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【 CASE REPORT 】

Slowly Progressive Male Alport Syndrome Evaluated by Serial Biopsy: Importance of Type IV Collagen Staining

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Abstract:
A slowly progressive middle-aged man initially diagnosed with thin basement membrane nephropathy based on extensive thinning of the glomerular basement membrane (GBM) was subsequently diagnosed with Alport syndrome (AS) by a serial renal biopsy eight years later. The ultrastructural analysis of the second biopsy indicated thickening and wrinkling with mild reticulation in the GBM, consistent with AS. However, a retrospective analysis of the first biopsy revealed mild attenuation of type IV collagen α5 chain staining, suggesting a potential diagnosis of AS, despite the lack of ultrastructural features of AS. We herein report the clinical usefulness of type IV collagen staining in the early diagnosis of AS.

Key words: Alport syndrome, renal biopsy, thin basement membrane nephropathy, type IV collagen

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Introduction

Alport syndrome (AS) is a progressive hereditary glomerular disease that results in end-stage renal disease. AS can be associated with hearing loss and eye lesions (1). In contrast to AS, thin basement membrane nephropathy (TBMN) is a benign disease that does not result in an impaired renal function. Therefore, AS should be differentiated from TBMN in practice (2).

Two types of AS have been identified: X-linked AS (XLAS), caused by a mutation on the type IV collagen α5 chain (COL4A5); and autosomal recessive AS (ARAS) or autosomal dominant AS (ADAS), caused by a mutation on the type IV collagen α3 (COL4A3) or α4 chain (COL4A4). Each type of AS has a different prognosis, with a slower disease progression for ADAS than XLAS in men and than ARAS regardless of sex. By comparison, TBMN is defined by thinning observed in ≥50% of glomerular basement membranes (GBMs), with the absence of any GBM thickening, wrinkling, reticulation and lamellation (3). Although TBMN generally has a good prognosis, poor prognostic outcomes have recently been reported for cases originally assumed to be TBMN (4). These cases are often diagnosed later as ADAS (5). As such, the early differentiation of TBMN from AS is important but often not straightforward.

We herein report a patient in whom the observation of thinning in the GBM led to a diagnosis of TBMN on a first renal biopsy. However, a subsequent renal biopsy, performed due to exacerbation of urinary findings over a period of eight years, confirmed a diagnosis of AS based on ultrastructural abnormalities in the GBM. Retrospective analyses of the first biopsy specimen, including type IV collagen staining, led to an early diagnosis of AS. Based on our experience, we propose that type IV collagen staining might be useful for the early diagnosis of atypical AS.

Case Report

The patient was a 49-year-old Japanese man. His mother

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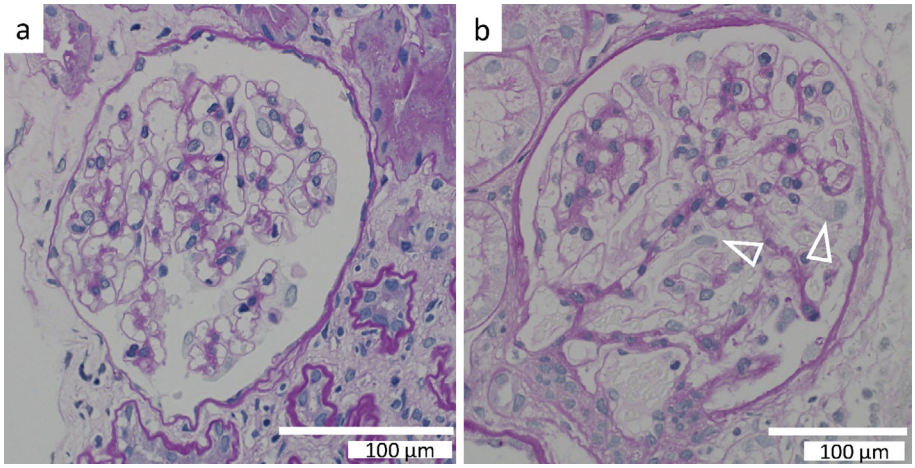

