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# Clinical and Histological Features of Phospholipase A2 Receptor-Associated and Thrombospondin Type-I Domain-containing 7A-Associated Idiopathic Membranous Nephropathy: A Single Center Retrospective Study from China

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Data Collection B  
Statistical Analysis C  
Data Interpretation D  
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**Background:** M-type phospholipase A2 receptor (PLA2R) was identified as the major target antigen in idiopathic membranous nephropathy (IMN). Another target antigen, namely thrombospondin type-1 domain-containing 7A (THSD7A), was recently detected in approximately 10% of non-PLA2R-associated IMN. In this single center retrospective study, clinical and histological features of PLA2R-associated and THSD7A-associated IMN patients were evaluated.


**Material/Methods:** A total of 192 IMN patients, who were receiving no glucocorticoids or immunosuppressant before renal biopsy, were enrolled in this study and followed for a median duration of 25.5 months. IMN with enhanced glomerular PLA2R and THSD7A staining by immunohistochemistry (IHC) were designated as PLA2R-associated IMN and THSD7A-associated IMN respectively. Serum anti-PLA2R and anti-THSD7A antibodies levels were assessed by enzyme linked immunosorbent assay and indirect immunofluorescence testing in PLA2R-associated and THSD7A-associated IMN.

**Results:** Of 192 IMN patients, 164 patients (85.4%) had PLA2R-associated IMN and 3 patients (1.6%) had THSD7A-associated IMN. Compared with non-PLA2R-associated IMN patients, the 24-hour urinary protein levels were significantly higher ( $P=0.008$ ), whereas, the proteinuria remission rates were significantly lower ( $P=0.03$ ) in PLA2R-associated IMN patients. No pathological differences were found between PLA2R-associated IMN and non-PLA2R-associated IMN. Among 3 THSD7A-associated IMN patients, 1 patient had elevated serum anti-THSD7A antibody levels, which was below detectable levels after achieving partial proteinuria remission with combined glucocorticoid and cyclosporine treatment.

**Conclusions:** Compared with non-PLA2R-associated IMN patients in our cohort, PLA2R-associated IMN patients presented with more severe proteinuria and lower remission rates after treatment, with no distinct histological differences. Glomerular expression of PLA2R could be a useful marker to indicate the severity, treatment response, and prognosis of IMN.

**MeSH Keywords:** **Glomerulonephritis, Membranous • Immunohistochemistry • Receptors, Phospholipase A2**

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## Background

Membranous nephropathy, an organ-specific autoimmune disease, is the leading cause of nephrotic syndrome among Caucasian adults [1]. In the Chinese population, the prevalence of membranous nephropathy has doubled over the past 10 years [2]. About 80% of cases of membranous nephropathy are considered as idiopathic membranous nephropathy (IMN), whereas in the remaining cases, the disease could be related to a plethora of secondary causes such as infections, autoimmune diseases, cancer, or exposure to drugs or toxic agents [3]. Proteinuria is considered a hallmark of IMN. Spontaneous proteinuria remission occurs in one-third of IMN patients, and one-third of IMN patients will have persistent proteinuria, in which a significant number of patients will progress to end-stage renal disease (ESRD) within 5–15 years. Although the level of proteinuria is valuable to guide the treatment regimens [4,5], it can only indirectly reflect auto-immune activity and disease severity [6]. Therefore, new biomarkers for better reflection of immunologic disease activity and severity are needed.

In 2009, Beck et al. first described M-type phospholipase A2 receptor (PLA2R) as the major membranous nephropathy target antigen. IMN patients with elevated serum anti-PLA2R antibodies levels (approximately 70% of total IMN patients) or IMN patients with enhanced glomerular PLA2R deposits (approximately 80% of total IMN patients) were designated as PLA2R-associated IMN [7]. The underlying causes of non-PLA2R-associated IMN might be related to other IMN target autoantigens such as the recently identified thrombospondin type-1 domain-containing 7A (THSD7A) [8]. It has been reported that 10.5–16.0% of non-PLA2R-associated IMN membranous nephropathy patients had elevated circulating THSD7A autoantibodies levels or enhanced glomerular THSD7A deposits [9,10].

