Evolution of glomerular basement membrane lesions in a male patient with Alport syndrome: ultrastructural and morphometric study

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Introduction

In spite of the fact that Alport syndrome (AS) is the best characterized haematuric hereditary nephritis, many questions regarding genetic and phenotypic aspects remain [1].

Albeit the eponym of 'Alport syndrome' defines a progressive hereditary haematuric nephritis, with sensorineural deafness, ocular abnormalities, and characteristic ultrastructural lesions of the glomerular basement membrane (GBM), it is actually used for patients with progressive haematuric non-immune glomerulonephritis, even in the absence of two or three of the four clinical required features.

The ultrastructural abnormalities of the lamina densa of the GBM suggested that the phenotypic expression of the genetic defect was a structural alteration of basement membranes (BM) of the glomerular capillary and of other organs which are impaired in AS [2,3].

Recently, six alpha-amino acid chains of type IV collagen have been identified in the GBM and in other BM; the interaction among the alpha-chains of type IV collagen results in distinct type IV collagen supramolecular structures that constitute the specific network of the GBM.

Now, it has been documented that the common X-linked AS is due to a primary defect in COL4A5 gene, which has been mapped on chromosome Xq22–23 [4] and encodes the alpha-5(IV) chain. The autosomal recessive form has been ascribed to defects in COL4A3 and COL4A4, mapped on chromosome 2 and encoding for alpha-3 and -4(IV) chains [5].

Autosomal dominant forms may exist in some patients [6].

Ultrastructural characteristic GBM lesions, which are only pathognomonic if diffuse [7], are present in about 60–80% of patients and appear similar in the different clinical and genetic forms of AS, related to anomalies in type IV collagen structure of the lamina densa.

The ultrastructural lesions of the GBM in AS have been well described since the early 1970s [3,7–10], and correlations between the GBM features and clinical data have also been reported [11]. Herein we report the development of the glomerular lesions during the clinical course of the disease, documented by three kidney biopsies from a male patient, obtained at three points from the childhood to 21 years of age, at which time dialysis therapy was initiated.

Subject and methods

A 6-year-old male patient (RA) presented an episode of macroscopic haematuria which was followed by persistent microscopic haematuria and very slight proteinuria. A first renal biopsy was performed at age 9. Microscopic haematuria persisted and proteinuria increased up to 1–4 g/l. The patient was evaluated again at age 18 and a second renal biopsy was performed. At age 21 he developed hypertension and chronic renal failure (serum creatinine 7.9 mg%, BUN 130 mg%); a third renal biopsy was performed at this time.

Ocular abnormalities were never present and at age 21, a slight sensorineural hypoacusia at high frequency tones (8000–10000 Hz) developed. It recently became apparent that the patient had a family history of renal disease: maternal aunt and one maternal female cousin are affected by AS and his mother died in end-stage renal failure (AS?). In addition, a maternal aunt died at age 28 for unknown reasons (AS?).

The renal specimens were processed for light-, immunofluorescence- and electron-microscopy, according to standard techniques. Since the renal tissue obtained at age 9 was not processed for electron-microscopy, the paraffin-embedded specimen was processed for electron-microscopy.

The GBM of each glomerulus on the electron micrographs was measured with an ocular micrometer at a final magnification of 6000. Three to ten points in each capillary loop
were evaluated. The width of the GBM was measured between the endothelial cytoplasmic membrane and the outer lining of the lamina rara externa, underneath the cytoplasmic membrane of the epithelial foot processes. Measurements were taken on peripheral capillary walls, avoiding mesangial and tortuous portions of the GBM, as well as those with abnormal thickness of the lamina rara interna. The number of measurements performed with the first, second, and third biopsies were 172 widths (7 glomeruli), 212 GBM widths (4 glomeruli) and 80 GBM widths (6 glomeruli) respectively.

Results

First renal biopsy
Thirty glomeruli were examined by light-microscopy. Two glomeruli were normal and the others showed only a mild mesangial hypercellularity. The interstitium and tubules were normal.

In the tissue for immunofluorescence, glomeruli were absent in the sample processed for immunofluorescence microscopy.

