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Clinical Report

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The variable course of women with X-linked Alport Syndrome

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Abstract
X-linked Alport syndrome (XLAS) arises from mutations in the *COL4A5* gene encoding the α5-chain of type IV collagen and is associated with hematuria, ocular abnormalities and high-tone sensorineural hearing loss. Nearly all affected males have decreased kidney function resulting in end-stage renal disease (ESRD) as early as the second decade of life. It was long thought that affected females had a benign outcome; however, in recent decades, it has become quite clear that they too are at risk for developing nephrotic syndrome, decreased kidney function and ESRD. We report two young females presenting with microscopic hematuria and proteinuria diagnosed with XLAS on renal biopsy. Both developed nephrotic-range proteinuria and progressive renal insufficiency. Additionally, both developed extra-renal manifestations of XLAS. The ultrastructural and immunofluorescence features on kidney biopsy were instrumental in making the diagnosis of heterozygous XLAS as neither patient had a family history of AS.

Keywords: end-stage renal disease; nephrotic syndrome; X-linked Alport syndrome

Introduction

X-linked Alport syndrome (XLAS) arises from mutations in the *COL4A5* gene encoding the α5-chain of type IV collagen (IV) and accounts for ∼80% of families with AS. Features include microscopic hematuria, anterior lenticonus, high-tone sensorineural deafness and progressive renal insufficiency leading to end-stage renal disease (ESRD) [1, 2]. In his initial description of AS in 1927, Alport noted that the 'male members of a family tend to develop nephritis and deafness and do not as a rule survive', while 'the females have deafness and hematuria and live to old age' [3]. Since then it has become evident that heterozygous XLAS females are, in fact, not 'benign carriers', but rather have widely variable disease outcomes and are at risk of developing nephrotic syndrome, decreased kidney function, and can progress to ESRD [4–6]. In addition to an unpredictable clinical course, the kidney biopsy specimens of heterozygous XLAS females often have widely variable appearances.

The ultrastructural features on kidney biopsy that are diagnostic of AS consist of (i) variable thickening and thinning of the glomerular basement membrane (GBM); (ii) splitting or lamellation of the GBM; (iii) 'basket weaving' of the GBM and (iv) foot process fusion in regions of an abnormal GBM [1, 2]. In males with XLAS, these changes become more prominent with increasing age, while in females the extent and impact of aging on GBM thickening are variable and unpredictable [4, 7, 8]. Furthermore, the earliest ultrastructural finding in AS is diffuse thinning of the GBM, and this can result in girls or women being misdiagnosed with thin basement membrane nephropathy (TBMN) [2, 6].

The use of immunohistochemistry is additionally helpful in diagnosing XLAS [1, 2, 6] as immunostaining for α3(IV), α4(IV) and α5(IV) collagen demonstrates the complete absence of these collagen chains in the GBM, distal tubular basement membrane (dTBM) and Bowman's capsule in essentially all males with XLAS, whereas women who are heterozygous carriers of XLAS demonstrate a segmental or 'mosaic' absence due to variable X-chromosome inactivation [9]. These immunohistologic features help to distinguish XLAS from autosomal-recessive AS (ARAS), where expression of α5(IV) collagen by immunostaining is negative in the GBM but positive in the dTBM and Bowman's capsule. While genetic testing can also aid in the diagnosis of XLAS, it can be quite expensive and is not always readily available, and thus, the renal biopsy findings become essential in diagnosing XLAS.

We report two young females who presented with microscopic hematuria and proteinuria and developed nephrotic-range proteinuria and progressive renal insufficiency. At presentation, neither patient had a family history of AS or extra-renal manifestations of XLAS. Renal biopsy led to the diagnosis of XLAS based on the ultrastructural and immunofluorescence (IF) features.