The aim of this study was to evaluate the clinical and histological features of PLA2R-associated and THSD7A-associated IMN in a retrospective cohort of 192 Chinese IMN patients.

## Material and Methods

### Patients and samples

This study was approved by the Institutional Review Board of Xin Hua Hospital affiliated to Shanghai Jiao Tong University School of Medicine. All study-related procedures were performed in accordance to the Declaration of Helsinki. All patients provided written informed consent.

There were 218 patients with biopsy-proven MN at Xin Hua Hospital from January 2010 to January 2017 who were initially included in this study. Secondary MN was found in 26

patients, including 7 patients with SLE, 12 patients with HBV, and 7 patients with malignancies. The other secondary causes of MN, such as sarcoidosis, Sjogren syndrome, HCV, and HIV, were not found in our cohort of patients. Thus, the remaining 192 patients were diagnosed as IMN. Patients receiving glucocorticoid nor immunosuppressant treatment prior to renal biopsy were not included in this study.

Clinical data were obtained from all patients at the time of renal biopsy, and included gender, age, disease duration, and the presence of hypertension. Serum specimens and urine samples were also collected to assess urinalysis, 24-hour proteinuria, serum albumin, serum creatinine, estimated glomerular filtration rate [eGFR, calculated by The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) test] [11], and serologic testing including complement components 3 and 4 (C3 and C4), anti-nuclear antibody (ANA), and hepatitis B/C surface antigen at baseline and during follow-up.

### Routine renal pathological examinations

Renal biopsy samples were processed for light, immunofluorescence (IF), and electron microscopy (EM). Paraffin-embedded tissues for light microscopy were sectioned at 3 µm for routine staining. All light microscopy samples were stained with hematoxylin and eosin (H&E), Jones methenamine silver, Masson trichrome, and periodic acid-Schiff reagent. For direct immunofluorescence, renal tissues were snap frozen in liquid nitrogen and cut into 3-µm sections. The antibodies to IgG, IgA, IgM, C3, C4, C1q, and fibrinogen in the biopsy specimens were examined. Tubular atrophy/interstitial fibrosis of parenchyma was classified as either as absent (T0), mild (T1) <25%, moderate (T2) 25–50%, or severe (T3) >50% [12]. For EM, Ehrenreich and Churg classification was used for the ultrastructural staging of membranous nephropathy.

### Glomerular staining of PLA2R, THSD7A, and IgG4

Renal biopsy specimens were paraffin-embedded and cut into 3-µm sections for immunohistochemistry (IHC) staining. IHC staining of THSD7A, PLA2R, and IgG4 were performed according to the same protocol as reported previously, and the intensity of glomerular THSD7A, PLA2R, and IgG4 expression was assessed using the method as previously described [13,14]. Rabbit polyclonal anti-PLA2R (Sigma-Aldrich, Germany), rabbit polyclonal anti-THSD7A (Sigma-Aldrich, Germany), and mouse monoclonal anti-IgG4 (Southern Biotech, United Kingdom) were used as the primary antibodies.

### Circulating anti-PLA2R antibodies and anti-THSD7A antibodies

Circulating anti-PLA2R antibodies were tested using enzyme linked immunosorbent assay (ELISA) (EUROIMMUN Lübeck,

**Table 1.** Baseline characteristics of the membranous nephropathy cohort.

	Total n=218	IMN n=192	SMN n=26	P value
Age	54.67±15.39	54.54±15.62	55.70±13.51	0.74
Gender (Male %)	129 (59.2)	117 (60.9)	12 (46.2)	0.23
Hypertension (%)	122 (55.9)	108 (56.3)	14 (53.8)	0.71
Hematuria (%)	68 (31.2)	56 (29.2)	12 (46.2)	0.16
Proteinuria (mg/24 h)	5070.11±2564.60	5022.87±2557.38	5464.50±2648.67	0.44
Serum albumin (g/L)	26.24±5.71	26.28±5.70	25.93±5.94	0.78
Serum creatinine (μmol/l)	73.16±26.78	71.55±25.73	86.60±31.91	0.01
eGFR (ml/min/1.73 m <sup>2</sup> )	94.22±23.92	95.29±23.23	85.29±28.04	0.06

IMN – idiopathic membranous nephropathy; SMN – secondary membranous nephropathy; eGFR – estimated glomerular filtration rate.