Figure 1. Light microscopy findings for the first (a) and second (b) renal biopsy specimens. (a) No proliferative or sclerotic changes are observed in the glomeruli (PAS stain, ×400). (b) The glomeruli are slightly hypertrophic with swelling of the podocytes (arrowhead) (PAS stain, ×400).

had childhood hearing loss, but no hematuria and no renal dysfunction were identified. His older brother had had hematuria since childhood and a decreased renal function [an estimated glomerular filtration rate (eGFR) 15 mL/min/1.73 m^2] without hearing loss or fleck retinopathy. The patient had experienced hematuria since childhood, with proteinuria identified at 31 years old. Both hematuria and proteinuria persisted thereafter, and the patient was referred to our department at 41 years old.

A clinical examination revealed proteinuria (0.46 g/gCr), hematuria, and eGFR of 74.1 mL/min/1.73 m^2 with hypertension and hyperuricemia. TBMN was diagnosed based on the findings of the first renal biopsy (Fig. 1a, 2a, b). After the first biopsy, several anti-hypertensive agents and diuretics were administered, and urate-lowering agents and lipid lowering agents were added (Supplementary material). At 48 years old, urinary protein increased (2 g/gCr), and the eGFR decreased (50.5 mL/min/1.73 m^2). The second renal biopsy was performed at 49 years old, leading to a revised diagnosis of AS (Fig. 1b, 2c, d).

Findings on the first renal biopsy

On light microscopy, 5 of 25 glomeruli showed global sclerosis, and 1 had adhesions (Fig. 1a). Electron microscopy findings were suggestive of TBMN, namely GBM thinning and no evidence of AS, such as thickening of the GBM (Fig. 2a, b).

Findings on the second renal biopsy

On light microscopy, global sclerosis in 1 out of 10 glomeruli, 1 periglomerular fibrosis, and adhesions were observed. The glomeruli were slightly hypertrophic, with swelling of the podocytes (Fig. 1b), and showed mild interstitial fibrosis and tubular atrophy. Although GBM thinning was the main presenting feature on electron microscopy, thickening, wrinkling, collapse (Fig. 2c), and irregularity (Fig. 2d) of the lamella densa in the GBM, findings suggestive of AS, were observed.

Type IV collagen staining

Retrospective analyses of the first biopsy specimen were performed. Type IV collagen staining revealed partial attenuation of type IV collagen α5 chain [α5(IV)] in the GBM, consistent with AS (Fig. 3d), with α2(IV) staining restricted to the mesangial region (Fig. 3e). In the second renal biopsy specimen, attenuation of α5(IV) staining in the GBM was more noticeable (Fig. 3g), and α2(IV) staining was positive along the GBM in addition to the mesangial region (Fig. 3h) compared with the first biopsy (Fig. 3d, e).

Genetic analyses

We performed genetic analyses via next-generation sequencing using targeting hybrid-capture methods that covered all of the exon and the adjacent introns within 10 bases. However, no mutations were identified in either *COL 4A3*, *COL4A4*, or *COL4A5*.

Discussion

We herein report a slowly progressive man with AS evaluated by a serial biopsy. Although ultrastructural features of AS were not observed on the first biopsy, attenuation of α5 (IV) staining in the GBM was observed, indicating a diagnosis of AS. Our findings underscore the potential utility of type IV collagen staining when AS is suspected in cases of TBMN.

No mutation was identified in either *COL4A3*, *COL4A4*, or *COL4A5*. For XLAS, approximately 10% of patients with no identified mutation have been described in the literature (6). There is a possibility of deep intronic mutations (7). Based on the histological findings of electronic microscopy and type IV collagen staining on the second biopsy, the patient had a potential diagnosis of XLAS with a mild genotype but not with a truncating mutation. However,