Case Report 1

Clinical history and initial laboratory data

A 25-year-old white female presented in 2006 at age 19 with nephrotic syndrome. Her serum creatinine (SCr) was 0.9 mg/dL (79.6 μmol/L) with an estimated glomerular

filtration rate (eGFR) [10] of 74 mL/min/1.73 m^2, serum albumin (SAlb) was 2.9 g/dL (29 g/L) and a urinalysis had 4+ protein and large blood by dipstick. A 24-h urine protein was 6.4 g/day. She had no history of gross hematuria, hearing difficulty or visual changes. Family history was negative for proteinuria, renal failure or AS, but was positive for microscopic hematuria in her maternal grandmother and aunt. A kidney biopsy was performed for the evaluation of nephrotic syndrome and microscopic hematuria.

Kidney biopsy

Light microscopy (LM) contained six glomeruli, one of which was globally sclerotic. The remaining glomeruli had preserved architecture with delicate basement membranes, patent glomerular capillaries and normal mesangial matrix and cellularity (Figure 1A). Tubular atrophy and interstitial fibrosis involved 5% of the cortex, and interstitial foams cells (Figure 1B) affected 10–15% of the cortex. IF demonstrated weak mesangial staining for IgM (trace–1+) and C3 (trace). The tissue was stained with antibodies to the alpha 1, 3 and 5 chains of type IV collagen. This revealed 1–2+ segmental/mosaic staining of the GBM with the alpha 3 chain of type IV collagen (Figure 2). Staining for the alpha 5 chain of type IV collagen also showed 1+ segmental/mosaic staining of the GBM (Figure 3). There was no staining of the dTBM for either the alpha 3 or the alpha 5 chains of type IV collagen. Electron microscopy (EM) demonstrated diffusely thinned GBMs, measuring 124 nm in average thickness (Figure 4), but there was no lamellation or splitting. Segmental foot process effacement involved 50% of the total glomerular capillary surface area.

Diagnosis

Heterozygous XLAS was diagnosed based on the ultrastructural findings and the segmental/mosaic IF staining of the GBM and Bowman's capsule and the lack of staining of the dTBM for the alpha 3 and alpha 5 chains of type IV collagen.

Clinical follow-up

An angiotensin-converting enzyme inhibitor (ACEi) was started after the biopsy. In 2012, her SCr rose to 1.6 mg/dL (141.4 μmol/L) (eGFR—38 mL/min/1.73 m^2), and she remained nephrotic (urine protein creatinine ratio 3.7 g/g) with microscopic hematuria. Additionally, she developed

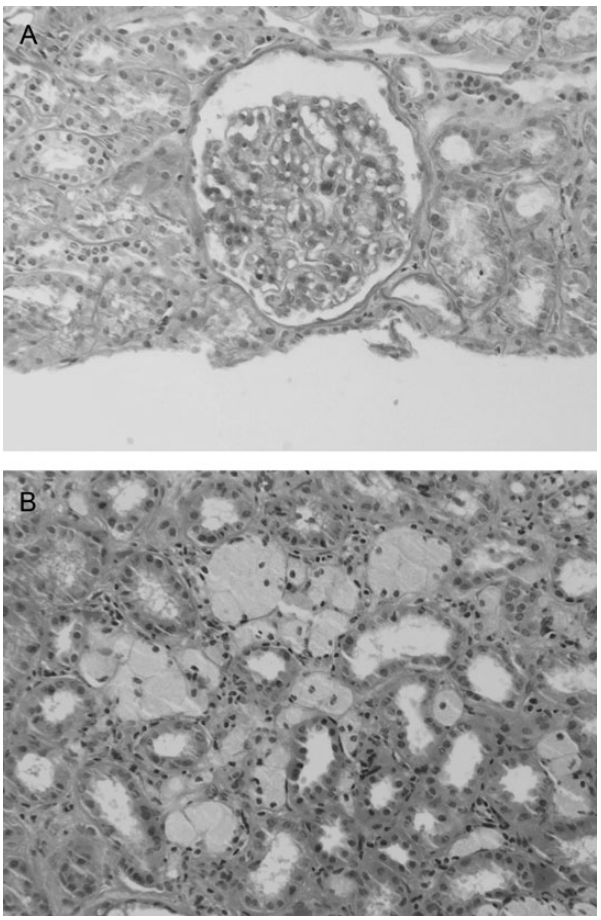

Fig. 1. (**A**) Glomeruli appear unremarkable with normal mesangial matrix and cellularity, open capillary lumina and the glomerular capillary walls appear normal in thickness, texture and contour (Periodic acid-Schiff stain; original magnification, ×80). (**B**) The interstitium is remarkable for foam cells, which appear as isolated cells and in aggregates (hematoxylin and eosin stain; original magnification, ×80). All images are from Case Report 1.