Germany) according to the instructions of the manufacturer [15]. The ELISA results were considered as negative for the levels <20 RU/mL and positive for the levels >20 RU/mL.

The detection of circulating antibodies against THSD7A was performed using indirect immunofluorescence test (IFT) (EUROIMMUN AG, Lübeck, Germany). Serum samples were diluted at 1: 10 and those positive at 1: 10 dilution would further be diluted according to the manufacturer's protocol.

The results of the anti-PLA2R antibodies and anti-THSD7A antibodies of were compared with those of 6 healthy controls samples.

### Definition

In this study, complete remission (CR) was defined as urinary protein excretion <0.3 g/day. Partial remission (PR) was defined as proteinuria <3.5 g/24-hours with a decrease of proteinuria <50% from baseline and stable renal function. A relapse was defined by the reappearance of proteinuria >3.5 g/24-hours during follow-up after partial or complete remission.

IMN with enhanced glomerular PLA2R staining by IHC was designated as PLA2R-associated IMN. IMN with enhanced glomerular THSD7A staining by IHC was designated as THSD7A-associated IMN.

### Statistical analysis

All statistical analyses were performed using the SPSS 17.0 software package (SPSS Inc., Chicago, IL, USA). Frequencies were reported for categorical data, mean and standard deviation (SD) for normally distributed variables, and median and range or interquartile range for skewed continuous data.

Continuous data were compared using the Student's *t*-test or the Mann-Whitney test, as appropriate. Categorical data were compared using the  $\chi^2$  test. All *P* values were 2-tailed, with *P*<0.05 was considered statistically significant.

