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The Nongenetic Diagnosis of Thin Basement Membrane Nephropathy

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Three disorders, thin basement membrane nephropathy (TBMN), immunoglobulin A nephropathy (IgAN), and Alport syndrome (AS), account for the majority of children and adults with persistent glomerular hematuria. Although there is some clinical overlap between these conditions, they can be distinguished on the basis of family history, extrarenal findings, routine immunofluorescence, and glomerular basement membrane ultrastructure or type IV collagen chain composition. This distinction is important because of the very different prognoses of these conditions.
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Microscopic hematuria is a common presenting complaint in nephrology clinics, particularly those caring for children and adolescents. Three glomerular disorders, IgA nephropathy (IgAN), thin basement membrane nephropathy (TBMN), and Alport syndrome (AS), account for most individuals with persistent microscopic hematuria in the pediatric population, with other glomerulopathies such as membranoproliferative glomerulonephritis occurring occasionally. These disorders also are responsible for a large proportion of adults with glomerular hematuria. The clinical features of IgAN, TBMN, and AS overlap, and TBMN and AS also share some histologic abnormalities. This article discusses the use of clinical, pedigree, and morphologic data to differentiate the major causes of persistent microscopic hematuria of glomerular origin.

Clinical Features

Hematuria

Persistent microscopic hematuria is characteristic of IgAN, TBMN, and AS. Superimposed episodic macroscopic hematuria, often associated with acute infection, can occur in all three conditions, although macroscopic hematuria is unusual after adolescence in patients with AS (Table 1).[1]

AS is X-linked in approximately 80% of cases. Consequently, affected males inevitably exhibit severe disease, whereas the spectrum of severity in affected female heterozygotes is diverse. Essentially all males with AS have persistent microscopic hematuria so that intermittent hematuria in a male is unlikely to be caused by AS. More than 90% of females with X-linked AS have microscopic hematuria, although it may be intermittent.[2] All males and females with autosomal-recessive AS (who account for about 15% of AS patients) have persistent microscopic hematuria.

Approximately 50% to 60% of heterozygous carriers of autosomal-recessive AS mutations show microscopic hematuria that may be persistent or intermittent.[3,4] Many patients who have clinical and histologic features of TBMN are carriers of autosomal-recessive AS mutations. It is not surprising then that microscopic hematuria in patients with TBMN typically is persistent but also may be intermittent.

Proteinuria

Although the great majority of individuals with TBMN have isolated microscopic hematuria, proteinuria has been observed occasionally. Sometimes these patients have unrecognized AS. Proteinuria is common in AS, developing eventually in all affected males, and in about 75% of affected females.[2] Thus, the diagnosis of TBMN should be questioned in any individual who presents with substantial proteinuria in addition to microscopic hematuria, or who develops proteinuria during follow-up observation.

Renal Insufficiency

Renal insufficiency, similar to proteinuria, has been described in some patients with TBMN. As with proteinuria, it is not clear whether renal insufficiency is part of the spectrum of clinical severity in TBMN, or whether these patients actu-

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Table 1 AS, TBMN, and IgAN: Comparison of Clinical and Pathologic Features

	AS	TBMN	IgAN
Family history of hematuria	Common	Common	Rare
Family history of ESRD	Common	Rare	Rare
Deafness	Common	Rare	Rare
Proteinuria	Common	Rare	Common
Hypertension	Common	Rare	Common
Ocular findings	Common	Rare	Rare
Light microscopy (early)	Normal, or mild mesangial Proliferation	Normal	Mesangial proliferation
Routine immunofluorescence	Normal	Normal	Mesangial IgA, C3
Collagen IV histochemistry	Usually abnormal	Always normal	
GBM ultrastructure			
Width	Age & sex-dependent Thin and thick	Independent of sex & age Diffusely thin	Normal
Lamellation	Diffuse (esp. in males > 10)	Focal or absent	Absent
Epithelial scalloping	Frequent	Infrequent	Absent
Intramembranous inclusions	Frequent	Infrequent	Absent

ally have AS. In this context, it is important to remember that 100% of males and 30% of females with X-linked AS have developed end-stage renal disease by the age of 60.[2]

Deafness

Hearing loss of any kind is unusual in patients with TBMN. In contrast, sensorineural deafness is detectable in about 90% of males and 10% of females with X-linked AS by age 40.[2] Certain features of the hearing loss of AS may be helpful diagnostically. The hearing loss of AS never is present at birth but usually develops in late childhood or adolescence, is always bilateral, and preferentially affects high-frequency sounds.

Ocular Findings

Anterior lenticonus, perimacular flecks, posterior corneal dystrophy, and recurrent corneal erosions may be observed alone or in combination in patients with AS, and when present can confirm the diagnosis.[5,6] On the other hand, these abnormalities are not seen in patients with microscopic hematuria caused by other conditions, such as TBMN and IgAN.

