The Renal Lesions of Alport Syndrome

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ABSTRACT

Alport syndrome is a hereditary, progressive, hematuric nephropathy characterized by glomerular basement membrane abnormalities with frequent hearing defects and ocular anomalies. The disease is associated with mutations in genes encoding the α3, α4, or α5 chains of type IV collagen, COL4A3, or COL4A4 in the autosomal forms of the disease, COL4A5 in the more frequent X-linked variety. Ultrastructural changes in the glomerular basement membrane and frequent abnormal expression of type IV collagen chains in renal and skin basement membranes are crucial elements for the diagnosis of Alport syndrome, determination of the mode of inheritance, and genetic counseling. Animal models have provided invaluable tools to study the mechanisms leading to progressive deterioration of the glomerular basement membrane and ultimately to renal failure, and to evaluate benefits of potential targeted therapies.


CASE REPORT

A 10-yr-old boy was seen because of a 3 d episode of macroscopic hematuria occurring shortly after an upper respiratory tract infection. BP and a physical examination were normal. Blood cell count and serum creatinine level (65 μmol/L) were in the normal range. Urine analysis showed 100mg/d microalbuminuria and 10⁶ red blood cells per mn on Addis count. Kidneys were normal by ultrasonography. Discrete hearing loss was detected by audiometry.

His mother, a 37-yr-old female, was followed in the adult nephrology unit because of persistent microscopic hematuria discovered on routine urine analysis during a medical screen before employment. She never developed proteinuria or micro-albuminuria, and her GFR was normal. Her husband was said to be healthy.

Because of the possibility of Alport syndrome, a skin biopsy was performed in the boy. It was not informative, as the expression of the α5 chain of type IV collagen on the epidermal basement membrane (BM) was normal. On renal biopsy, podocyte hypertrophy was seen by light microscopy. No specific immune deposits were detected by immunofluorescence. However, using antibodies against type IV collagen chains, a complete lack of staining of the glomerular basement membrane (GBM) for the α3, α4, and α5(IV) chains was observed, contrasting with the normal α5(IV) labeling of Bowman capsule and collecting duct BMs. On ultrastructural examination, irregular alternation of thick and split, and of thin GBM segments was observed. These morphologic findings led to the diagnosis of autosomal recessive Alport syndrome.

Alport syndrome (AS) is a hereditary disease of the GBM characterized by the familial occurrence of progressive, hematuric nephropathy with sensorineural deafness. It accounts for 0.3 to 2.3% of all patients reaching end-stage renal disease (ESRD). The disease is genetically heterogeneous, as it is associated with mutations in one of the genes, COL4A3, COL4A4 on chromosome 2, or COL4A5 on chromosome X, encoding the α3(IV), α4(IV), and α5(IV) chains of type IV collagen. In the glomerulus, these three chains are synthesized by the podocyte.

The α(IV) chains have a long collagenous domain, with a glycine every third amino acid, and a noncollagenous domain at the C-terminal. They associate to form triple helical type IV collagen molecules that assemble into three different networks to constitute the backbones of BMs. The α1–α1–α2(IV) network is a component of all BMs whereas the α3–α4–α5(IV) network is present in the GBM, in the ear, eye, and lung BMs; and the α5–α5–α6(IV) in the skin and smooth muscle cell BMs.¹

Whatever the gene mutated, macroscopic or microscopic hematuria, initially isolated, is the major sign of the disease. Other signs—proteinuria, increasing with age, renal failure and its rate of progression, and the presence and severity of extrarenal symptoms (deafness, ocular changes)—vary with the

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type of mutation, and the sex of the affected patient in X-linked AS.

Renal Biopsy
In early biopsy specimens, renal tissue appears normal by light microscopy. However, podocyte hypertrophy (Figure 1A) and stiffness of the capillary wall (Figure 1B) are frequently observed, associated or not with the presence of tubular red blood cell casts. Then, focal and segmental thickening of the GBM may be seen on silver stains (Figure 1C), with progressive enlargement of the mesangial stalks. Development of segmental, then diffuse, glomerular sclerosis in an increasing number of glomeruli leads to complete sclero-hyalinosis of the tuft. Foci of tubulointerstitial lesions, which may precede clear-cut glomerular changes, increase in size and severity.