## Results

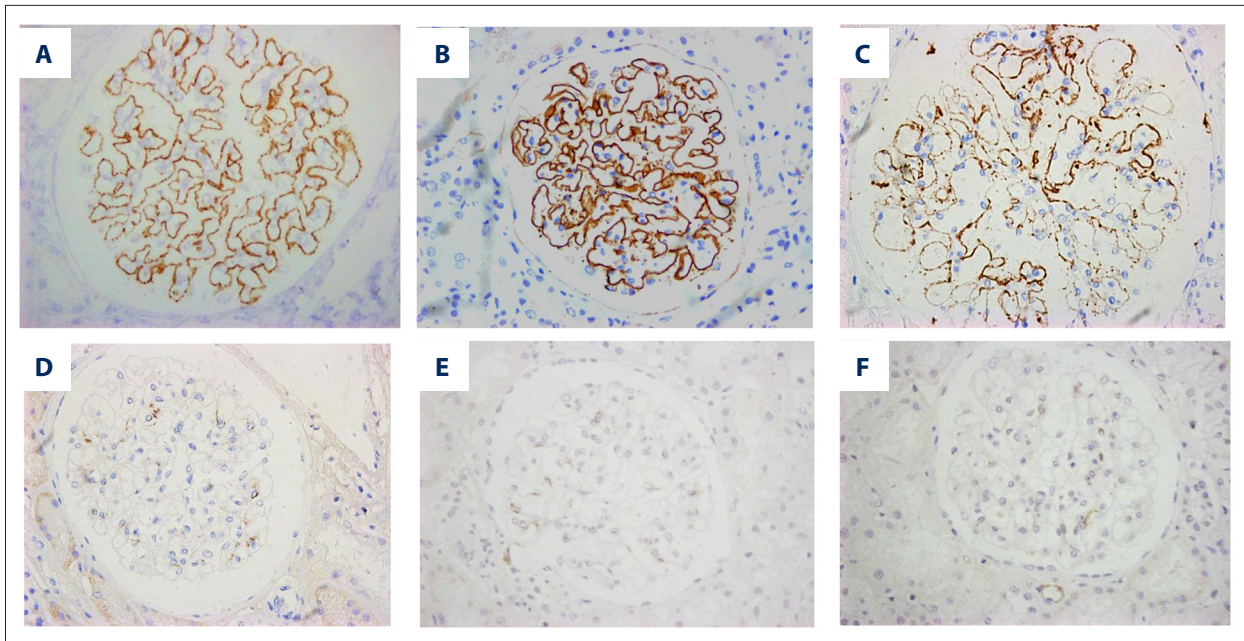
### Patient cohorts

Clinical characteristics of 192 patients with biopsy-proven IMN are shown in Table 1. Compared with 26 membranous nephropathy patients with secondary causes diagnosed in our center, we found that IMN patients had lower serum creatinine at baseline (*P*<0.05). There were no significant differences in age, gender, proportion of hypertension, proteinuria, hematuria, and serum albumin between idiopathic and secondary membranous nephropathy patients.

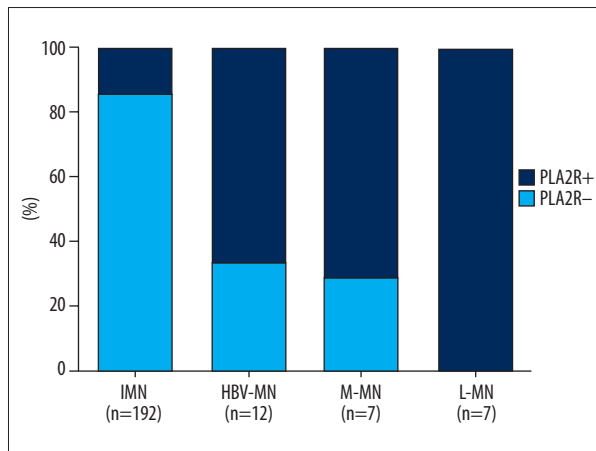
### Comparison of clinical and histological characteristics of PLA2R-associated and non-PLA2R-associated IMN patients

85.4% of IMN patients (164 out of 192 patients) had enhanced glomerular PLA2R expression (Figure 1A, 1D as controls, Figure 2). Serum samples of 70 out of 192 IMN patients were available for anti-PLA2R antibody testing. As shown in Table 2, among 60 PLA2R-associated IMN patients, 46 patients (76.7%) had elevated serum anti-PLA2R antibodies. Among the 10 non-PLA2R-associated IMN patients, we detected elevated serum anti-PLA2R antibodies in 1 patient (10%).

We compared the clinical and histological differences between PLA2R-associated and non-PLA2R-associated IMN patients and as shown in Table 3; the 24-hour urinary protein level and the proportion of patients with hypertension were significantly



**Figure 1.** Glomerular staining of PLA2R, THSD7A, and IgG4 in membranous nephropathy. PLA2R, THSD7A, and IgG4 were detected as granular deposits in the subepithelial space (A–C, magnification 400×); Staining in renal biopsy tissues of patients with minimal change disease were used as controls (D–F, magnification 400×).



**Figure 2.** The proportion of PLA2R-associated idiopathic membranous nephropathy (IMN) patients in the IMN cohort. HBV-MN – hepatitis B-associated membranous nephropathy; M-MN – malignancy-associated membranous nephropathy; L-MN – lupus-associated membranous nephropathy.

higher, whereas serum albumin was significantly lower, in the PLA2R-associated IMN patient group, compared with those in the non-PLA2R-associated IMN patient group ( $P < 0.05$ ). There

were no significant differences between the 2 groups in terms of age, gender, serum creatinine levels, eGFR, and pathological staging of membranous nephropathy. The proportion of enhanced glomerular IgG4 deposits (Figure 1C, 1F as controls) was not significantly different between PLA2R-associated and non-PLA2R-associated IMN patients.