Electron microscopy, performed on seven glomeruli, revealed a diffuse and marked attenuation of the GBM thickness, involving more than 50% of the often dilated capillary loops (mean value $\pm$ SD = 162.4 $\pm$ 59.2 nm, ranging from 57.05 to 343.24 nm) (Figures 1 and 2).

Second renal biopsy
Light-microscope examination of 27 glomeruli revealed a mild and irregular enlargement of mesangial areas with increase in cells and matrix. Small foci of atrophic tubules were present.

Immunofluorescence was completely negative. Electron-microscopy performed on four glomeruli, showed a diffuse, marked and irregular thickening of GBM (mean values $\pm$ SD = 458.16 $\pm$ 209.4 nm, ranging from 91.53 to 1031.68 nm), accompanied by severe structural distortion of the lamina densa. The most important finding was the presence of diffuse splitting, fragmentation and reticulation of the lamina densa, giving a ‘basket-weave’ appearance to the GBM. Minute electron-dense granules were present in the

Fig. 1. Alport syndrome. First renal biopsy. Male patient. 9 years. Electron-micrograph showing diffuse thinning of the glomerular basement membrane. $\times 4400$. 
clear spaces enclosed by the lamellae (Figure 3a). The inner and outer borders of these thick segments appeared irregular and knobby. Some capillaries showed thinning of the GBM, up to 90 nm (Figure 3b), and occasional gaps were also observed.

Third renal biopsy

Ten glomeruli were examined by light-microscopy. Mesangial sclerosis was always present and four glomeruli were obsolescent. Interstitial fibrosis and tubular atrophy were evident with abundant interstitial inflammatory infiltrates.

No glomeruli were available for immunofluorescence.

Six glomeruli were examined by electron-microscopy, two of which were obsolescent. The GBM was irregularly thickened (mean values ± SD = 438.73 ± 208.5 nm, ranging from 94.86 to 1366.45 nm). Such values were similar to those detected in the second biopsy, but the lamina densa seemed more compact, since its fine reticulation and splitting were reduced. Sparse capillaries with focal attenuation of the GBM, occasionally smaller than 100 nm, were still present.

Discussion

Our ultrastructural and morphometric studies of the glomeruli of a male patient with AS, from whom to three renal biopsies were obtained from 9 to 21 years of age, clearly show the evolution of the GBM lesions from thin GBM to thickened GBM, as a result of the typical splitting and reticulation of the lamina densa.

It is well known that AS must be considered in any young person with microscopic haematuria, with or without other associated clinical abnormalities, and even in the absence of a family history of nephropathy [12].

At 9 years of age our patient underwent renal biopsy because of persistent microscopic haematuria and very slight proteinuria. The electron-microscopic examination of the kidney biopsy suggested the diagnosis of thin membrane nephropathy. No other males in his family were affected; however, related females developed ESRD. Thus, thin GBM does not seem to imply a favourable prognosis.

A good correlation between thin glomerular basement membrane and haematuria seems to exist [13], but the basis for haematuria remains unknown and breaks in a thin GBM have never been demonstrated. Moreover, thin GBM has been reported in 5–10% of normal kidneys [14]; it has been found in association with other glomerulopathies [15] and in nephrotic syndrome, with or without microscopic haematuria [16]. Therefore we agree with those authors who recently argued against an association of thin GBM with haematuric glomerular disease [17,18].

A relationship between thin membrane nephropathy and AS remains ambiguous even though AS and thin glomerular basement membrane nephropathy have been reported [19] within families. The irregular thickening of the GBM with a basket-weave appearance of the lamina densa has been suggested by some authors...
Fig. 3a,b. Alport syndrome. Second renal biopsy. Same patient, 18 years. a. The glomerular basement membrane (G) shows extensive lamellation and reticulation of the lamina densa (thick arrow). C, capillary lumen; P, podocyte; E, endothelial cell. ×11 800. b. Another capillary loop shows segments of reticulation of the lamina densa (thick arrow) alternating with focal thinning (thin arrow) of the glomerular basement membrane (G). C, capillary lumen; P, podocyte; B, Bowman's capsule. ×6800.

to have evolved from GBM of normal or thin texture [20]. Splitting and lamellation of the lamina densa of the GBM seem to be the most relevant pathological changes in AS, and it is likely that the progression of GBM lesions means progression of the nephropathy. The genetic heterogeneity of the disease and even the different mutations in COL4A5 gene may be responsible for the phenotypic heterogeneity.

