Pathology of Thin Basement Membrane Nephropathy

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Thin basement membrane nephropathy (TBMN) is a glomerular disorder characterized clinically by isolated hematuria and pathologically by diffuse thinning of the glomerular basement membrane (GBM) on ultrastructural examination. The pathologic diagnosis of TBMN is problematic, in part because of the wide range of GBM thicknesses in the normal population. GBM thickness varies with age, sex, and the different methods of tissue preparation and measurement. In addition, there are no standardized diagnostic criteria defining the degree or extent of GBM thinning required for the diagnosis of TBMN. GBM thinning is often seen in other glomerulopathies, where it may represent an overlap with TBMN or may be secondary to GBM damage and remodeling. Importantly, TBMN must be differentiated from the GBM thinning seen in some renal biopsy specimens from boys and female heterozygotes with X-linked Alport syndrome because of the very different prognoses of these two conditions.
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Thin basement membrane nephropathy (TBMN) is the glomerular disorder characterized clinically by isolated hematuria and pathologically by a diffusely thinned glomerular basement membrane (GBM). This condition often is referred to as *benign familial hematuria* because of its familial aggregation and lack of progression.

The genetic basis of TBMN is heterogeneous and includes heterozygous mutations in the *COL4A3* or the *COL4A4* genes, which encode the α3 and α4 chains of type IV collagen, respectively.[1-3] Mutations usually result in missense, splice site, or frame shift changes. Most cases show an autosomal-dominant pattern of inheritance, although sporadic cases also occur. Because homozygous or compound heterozygous mutations in *COL4A3* or *COL4A4* result in autosomal-recessive Alport syndrome, some investigators consider TBMN to be the carrier state of this form of Alport disease,[4] and the distinction between TBMN and Alport syndrome becomes less absolute. However, some kindreds with TBMN do not show linkage to the *COL4A3/COL4A4* locus, suggesting the existence of further, still to be identified, chromosomal loci.[5]

TBMN is aptly named for its defining morphologic characteristic, diffuse thinning of the GBM, which is evident only at the ultrastructural level. The pathology of TBMN appears deceptively simple, but in practice the diagnosis of TBMN is often difficult. The major problems are the absence of guidelines on how to measure the GBM thickness, and of standardized criteria to define GBM thinning. GBM thickness normally varies with age and can be influenced by the type of tissue fixation, embedding medium, and method of measurement. TBMN must be differentiated from the thinned GBM seen in some patients with Alport syndrome. It also must be distinguished from the nonspecific localized thinning that can occur in the course of GBM remodeling in diverse glomerular diseases. Interpretation of the literature is hampered by the lack of genotype-phenotype correlations in early studies and the difficulty in distinguishing the overlapping clinical and pathologic features of TBMN from those of Alport syndrome. In addition, in part because TBMN is so common (it affects up to 5% of the general population[6]), it may occur as an incidental finding in renal biopsies performed for other unrelated renal diseases. This article outlines the approach to the renal biopsy diagnosis of TBMN in the context of these issues.

Pathologic of TBMN

In 1973, Rogers et al[7] were the first to link familial hematuria to thinning of the GBM in a family with isolated persistent microscopic hematuria over 4 generations. By light microscopy, the glomeruli and tubulointerstitium of an affected family member appeared normal. Direct immunofluorescence examination showed no immunoglobulin deposition. The GBM was reported to show "marked thinning" by electron microscopy with "irregular narrowing of the lamina densa" that varied from segmental to circumferential. Com-

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parison with the size of latex beads measuring 312 nm introduced onto the tissue sections indicated the GBM thickness was 150 nm. The rare breaks in the GBM that were observed were proposed as the source of entry of red blood cells into the urine. Since this historic report, numerous clinicopathologic studies have confirmed the association of benign familial hematuria with the renal biopsy findings of diffuse GBM thinning.[8-19]

What is Normal GBM Thickness and How Should it be Measured?

