## **Supplementary Material**

## Overexpression of Plasmalemmal Vesicle-Associated Protein-1 Reflects Glomerular Endothelial Injury in the Cases of Proliferative Glomerulonephritis With Monoclonal IgG Deposits

Running Head: Glomerular PV-1 overexpression in PGNMID

**Authors:** Anri Sawada, MD<sup>1,2\*#</sup>, Kunio Kawanishi, MD, PhD<sup>3\*#</sup>, Yuto Igarashi, MD<sup>4</sup>, Sekiko Taneda, MD<sup>2</sup>, Motoshi Hattori, MD<sup>5</sup>, Hideki Ishida, MD<sup>4,6</sup>, Kazunari Tanabe, MD<sup>4</sup>, Junki Koike, MD<sup>7</sup>, Kazuho Honda, MD<sup>8</sup>, Yoji Nagashima, MD<sup>2</sup>, Kosaku Nitta, MD<sup>9</sup>

## Affiliations:

 <sup>1</sup>Department of Analytic Human Pathology, Nippon Medical School, Tokyo, Japan. <sup>2</sup>Department of Surgical Pathology, Tokyo Women's Medical University Hospital, Tokyo, Japan.
<sup>3</sup>Department of Experimental Pathology, Faculty of Medicine, University of Tsukuba, Ibaraki, Japan.
Department of <sup>4</sup>Urology, <sup>5</sup>Pediatric Nephrology, <sup>6</sup>Organ Transplant Medicine, <sup>9</sup>Nephrology, Tokyo Women's Medical University, Tokyo, Japan.

<sup>7</sup>Department of Pathology, St. Marianna University School of Medicine, Kawasaki, Kanagawa, Japan. <sup>8</sup>Department of Anatomy, Showa University School of Medicine, Tokyo, Japan.

\*Correspondence: <u>kukawanishi@md.tsukuba.ac.jp</u> (lead contact) or <u>anri-sawada@nms.ac.jp</u>, Department of Experimental Pathology, Faculty of Medicine, University of Tsukuba, 1-1-1, Tennodai, Tsukuba, Ibaraki Japan. Tel: +81-29-853-3944 <sup>#</sup>A.S. and K.K. contributed equally to this work.

Supplementary Figure 1-5.

Supplementary Table 1-3.



Supplementary Figure 1. PGNMID is characterized by glomerular overexpression of PV-1.

IgA nephropathy (A–D) showed no glomerular PV-1 expression (D). An allograft biopsy of the case revealed IgA nephropathy (E–H) without PV-1 expression in glomeruli (H) on the post operative day 1463, however, PGNMID (I–L) was complicated and PV-1 turned to be positive (L, dot circle) at the onset of on the post operative day 1549. (A, E, I) Periodic acid-schiff staining, black bars showed 50 μm.



Area 54.06, Integrated density 92.62 Integrated density / area = 1.71

Area 44.57, Integrated density 168.30 Integrated density / area = 3.78 Area 53.37, Integrated density 322.11 Integrated density / area = 6.02

## Supplementary Figure 2. Measurements of glomerular PV-1 intensity.

The glomerular PV-1 intensities (integrated density /  $\mu m^2$ ) were measured with Image J.



Supplementary Figure 3. The correlations between the PV-1 intensity and proteinuria or renal prognosis in PGNMID.

(A) Spearman r between the glomerular PV-1 intensity and proteinuria are -0.3754 (p=0.0131) (non nephrotic < 3 g/gCr) and -0.1117 (p=0.6034) (nephrotic > 3 g/gCr), respectively. There is no significant difference either between the non nephrotic and nephrotic group (B) or between the non ESRD and ESRD group (C). (n = 22 patients: one patient was excluded from the analysis, because of lacking data for urinary protein (g/gCr) at the biopsy in the database; Mean with SD, Mann–Whitney test).



Supplementary Figure 4. Low vacuum scanning electron microscopy analysis.

Negative control stain with isotype control for PV-1 antibody (A-C) and those LVSEM image (D-F). Periodic acid-methenamine silver (PAM) stain (G-I) and those LVSEM images (J-R). Asterix and arrow indicate the same lesions. Bars indicate 20 µm.