Histologic Features

Renal biopsy findings in patients with TBMN are discussed in detail elsewhere. Differentiation of TBMN from IgAN is straightforward, based on the presence of mesangial proliferation (light microscopy), mesangial IgA deposits (immunofluorescence), and mesangial electron-dense deposits (electron microscopy) in the latter disorder.

AS and TBMN may be difficult to distinguish on the basis of routine renal biopsy evaluation, especially in young people. Light microscopic findings may be minimal in both conditions. Similarly, immunofluorescence typically is negative, or shows nonspecific immunoprotein deposition, in both AS and TBMN. Glomerular basement membrane (GBM) attenuation, with focal areas of lamina densa separation, is seen commonly in children with AS, making differentiation from TBMN difficult. With time, particularly in males, the characteristic features of AS, such as GBM thickening, multilamellation and basket-weaving of the lamina densa, epithelial scalloping, intramembranous cytoplasmic inclusions, and podocyte foot process fusion, become progressively apparent, and diagnostic uncertainty diminishes.[7]

Despite the similarities between early AS and TBMN, an accurate diagnosis of AS can be made in most patients if clinical, pedigree, and histologic findings are considered together.[8] An additional diagnostic tool that can be very helpful is specific immunostaining for the α chains of type IV collagen.

Type IV Collagen Immunostaining

Type IV collagen, the predominant collagenous constituent of basement membranes, comprises a family of 6 α chains, designated α1(IV) through α6(IV) (Fig 1).[9] The α1(IV) and α2(IV) chains are present in all basement membranes, whereas the α3(IV) through α6(IV) chains show restricted expression. Type IV collagen α chains form trimers that associate into networks through several intermolecular interactions. There are at least 3 type IV collagen networks in mammalian basement membranes: a ubiquitous network composed of $\alpha1(IV)_2\alpha2(IV)_1$ trimers, and networks made up of $\alpha3(IV)_1\alpha4(IV)_1\alpha5(IV)_1$ trimers and $\alpha5(IV)_2\alpha6(IV)_1$ trimers that are restricted in distribution. The $\alpha3(IV)_1\alpha4(IV)_1\alpha5(IV)_1$ network predominates in the mature GBM, and also is present in Bowman's capsule and the basement membranes of the distal and collecting tubules.

AS results from mutations in the genes that correspond to the α3(IV), α4(IV), and α5(IV) chains—*COL4A3*, *COL4A4*, and *COL4A5*, respectively. Mutations in *COL4A5*, located on the X chromosome, cause X-linked AS, whereas autosomal-