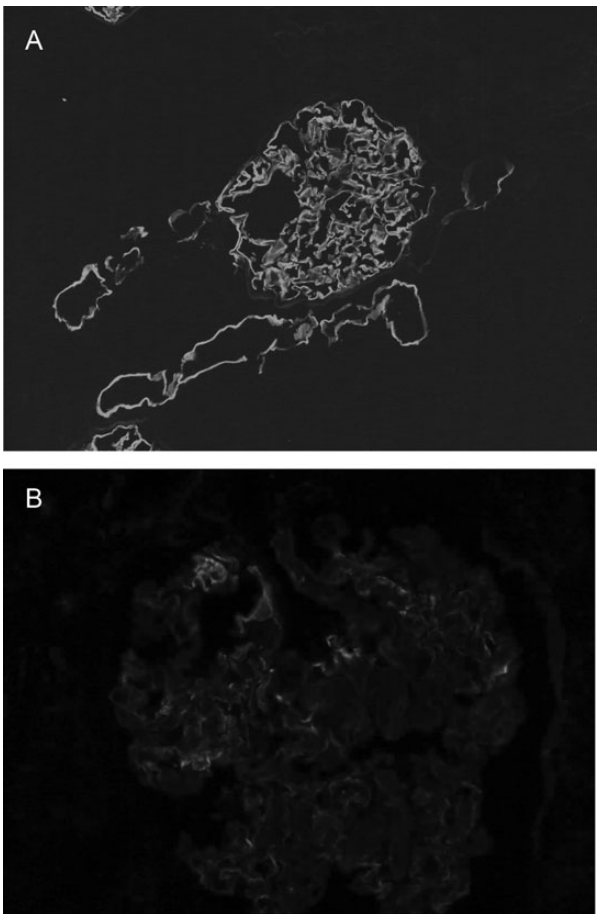

Fig. 2. (**A**) IF micrograph showing staining of normal tissue (positive control) for the alpha 3 chain of type IV collagen. There is linear staining of the entire GBMs and dTBMs. (**B**) On high power, a glomerulus displays weak and segmental positivity in the GBMs for the alpha 3 chain of collagen IV and loss of staining in the dTBM. This image is from Case Report 1.

