Morphological alterations of podocytes in Alport syndrome investigated by low vacuum scanning electron microscopy

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Introduction and Objectives

Patients with Alport syndrome (AS) present with glomerulonephropathies with haematuria and proteinuria, resulting in progressive end stage renal disease. In contrast, patients with thin basement membrane nephropathy (TBMN) exhibit haematuria and a good renal prognosis. AS and TBMN are both disorders of the glomerular basement membranes (GBMs), but the role of podocytes in the progression of both diseases is not clear.

We previously reported that the evaluation by low vacuum scanning electron microscopy (LVSEM) using renal paraffin sections is useful for the renal histological diagnosis[1-4], especially for the evaluations of podocytes, and GBMs. Under LVSEM, characteristic morphological differences in GBMs between AS and TBMN were easily observed through the overlying cellular components without removal[4].

Here, we investigate the three-dimensional morphological alterations of podocytes in AS comparing with those in TBMN by LVSEM.

Methods

Cases:

A total of 4 (3 males and 1 female) and 6 (1 male and 5 females) patients with AS and TBMN, respectively, were identified.

Histopathological diagnoses of AS and TBMN were made beforehand by renal biopsy with transmission electron microscopy and immunofluorescence staining of collagen type IV α5 (IV) and α2 (IV) chains.

LVSEM observations:

Renal biopsy paraffin sections were stained with platinum-blue at pH9 (TI-blue staining kit, Nisshin EM Co. Ltd., Tokyo) for observations of podocytes, or periodic acid methenamine silver (PAM) for those of GBMs. They were directly observed at magnifications between ×50 and ×10,000 with a LVSEM (Hitachi TM-1000, Hitachi High Technologies Co. Ltd., Tokyo) in the backscattered electron mode.

Conclusions

Round-shaped podocyte cell bodies and irregularities in foot processes interdigitation in AS were recognized more often than that in TBMN. These morphological disorders of podocytes in AS may be related to the development of proteinuria in AS.

Acknowledgment

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References

1. Inaga S et al., *Arch Histol Cytol* 72:101-106,2009.
2. Inaga S et al., *Arch Histol Cytol* 73:113-125,2010
3. Okada S et al., *Biomed Res (Tokyo)* 35:227-236,2014
4. Okada S et al., *Biomed Res (Tokyo)* 35:345-350,2014

Results

Characteristics of cases.

No	Sex	Age at onset	Age at RBx	Initial Findings	Familial History	Proteinuria at RBx	Sediment RBC	BUN (mg/dl)	Cr. (mg/dl)
AS-1	M	3	16	micro H.	H: mother	2+	30-49	15	0.71
AS-2	F	1	3	macro H.	H: mother	1+	100<	14	0.24
AS-3	M	8	14	micro H.	H: daughters	+/-	10-19	13	0.61
AS-4	M		17	micro H.	AS: sibling				
TBMN-1	F	13	19	micro H.	CGN: sibling	+/-	30-49	11	0.21
TBMN-2	M	6	6	proteinuria	TBMN: mother	+/-	100<	16	0.31
TBMN-3	F		13		kidney disease: mother	-	100<	10	0.50
TBMN-4	F		9		H: mother, grandmother	-	10-19	10	0.40
TBMN-5	F	4	11	micro H.	none	1+	100<	18	0.40
TBMN-6	F		8	micro H.	none	1+	50-99	12	0.30

H: hematuria, AS: Alport syndrome, CGN: chronic kidney disease. TBMN: thin basement membrane disease