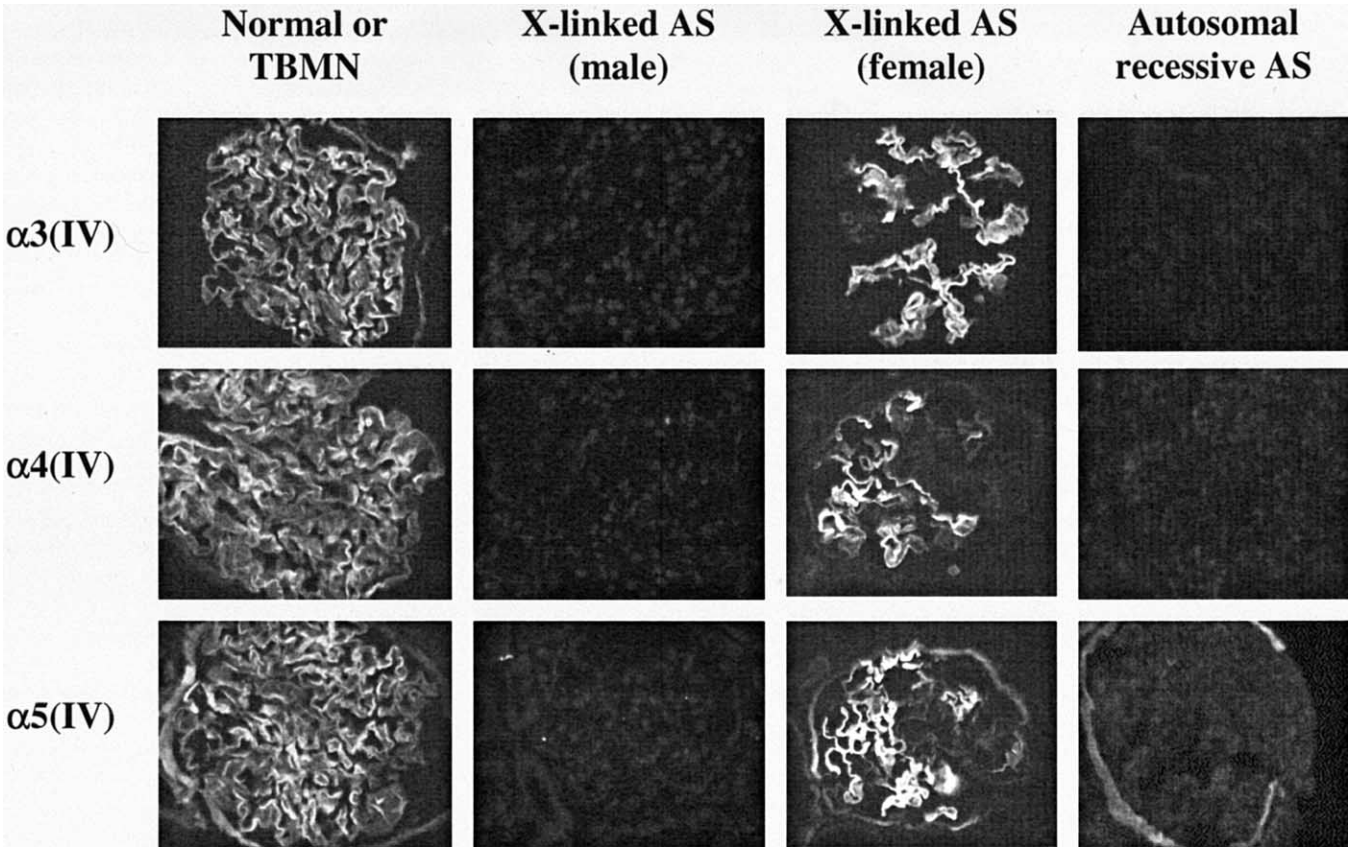

Figure 1 Typical results of immunostaining for type IV collagen α3, α4, and α5 chains in glomeruli of normal, TBMN, and AS renal biopsy specimens. The α3(IV), α4(IV), and α5(IV) chains are distributed normally in TBMN. These chains are entirely absent from renal basement membranes in approximately 80% of males with X-linked AS, and are present in a mosaic distribution in about 70% of females with X-linked AS. In most individuals with autosomal-recessive AS, the α3(IV) and α4(IV) chains are absent from all renal basement membranes, whereas the α5(IV) chain is present in Bowman's capsules and the distal/collecting tubule basement membranes, but absent from GBMs.

recessive AS arises from mutations in both alleles of either *COL4A3* or *COL4A4*. The ultimate effect of most of these mutations is to prevent or severely impair the formation of $\alpha3(IV)_1\alpha4(IV)_1\alpha5(IV)_1$ trimers such that the corresponding network is absent from the affected basement membranes.

This phenomenon can be exploited for diagnostic purposes (Table 2). Most patients with AS have abnormalities in the expression patterns of the α3(IV), α4(IV), and α5(IV) chains in their kidneys, and of the α5(IV) chain in their skin.[10-12] Unfortunately, normal expression of these chains does not exclude the diagnosis of AS because about 20% of males and 30% of females with X-linked AS, and an uncertain percentage of patients with autosomal-recessive AS, have basement membranes in which the expression of these proteins is indistinguishable from normal.

To date, there have been no definitive reports of patients with abnormal basement membrane expression of the α3(IV), α4(IV), or α5(IV) chains who have not developed progressive renal disease, or who have not had a family history of end-stage renal disease. Conversely, individuals with isolated microscopic hematuria, a negative family history of end-stage renal disease, and diffusely attenuated GBM almost invariably have exhibited normal basement membrane expression of these proteins. Based on current information then, abnormal basement membrane expression of the α3(IV), α4(IV), and α5(IV) chains is diagnostic of AS. Normal basement membrane expression of these chains supports a diagnosis of TBMN but cannot definitively exclude AS.

The contrasting natural histories of AS and TBMN may reflect the contrasting effects of complete (AS) and partial (TBMN) loss of the $\alpha3(IV)_1\alpha4(IV)_1\alpha5(IV)_1$ network from renal basement membranes. Complete loss of this network from GBM appears to induce, through mechanisms as yet undefined, increased synthesis and deposition of a variety of extracellular matrix proteins that contribute to GBM thickening and glomerulosclerosis, including the α1(IV) and α2(IV) chains, types V and VI collagen, laminin α2 chain, and fibronectin.[13-16] These responses apparently do not occur or are muted when deposition of the $\alpha3(IV)_1\alpha4(IV)_1\alpha5(IV)_1$ network is decreased but not absent, as is probably the case in individuals with TBMN caused by heterozygous mutations in *COL4A3* or *COL4A4* who are left with one functional allele of the affected gene.