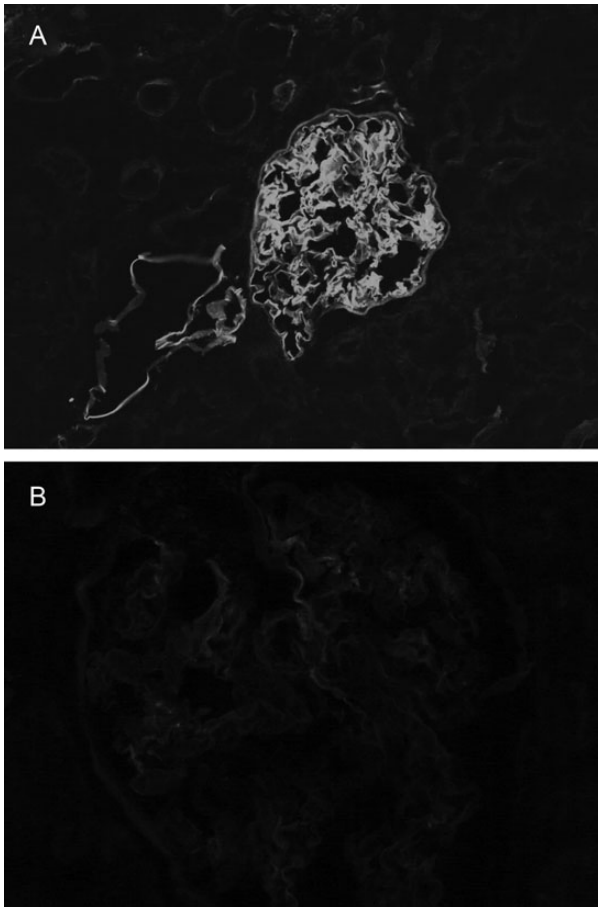

Fig. 3. (**A**) IF micrograph showing staining of normal tissue (positive control) for the alpha 5 chain of type IV collagen. Similar to alpha 3, there is linear staining of the entire GBMs and dTBMs. The alpha 5 chain of type IV collagen also stains Bowman's capsule. (**B**) On high power, a glomerulus displays weak and segmental positivity for the alpha 5 chain of type IV collagen in the GBMs, and the absence of staining in Bowman's capsule and the dTBMs. Image is from Case Report 1.

progressive high-tone hearing loss and now requires hearing aids.

Case Report 2

Clinical history and initial laboratory data

A 36-year-old white female presented in 1990 at age 14 with microscopic hematuria and nonnephrotic proteinuria, and had a normal SCr of 0.8 mg/dL (70.7 μmol/L) (eGFR—98 mL/min/1.73 m^2). A kidney biopsy demonstrated TBMN, but there was no other evidence of AS. Her urinalysis findings persisted, but her kidney function remained normal so no further work-up was pursued. The family history was negative for AS, TBMN, microscopic hematuria, proteinuria or renal disease. In 2007, she had proteinuria of 500 mg/day which by 2008 had progressed to 4 g/day with a SAlb of 3.6 g/dL (36 g/L) and a SCr of 0.8 mg/dL (70.7 μmol/L) (eGFR —84 mL/min/1.73 m^2), and the urinalysis continued to demonstrate microscopic hematuria. A serological evaluation (rheumatoid factor, C3 and C4 levels, myeloperoxidase and proteinase 3 anti-neutrophil cytoplasmic antibody, hepatitis B surface antigen, hepatitis C antibody, HIV, anti-nuclear antibody, anti-Smith antibodies and anti-ribonucleoprotein antibodies) was negative. She had no history of gross hematuria, hearing difficulty or visual changes. She underwent a second kidney biopsy in 2008 for the evaluation of nephrotic-range proteinuria and microscopic hematuria.

