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CORRESPONDENCE

Crescentic and necrotising glomerulonephritis: a rare histological manifestation of Alport syndrome

Alport syndrome (AS) is a generalised inherited disease characterised by haematuria, progressive renal failure, sensorineural deafness and ocular abnormalities.[1] X-linked AS, resulting from mutations of the type IV collagen α5 (COL4A5) gene encoding the type IV collagen α5 chain, accounts for 85% of AS. To date, nearly 700 *COL4A5* mutations have been reported, with about 45% of them being missense mutations. The remainder of the patients with AS have autosomal recessive, or rarely, an autosomal dominant inheritance, both of which result from mutations in the COL4A3 or COL4A4 gene.[2] Diagnosis of AS relies on clinical presentation, immunohistochemical analysis of the collagen α (IV) chains in the skin and/or renal biopsy specimen, ultrastructural changes of the glomerular basement membrane (GBM) and genetic molecular analysis.

An 11-year-old white male patient presented with palpebral oedema beginning 2 months before his first visit, with moderate oedema and normotension being observed in the outpatient clinic. Screening laboratory tests showed serum proteins 3 mg/dL, albumin 2.6 mg/dL, proteinuria >6 g/24 h, total cholesterol 450 mg/dL (low density lipoprotein 309 mg/dL), urinary red blood cells (+++), serum creatinine 0.97 mg/dL and creatinine clearance 86 mL/min/1.73 m^2. The complement components levels were within the normal range and serological test results were negative (P-antineutrophil cytoplasmic autoantibody (P-ANCA), C-ANCA, antinuclear antibody, anti-DNA and, anti-GBM). After 4 weeks of empirical treatment with prednisone (80 mg/day) and a course of pulses of methylprednisolone (three doses), due to rapidly progressive glomerulonephritis being suspected, the patient was posted for a renal biopsy. This patient was the only child of a marriage without a history of consanguinity or renal diseases.

A formalin-fixed paraffin-embedded biopsy was carried out, 3 μm sections were cut and stained by H&E and the periodic acid Schiff reaction and Masson's trichrome methods were used. Pieces of renal cortex were processed for electron microscopy, and semi-thin sections were stained with toluidine blue and examined by light microscopy in order to select the appropriate glomeruli. Next, 50 nm sections were cut, stained with uranyl acetate and lead citrate and examined with a Zeiss Leo 906 E electron microscope equipped with a Megaview III digital camera (Oberkochen, Germany). For routine immunofluorescence evaluation of the renal biopsy, one fragment was embedded in optimal cutting temperature medium and snap frozen in liquid nitrogen. Then, 5 mm thick frozen sections were cut and sequentially fixed in acetone, after which they were washed with phosphate buffered saline (PBS), stained with fluorescein isothiocyanate-conjugated antisera (Kallestad, Austin, Texas, USA) against human immunoglobulin G (IgG), IgM, IgA, C3, C1q and κ and λ light chains diluted 1:20, washed in PBS and mounted with buffered glycerol. Furthermore, the expression of the α1 chain, α3 chain and α5 chain collagen type IV was analysed using an Alport Syndrome Staining Kit (ALPOC diagnostics TM). Negative (without primary antibody) and positive (normal kidney) controls were also analysed.

Histological examination revealed cellular crescents in four out of six glomeruli, with extracapillary proliferation also being present (figure 1A). Approximately 50% of glomeruli revealed segmental necrosis (figure 1B) and segmental mesangial expansion with irregular enlargement of mesangial stalks. Interestingly, lipid-laden foam cells, considered to be a marker of AS, were rarely found. Tubules were

