

Phospholipase A2 receptor staining in pediatric idiopathic membranous glomerulopathy

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Abstract

Background Membranous glomerulopathy, though typically a disease of adults, does occur in children. Antiphospholipase A2 receptor (PLA2R) autoantibodies have recently been implicated as a causative agent in most cases of adult primary (idiopathic) membranous glomerulopathy. PLA2R staining of renal biopsies in two recent large case series of adults with primary membranous glomerulopathy showed a sensitivity of approximately 75 % for detecting primary membranous glomerulopathy. To our knowledge, this is the largest study of its kind to assess PLA2R staining in a pediatric population. **Methods** Forty-one consecutive cases of pediatric membranous glomerulopathy were identified from our database, and clinical follow-up was performed to confirm primary membranous glomerulopathy. Twenty-two patients met inclusion criteria and are the subject of this report.

Results Granular, capillary loop immunofluorescence staining for immunoglobulin G (IgG) was present in 100 % of patients, and C3 staining was present in 77 %. PLA2R staining was identified in ten patients, providing a sensitivity of 45 % [confidence interval (CI) 25–67 %]. Bovine serum albumin staining was performed in all PLA2R-negative cases and showed no positivity. Morphologic findings associated with negative PLA2R staining included segmental membranous lesions, mesangial and subendothelial deposits, C1q and “full-house” staining, and lower-stage lesions by electron microscopy. At 38 months’ average follow-up, all patients were still considered as having primary membranous

glomerulopathy, with none developing a clinically detectable secondary etiology.

Conclusions PLA2R staining sensitivity is much lower in the pediatric than the adult primary membranous glomerulopathy population. This finding suggests a more diverse and currently incompletely described set of etiologies for this disease in this group.

Keywords Phospholipase A2 receptor · PLA2R · Membranous glomerulonephritis · Membranous nephropathy · Membranous glomerulopathy · IgG4

Introduction

Membranous glomerulopathy was first described in 1946 by Bell [1] and separated into a unique disease in 1957 by Jones [2]. The presence of glomerular immunoglobulin deposits was discovered by Mellors et al. in 1957, whereas electron microscopic features of the disease were first reported by Movat and McGregor in 1959 [3, 4]. A breakthrough occurred in 1959 when Heymann and colleagues [5] identified an inducible disease (Heymann nephritis) in rats that was virtually identical, both clinically and morphologically, to membranous glomerulopathy in humans. Makker et al. later implicated megalin, a 600-kDa glycoprotein, as the causative antigen [6]. Recently, Beck et al. found that 70 % of adult patients with primary membranous glomerulopathy expressed a specific M-type phospholipase A2 receptor (PLA2R) on podocytes [7]. PLA2R appears to be the predominant causative antigen of primary membranous glomerulopathy in adults [7]. In previous reports, the presence of PLA2R autoantibodies in serum were shown to have a sensitivity of 70–82 %, and tissue staining for PLA2R showed a sensitivity of 74 % [7, 8]. However, in those studies, the majority of patients were adults or older adolescents. Early reports suggest promising serologic tests for diagnosis and monitoring of primary membranous glomerulopathy

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[7, 9, 10]. Studies in our lab demonstrate the importance and utility of immunofluorescence staining for PLA2R on all newly diagnosed cases of membranous glomerulopathy.

Primary membranous glomerulopathy is a rare entity in children, representing 1–2 % of nephrotic syndrome patients seen in this population [11, 12]. Historically, membranous glomerulopathy in children has been associated with secondary causes, such as systemic lupus erythematosus, hepatitis B infection, congenital syphilis, etc [13, 14]. Recently, Debiec et al. described neutral endopeptidase as a causative alloantibody in neonatal membranous glomerulopathy and bovine serum albumin antibodies as a cause of secondary membranous glomerulopathy in young children [15, 16]. However, there is no information available on a potential role for PLA2R in the pediatric population, and morphologically, there are no findings with a known high sensitivity or specificity for differentiating primary and secondary forms of this disease.

Methods

Patient selection

This study was approved by Schulman institutional review board with data collected according to approved protocols. Our database was searched from 2005 to 2012 for patients aged 17 and younger with membranous glomerulopathy and negative antinuclear antibody (ANA) serology. Forty-one consecutive cases were identified. Clinical follow-up was performed on all patients to determine whether they had an active disease that is a known secondary etiology of membranous glomerulopathy. Either no or inadequate follow-up occurred in 19 patients. Twenty-two patients showed negative serologic testing for ANA, antineutrophil cytoplasmic autoantibodies (ANCA), hepatitis B or C virus (HBV, HCV), sarcoidosis, or syphilis and showed no evidence of neoplasm. All cases had light microscopic, immunofluorescence, and electron microscopic results available.