Figure 1. Alport syndrome. (A–C) Light microscopy. (A) Podocyte hypertrophy on an early biopsy specimen (Trichrome-light green x250). (B) Rigidity and moderate thickening of the glomerular basement membrane (GBM), and segmental glomerular sclerosis (Trichrome-light green x250). (C) Irregular thickening of the GBM (Silver methenamine x450). (D–F) Electron microscopy. (D) Irregular distribution of thick and split, and of thin GBM segments (Uranyl acetate-lead citrate x7000). (E) Diffusely thin GBM (Uranyl acetate-lead citrate x 11000). (F) Immunogold microscopy. Anti-α1(IV) chain antibody: Distribution of the gold particle within the full thickness of the GBM (x8000). (G–L) Immunofluorescence: Distribution of the α5(IV) chain in the kidney (G–I) and the skin (J–L) basement membranes. In control (G, J) the antibody stain the GBM, the capsular BM, the distal tubule BM and the epidermal BMs. In a male patient affected with X-linked Alport syndrome (H, K), no labeling is observed (there is a nonspecific diffuse fluorescence of the epidermis). In a patient affected with autosomal recessive Alport syndrome (I, L), the GBM is unstained but the capsular and collecting duct BMs are labeled as well as the epidermal BM.
Clusters of interstitial foam cells are frequently found in proteinuric patients, and their number usually decreases in ESRD kidneys. No significant arterial changes are initially observed.

Immunofluorescence is initially negative; however, faint and irregular deposits of IgG, IgM, and/or C3 may be observed. More frequently, granular C3 deposits are irregularly distributed on the glomerular tuft and the afferent arterioles. With progression of the lesions, deposits of IgM, C1q, and C3 are seen in glomerular segmental lesions.

None of the previous anomalies are specific. Identification of ultrastructural changes of the GBM was thus of utmost importance as it provided a specific marker of the disease and suggested that AS is a disorder of the GBM. Typically, the renal lesion is characterized by diffuse thickening and splitting of the GBM with strikingly irregular outer and inner contours. But, most often, especially in young patients or in females with X-linked AS, irregular alternation of thick and abnormally thin GBM is observed (Figure 1D). Moreover, diffusely thin GBM, with smooth inner and outer contours, is the only GBM changes observed in 10 to 20% of patients, mostly in children, but also in adults with typical AS (Figure 1E).

**X-Linked Alport Syndrome**

X-linked AS accounts for about 85% of AS families. In males, the disease is severe. Proteinuria usually appears in childhood and increases steadily with age, with possible development of nephrotic syndrome, and constant progression to ESRD. Progressive hearing loss initially affecting high and middle frequencies, was observed in 79% of the male patients affected with X-linked AS, irregular alternation of thick and abnormally thin GBM is observed (Figure 1D). Moreover, diffusely thin GBM, with smooth inner and outer contours, is the only GBM changes observed in 10 to 20% of patients, mostly in children, but also in adults with typical AS (Figure 1E).

In autosomal recessive form of AS, associated with homozygous or compound heterozygous mutations in either COL4A3 or COL4A4, the disease is as severe in females as in males, and early progression toward ESRD—hearing defect, and retinopathy—are frequent. Clinical and morphologic features are comparable to the X-linked form of AS. This mode of inheritance must be suspected in consanguineous families, when the disease is severe in a young female, and/or when the father of an affected male shows microhematuria. Sometimes X-linked inheritance can be ruled out by haplotype analysis (when two affected brothers have inherited a different COL4A5 allele from their mother). Immunohistochemical study of skin and renal BMs may be normal, but shows, in most cases, abnormal pattern typical of this mode of inheritance: the GBM does not stain for a3-a4-a5 chains because of the lack of a3 or a4 (IV), which precludes the assembly of the a3-a4-a5 protomer, whereas the Bowman capsule BM (Figure 1I) as well as the skin BM normally express the a5-a6(IV) network (Figure 1I).

The spectrum of phenotype in related individuals carrying heterozygous mutations is very wide. Some individuals are completely free of symptoms, whereas many others display intermittent or permanent microhematuria, associated with thin GBM in rare cases studied, without proteinuria and with stable renal
function. These latter features are typical of "familial benign hematuria." Rarely related individuals display an intermediate phenotype with hemorrhagic disease with some level of proteinuria and/or renal failure.8

At the end of the spectrum, heterozygous COL4A3 or COL4A4 mutation is associated with autosomal dominant AS, which involves only a few families. In this form, male to male transmission can be observed, the renal disease progresses slowly in males as well as in females and, when documented, the electron microscopy shows thick, thick and thin, or diffusely thin GBM, and the hearing loss, when present, is a late event.9 The expression of type IV collagen is normal in the few cases that have been studied.9

Diagnosis of AS
The diagnosis is straightforward when clinical renal and extra-renal symptoms as well as family history are typical of AS. However, the lack of family history does not exclude the diagnosis of AS, as approximately 10% of COL4A5 mutations occur de novo.10 In this case, the presence of deafness should raise suspicion, and the finding of ocular abnormalities may lead to the diagnosis. Lack of α5(IV) expression in skin BM allows rapid and definitive diagnosis and genetic counseling, as it affirms X-linked transmission. However, the lack of extra-renal symptoms as well as the normal expression of the α5-α6 (IV) network does not rule out the diagnosis. In the presence of sporadic persistent hematuria, associated with proteinuria without any other symptoms suggesting AS, renal biopsy is frequently the first investigation performed. Examination of the renal tissue can exclude other hematuric glomerular disease, the most frequent being IgA nephropathy, and often shows typical ultrastructural abnormalities of the GBM when electron microscopy is performed, although exclusively thin BM can be observed. Glomerular hematuria, with proteinuria and deafness, can also be observed in MYH9 related disease, with familial macrothrombocytopenia variably associated with glomerulopathy and deafness,11 as well as in mitochondrial diseases.