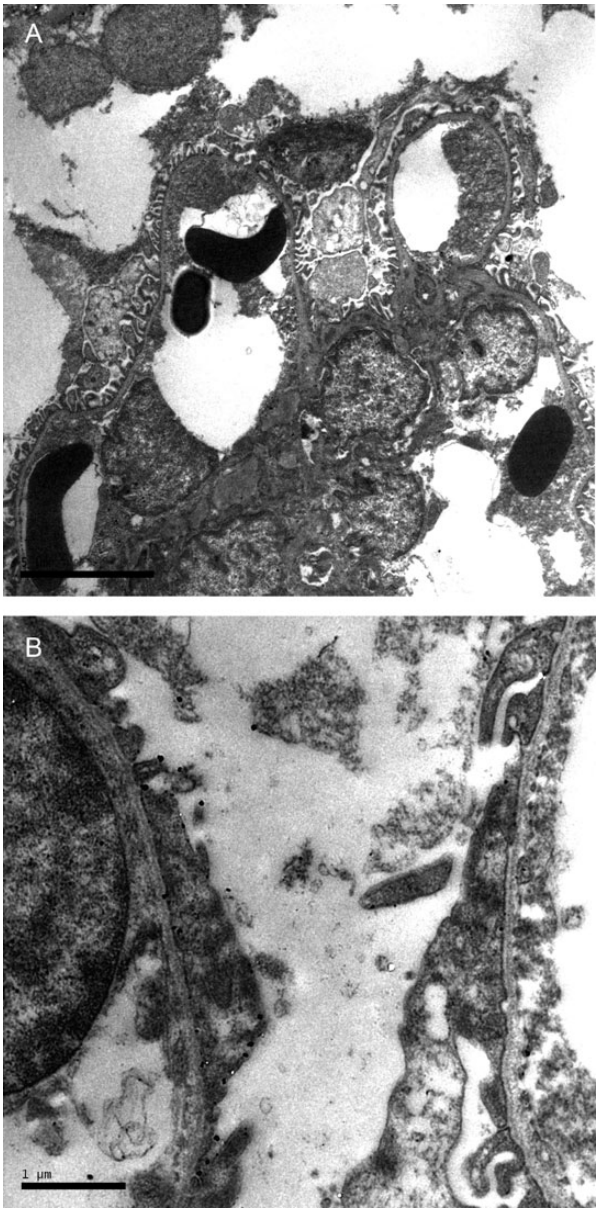

Fig. 4. (**A**) By EM, GBMs appear uniformly and diffusely thinned without electron-dense deposits. The foot processes appear preserved. The mesangium contains rare, small electron-dense deposits. (**B**) GBMs appear uniformly and diffusely thinned without lamellation or splitting. There is focal foot process effacement in a glomerulus. All images are from Case Report 1.

Kidney biopsy

The biopsy contained eight glomeruli by LM and two were globally sclerotic. The remaining six glomeruli appeared normal. Small foci of tubular atrophy and interstitial fibrosis affected 10% of the total cortex. IF demonstrated segmental/mosaic staining of the GBM and Bowman's capsule with the alpha 3 and alpha 5 chains of type IV collagen. There was no staining of the dTBM for either the

alpha 3 or alpha 5 chains of type IV collagen. EM demonstrated variable thinning and thickening of the GBMs, averaging 265 (range 157–427) nm with no splitting or lamellation. Foot process effacement was focal.

Diagnosis

Heterozygous XLAS was diagnosed based on the ultrastructural findings and the segmental/mosaic IF staining of the GBM and Bowman's capsule and the lack of staining of the dTBM for the alpha 3 and alpha 5 chains of type IV collagen.

Clinical follow-up

She was placed on an ACEi. Nonetheless, in 2012, her SCr had risen to 1.1 mg/dL (97.2 μmol/L) (eGFR—54 mL/min/1.73 m^2), she remained nephrotic with proteinuria of 4–6 g/day and continued to have microscopic hematuria. Additionally, formal hearing test demonstrated high-frequency hearing loss and an ophthalmologic evaluation showed anterior lenticonus.

Discussion

We report two young females presenting with microscopic hematuria and proteinuria in whom the diagnosis of XLAS was made possible based on the ultrastructural and IF features on kidney biopsy. At presentation, neither patient had a family history of AS or exhibited extra-renal manifestations of AS. Both patients developed nephrotic-range proteinuria and had progressive renal insufficiency, and both patients went on to manifest extra-renal manifestations of XLAS.

The diagnosis of XLAS in women presenting with microscopic hematuria and proteinuria can be challenging as the presentation and early ultrastructural features could easily be misdiagnosed as TBMN as demonstrated in our patients [1, 6, 11]. In Case 1, there was a family history of microscopic hematuria and her EM demonstrated diffuse thinning of the GBMs, and Case 2 was actually misdiagnosed with TBMN at a young age. TBMN accounts for most cases of what has been called benign familial hematuria. TBMN is often familial, with a history of hematuria being noted in up to 60% of family members [12, 13]. Several mutations of the type IV collagen genes COL4A3 and COL4A4 have been identified in patients with TBMN, but such mutations are not present in all families; and in some patients, TBMN represents a carrier state for ARAS [1, 11–13]. As demonstrated by our cases, the correct diagnosis can be made possible as a result of the immunohistochemical features on renal biopsy that underscores the importance of assessing for α3(IV), α4(IV) and α5(IV) collagen in these biopsies [6]. Genetic testing can also help in differentiating XLAS from TBMN, but it is quite expensive and is not always readily available [6].