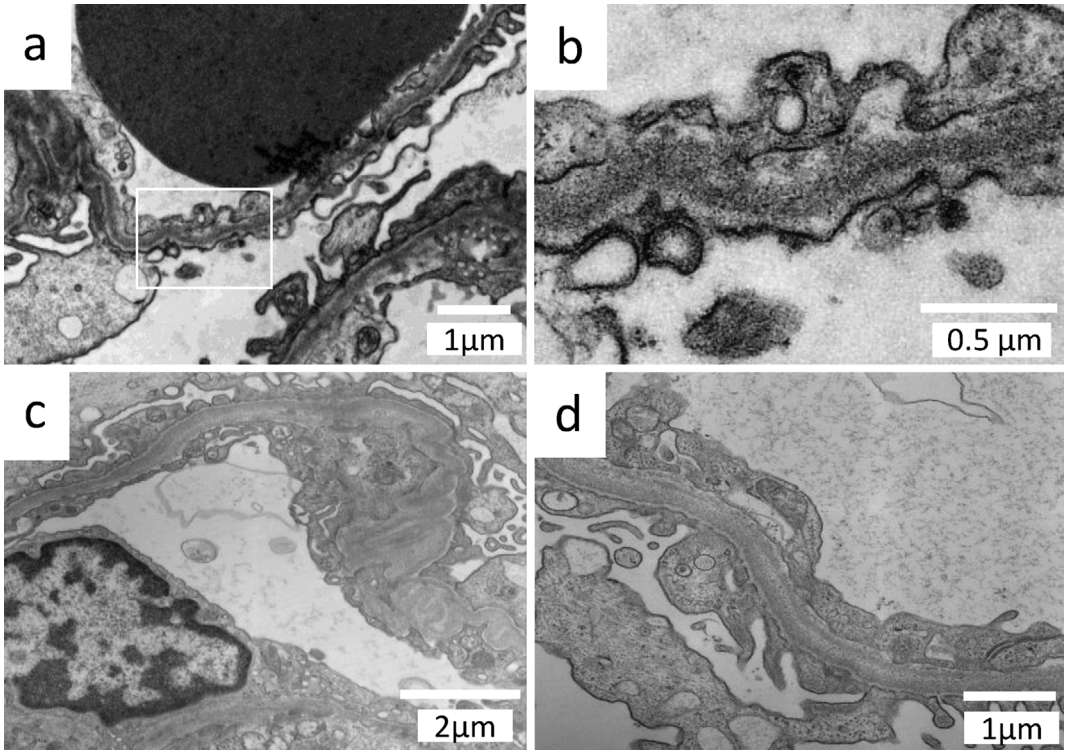

Figure 2. Electron micrographs of the first (a, b) and second (c, d) renal biopsy specimens. (a) Diffuse thinning of the glomerular basement membrane (GBM) is observed (×5,000). (b) High-power field image of the square in a. (c) Irregular thinning, thickening, wrinkling, and collapse in the GBM are observed (×12,000). (d) Irregular lamella densa in the GBM is visible (×30,000).

we were unable to exclude the possibility of ADAS.

One of the diagnostic criteria for AS is an abnormality in type IV collagen staining. In contrast, TBMN is normally diagnosed by thinning of the GBM, without the assessment of type IV collagen staining. In our case, the initial diagnosis of TBMN was based on the ultrastructural features of thinning GBM without thickening, wrinkling, reticulation, or lamellation. Type IV collagen staining on the first biopsy specimen indicated that the α5(IV) expression was reduced without any apparent increase in α2(IV) expression in the GBM, showing a partial mosaic pattern (Fig. 3f). The extent of these changes was more marked on the second biopsy (Fig. 3i). Based on our findings, we speculate that abnormality in type IV collagen staining appears earlier than abnormalities in ultrastructural features of the GBM.