Routine biopsy processing techniques

Standard renal biopsy processing techniques were used, including light, immunofluorescence, and electron microscopy [17, 18]. All light microscopy samples were stained with hematoxylin and eosin (H&E), Jones methenamine silver, Masson trichrome, and periodic acid-Schiff reagent. All direct immunofluorescence sections were cut at 5 μ m and reacted with fluorescein-tagged polyclonal rabbit anti-human antibodies to immunoglobulin (Ig)G, IgA, IgM, C3, C1q, fibrinogen, and κ - and λ -light chains (Dako, Carpinteria, CA, USA) for 1 h, rinsed, and a coverslip was applied using aqueous mounting media. Select cases were stained for fluorescein-tagged polyclonal mouse anti-human antibodies to IgG1, IgG2, IgG3, and IgG4 (Sigma-Aldrich, St. Louis, MO, USA). All PLA2R-

negative cases were stained for bovine serum albumin (Life Technologies, Carlsbad, CA, USA). For electron microscopy, thin sections were examined in a Jeol JEM-1011 electron microscope (Jeol, Tokyo, Japan). Photomicrographs were routinely taken at $\times 5,000$, $\times 12,000$, and $\times 20,000$ magnifications. Electron photomicrographs were used to stage the cases of membranous glomerulopathy according to the classification of Ehrenreich and Churg [19]. Electron photomicrographs were also utilized to identify mesangial and subendothelial deposits. For this report, the term “mesangial deposits” refers to deep mesangial deposits within the mesangial matrix and internal to identifiable paramesangial basement membranes [20].

PLA2R immunofluorescence

PLA2R was detected as in our previous study [10] using the routinely prepared, formalin-fixed, paraffin-embedded renal biopsy tissues using rabbit polyclonal anti-PLA2R antibodies (Sigma-Aldrich) at a dilution of 1:50, followed by highly cross-adsorbed Alexa Fluor 488 goat anti-rabbit IgG (Life Technologies, Carlsbad, CA, USA) at a dilution of 1:100. Each case was run with a positive and negative (secondary antibody only) control. The stain was evaluated by standard immunofluorescence microscopy using a Leica L5 filtercube. It was judged to be positive if there was granular capillary loop staining along glomerular capillary loops. Each section was scored on a scale of 0–3+. All PLA2R-stained sections were evaluated by at least two renal pathologists.

Results

Though 41 consecutive pediatric patients with membranous glomerulopathy were initially identified, follow-up information confirming the primary nature of their disorder was only obtainable for 22 patients. Patient age ranged from 4 to 17 years, with a 1:1 M:F ratio. Average serum creatinine level at time of biopsy was 0.72 mg/dl (0.2–1.3 mg/dl), with average proteinuria of 6.8 gm/day (1.4–18.4 gm/day) (Table 1). Granular capillary loop immunofluorescence staining of glomeruli for PLA2R was present in ten of 22 patients (Fig. 1). Thus, sensitivity of positive PLA2R glomerular staining was 45 % [confidence interval (CI) 25–67 %] for detecting primary membranous glomerulopathy (Fig. 1). All PLA2R-negative biopsies were examined for the presence of antibodies against bovine serum albumin, and all were negative. IgG subclass staining was attempted in all cases. Eight cases had tissue with glomeruli present for evaluation (Table 2). Three of six PLA2R-negative patients showed IgG4 dominance/codominance, whereas both PLA2R-positive patients showed IgG4 dominance/codominance. Of the remaining three PLA2R-negative patients, two showed IgG3 dominance/codominance and one IgG1 dominance. On immunofluorescence, capillary-wall IgG was positive ($\geq 2+$) in all patients

(two displayed segmental staining); capillary-wall C3 was positive in 77 % (17/22). Kappa and lambda light-chain staining were present in all cases. Capillary-wall IgA and IgM staining were present in 9 % (2/22) and 14 % (3/22), respectively. C1q was present in 18 % (4/22), all of whom were PLA2R negative.

By electron microscopy, all patients showed subepithelial immune complex deposits, with nine showing stage 1 deposits, four showing stage 2, and nine showing stage 3 [19]. Seven of nine patients with stage 3 deposits showed positivity for PLA2R. Eleven patients (50 %) showed mesangial deposits, 82 % of which (9/11) were also PLA2R negative (Table 3). Follow-up was performed on all cases to ascertain whether the patients had developed any secondary cause of membranous glomerulopathy in the interim. An average of 38 months follow-up was achieved in 17 patients, with all reporting continuation of idiopathic, primary disease and none having developed a secondary etiology (Table 2).