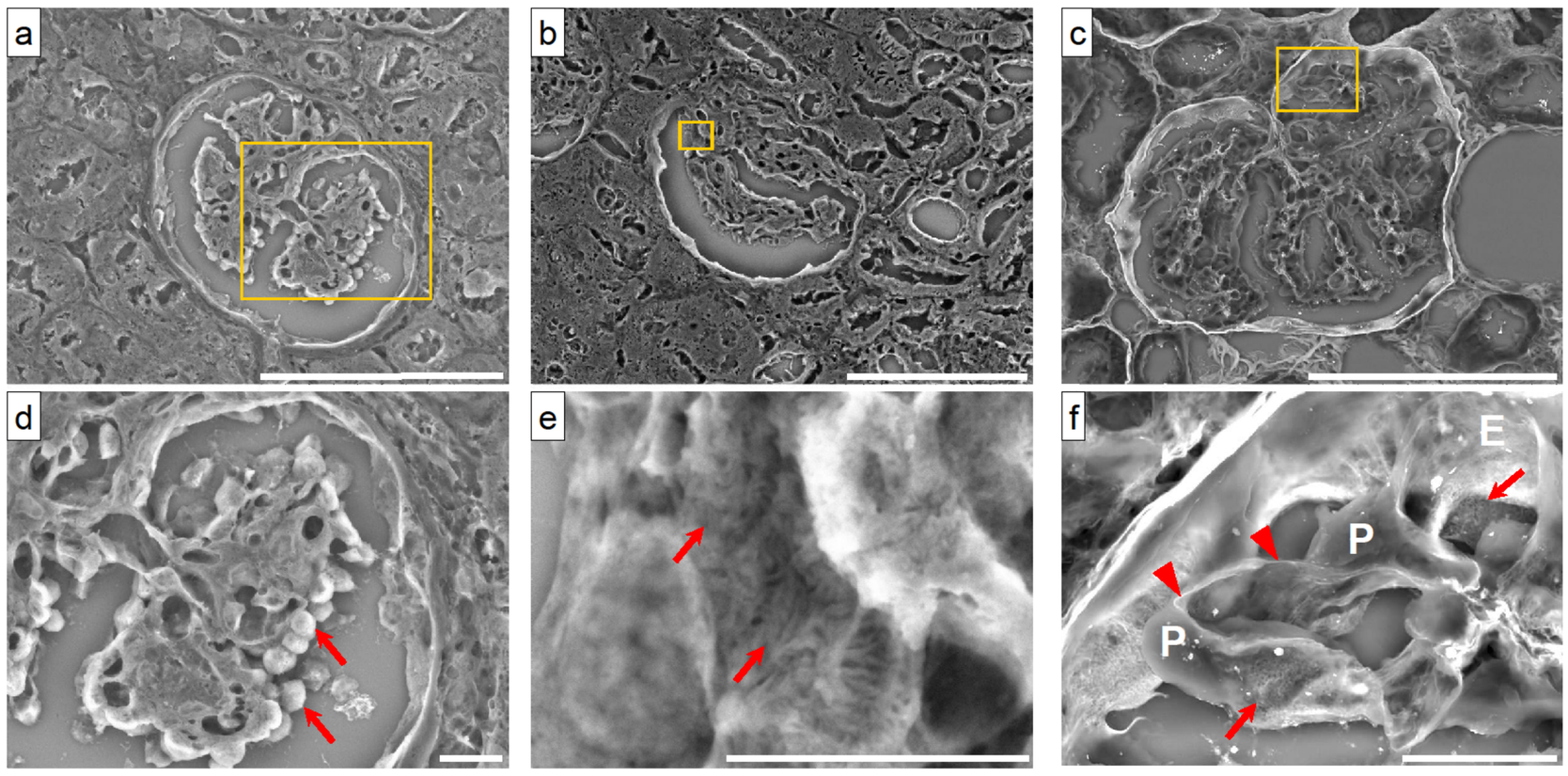

Fig.1. LVSEM images of Pt-blue- or PAM-stained glomeruli in Alport syndrome.
a, b, d, e) Pt-blue-stained glomeruli in case AS-2 (a, d) and in case AS-3 (b, e), respectively. d, e) Higher-magnification images of the square shown in Fig.1a or 1b, respectively. c, f) PAM-stained glomerulus in case AS-2. f) A Higher-magnification image of the square shown in Fig.1c.
a, b, d, e) The surface view of podocytes and their foot processes are observed clearly from the Bowman's space. Round-shaped podocyte cell bodies (d: arrows), and irregularities in foot process interdigitation (e: arrows) are noted. c, f) The GBMs showing the coarse meshwork structures (arrows) can be distinctly observed through the thinner parts of podocytes or endothelial cells. GBMs observed at the cut side view in this case are very thin and uneven (arrowheads).
P: podocyte, E: endothelial cell. Bars: 100 μm (a, b, c), 10 μm (d, e, f)