Table 2 Expression of Type IV Collagen α Chains in Normal Kidneys, TBMN, and AS

	Normal	TBMN	X-linked AS		Autosomal recessive AS‡	Autosomal dominant
			Male*	Female†		
GBM						
α3(IV)	Positive	Positive	Negative	Mosaic	Negative	Positive
α4(IV)	Positive	Positive	Negative	Mosaic	Negative	Positive
α5(IV)	Positive	Positive	Negative	Mosaic	Negative	Positive
TBM (distal and collecting)						
α3(IV)	Positive	Positive	Negative	Mosaic	Negative	Positive
α4(IV)	Positive	Positive	Negative	Mosaic	Negative	Positive
α5(IV)	Positive	Positive	Negative	Mosaic	Positive	Positive
Bowman's capsule						
α3(IV)	Positive	Positive	Negative	Mosaic	Negative	Positive
α4(IV)	Positive	Positive	Negative	Mosaic	Negative	Positive
α5(IV)	Positive	Positive	Negative	Mosaic	Positive	Positive
Epidermal BM						
α5(IV)	Positive	Positive	Negative	Mosaic	Positive	Positive

*Approximately 20% of males with X-linked AS exhibit positive immunostaining of renal basement membranes for the α3(IV), α4(IV) and α5(IV) chains and epidermal basement membranes for the α5(IV) chain.

†Approximately 30% of females with X-linked AS exhibit positive, uninterrupted immunostaining of renal basement membranes for the α3(IV), α4(IV), and α5(IV) chains and epidermal basement membranes for the α5(IV) chain.

‡Some patients with autosomal-recessive AS exhibit positive immunostaining of renal basement membranes for the α3(IV), α4(IV), and α5(IV) chains.

References

1. Gubler M, Levy M, Broyer M, et al: Alport's syndrome: A report of 58 cases and a review of the literature. Am J Med 70:493-505, 1981
2. Jais JP, Knebelmann B, Giatras I, et al: X-linked Alport syndrome: Natural history and genotype-phenotype correlations in girls and women belonging to 195 families: A "European Community Alport Syndrome Concerted Action" study. J Am Soc Nephrol 14:2603-2610, 2003
3. Boye E, Mollet G, Forestier L, et al: Determination of the genomic structure of the COL4A4 gene and of novel mutations causing autosomal recessive Alport syndrome. Am J Hum Genet 63:1329-1340, 1998
4. Heidet L, Arrondel C, Forestier L, et al: Structure of the human type IV collagen gene *COL4A3* and mutations in autosomal Alport syndrome. J Am Soc Nephrol 12:97-106, 2001
5. Colville DJ, Savige J: Alport syndrome: A review of the ocular manifestations. Ophthalmic Genet 18:161-173, 1997
6. Colville D, Savige J, Morfis M, et al: Ocular manifestations of autosomal recessive Alport syndrome. Ophthalmic Genet 18:119-128, 1997
7. Rumpelt H-J: Hereditary nephropathy (Alport syndrome): Correlation of clinical data with glomerular basement membrane alterations. Clin Nephrol 13:203-207, 1980
8. Mazzucco G, Barsotti P, Muda AO, et al: Ultrastructural and immunohistochemical findings in Alport's syndrome: A study of 108 patients from 97 Italian families with particular emphasis on COL4A5 gene mutation correlations. J Am Soc Nephrol 9:1023-1031, 1998
9. Hudson BG, Trygvasson K, Sundaramoorthy M, et al: Alport's syndrome, Goodpasture's syndrome, and type IV collagen. N Engl J Med 348:2543-2556, 2003
10. Gubler MC, Knebelmann B, Beziau A, et al: Autosomal recessive Alport syndrome: Immunohistochemical study of type IV collagen chain distribution. Kidney Int 47:1142-1147, 1995
11. Nakanishi K, Yoshikawa N, Iijima K, et al: Immunohistochemical study of α1-5 chains of type IV collagen in hereditary nephritis. Kidney Int 46:1413-1421, 1994
12. van der Loop FTL, Monnens LAH, Schroder CH, et al: Identification of COL4A5 defects in Alport syndrome by immunochemistry of skin. Kidney Int 55:1217-1224, 1999
13. Kashtan CE, Kim Y: Distribution of the α1 and α2 chains of collagen IV and of collagens V and VI in Alport syndrome. Kidney Int 42:115-126, 1992
14. Miner JJ, Sanes JR: Molecular and functional defects in kidneys of mice lacking collagen α3(IV): Implications for Alport syndrome. J Cell Biol 135:1403-1413, 1996
15. Kalluri R, Shield CF, Todd P, et al: Isoform switching of type IV collagen is developmentally arrested in X-linked Alport syndrome leading to increased susceptibility of renal basement membranes to endoproteolysis. J Clin Invest 99:2470-2478, 1997
16. Kashtan CE, Kim Y, Lees GE, et al: Abnormal glomerular basement membrane laminins in murine, canine, and human Alport syndrome: Aberrant laminin alpha2 deposition is species independent. J Am Soc Nephrol 12:252-260, 2001