GBM thickness varies with age and is influenced by methods of tissue preparation and measurement. Because the trilaminar GBM is composed of a central lamina densa, an inner lamina rara interna, and an outer lamina rara externa, there is general agreement that the most accurate means of determining GBM thickness is to measure the distance between the outer limits of the endothelial cell and the podocyte cell membranes. Measuring the electron-dense portion of the GBM accounts only for the lamina densa and not the more electron-lucent laminae rarae. The GBM thickness is most uniform over the peripheral capillary filtering surface, but may appear thickened, folded, and redundant where it reflects over the mesangium. Thus, GBM thickness should be measured only in the peripheral capillaries. Because the GBM is curved, measurements are most accurate when they are made where the GBM is cut in cross-section, rather than tangentially.

The methods of GBM measurement are variable and include the introduction of latex beads of known diameter onto the tissue sections, the *orthogonal intercept* method which superimposes a grid of perpendicularly oriented lines of known dimension onto the electron micrograph, and simple measurement with a magnification graticule or metric ruler placed over the electron microscope prints. Once the GBM thickness has been measured in millimeters from the electron microscopic print, its value in nanometers can be calculated by correcting for the magnification of the photographic negative and the factor of enlargement from the negative to the print according to the following equation:

Thickness (nm) = thickness on print (mm) ÷ (magnification × enlargement factor)

For example, if the GBM thickness (T) measures 1.5 mm on an electron microscopic print photographed at 2,000× magnification and if the print represents a 2.5-fold enlargement from the negative, then T = 1.5 mm ÷ (2,000 × 2.5) or 300 nm. To simplify matters, the digital cameras on many electron microscopes are now equipped with computer software that calculates the distance between 2 points for any given magnification.

There is no consensus on how many measurements of GBM width should be made per biopsy specimen, how many glomerular capillaries and glomeruli should be sampled, and whether the arithmetic or harmonic mean should be used. However, there is general agreement that the mean from multiple representative measurements made over multiple capillaries of multiple glomeruli is most accurate.

Normal GBM thickness varies with age and sex, and the values reported in the literature differ widely. Osawa et al[20] studied a series of normal adult biopsy (n = 7) and autopsy (n = 14) specimens. An average of 587 GBM measurements were made for each biopsy and 254 for each autopsy specimen. In each case, the thickness was measured from the endothelial to the epithelial cytoplasmic borders, avoiding tangentially cut areas, and only in the peripheral capillaries. By using these criteria, the mean GBM thickness (arithmetic mean) for all cases was calculated to be 315 nm with a range of means from 239 to 453 nm. However, the mean GBM thickness in individual glomeruli ranged from 225 to 500 nm, indicating even wider interglomerular variability. Furthermore, these investigators showed that the distribution of GBM thickness is relatively Gaussian in some individual glomeruli, whereas in others it is much more irregular.

In 1981, Haynes[21] outlined his methods for ultrastructural analysis of the normal human glomerulus. By overlaying a grid of lines of known dimensions, measurements were taken at positions where the GBM clearly was bordered by epithelial and endothelial cytoplasm. For normals aged 11 to 26 years, the mean GBM thickness was 394 nm (range, 372-632 nm).[21]

The orthogonal intercept technique is a modification of the method originally described by Weibel and Knight[22] for studying the thickness of alveolar membranes. A grid of perpendicularly oriented lines is superimposed on the electron micrograph, and the GBM thickness is measured at any point where a line intersects the interface between endothelial cytoplasm and membrane. The inverse length is then used to calculate the harmonic mean. The advantage of this method is that tangential sections may be included in the measurements and are accounted for by the formula. Multiplication by a correction factor yields the mean GBM thickness. The mathematic basis of this method is quite complicated, and the reader is referred to the detailed descriptions.[23,24] By using this method with an average intercept number of 164, the mean GBM thickness was 326 nm in a group of 59 normal female kidney donors and 373 nm in 59 normal male donors.[25] These investigators also showed that the GBM thickness increases with age until age 40 and then plateaus.