Discussion

We examined the pertinent clinical, morphologic, and PLA2R staining patterns in a group of 22 pediatric primary membranous glomerulopathy patients. In ten patients, PLA2R staining

was positive; the remaining 12 showed no staining, providing a sensitivity of 45 % for detecting primary membranous glomerulopathy in the pediatric population. This finding is in contrast to similar studies in primarily adults, where 70–82 % sensitivity was achieved [7, 8, 10, 21]. This suggests that the majority of primary pediatric membranous glomerulopathy patients do not have antibodies to PLA2R antigen as the etiology of their disease. This is critical information for several reasons. First, this suggests that membranous glomerulopathy in the pediatric population has a more diverse set of etiologies than what would be expected from adult studies of this disease and that these etiologies are incompletely described [7, 8, 10]. Also, as better disease monitoring and treatment may one day depend on the underlying autoantibody/etiology in membranous glomerulopathy, a more complete understanding of the different etiologies of this disease is crucial. Lastly, this study suggests that in the majority of pediatric primary membranous glomerulopathy patients, the underlying etiology is currently unknown.

Clinically, we saw similar age and gender distribution, serum creatinine at time of biopsy, and 24-h urine protein levels in both PLA2R-positive and -negative patients. Similarly, by light microscopy no specific features were identified that were more strongly associated with either group. However, by immunofluorescence, C1q staining was identified in four PLA2R-negative patients and was always associated with the presence of mesangial deposits; it was not present in PLA2R-positive patients. Additionally, one patient with C1q staining exhibited full-house staining; however, at 43 months' follow-up, this patient was still ANA negative and was classified as having primary membranous glomerulopathy. Eleven patients showed mesangial deposits, eight of whom were PLA2R-negative patients; a single patient displayed subendothelial deposits and was PLA2R negative. In adult membranous glomerulopathy, mesangial and subendothelial deposits have been reported as a "soft sign" of secondary etiologies [13]. Also, whereas C1q staining and mesangial deposits are not sensitive or specific for lupus or other autoimmune disease, these findings do raise the possibility that some patients with negative PLA2R staining may represent atypical or early stages of clinically undetectable autoimmune disease. However, follow-up occurred for three of these four patients; none showed progression to a clinically detectable autoimmune disease over an average 35 months.

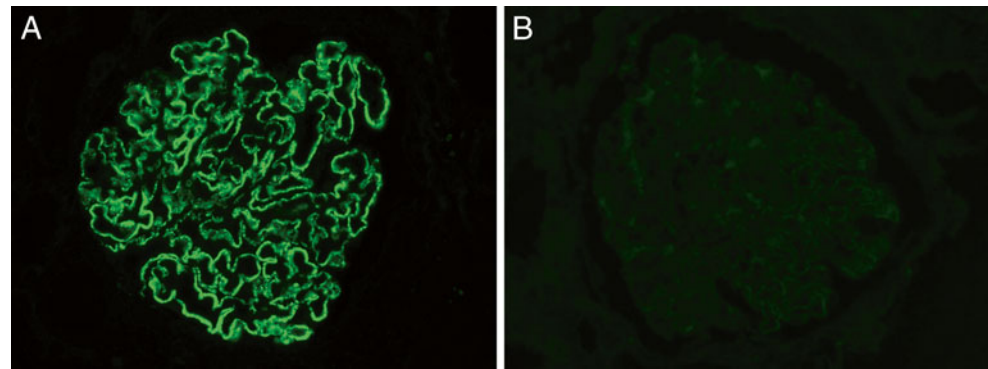
Patients who showed positivity for IgA and/or IgM in addition to IgG were similar in PLA2R-positive and -negative patients, (four total; two PLA2R +, two PLA2R -); however, all of these patients displayed mesangial deposits. Also, by electron microscopy, nine patients showed stage 3 deposits; seven of them were PLA2R positive. Segmental immune complex deposition was seen in two patients, one male and one female, ages 10 and 17 years, respectively. Both patients showed negative staining for PLA2R, mesangial deposits, and

Table 1 Clinical details

Patient	Age	Gender	Creatinine (mg/dl)	24-h urine protein (gm)
1	13	M	0.9	3.1
2	17	M	0.9	7.1
3	14	M	NA	NA
4	16	F	0.5	8.3
5	12	F	0.3	9.3
6	14	F	0.5	4.8
7	17	F	0.6	6.0
8	17	M	0.7	5.2
9	14	F	0.5	3.8
10	16	M	0.7	2.1
11	15	M	0.6	3.5
12	14	M	0.7	9.9
13	10	M	0.3	3.0
14	15	F	1.0	9.2
15	12	F	0.6	NA
16	4	F	0.2	NA
17	17	M	1.2	5.7
18	15	F	1.3	6.0
19	15	M	1.3	15.0
20	14	M	1.0	18.4
21	16	F	NA	NA
22	10	M	0.5	1.4

NA data not available

Fig. 1 Antiphospholipase A2 receptor (PLA2R) antibody staining pattern in a glomerulus. **a** Granular capillary-wall 3+ staining for anti-PLA2R antibodies in a glomerulus (PLA2R stain, $\times 400$ original magnification). **b** Negative staining for anti-PLA2R antibodies in a glomerulus (PLA2R stain, $\times 400$ original magnification)



earlier-stage deposits (stage 1 and 2); one showed staining for C1q (1+). On follow-up, neither patient had developed secondary causes for their membranous glomerulopathy.