All IMN patients were followed with a median follow-up duration of 25.5 months (range: 14.5, 46). Information about therapeutic interventions was collected, including the use of ACE inhibitors/ARBs and corticosteroids or other immunosuppressive

**Table 2.** Comparison of the prevalence of serum anti-PLA2R antibody and glomerular PLA2R deposition of idiopathic membranous nephropathy group.

Glomerular deposition	Serum PLA2R-Ab (+)	Serum PLA2R-Ab (-)	Total
PLA2R (+)	46 (76.7%)	14 (23.3%)	60 (85.7%)
PLA2R (-)	1 (10%)	9 (90%)	10 (14.3%)
Total	47 (67.1%)	23 (32.9%)	70 (100%)

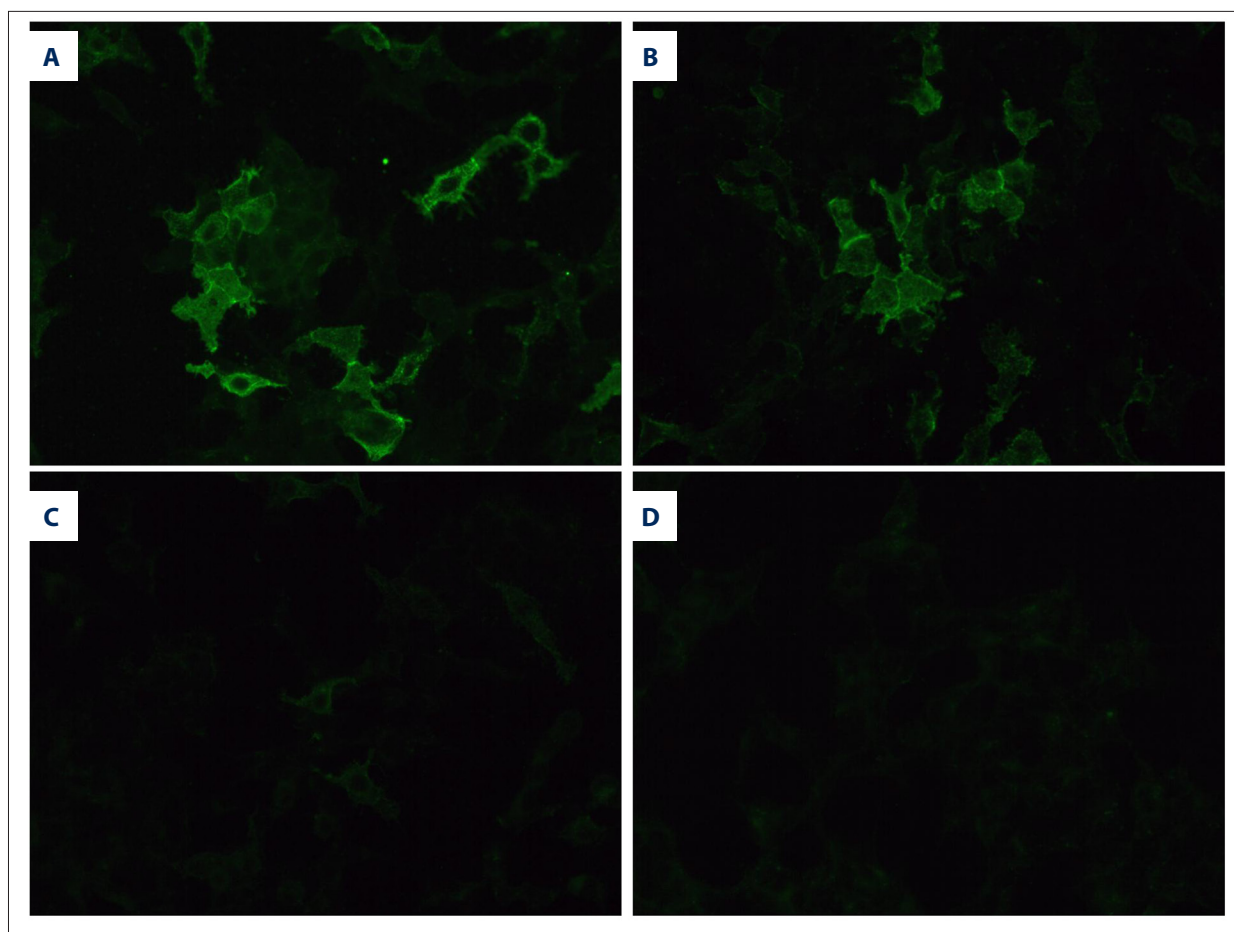
PLA2R – M-type phospholipase A2 receptor; PLA2R-Ab – anti-phospholipase A2 receptor antibody.