The definition of normal GBM thickness is more problematic in the pediatric population. Vogler et al[26] calculated normal GBM thickness in an autopsy study of 37 children without known renal disease by using latex microbeads 500 nm in diameter as comparative markers. GBM measurements were taken at the thinnest points to minimize error introduced by tangential sectioning. The GBM thickness was found to increase progressively with age until preadolescence (≈11 years of age), at which time it reached the adult width of about 300 nm. The growth rate was maximal at 2 years of age and thereafter decreased. The GBM thickness ranged from 132 to 208 nm at ages 2 days to 1 year, and increased to 208 to 245 nm at ages 1 to 6 years, and 244 to 307 nm at ages 6 to 11 years.[26] In addition to being thin, the GBM of immature glomeruli may show apparent splitting of the lamina densa, which represents the individual basement membranes produced by the endothelial cell and the podocyte before their fusion into a single membrane.

Measurements of GBM thickness also are influenced by the choice of embedding compound for electron microscopy. Osawa et al[20] found thicker measurements using Vestopal, a polyester-polystyrene copolymer compared with Ethacrylate and Araldite epoxy resins. Haynes[21] described thicker measurements using the low-viscosity Spurr's resin compared with Vestopal and Methacrylate. We consistently have observed that GBMs appear thinner in reprocessed formalin-fixed tissue that has been deparaffinized for electron microscopy (as a salvage technique for inadequate tissue) than in glutaraldehyde-fixed, Epox-embedded tissue. This difference probably relates to increased tissue dehydration with the former method. For this reason, it is not possible to diagnose TBMN accurately in deparaffinized, reprocessed tissue.

Optimally, individual renal pathology laboratories should establish their own criteria for normal GBM thickness based on their fixation, embedding, and measurement techniques. Because biopsy specimens of normal kidneys are rare, some studies have used cases with either minimal change disease or mesangial proliferative glomerulonephritis as the control populations, with normal mean GBM thickness ranging from 287 to 399 nm.[8,10,17,27,28] As a result, the threshold used to define thin in various studies is highly variable, ranging from 200 to 330 nm.[12,13,17,28-31]

In summary, it is best to measure the GBM thickness from the endothelial to the podocyte cell membranes in peripheral capillary walls (not overlying the mesangial reflection). Multiple representative measurements from many capillaries should be recorded, and the arithmetic or harmonic means calculated. The results must be interpreted based on the normal values for the patient's age and sex. Mean adult GBM thickness is approximately 370 ± 50 nm in men and 320 ± 50 nm in women.[25] GBM thickness is approximately 150 nm at birth, 200 nm at 1 year, and approaches adult thickness by 11 years of age.[26]

How Thin is Thin?

Given the wide variation of GBM thickness in the normal population, it has been difficult to define what constitutes thinning of the GBM in a particular individual. For practical purposes, the World Health Organization has promulgated a threshold of 250 nm for adults and 180 nm for children aged 2 to 11 years.[32] Some investigators prefer a threshold of 200 nm in adults, especially in women.[33] There is general agreement that the thinning should affect at least 50% of the total glomerular surface area.

The Columbia University Nephropathology Laboratory has set its own standards for normal based on tissue fixed in 2.5% glutaraldehyde and embedded in Epoxy Resin (Epox 812; Fullam Inc., Latham, NY). Mean GBM thickness is 350 ± 50 nm for adult males and 300 ± 40 nm for adult females. By using a definition of thin that requires greater than 2 SDs below the mean value, we thus define thin GBM as less than 250 nm in adult males and less than 220 nm in adult females. In addition, we require greater than 50% involvement of glomerular capillaries for the diagnosis of TBMN. In the case

of biopsies performed for hematuria and showing isolated GBM thinning that involves less than 50% of the glomerular capillaries, this ultrastructural abnormality is duly noted as *segmental GBM thinning* in the electron microscopic report, although its significance remains unclear at this time.

Light Microscopic Findings in TBMN

By light microscopy, the majority of renal biopsy specimens have little or no pathologic alterations. Usually, the glomeruli appear unremarkable. Light microscopy is too insensitive to detect thinning of the basement membrane accurately, and in most cases the GBM appears normal in thickness, texture, and contour. In some cases, delicate attenuation of the GBM can be appreciated by Jones methenamine silver or periodic acid-Schiff stain, suggesting GBM thinning, but this must be confirmed by electron microscopy (Fig 1A). The mesangium usually is normal in size and cellularity. In a minority of cases, however, the mesangium may show mild segmental or global expansion by either increased cell number, increased matrix material, or both (Fig 1B).[10,11,13,29,30] Erythrocytes may be identified free within the urinary space.

