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X-linked Alport Syndrome with Type IV Collagen *α*5 Chain Staining Revealing Normal Expression in the Glomerular Basement Membrane and Negative on Bowman's Capsule and Distal Tubular Basement Membrane: A Case Report

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X-linked Alport syndrome is a hereditary progressive renal disease resulting from the disruption of collagen *α*3*α*4*α*5 (IV) heterotrimerization caused by pathogenic variants in the *COL4A5* gene. This study aimed to report a male case of X-linked Alport syndrome with a mild phenotype accompanied by an atypical expression pattern of type IV collagen *α*5 [*α*5 (IV)] chain in glomerulus. A 38-year-old male presented with proteinuria (2.3 g/day) and hematuria. He has been detected urinary protein and occult blood since childhood. A renal biopsy was performed at the age of 29 years; however, a diagnosis of Alport syndrome was not considered. A renal biopsy 9 years later revealed diffuse thinning and lamellation of the glomerular basement membrane. A staining for *α*5 (IV) revealed a normal expression pattern in the glomerular basement membrane and a complete negative expression in Bowman's capsule and distal tubular basement membrane. Using next-generation sequencing, we detected a *COL4A5* missense variant within exon 35 (NM_000495.5: c.3088G>A, p. G1030S). The possibility of X-linked Alport syndrome should be considered when negative expression of *α*5 (IV) staining on Bowman's capsule was observed.

Keywords: Bowmans's capsules; distal tubular basement membrane; glomerular basement membrane; type IV collagen *α*5; X-linked Alport syndrome
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Introduction

Alport syndrome (AS) is an inherited progressive renal disease accompanied by sensorineural hearing loss and ocular abnormalities (Nozu et al. 2019). X-linked Alport syndrome (XLAS) constitutes approximately 80-85% of AS cases. It is caused by pathogenic variants in the *COL4A5* gene on chromosome Xq26-48, which encodes the type IV collagen *α*5 [*α*5 (IV)] chain (Kashtan 1998; Nozu et al. 2019). Ninety percent of XLAS male patients develop end-stage renal disease by the age of 40 years (Jais et al. 2003). However, milder phenotypes have recently been recognized (Demosthenous et al. 2012; Hashimura et al. 2014; Kamura et al. 2020). Herein, we report a case of a male patient with XLAS with a mild phenotype accompanied by an atypical expression pattern of *α*5 (IV) in glomerulus.

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Case Presentation

A 38-year-old man presented with hematuria and proteinuria. The family history revealed that his mother and grandmother had also hematuria and proteinuria. His children had no abnormal urinary findings. There was no family history of end-stage renal disease or hearing loss. The patient had no allergies and received no medication, and had a medical history of mixed mild hearing loss (25-30 dB) and pneumothorax. The patient had no ophthalmic diseases. He has been detected urinary protein and occult blood since childhood. He underwent a renal biopsy at the age of 29 years and was diagnosed with minor glomerular abnormalities. He underwent no specific treatment. Thereafter, the patient was referred to our hospital because the hematuria and proteinuria persisted. Urinalysis revealed grade 3+ proteinuria, a urine protein level of 2.3 g/day, and hematuria (sediment red blood cells, 30-49 per high-power field). Blood tests revealed blood urea nitrogen of 15.3 mg/dL, creatinine of 0.87 mg/dL, total protein of 7.4 g/dL, and