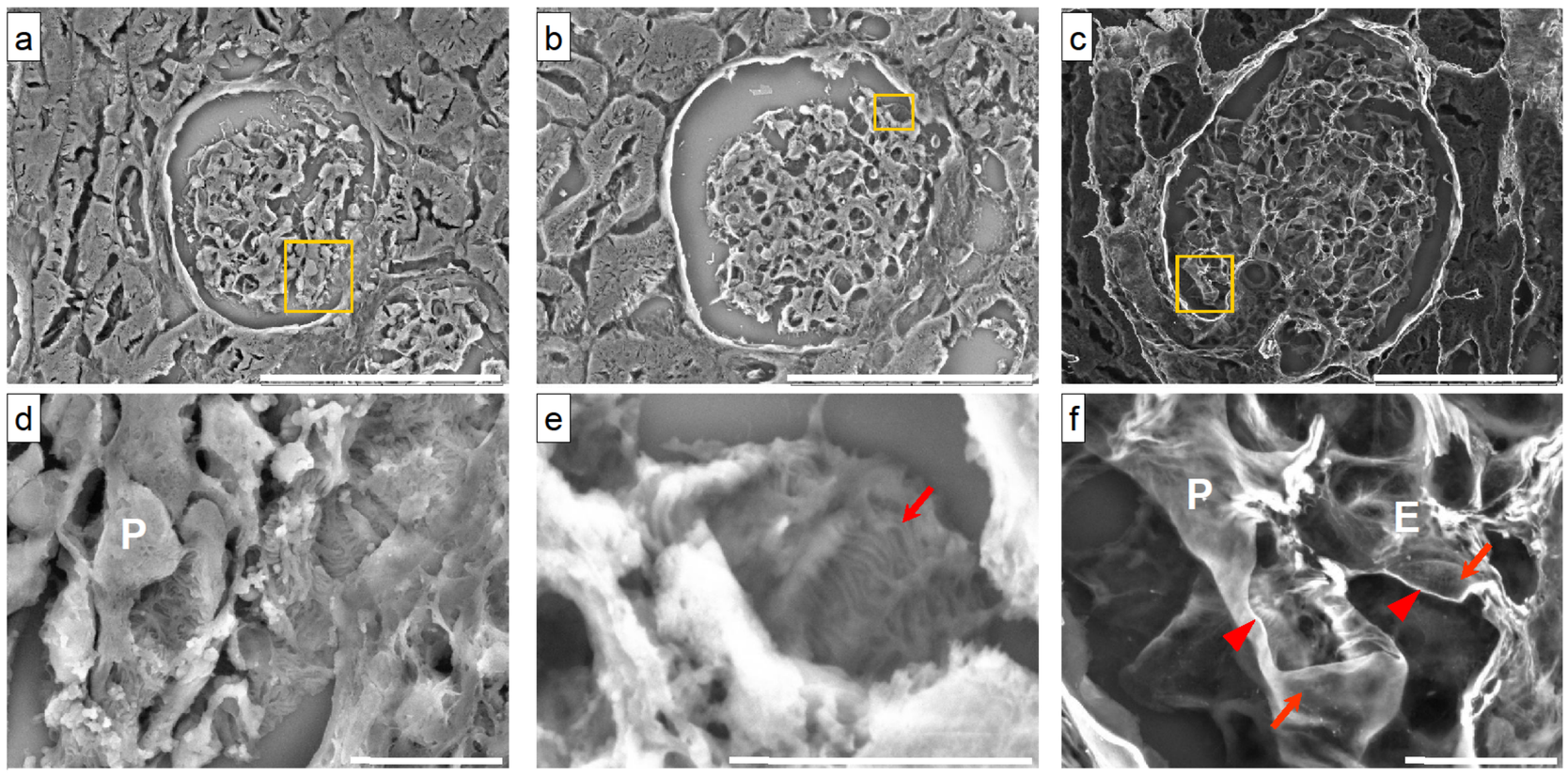

Fig. 2. LVSEM images of Pt-blue- or PAM-stained glomeruli in thin basement membrane disease.
a, b, d, e) Pt-blue-stained glomeruli in case TBMN-4. d, e) Higher-magnification images of the square shown in Fig. 2a or 2b, respectively. c, f) PAM-stained glomerulus in case TBMN-5. f) A Higher-magnification image of the square shown in Fig. 2c.
a, b, d, e) The surface view of podocytes and their foot processes are observed from the Bowman's space. Podocytes show normal shaped cell bodies and foot processes (a, d) showing normal interdigitation (e: arrow). c, f) GBMs can be distinctly visible as thin and sheet-like appearances through the thinner parts of podocytes or endothelial cells (arrows). GBMs exhibit thin linear patterns at the cut side view (arrowheads).
P: podocyte, E: endothelial cell. Bars: 100 μm (a, b, c), 10 μm (d, e, f)

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