Focal glomerulosclerosis occurs in 5% to 25% of glomeruli, especially when aging or hypertension are present (Fig 1C).[28,34] In the older adult, coexistent arterionephrosclerosis of aging or hypertension, with secondary patchy arteriosclerosis, tubular atrophy, and interstitial fibrosis are not uncommon. It is controversial whether TBMN ever progresses to global glomerulosclerosis. In a Dutch study, global glomerulosclerosis in TBMN involved 13% ± 17% of glomeruli and correlated with the subsequent development of hypertension, proteinuria, and progression to renal insufficiency, suggesting a nonbenign course.[35] Because the mean age of participants in the study was 36 years and none had hypertension at the time of biopsy examination, the global glomerulosclerosis could not be attributed to aging or hypertension. In these patients, Alport syndrome was excluded by the absence of hearing loss and ophthalmologic disease, by the normal GBM staining for the α3 subunit of collagen IV, and by repeat biopsy specimens taken after a median of 10 years that confirmed persistent GBM thinning with minimal reduplication. Unlike in Alport syndrome, repeat biopsy examinations in these patients all showed increased focal global glomerulosclerosis with patchy tubular atrophy and interstitial fibrosis, but without focal segmental glomerulosclerosis or interstitial foam cells.[35] In rare instances in which patients have both TBMN and the nephrotic syndrome, segmental sclerosis including a tip lesion have been reported.[36] Such cases probably represent the coincidental superimposition of idiopathic nephrotic syndrome on TBMN.