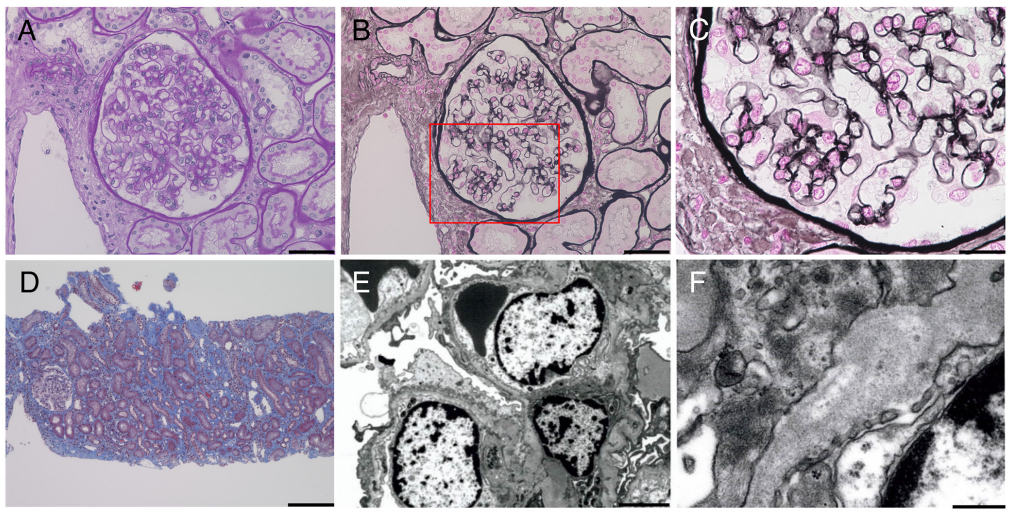

Fig. 1. Renal biopsy specimen by light microscopy.
(A) Several glomeruli revealed mild mesangial proliferation. Periodic acid-methenamine silver staining (400 × magnification, scale bar is 50 μm). (B), (C) The glomerular basement membrane (GBM) revealed partial irregular thickening. Periodic acid-methenamine silver staining (B, 400 × magnification, scale bar is 50 μm; C, 1,000 × magnification, scale bars is 50 μm). (D) Diffuse interstitial nephritis and interstitial fibrosis were identified. Masson trichrome staining (100 × magnification, scale bars is 200 μm). (E), (F) Electron microscopy revealed diffuse thinning and lamellation of the GBM. There were no immune complex deposits (E, 4,000 × magnification, scale bar is 30 μm; F, 30,000 × magnification, scale bar is 4 μm).

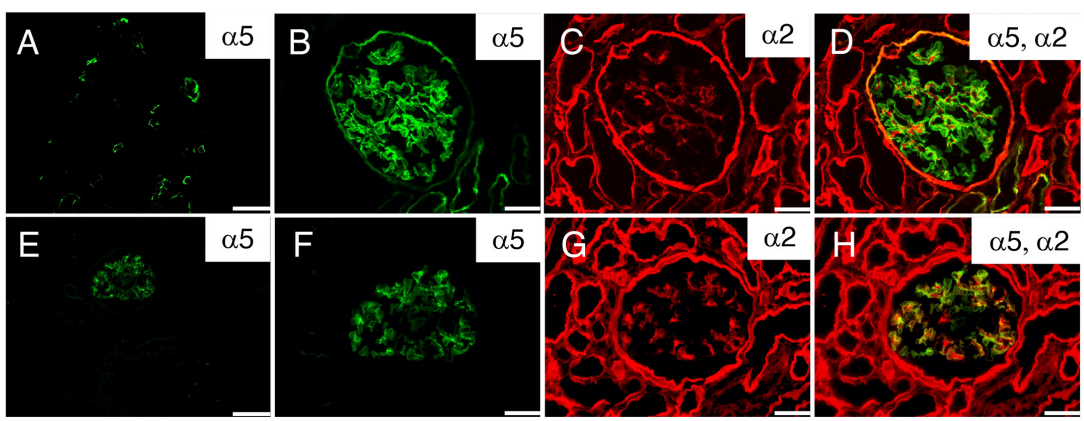

Fig. 2. Immunohistochemical staining of type IV collagen α5 [α5 (IV)] and α2 chains [α2 (IV)] in the control kidney (A-D) and this patient (E-H) (A, E, 100 × magnification, scales bars are 100 μm; B, C, D, F, G, H, 400 × magnification, scale bars are 50 μm).
In the control kidneys, α5 (IV) staining was positive expression in the glomerular basement membrane (GBM), Bowman's capsule (BC), and distal tubular basement membrane (dTBM) (A, B), and α2 (IV) staining was positive in the mesangial region, BC, and TBM (C, D). In this case, α5 (IV) staining revealed a normal expression pattern in the GBM and a complete negative expression in BC and dTBM (E, F), while α2 (IV) staining were positive in the mesangial region, BC, and TBM (G). The merged image revealed a normal expression pattern in the GBM (H).

albumin of 4.2 g/dL.