The missed diagnosis of AS might have resulted from various factors. TBMN is diagnosed based on an observation of thinning in 50% or more of the GBM, as revealed on electron microscopy, but with no thickening, wrinkling, reticulation, nor lamellation in the GBM (3). However, the presence of diffuse GBM thinning is the only finding in 10-20% of patients with AS, particularly in children (8). Notably, focal thinning and mild irregularity of the GBM are the only findings observed in a six-week-old XLAS model mouse with a *COL4A5* mutation R471 X (9). Based on these previous reports, TBMN may simply be the clinical presentation of early stages of AS with slower disease progression. Our observation of attenuation of α5(IV) staining without an apparent compensatory increase in α2(IV) staining in the GBM on the first renal biopsy might be consistent with the ultrastructural findings of thinning of the GBM in the early stages of AS (8). Pathologically, in AS, a decrease in the α5(IV) expression initially leads to thinning of the GBM, with a subsequent compensatory increase in α1(IV) and α2(IV), which are normally only expressed in the GBM during the embryonic period. This change might result in thickening, wrinkling, reticulation, and lamellation in the GBM. The slowly progressive AS in our case, described by a serial biopsy, is consistent with previous reports (3, 8).

Generally, in XLAS, patients with negative staining of α5 (IV) are considered to be typical, whereas patients with normal or attenuated staining of α5(IV) are thought to be atypical. Among a cohort of 25 men with XLAS, negative α5 (IV) staining was reported in 40% (10). Staining of α5(IV) in the GBM is typically negative in men with XLAS and all patients with ARAS (5), as the α3-4-5 collagen network does not form. However, even in cases of AS, there are some patients whose α5(IV) expression is partially reduced. In these cases, we should consider three possible patterns of AS when making a diagnosis. The first is the pattern in women with XLAS with a normal or reduced expression of α5(IV) due to an X-linked inactivation mechanism (11). The second is the pattern in men with XLAS, in whom α5(IV) is incompletely produced due to a substitution of *COL4A5*, as well as ARAS patients with a substitution of *COL4A3* or *COL4A4*. Notably, positive α5(IV) staining has been identi-