PV-1 intensities and (A) monoclonal deposition type in PGNMID, (B) International Society of Nephrology (ISN) / Renal Pathology Society (RPS) Classification in lupus nephritis, and (C) IgG deposition negative (-) or positive (+) in antineutrophil cytoplasmic antibody (ANCA) associated vasculitis (AAV) (Mean with SD, Mann–Whitney test).

Characteristics	Value		
	IgA nephropathy	MPGN	
Total case number	54	5	
Age, year, median (range)	36 (16-72)	57 (14-76)	
Sex, male, n (%)	31 (57.4)	3 (60)	
U-pro, g/day or g/g.cr, median (range)	0.5 (0.03-5.9)	3.46 (0.91-7.2)	
sCr, mg/dl, median (range)	0.81 (0.52-2.02)	1.27 (0.58-2.4)	
U-OB, n (%)	48 (88.9)	1 (20)	
Other, n (%)		Туре	
		I, 1 (20)	
		III, 3(60)	
		unknown, 1 (20)	

Supplementary Table 1. IgA nephropathy and MPGN characteristics

MPGN, membranoproliferative glomerulonephritis; U-pro, urine protein; sCr, serum creatinine; U-OB; urine occult blood.

Supplementary	Table 2. I	Lupus nephritis,	MCD and	FSGS	characteristics
---------------	------------	------------------	---------	------	-----------------

Characteristics	Value			
	Lupus nephritis	MCD	FSGS primary	FSGS secondary
Total case number	51	21	21	6
Age, year,	36	14	21	48.5
median (range)	(16-66)	(3-64)	(1-68)	(13-68)
Sex, male, n (%)	8 (15.7)	13 (61.9)	11 (52.4)	4 (66.7)
U-pro, g/day or g/g.cr,	0.97	2.34	5.35	1
median (range)	(0.01-10.6)	(0.05-23.1)	(0.49-38.3)	(0.63-2.07)
sCr, mg/dl,	0.64	0.45	0.9	1.56
median (range)	(0.4-1.87)	(0.23 - 1.47)	(0.15 - 1.88)	(0.7-5.1)
U-OB, n (%)	23 (45.1)	5 (23.8)	12 (57.1)	1 (16.7)
Other, n (%)	ISN/RPS Class			
	I, 1 (2)			
	II, 5 (9.8)			
	III, 13 (25.5)			
	IV, 16 (31.4)			
	V, 16 (31.4)			

MCD, minimal change disease; FSGS, focal segmental glomerulosclerosis; ISN/RPS Class,

International Society of Nephrology (ISN) / Renal Pathology Society (RPS) Classification

Characteristics	Value				
	MN primary	MN secondary	C3 glomerulopathy	AAV	
Total case number	15	9	6	16	
Age, year,	68	60	16.5	68	
median (range)	(22-74)	(6-78)	(11-73)	(14-84)	
Sex, male, n (%)	9 (60)	4 (44.4)	3 (50)	8 (50)	
U-pro, g/day or					
g/g.cr,	2.66	5.14	0.53	0.61	
median (range)	(0.08-19.7)	(0.8-15.5)	(0.04-2.8)	(0.4-3.86)	
sCr, mg/dl, median	0.84	0.6	0.61	1.46	
(range)	(0.53-7.0)	(0.4-1.27)	(0.43-1.55)	(0.78-9.72)	
U-OB, n (%)	9 (60)	7 (77.8)	6 (100)	16 (100)	
Other,	PLA2R,		C3 glomerulonepritis,	MPO-ANCA,	
n (%)	4 (26.7)		4 (66.7)	16 (100)	
	THSD7A,		Dense deposit disease,		
	0 (0)		2 (33.3)		

Supplementary Table 3. MN, C3 glomerulopathy and AAV characteristics

MN, membranous nephropathy; AAV, antineutrophil cytoplasmic antibody (ANCA) associated vasculitis; PLA2R, phospholipase A2 receptor; THSD7A ,Thrombospondin type-1 domain-containing 7A; MPO, myeloperoxidase.