A renal biopsy was performed. There were 15 glomeruli, seven of which were globally sclerotic. Several glomeruli showed mild mesangial proliferation (Fig. 1A). The glomerular basement membrane (GBM) showed partial irregular thickening (Fig. 1B, C). Diffuse interstitial nephritis and interstitial fibrosis were also identified (Fig. 1D). Immunofluorescence staining revealed granular mesangial IgM deposits. Electron microscopy revealed diffuse thinning and lamellation of the GBM (Fig. 1E, F). No immune-complex deposits were observed. Based on these findings, we suspected AS and peformed immunohistochemical staining of α5 (IV) and type IV collagen α2 chains [α2 (IV)]. Fig. 2 shows the immunohistochemical distribution of α5 (IV) and α2 (IV) in the control kidney (Fig. 2A-D) and this case (Fig. 2E-H). In the control kidney, α5 (IV) staining was positive expression in the GBM, Bowman's capsule (BC), and distal tubular basement membrane (dTBM) (Fig. 2A, B), and α2 (IV) staining was positive in the mesangial region, BC, and TBM (Fig. 2C, D). In this case, α5 (IV) staining revealed a normal expression pattern in the GBM and a complete negative expression in BC and dTBM (Fig. 2E, F), while α2 (IV) staining were positive in the mesangial region, BC, and TBM (Fig. 2G). The merged image revealed a normal expression pattern in the GBM (Fig. 2H). Using next-generation sequencing, we detected a *COL4A5* missense variant within exon 35 (NM_000495.5: c.3088G>A, p. G1030S) (Fig. 3).

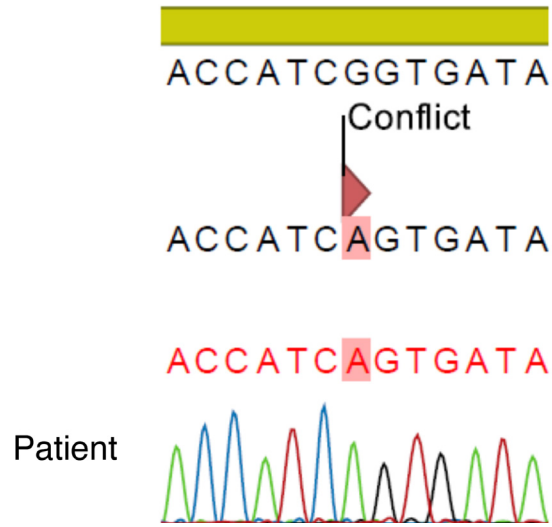

Fig. 3. Next-generation sequencing confirmed a *COL4A5* missense variant within exon 35 (NM_000495.5: c.3088G>A, p. G1030S).

Discussion

Herein, we report a male patient with XLAS complaining of hematuria and proteinuria who was diagnosed by a second renal biopsy and genetic testing. Type IV collagen is composed of six chains, α1 to α6, three of which form a triple helical structure through associations between their carboxy-terminal NC1 domains, accompanied by folding of the collagenous domains into triple helices: α1α1α2 (IV),

Table 1. The summarized table of mutations of X-linked Alport syndrome patients with positive type IV collagen α5 [α5 (IV)] chain expression mentioned in this report.

Authors	Exon	Function	Nucleotide	Amino acid change	α5 (IV) expression in kidney tissue
Hashimura et al. (2014)	12	Non-truncating	c.679G>A	p.G227S	normal
Hashimura et al. (2014)	16	Non-truncating	c.893G>A	p.G298D	reduced
Hashimura et al. (2014)	25	Non-truncating	c.1781G>A	p.G594D	normal
Hashimura et al. (2014)	16	Non-truncating	c.929G>A	p.G310E	reduced
Hashimura et al. (2014)	23	Non-truncating	c.1526G>A	p.G509D	reduced
Hashimura et al. (2014)	38	Non-truncating	c.3410G>A	p.G1137D	reduced
Hashimura et al. (2014)	7	Non-truncating	c.414G>A	p.G138D	reduced
Hashimura et al. (2014)	41	Non-truncating	c.3731C>T	p.G1244D	reduced
Hashimura et al. (2014)	17	Non-truncating	c.955G>A	p.G319A	reduced
Hashimura et al. (2014)	21	Non-truncating	c.1347_1355 del 9 bp	9 bp del	normal
Hashimura et al. (2014)	19	Non-truncating	c.1066_1101 del 36 bp	36 bp del	normal
Hashimura et al. (2014)	2-8	Non-truncating	exon 2-8 del	384 bp del	reduced
Hashimura et al. (2014)	9	Non-truncating	c.546+2_3 InsT	81 bp del (exon 9 skip)	normal
Hashimura et al. (2014)	44	Truncating	c.3809-2A>T	144 bp del (exon 44 skip)	mosaic
Hashimura et al. (2014)	25	Non-truncating	c.1912G>A	p.G638S	mosaic
Kamura et al. (2020)	30	Non-truncating	c.2413G>A	p.G805A	positive (NA)
Martin et al. (1998)	35	Non-truncating	c.3088G>A	p.G1030S	NA
Our case	35	Non-truncating	c.3088G>A	p.G1030S	normal