While it was initially thought that females with XLAS had a benign renal course with a normal life span [3, 14], in the late 1960's and early 1970's, case reports began demonstrating that females, like affected males with XLAS, can have a progressive course leading to ESRD at a young age [15, 16]. In 1985, Grünfeld *et al*. [4] found that 39% (14 of 36 patients) of patients with AS progressed to ESRD. In 64% (9 of 14 patients) of these patients, ESRD was reached before 35 years of age. In 2003, Jais *et al*. [5] published the largest study to date looking at the natural history of XLAS in 288 female carriers from 195 families. They found that 18% (51 patients) of women progressed to ESRD over the course of the follow-up. Progression to ESRD occurred between the ages of 19–30 years in 27% (14 of 51 patients), 31–40 years in 31% (16 of 51 patients) and after 41 years of age in 41% (21 of 51 patients) of women. By comparison, while only 12% of women had progressed to ESRD by age 40 years, 90% of men had reached ESRD by age 40. However, 30–40% of female patients followed to age 60 or greater had progressed to ESRD. These studies, as with our cases, highlight the fact that, even in female carriers with XLAS, the risk of progressive renal disease increases with age.

A number of risk factors for progressive renal diseases have been identified in females with AS. Grünfeld *et al*. [4] found that the presence of nephrotic syndrome, gross hematuria in childhood and diffuse GBM thickening by EM were prognostic of progression to ESRD. Jais *et al*. [5] also found that proteinuria was of prognostic significance in women with XLAS. They found that no women without proteinuria developed ESRD, while in women with proteinuria ESRD occurred in 20% by age 40 and 30% by age 60. Thus, the development of and progressive increase in proteinuria are prognostic of progression to ESRD in women with XLAS.

Unfortunately, there is no specific therapy available for the treatment of AS. However, it has been shown that early initiation of ACEi in XLAS, even prior to the development of overt proteinuria, significantly delays the progression to ESRD and improves life expectancy in both men [17] and women [18]. Temme *et al*.[18] found that, in females with XLAS, only 5.4% of patients receiving an ACEi or angiotensin receptor blocker (ARB) progressed to ESRD compared with 25% not on an ACEi/ARB. Furthermore, they found that the age of the onset of ESRD was significantly later in those patients on an ACEi/ARB. By age 60, ∼15% of patients on an ACEi/ARB had reached ESRD compared with 55% of patients not on treatment. As a result, it has been recommended that patients with XLAS be followed yearly by a nephrologist, and that early initiation of ACEi/ARB is recommended in patients with microalbuminuria, proteinuria or hypertension [11, 18].

It has been suggested that the variable phenotype in females with XLAS might be due to different X-chromosome inactivation patterns [19, 20]. In a normal female, we would expect 50% of the active X-chromosome to be of maternal origin and 50% to be of paternal origin [21]. If this always held true in females with XLAS, then we would expect only half of the cells to express the mutant *COL4A5* gene and this would explain the less severe clinical course often expected in women. It has since been recognized that a subset of X-linked genes can escape silencing by X-inactivation [21, 22]. Based on this observation, Vetrie *et al*. [19] suggested that selection against cells expressing the mutant AS allele in females with XLAS might result in a less severe disease, whereas inactivation of a high proportion of normal X-chromosomes in the critical tissues could lead to a more severe clinical manifestation. They were unable to find a correlation between X-inactivation measured in lymphocytes with AS severity in a group of 43 women, but could not exclude that this mechanism was operating in the basement membranes of the kidney, ear and cells within they eye. Since that study, there have been multiple case reports showing skewing of X-inactivation in favor of the mutant COL4A5 resulting in a severe Alport phenotype in heterozygous females [20, 23, 24]. Rheault *et al*. [25] studied skewed X-inactivation ratios on disease outcomes in the transgenic mouse

model and found that preferential inactivation of the mutant *COL4A5* gene improved survival and surrogate outcome measures of urine protein and plasma urea nitrogen. They concluded that X-inactivation patterns may offer prognostic information and could potentially guide treatment strategies including gene replacement therapy or manipulation of X-inactivation choice in the future.

In conclusion, the presentation of XLAS in women is variable and may not always be apparent based on initial history and physical findings. The renal manifestations may be the first indication of XLAS, and the renal biopsy findings become critical in leading to the diagnosis of XLAS. The prognosis for women with XLAS is not as benign as once thought and the presence of proteinuria portends an unfavorable prognosis similar to that in men with XLAS. Early detection and treatment of women with XLAS are important and close long-term follow-up is essential.

Conflict of interest statement. None declared.

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