
Mesangial EDD, %	4/54 (7.4)	11/75 (14.7)	0.204
Subendothelial EDD, %	0/54 (0.0)	2/74 (2.7)	0.508

that there was no statistically significant difference in treatment response between the two groups. As this study was a multicenter retrospective study and treatment regimens were not uniform, some patients were using steroid monotherapy, which is currently not recommended by KDIGO guidelines [19]. The prevalence of malignancy in NELL1-positive MN is variable [28]. Caza

et al. [29] reported that NELL1-associated MN was the most common type of MN with a malignancy association. However, studies from China and Japan with NELL1-associated MN have not identified tumors [24, 28, 30]. In our study, 2 of the 58 patients with NELL1-positive SMGN had tumors at the time of diagnosis. However, there was no statistically significant difference in the



incidence of cancer between the NELL1-positive and negative groups. The relationship between NELL1-positive MN and tumors may be geographically influenced. In addition, some studies have shown that the NELL1-positive MN were associated with drugs, infections, autoimmune disease, and sarcoidosis [28, 30, 31]. Therefore, the role of NELL1 should be examined in further studies.

There were some limitations in this study. First, we did not detect anti-NELL1 antibodies in serum. Second, PLA2R, THSD7A, NELL1, and IgG subclass staining were not available in all cases. Third, follow-up was not available for all the patients, and the treatment regimens were not uniform. Finally, the clinical data of a few patients were incomplete.

## Conclusion

SMGN is a relatively rare pathological type, accounting for 0.17% of total kidney biopsies and 0.51% of MN in adults. Majority of patients with isolated SMGN were female, with a median age of 41.5 years, 33.1% had full nephrotic syndrome, IgG1 subclass predominance, 43.0% with NELL1-positive, and mainly stage I or II MN (83.8%). NELL1 is the target antigen of SMGN in adults.

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## Statement of Ethics

This study protocol was reviewed and approved by the Ethics Committee of King Medical Diagnostics Center, Guangzhou, China (approval number: 2021029). The patient informed consent was waived in view of the retrospective nature of the study. This study was performed fulfilling the principles of the Helsinki Declaration.

## Conflict of Interest Statement

No potential conflict of interest relevant to this article was reported.

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## Author Contributions

All authors have reviewed and contributed to this manuscript. Shuangshuang Zhu was involved in study design, diagnosis of SMGN, data collection, follow-up, statistical analysis, drafting and revising the manuscript. Xiaotao Hou and Bei Luo were involved in electron microscopy filmmaking and evaluation. Shuling Yue and Lin Wang were involved in the diagnosis of SMGN. Xiaoting Liu, Kongshan Li, and Qiming Liang were involved in performing LM and IF. Xiaomeng Xu and Zhen Song were involved in revising the manuscript. Zheyu Zhou was involved in data collection. Lei Zheng and Wenfang Chen were involved in designing the study and revising the manuscript.

## Data Availability Statement

Data are not publicly available due to ethical reasons. Further inquiries can be directed to the corresponding author.

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