del, deletion; Ins, insertion; NA, not available.

α3α4α5 (IV), and *α5α5α6* (IV) (Kashtan 2000; Hudson 2004). *α3α4α5* (IV) was present in the GBM, BC, and dTBM, while *α5α5α6* (IV) was present in the BC and dTBM. In XLAS patients, mutations in the *α5* (IV) chain, *α3α4α5* (IV), and *α5α5α6* (IV) networks are broken depending on the amino acid type and substitution position (Hudson 2004). XLAS male patients typically show a complete absence of *α5* (IV) in the GBM (Kashtan and Michael 1996). However, over 20% of XLAS male patients demonstrate *α5* (IV) expression, which is associated with a mild renal course (Hashimura et al. 2014). To date, over 1,000 different *COL4A5* gene variants have been identified in the Human Gene Mutation Database. Missense varians are the most common type of *COL4A5* gene varians, which often result in the substitution of glycine with a larger or more highly charged amino acid (International Alport Mutation Consoritium et al. 2014). A point mutation, such as a glycine substitution within the collagenous domain, does not affect the construction of the NC1 domain (Hashimura et al. 2014). Male XLAS patients with *α5* (IV) positivity possessed non-truncating variants or somatic mosaic variants, which were more likely to be located in exons 1-25 (Hashimura et al. 2014). This suggests that missense variants before exon 25 are less likely to break the triple helical structure. To the best of our knowledge, only one case of a *COL4A5* missense variant within exon 35 (G1030S) has been previously reported (Martin et al. 1998). However, *α5* (IV) protein expression, as was found in this case, has not been investigated.

We summarized the table of mutations of XLAS patients with positive *α5* (IV) chain expression mentioned in our report (Table 1). We identified a normal expression pattern of *α5* (IV) in the GBM and a complete negative expression in BC and dTBM. There are several possible reasons for this finding. First, G1030S may disrupt the structure of the *α5α5α6* (IV) network but not that of the *α3α4α5* (IV) network. Some missense variants may affect the structure of the *α3α4α5* triple helical network, but its degradation is slow, resulting in reduced amounts of the *α3α4α5* (IV) network or abnormal *α3α4α5* (IV) network formation (Kashtan 2000). Second, there may be mutations in *α6* (IV) in addition to G1030S. Mutations in both *α5* (IV) and *α6* (IV) may lead to a complete lack of the *α5α5α6* (IV) network. The *COL4A6* gene is paired with *COL4A5* head-to-head, and located on chromosome Xq22.3, which encodes the *α6* (IV) chain and is expressed in BC, epidermis, and smooth muscle (Zhou et al. 2021). Mutations in *α6* (IV) alone do not appear to cause AS, except in those with diffuse leiomyomatosis (Kashtan 2000; Nozu et al. 2017). AS-diffuse leiomyomatosis has been reported in approximately 30 families (Zhou et al. 2021). However, this patient had no clinical signs of leiomyomatosis, such as dysphagia, vomiting, retrosternal pain, dyspnea, or cough (Kashtan 2000). Further genetic testing of *COL4A6* is required. Third, we should consider the possibility of male XLAS with somatic mosaicism, which was rare (Bu et al. 2019).

In conclusion, we should consider the possibility of XLAS when negative expression of *α5* (IV) staining on BC was observed.

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Conflict of Interest

The authors declare no conflict of interest.

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