The mode of inheritance of the disease (and therefore the genetic counseling) may be difficult to assess, and beyond cases of COL4A5 neomutations, the genealogy sometimes does not allow definitive conclusions. The pattern of expression of type IV collagen chains, when abnormal, is very helpful to address this issue. However, normal distribution of type IV collagen chains in skin and kidney basement membranes may be observed in AS, and, in some families, the mode of inheritance cannot be definitively assessed. This is frequently the case when young children present with sporadic hematuria and diffusely thin GBM, which may correspond either to AS or familial benign hematuria, and in families with only females affected with microhematuria.

COL4A3, COL4A4, and COL4A5 contain respectively 48, 52, and 53 exons. Direct sequencing of all coding exons from genomic DNA allows the finding of about 80% of mutations, but is laborious, time consuming, and expensive. Indirect diagnosis by haplotype analysis can be performed in informative families when expression of type IV collagen has established definitive diagnosis and mode of inheritance. Sequencing is currently performed for diagnostic purposes when the skin and kidney expression of the α(IV) chains is normal, and/or when there is an issue of genetic counseling. However, sequencing does not always provide a definitive diagnosis: for example, the finding of a single COL4A3 or COL4A4 mutation in a young child with normal α(IV) chains expression may correspond to a recessive form in which the second mutation was not found, to familial benign hematuria or to an autosomal dominant AS. Sometimes, only extended follow up will reveal the patient’s phenotype. Figure 2 schematizes the algorithm that can be proposed for diagnosis of AS.

Pathophysiology and Therapeutic Approaches
The basic defect in Alport syndrome is either the lack, in the mature GBM, of the α3-α4-α5(IV) network and its failure to replace the α1-α2 network, which is known to be less resistant to proteolysis, or the presence of a defective α3-α4-α5(IV) network. There are several animal models for AS, in dogs and mice that faithfully recapitulate autosomal and X-linked forms of the disease. They have brought novel data to the understanding of the mechanisms responsible for the progression of AS nephropathy and in
the elaboration of future therapies. The re-expression of the \(\alpha3(IV)\) chain in \(Col4a3^{-/-}\) mice, for example, was shown to restore the expression of \(\alpha4\) and \(\alpha5\) (IV), thus demonstrating that the expression of all three \(\alpha3-\alpha4-\alpha5(IV)\) chains is required for network assembly. The downstream mechanisms responsible for progressive alteration of the GBM and renal failure are not fully understood. In young Alport mice, the ultrastructurally normal GBM is known to already be abnormally permeable. The concomitant accumulation of mrNAs encoding TGF\(\beta1\) and extracellular matrix components in human and mouse Alport podocytes are thought to reflect key events in renal disease progression. Blocking the TGF\(\beta1\) pathway prevents GBM thickening in Alport mice.

The role of metalloproteinases in Alport disease has been underlined by recent studies. Increased expression of MMP2, MMP3, and MMP9 has been described, both at the transcriptional and the protein level, in AS kidneys in humans, mice, and dogs. Such MMP up-regulation is not unique to Alport nephropathy. However, AS kidney basement membranes were shown to be more readily degradable in vitro by collagenase, elastase, and cathepsins, compared with normal kidney basement membranes, and this is thought to be due to the lack of the highly cross linked \(Col4a3\) and occurrence of proteinuria in a given before development of GBM injury. The downstream mechanisms responsible for progressive alteration of the GBM and renal failure are not fully understood. In young Alport mice, the ultrastructurally normal GBM is known to already be abnormally permeable. The concomitant accumulation of mrNAs encoding TGF\(\beta1\) and extracellular matrix components in human and mouse Alport podocytes are thought to reflect key events in renal disease progression. Blocking the TGF\(\beta1\) pathway prevents GBM thickening in Alport mice.

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Overt anti-GBM nephritis occurs in only 3 to 5\% of transplanted Alport patients. The risk of graft loss is very high, and treatment with plasmapheresis and cyclophosphamide has shown limited benefit. The risk of recurrence on subsequent transplantation is very high. This complication is more likely to occur in patients with deletions or frameshift mutations, who do not express the \(\alpha3\alpha4\alpha5(IV)\) GBM network. However, many patients with \(COL4A5\) deletion have been successfully transplanted, without developing anti GBM nephritis, and predictive factors for developing the disease are currently unknown.

DISCLOSURES

None.

REFERENCES