In children, the tubulointerstitial compartment is unremarkable or shows only minimal tubular atrophy and interstitial fibrosis.[18] In adults, the amount of tubulointerstitial disease is expectedly more variable, with tubular atrophy and interstitial fibrosis involving from 0% to 60% of the sampled cortical tissue.[11,12,15] Aggregates of interstitial foam cells, a common finding in the more advanced stages of Alport syndrome, are not observed in TBMN. Depending on the severity
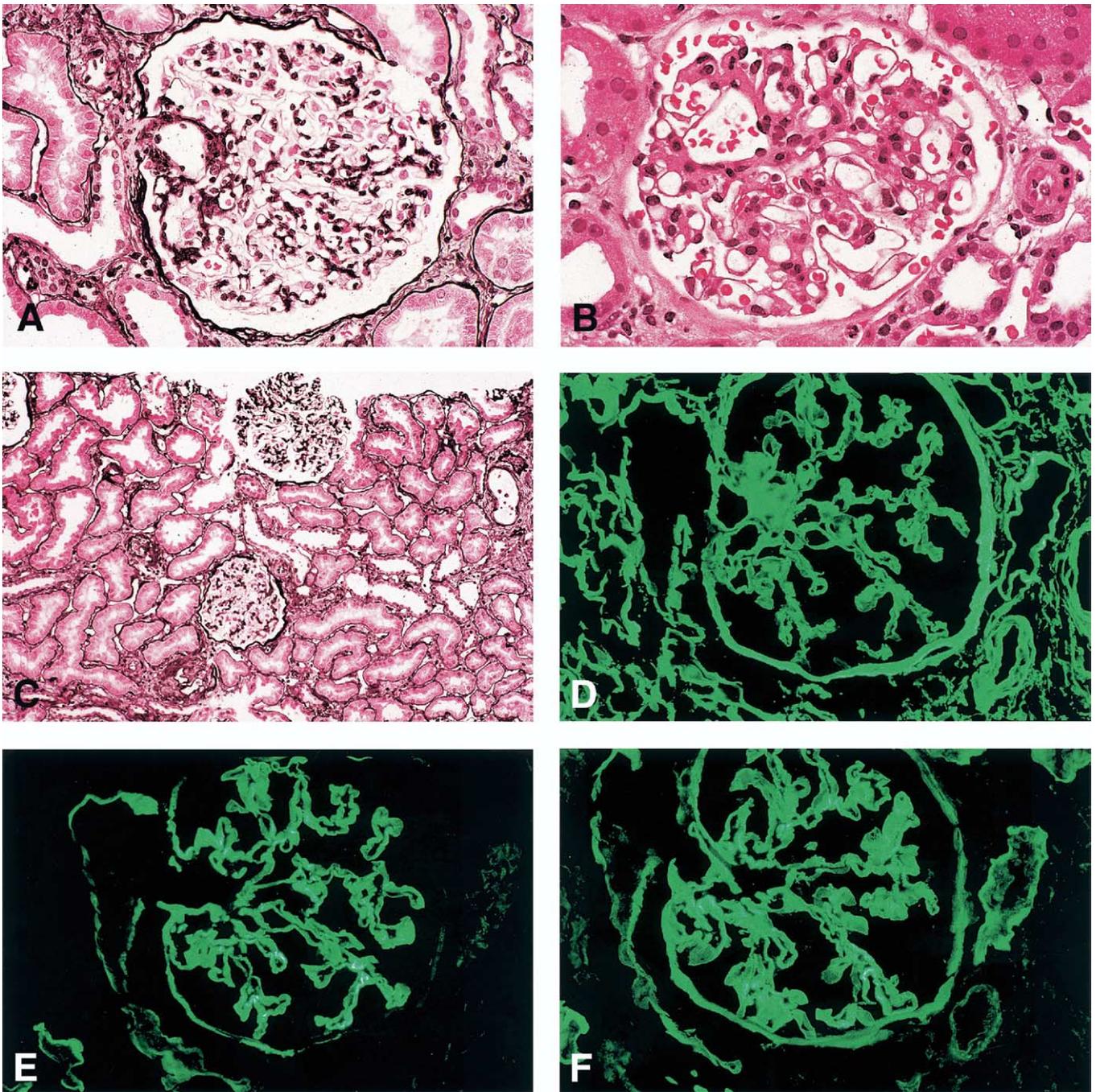
Figure 1 Light microscopic and immunofluorescence findings in TBMN. (A) A representative glomerulus is normocellular with fully patent capillaries. The GBMs appear attenuated, even by light microscopy, in this section stained with Jones methenamine silver (JMS) stain (magnification, 250×). (B) An example of a case with mild mesangial prominence by light microscopy. GBMs appear thin and delicate, with normal texture and contour. Erythrocytes are noted in the urinary space, consistent with a glomerular source of hematuria (hematoxylin-eosin stain, magnification, 400×). (C) A low-power view shows 2 globally sclerotic glomeruli. The other 2 glomeruli and the tubulointerstitial compartment appear unremarkable (JMS, magnification, 100×). (D) Immunostaining for the α1 subunit of collagen IV shows the normal distribution of positivity in all renal basement membranes and the mesangial matrix (magnification, 400×). (E) Immunostaining for the α3 subunit of collagen IV shows the normal distribution of global positivity in the GBM, as well as distal tubular basement membranes. Bowman's capsule stains negative over most of its circumference (in contrast to the positive staining of Bowman's capsule for α1 and α5) (magnification, 400×). (F) Staining for the α5 subunit of collagen IV shows retention of the normal global positivity in the GBM, with positivity in Bowman's capsule and distal tubular basement membranes (magnification, 400×).

of the hematuria, tubules may contain free intraluminal erythrocytes or red blood cell casts. The presence of dysmorphic red blood cells or casts helps differentiate in vivo hematuria from traumatic blood introduced into the tubules by the biopsy procedure. Red blood cell casts have been described in patients with TBMN who have loin pain and macroscopic hematuria.[34] Rarely, gross hematuria results in tubular injury and acute renal failure, analogous to the cases of acute tubular necrosis from hematuria in immunoglobulin (Ig)A nephropathy.[37,38] In these patients, the release of iron from hemoglobin probably produces toxic acute tubular injury through the generation of reactive oxygen species.[39]

Immunofluorescence Findings in TBMN

In most cases, direct immunofluorescence staining is negative for immunoglobulins and complement. In a minority of cases, there may be a weak (trace to 1+) and segmental mesangial positivity, usually for IgM or C3, and rarely for IgG or IgA.[8,13,15,30,36] In the absence of corresponding electron dense deposits at the ultrastructural level, this staining is considered nonspecific. Strong or diffuse staining for any immune reactants suggests a superimposed immune complex–mediated glomerular disease, the most common of which is IgA nephropathy.