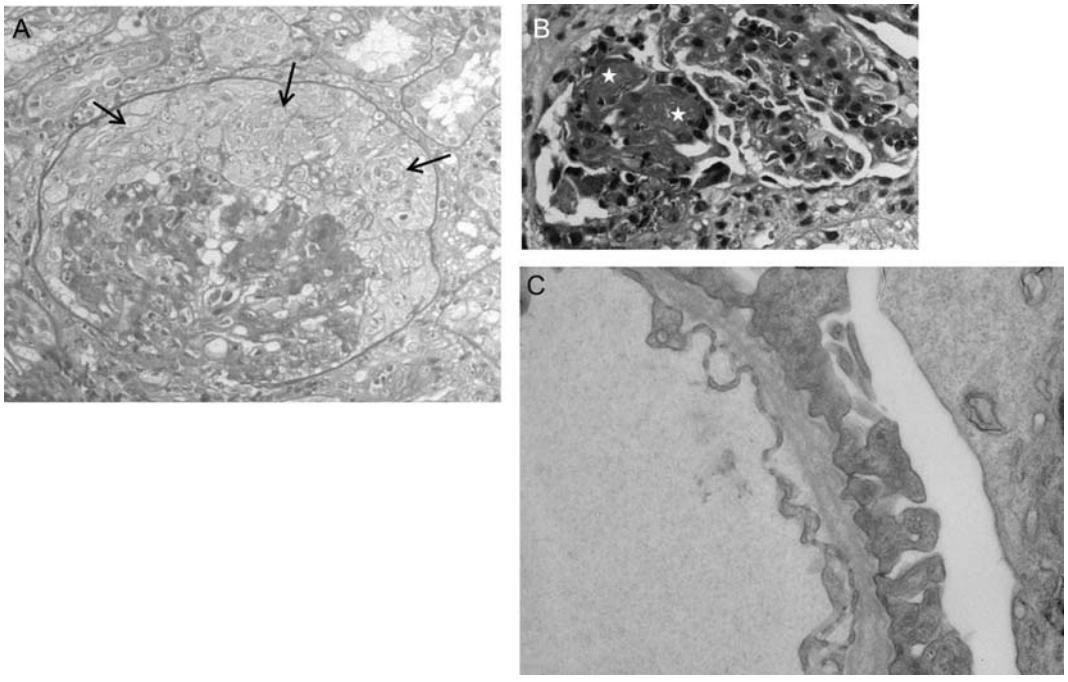

Figure 1 (A) The glomerulus in this periodic acid Schiff reaction-stained section shows extensive cellular crescent formation. Original magnification 400×. (B) In this Masson trichrome-stained section, the fibrin is indicated with an asterisk between the capillary loops. Original magnification 400×. (C) Classical ultrastructural findings of Alport syndrome: scalloping of subepithelial surface and multilamellation of glomerular basement membrane with podocyte foot process effacement. Original magnification 14 900×.