**Table 3.** Comparison of clinical histopathological characteristics and outcomes in idiopathic membranous nephropathy patients with positive or negative expression of glomerular phospholipase A2 receptor.

	Total	PLA2R+	PLA2R-	P value
n (%)	192	164 (85.4)	28 (14.6)	
Age	54.54±15.62	54.65±15.98	53.36±13.51	0.85
Gender (Male %)	116 (60.4)	104 (63.4)	12 (48)	0.48
Hypertension (%)	108 (56.3)	98 (59.8)	10 (35.7)	0.02
Hematuria (%)	56 (29.2)	50 (30.5)	6 (21.4)	0.33
Proteinuria (mg/24 h)	5946.37±936.25	5238.71±2515.38	3620.39±2539.18	0.01
Serum albumin (g/L)	24.27±4.19	25.89±5.46	28.53±6.56	0.02
Serum creatinine (μmol/l)	66.33±24.01	72.66±26.06	64.89±23.45	0.36
eGFR (ml/min/1.73 m <sup>2</sup> )	95.29±23.23	94.64±23.83	99.51±20.00	0.62
<b>Pathological parameters</b>				
Interstitial fibrosis				0.49
T0, T1 (%)	156 (81.3)	131 (79.9)	25 (89.3)	
T2 (%)	27 (14.1)	25 (15.2)	2 (7.1)	
T3 (%)	9 (4.7)	8 (4.9)	1 (3.6)	
<b>Immunohistological staining</b>				
IgG4 dominant (%)	153 (79.7)	137 (83.5)	16 (57.1)	0.28
<b>Ehrenreich-Churg stage</b>				
Stage I and I-II (%)	28 (14.6)	23 (14.0)	5 (17.9)	0.68
Stage II and II-III (%)	136 (70.8)	114 (69.5)	22 (78.6)	
Stage III and III-IV (%)	28 (14.6)	27 (16.5)	1 (3.6)	
<b>Treatments</b>				
ACE-I/ARB therapy (%)	20 (10.4)	14 (8.5)	6 (21.4)	0.10
<b>Immunosuppressive therapy</b>				
Steroid + CTX (%)	59 (30.7)	50 (30.5)	9 (32.1)	0.90
Steroid + CNI (%)	88 (45.8)	77 (47.0)	11 (39.3)	0.71
Steroid + MMF (%)	25 (13.0)	23 (14.0)	2 (7.1)	0.54
<b>Outcomes</b>				
Follow-up time (months)	25.5 (14.5, 46.0)	25.9 (13.6, 45.7)	23.3 (15.2, 35.5)	0.83
<b>Remission</b>				
Complete remission (%)	83 (43.2)	65 (39.6)	18 (64.3)	/
Partial remission (%)	81 (42.2)	72 (43.9)	9 (32.1)	/
No remission (%)	28 (14.6)	27 (16.5)	1 (3.6)	/
Relapse	45 (23.4)	41 (25.0)	4 (14.3)	0.31

PLA2R – M-type phospholipase A2 receptor; ACE-I – angiotensin-converting enzyme inhibitors; ARB – angiotensin II receptor blockers; CTX – cyclophosphamide; CNI – calcineurin inhibitors; MMF – mycophenolate mofetil.