One of the diagnostic criteria of AS is the detection of aspecific glomerular and tubulointerstitial lesions at light-microscopy, negative standard immunofluorescence, and characteristic ultrastructural lesions of the GBM [21].

Splitting of the basement membrane of tubules and of peritubular capillaries has been described as well, without specific significance [22] attributed to them.

The second renal biopsy was performed in our patient at 18 years of age, because of ascertained glomerular proteinuria besides haematuria. It showed the characteristic features of the lamina densa consistent with AS, and an increase in GBM thickness from 162 nm up to 458 nm, documented by 172 measurements in seven glomeruli from the firsty biopsy (9 years of age), and 212 measurements in four glomeruli of the second biopsy (18 years of age).

It has been shown that the normal GBM thickness increases progressively from 100 nm at birth to reach 300 nm after 3–5 years of age [23]. The mean GBM thickness of our patient was 162.4 nm at 9 years of age. The electron-microscopic studies of the first renal biopsy were performed on paraffin-embedded specimens, opportunely processed for electron-microscopy. In our experience (A. Cangiotti and S. Cinti, unpublished findings), paraffin-processed material is suitable for the electron-microscopic study of the GBM using paraffin blocks rather than paraffin slides, although the question is debated [24]. Moreover, the same embedding medium was used for the electron-
microscopic studies of the three different renal biopsies; thus the thickness of the GBM would not have been influenced by technical artefacts [25]. Therefore our morphological and morphometric data seem to demonstrate that the evolution of the GBM lesions results in the clinical progression of AS, the evolution from isolated microscopic haematuria to the associated proteinuria. Similar evolution of GBM lesions was reported in one Samoyed dog family, a well-known animal model of AS [26].

It is of interest that in previous studies, evidence of clear GBM lesions correlated with a more adverse prognosis [27]. It seems that progression of the GBM lesions corresponds to evolution of the renal disease. In addition, a correlation between the extent of lamellation of the lamina densa of the GBM and the level of proteinuria has been reported [11].

However, typical ultrastructural GBM lesions are not present in all patients with AS. There are cases in which the glomerular lesions might be focal rather than diffuse, or cases in which the lesion might develop later, or even might have disappeared with the progression of the nephropathy towards end-stage renal disease.

In fact, the third renal biopsy of our patient performed at 21 years of age because of rapid deterioration of glomerular filtration rate with heavy proteinuria showed a GBM thickness similar to that documented at the second biopsy (438 nm by 80 measurements in 4 glomeruli), but with a more homogeneous involvement of the lamina densa. The histological evaluation did not demonstrate a superimposed glomerulonephritis, as reported in other patients [28,29].

Therefore we think that the progression of the damage in the GBM due to type IV collagen defect, and identifiable with evolution of the ultrastructural GBM lesions, corresponds to progression of the nephropathy, until sclerosis and scarring of the glomerular and tubular structures.

In conclusion, ultrastructural and morphometric studies of three renal biopsies obtained at three different times from 9 to 21 years of age, from a male patient affected with AS, who exhibited isolated haematuria evolving to heavy proteinuria and ESRD, confirmed that: (a) thin GBM in isolated microhaematuria presents diagnostic difficulties; (b) a thin GBM may be consistent with a diagnosis of AS in young patients; (c) thickness and basket-weave features of the lamina densa of the GBM correlate with proteinuria and evolution of the nephropathy, and therefore indicate an adverse prognosis; (d) the absence of the typical splitting of the GBM’s lamina densa might be ascribed to the stage of the disease at the time of renal biopsy, in addition to scattered glomerular damage.

The mechanisms that produce the progressive GBM thickening and the progressive renal functional deterioration in AS are still unknown. However, different genetic defects involving the different alpha-chains of type IV collagen, which cause unphysiological rearrangements of collagen IV chains in the GBM, might be responsible for identical glomerular ultrastructural lesions.

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