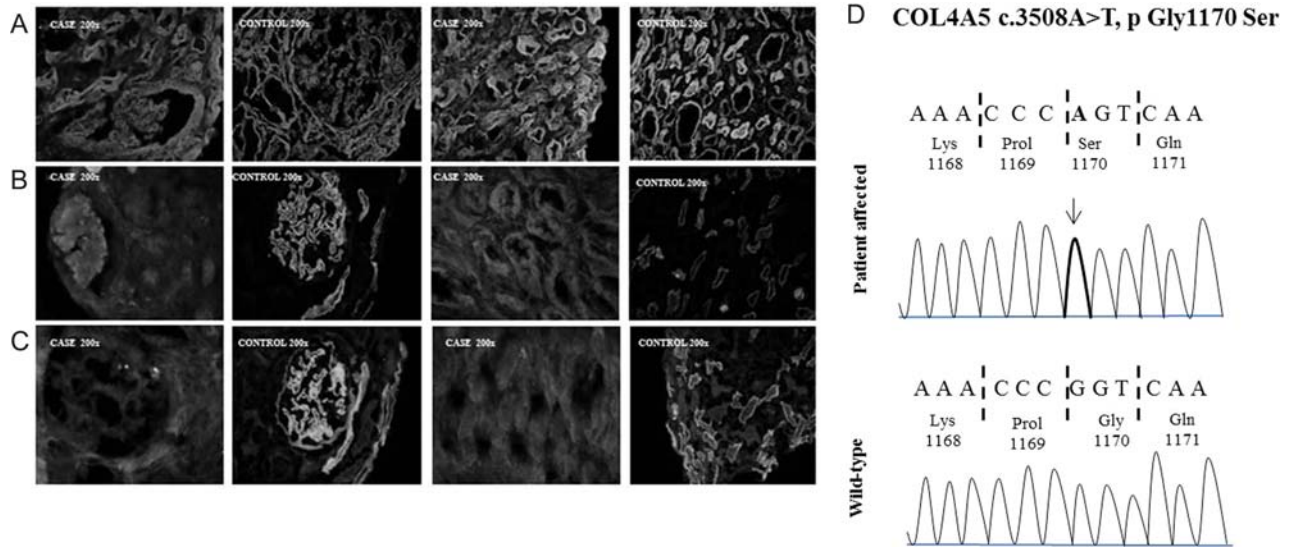

Figure 2 (A) α1 type IV chain. (B) α3 type IV chain. (C) α5 type IV chain. In the present case, there is no α3 or α5 collagen IV labelling, contrasting with α1 (IV) labelling of the glomerular basement membrane and tubular basement membranes. (D) Sequence chromatogram of COL4A5 in an affected patient with wild-type control. Nucleotides are shown and the corresponding amino acids are numbered. Sequence variants are arrowed.

normal except for the presence of occasional red blood casts, and no significant vascular changes were noted.

Ultrastructural examination of the glomerular capillary loops showed a diffuse and abnormal architectural organisation of the GBMs, characterised by frequent electronlucent areas with a frequently lamellated and 'basket-weave' appearance in glomeruli with or without crescents (figure 1C). No immune-type electron-dense deposits were identified in different renal compartments but there was an extensive effacement of podocyte processes. Immunofluorescence studies for IgG, IgA, IgM, C3 and C1q and κ and λ light chains revealed no significant deposition of these reactants in the glomeruli, tubular basement membranes or the interstitium. Results of the α3 or α5 collagen IV immunostaining were negative, contrasting with the α1(IV) labelling of GBM and tubular basement membranes (figure 2A–C).

Hypoacusia was confirmed by audiometry, and DNA analysis using whole exome sequencing confirmed that the proband carried the c.G3508A mutation in COL4A5, which is considered to be pathogenic and is known to cause X-linked AS (figure 2D). Oral cyclophosphamide was administered for 8 weeks, and 7 months after renal biopsy the patient had SCr 1.34 mg/dL, and proteinuria 324 mg/24 h, with dual antiproteinuric therapy (ACEI/ARB) being added.

This case illustrated a rare histological manifestation of AS, which is a hereditary disease of GBM deriving from a defect in the gene encoding for type IV collagen α-chain isoforms and clinically characterised by a progressive nephropathy often associated with sensorineural deafness and ocular abnormalities.[1 2] Among the different light microscopy patterns shown in AS, necrotising and crescentic glomerulonephritis are rarely found in this syndrome with only a few cases reported to date.[1–6] However, this pattern of glomerulonephritis is well known in transplant kidneys.[7] The presence of glomerular crescents and fibrinoid necrosis raises the possibility of other causes being responsible for the crescentic glomerulonephritis (CsGN), and a superimposed ANCA associated or pauci-immune type of CsGN cannot be ruled out, especially if we consider that approximately 20% of the pauci-immune type of CsGN may be associated with negative ANCA titres.[8–10] However, ultrastructural analysis showed characteristics of AS, as confirmed by immunodetection of collagen type IV chains. Finally, the mutation was pathogenic of X-linked AS as expected.[11]

In summary, a careful search for CsGN, particularly in paediatric populations, is justified because a nephrotic syndrome in childhood does not rule out a CsGN. In this context, the possibility of AS should be considered. Consequently, ultrastructural analysis represents an indispensable tool for a definitive nosological diagnosis.

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Notes