No consensus exists on the treatment and clinical management of pediatric patients with membranous glomerulopathy. This is likely due to the highly variable prognosis of membranous glomerulopathy and its rare occurrence in children [22–24]. However, Menon et al. suggested a treatment algorithm for membranous glomerulopathy occurring in childhood [22]. They suggest angiotensin blockade for those with

subnephrotic proteinuria and steroids for those with nephrotic syndrome. Alkylating agents are used when less aggressive treatment fails [22].

In conclusion, this study suggests that within the pediatric population, primary membranous glomerulopathy has a more diverse set of etiologies than in the adult population, with only 45 % of primary pediatric membranous glomerulopathy showing PLA2R receptor autoantibodies as their causative etiology. Additionally, whereas we found no morphologic finding that was absolutely consistent with PLA2R negativity, we did note

Table 2 Immunoglobulin (Ig)G subclass data and follow-up

Patient	PLA2R	IgG1	IgG2	IgG3	IgG4	Follow-up (months)	Follow-up								
1	+	NA				40	Idiopathic								
2	-	3+	3+	3+	3+	43	Idiopathic								
3	+	NA				NA	NA								
4	+	NA				45	Idiopathic								
5	+	NA				49	Idiopathic								
6	+	NA				7	Idiopathic								
7	-	NA				12	Idiopathic								
8	-	NA				NA	NA								
9	-	2+	1+	0	3+	28	Idiopathic								
10	-	NA				10	Idiopathic								
11	-	2+	0	0	3+	29	Idiopathic								
12	+	NA				45	Idiopathic								
13	+	2+	0	0	3+	44	Idiopathic								
14	-	NA				18	Idiopathic								
15	-	NA				NA	NA								
16	-	2+	0	2+	0	NA	NA								
17	+	NA				31	Idiopathic								
18	+	NA				68	Idiopathic								
19	+	3+	0	0	3+	NA	NA								
20	-	1+	0	3+	2+	43	Idiopathic								
21	-	NA				84	Idiopathic								
22	-	2+	0	1+	0	43	Idiopathic								

PLA2R phospholipase A2 receptor, IG immunoglobulin, NA data not available

Table 3 Immunofluorescence and electron microscopic findings

Patient	Electron microscopy			Immunofluorescence					
	SubEpi	Mes	SubEndo	PLA2R	IgG	IgA	IgM	C3	C1q
1	Yes	No	No	+	3+	0	0	2+	0
2	Yes	Yes	No	-	3+	2+	2+	3+	2+
3	Yes	Yes	No	+	3+	0	0	3+	0
4	Yes	No	No	+	3+	0	0	1+	0
5	Yes	Yes	No	+	3+	0	2+	3+	0
6	Yes	No	No	+	3+	0	0	1+	0
7	Yes	Yes	No	-	3+	0	0	2+	0
8	Yes	Yes	No	-	2+	0	0	0	0
9	Yes	Yes	Yes	-	3+	0	0	3+	0
10	Yes	No	No	-	2+	0	0	1+	0
11	Yes	No	No	-	3+	0	0	0	0
12	Yes	No	No	+	3+	0	0	3+	0
13	Yes	No	No	+	3+	0	0	2+	0
14	Yes	Yes	No	-	3+	0	0	0	1+
15	Yes	Yes	No	-	3+	0	0	1+	1+
16	Yes	Yes	No	-	3+	0	0	1+	0
17	Yes	No	No	+	3+	0	0	3+	0
18	Yes	Yes	No	+	3+	1+	0	0	0
19	Yes	No	No	+	3+	0	0	2+	0
20	Yes	No	No	-	3+	0	0	3+	0
21	Yes	No	No	-	3+	0	0	0	0
22	Yes	Yes	No	-	3+	0	2+	3+	1+

IG immunoglobulin, SubEpi subepithelial, Mes mesangial, SubEndo subendothelial, PLA2R phospholipase A2 Receptor

frequent association with mesangial and subendothelial immune complex deposits, C1q deposition, lesion segmentality, and lower electron microscopic lesion stage. Lastly, although morphologic findings in some patients with PLA2R-negative primary membranous glomerulopathy are suggestive of underlying secondary etiologies, on follow-up, we found no patient developed clinically identifiable, known secondary etiologies, with all patients still considered to have primary idiopathic membranous glomerulopathy.

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