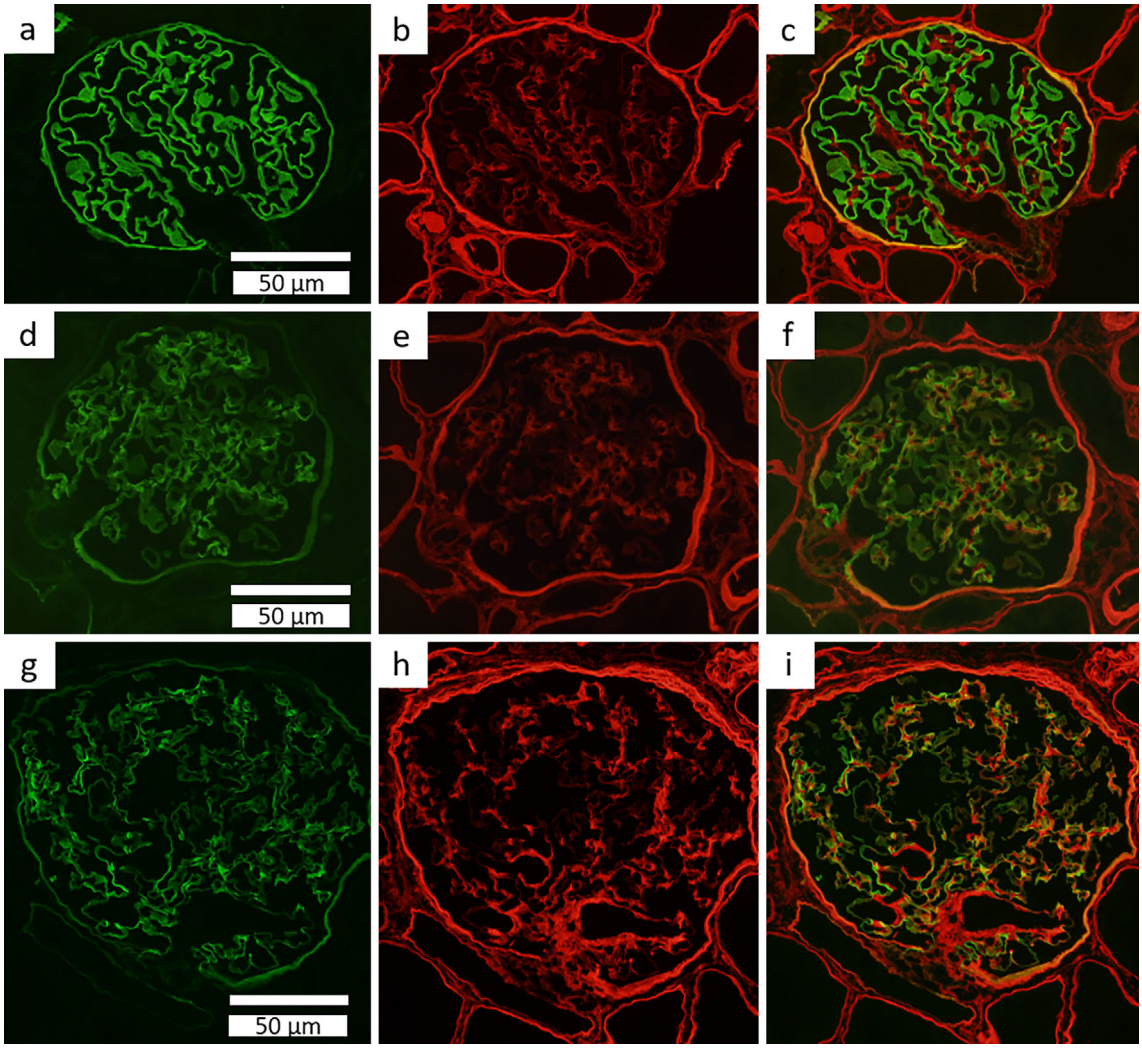

Figure 3. **Immunohistochemical staining of type IV collagen. Type IV collagen staining in the control (a-c), in the first biopsy specimen (d-f), and in the second biopsy specimen (g-i); α5(IV) staining (green) (a, d, g), α2(IV) staining (red) (b, e, h), and merged images for α5(IV) and α2(IV) (c, f, i) (original magnification, ×200). In a control kidney, α5(IV) shows positive staining in the glomerular basement membrane (GBM) (a), and α2(IV) shows positive staining in the mesangial region (b). In the first biopsy specimen, α5(IV) shows partially reduced staining in the GBM (d), while α2(IV) shows positive staining in mesangial region without an apparent increase in the GBM (e). In the second biopsy specimen, glomeruli were slightly hypertrophic (g-i). α5(IV) shows partially reduced staining in the GBM, which is more obvious than at the first biopsy (g), with partially positive α2(IV) staining in the GBM (h). In the first biopsy specimen, the merged image reveals a partial mosaic pattern in the GBM (f). In the second biopsy specimen, the merged image clearly reveals a mosaic pattern in the GBM (i).**

fied in ≥20% of men with XLAS who exhibit a mild disease phenotype (12). Another study reported a reduced α5(IV) expression in a patient with ARAS with a homozygous *COL 4A4* substitution who successfully delivered a child (13). The third is the pattern in patients with ADAS, in whom the α5(IV) expression reduced. Previous reports have indicated that α5(IV) is normal in patients with ADAS because one allele has a normal α3(IV) or α4(IV) expression (5). However, we recently reported a case in which α5(IV) was reduced due to a heterozygous *COL4A4* nonsense mutation (14). The formation of a network with sufficient type IV collagen requires α3-4-5 to maintain the function of the GBM. If the expression of α3(IV) or α4(IV) decreases, the expression of α5(IV) will inevitably decrease as well. Although no mutation was identified, our patient may have had XLAS with a substitution or ADAS, based on his family history. Furthermore, partial attenuation of the α5(IV) expression in Bowman's capsule was observed in our patient (Fig. 2d, f, g, i). Since the α5(IV) expression in Bowman's capsule is preserved in ADAS (14), our patient was presumed to have had XLAS with a substitution of *COL4A5*.

In conclusion, type IV collagen staining might be very useful for the early diagnosis of AS, especially in cases with GBM abnormalities for whom AS is a possibility.

Author's disclosure of potential Conflicts of Interest (COI).
Toshio Mochizuki: Honoraria, Otsuka Pharmaceutical.

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