**Figure 3.** Detection of serum anti-THSD7A antibody by indirect immunofluorescence test. (A). Immunofluorescence pattern of the positive control. (B) Immunofluorescence pattern with intense THSD7A positivity of the idiopathic membranous nephropathy (IMN) patient serum with 1: 10 dilution. (C) Immunofluorescence pattern with faint THSD7A positivity of the IMN patient serum with 1: 100 dilution. (D) Immunofluorescence pattern with negative THSD7A positivity of the IMN patient serum with 1: 10 dilution at the stage of disease remission.

agents that were prescribed during the follow-up period as shown in Table 3. At the end of the follow-up period, 164 patients (85.4%) achieved remission including 83 patients (43.2%) with CR and 81 patients (42.2%) with PR. Compared with non-PLA2R-associated IMN patients, the remission rate of proteinuria in the PLA2R-associated group was significantly lower ( $P=0.03$ ). Among 28 patients with no disease remissions, 5 patients progressed to end-stage renal disease (ESRD). After treatment, 45 cases relapsed and there were no significant differences between PLA2R-associated and non-PLA2R-associated IMN patient groups in terms of disease relapse.

#### The clinical and histopathological characteristics of THSD7A-associated IMN patients

Enhanced granular THSD7A deposits (Figure 1B, 1E as controls) were found in 3 out of 192 IMN patients (1.6%). Serologic testing for anti-THSD7A antibody using IFT showed that 1 out of

3 patients was positive for anti-THSD7A-Ab diluted at 1: 100 (Figure 3A as positive control, 3B, 3C). As shown in Table 4, all 3 THSD7A-associated IMN patients presented with nephrotic syndrome and received corticosteroids combined with immunosuppressive agents and all 3 patients achieved disease remission after glucocorticoid and immunosuppressant treatment. In 1 patient with elevated serum anti-THSD7A antibody levels at the point of PR, serum anti-THSD7A antibody was undetectable by IFT (Figure 3D).

#### Discussion

There were two main findings of this study. Firstly, in this retrospective cohort study of 192 Chinese IMN patients, there were discrepancies between PLA2R-associated IMN patients (85.4%) and non-PLA2R-associated patients (14.6%) in terms of the severity of proteinuria and disease remission rate. Compared

**Table 4.** The clinical histopathological characteristics and outcomes of THSD7A-Associated IMN patients.

No.	Age (yr)	Gender	Glomeruli PLA2R	Glomeruli THSD7A	Serum anti-THSD7A Ab	24 h-proteinuria (g/24h)	Alb (g/l)
1	62	Male	–	+	–	6.8	24.6
2	70	Male	–	+	+	6.49	21.9
3	65	Female	–	+	–	6.48	21.8

No.	Age (yr)	Gender	Scr (mmol/L)	GFR (ml/min/1.73 m <sup>2</sup> )	Glomerular lesions	Interstitial fibrosis	Glomeruli IgG4	Treatment	Outcomes
1	62	Male	72	91.34	II	T1	–	Pred+CTX	PR
2	70	Male	94	80.53	I	T1	+	Pred+CsA	PR
3	65	Female	51	95.19	I-II	T1	+	Pred+CsA	PR

PLA2R – M-type phospholipase A2 receptor; THSD7A – thrombospondin type-I domain-containing 7A; THSD7A-Ab – anti-thrombospondin type-I domain-containing 7A antibody; Pred – prednisolone; CTX – cyclophosphamide; CsA – cyclosporine; PR – partial remission; CR – complete remission.

with non-PLA2R-associated IMN, the 24-hour urinary protein level was significantly higher in PLA2R-associated IMN patients and the remission rate of proteinuria was significantly lower. Secondly, the prevalence of THSD7A-associated IMN patients was low in this Chinese IMN patient cohort (1.6%) and all 3 patients with enhanced glomerular THSD7A deposits had no concurrent PLA2R deposits in the kidney. In 1 patient with elevated serum anti-THSD7A antibody levels, after combined glucocorticoid and cyclosporine treatment for 6 months, serum anti-THSD7A antibody levels were below detectable levels, suggesting that serum anti-THSD7A antibody levels were correlated with disease severity.

We performed glomerular PLA2R IHC staining to identify PLA2R-associated IMN patients as previous reports suggested that screening for PLA2R staining in the glomeruli is more sensitive for the diagnosis of PLA2R-related MN as anti-PLA2R antibodies clearance is rapid in the blood compared to deposits in glomeruli. In addition, the level of antibodies may fluctuate with disease activity. Qin et al. [16] reported that in 572 Chinese IMN patients, the positive rate of enhanced glomerular PLA2R deposits were significantly higher (89.9%) than that of elevated serum anti-PLA2R antibodies (68.5%). In our study, among the 70 IMN cases with serum anti-PLA2R antibodies results, we found that the positive rate of enhanced glomerular PLA2R deposits were 85.7% (60 out of 70 cases) whereas only 67.1% (47 out of 70 cases) IMN patients had elevated serum anti-PLA2R antibodies. We also found that in our study the proportion of PLA2R-associated IMN patients designated by enhanced glomerular PLA2R deposits (85.4%) was similar to that of other Chinese IMN cohorts but was higher than that in other cohorts of Caucasian IMN patients [16–19],

which might be attributed to genetic differences or environmental variability.

Previous studies have not demonstrated significant differences in clinical parameters at the point of disease onset between PLA2R-associated and non-PLA2R-associated membranous nephropathy [17,18]. Our results, on the contrary, revealed that compared with non-PLA2R-associated IMN, the 24-hour urinary protein level was significantly higher, whereas, the serum albumin was significantly lower in PLA2R-associated IMN patients. These discordant findings might be explained by different population cohorts. In addition, the intensity of glomerular PLA2R deposits could change during different disease courses depending on the state of the immune response [16].

Previous studies have reported that high titers of anti-PLA2R antibody or the persistence of glomerular PLA2R antigen were associated with a lower rate of spontaneous or immunosuppressant induced remission, as well as a higher risk of relapse [16,20,21]. Our finding also showed that compared with non-PLA2R-associated IMN patients, the remission rate of proteinuria in the PLA2R-associated group was significantly lower, which was consistent with the results described in another recent Chinese IMN cohort [22]. The aforementioned results demonstrate that it is important to differentiate PLA2R-associated and non-PLA2R-associated membranous nephropathy by glomerular PLA2R staining or serum testing of anti-PLA2R antibody, as the clinical characteristics and treatment response between the 2 groups are different.

In our cohort of 192 IMN patients, glomerular THSD7A deposits were detected in 3 cases (1.6%), which is lower than the

prevalence reported in previous studies [9,10,23,24]. And in our cohort, no cases with double positivity of glomerular PLA2R and THSD7A deposits were found. Some studies have shown that patients with anti-THSD7A antibody may have a higher risk of cancer [9,25], whereas, Sharma et al. recently reported that malignancy might not have a close association with THSD7A-associated MN as compared with previous studies [26]. During our study follow-up period, we did not find malignancy-related evidence in the 3 THSD7A-associated IMN cases.

Several limitations to our study should be mentioned. First, this was a retrospective single center study with a relatively small sample size. Second, the serum anti-PLA2R antibodies results for IMN patients in this cohort were insufficiently obtained.

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## Conclusions

In our cohort of 192 Chinese IMN patients, PLA2R-associated IMN patients presented with more severe proteinuria and lower proteinuria remission rates after treatment, compared with non-PLA2R-associated IMN patients. Glomerular expression of PLA2R could be a useful parameter to indicate the severity, treatment efficacy and prognosis of IMN.

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## Conflict of interest

None.