Electron Microscopic Findings in TBMN

The renal biopsy diagnosis of TBMN can be made only by electron microscopy. The cardinal feature is thinning of the GBM in the absence of other significant glomerular alterations (Figs 2A and 2B). The reported mean GBM thickness in adults with TBMN varies widely (Figs 2C-2E).[10,12,14,28-31,36,40,41] In a compilation of series analyzing renal biopsy findings in 104 adults with TBMN, the mean GBM thickness was 250 to 300 nm in 22% of biopsy specimens, 200 to 250 nm in 34%, 150 to 200 nm in 38%, and less than 150 nm in only 6%.[10,12,28,30,31,36,40] Most studies report global glomerular involvement, but the changes appear segmental in some individuals.[10,13,15] Some studies have used a cut-off level of 50% or greater GBM involvement.[13,18]

Some investigators have described the attenuation as caused primarily by thinning of the lamina densa, whereas others have noted that the lamina densa appears accentuated.[15,29] We have observed that typically there is relative preservation of the lamina rara interna and externa (these are less affected), which makes the thinned lamina densa appear accentuated. The contour of the lamina densa generally is smooth and uniform. In a minority of cases, there may be very mild and focal splitting or lamellation, as well as foci of intramembranous rarefaction producing electron lucent lacunae.[10,15,18] These textural changes are extremely localized and far less developed than those seen in Alport syndrome. The lamina rara interna and externa also generally are uniform but can have a scalloped appearance (Fig 2F).[8]

Despite the characteristic clinical feature of hematuria in TBMN, ruptures in the GBM are rarely seen. However, a fortuitous plane of section may show an erythrocyte in midtransit across the GBM.[42] The difficulty in detecting gaps could be explained by the relatively small glomerular surface area sampled by 2-dimensional transmission electron microscopy. There are no reports of studies using scanning electron microscopy to quantitate the GBM gaps in TBMN. Based on the infrequency of detected gaps in this condition using transmission electron microscopy, it is likely that the GBM in TBMN is sufficiently thin for erythrocytes to be propelled through the gel-like structure at the heights of systolic pressure in susceptible areas, and that these then close over without the need for any fixed gaps.

The mesangium usually is unremarkable. The attenuated GBM maintains its normally folded contours where it is reflected over the mesangium (Fig 2E). There may be mild expansion of the mesangial matrix, and small scant mesangial electron densities are rare.[10,13,28] The podocytes usually have relatively intact foot processes. However, mild segmental effacement of foot processes may be observed overlying the more attenuated segments (Fig 2B).[11,12,28] If the patient has pronounced proteinuria and the foot process effacement is generalized or complete, minimal change disease or focal segmental glomerulosclerosis superimposed on TBMN should be considered.[36]

Little is known of the genotype-phenotype correlations in TBMN. As yet, no study has reported morphologic differences by either light or electron microscopy based on the mutant gene (*COL4A3* and *COL4A4*) or type of mutation (missense, splice site, frame shift, and so forth).[43-46]

Special Studies for Collagen IV Subtypes

It has been known for some time that the glomeruli from patients with Alport syndrome usually fail to stain with sera from patients with Goodpasture (or antiGBM) disease.[47] However, it was 25 years after this observation first was made before its molecular basis was elucidated. This absence of staining with Goodpasture antisera by indirect immunofluorescence often is used as an ancillary test to confirm the diagnosis of Alport syndrome. By contrast, the glomeruli of patients with TBMN retain positive staining with Goodpasture antisera, although with decreased intensity compared to normals.[48]

In recent years, the use of Goodpasture antisera has been superceded by commercially available monospecific antibodies to the α subunits of collagen IV.[49] These antibodies can be applied to frozen sections of renal or skin biopsy specimens (Table 1). The commercially available Wieslab kit (Wieslab AB, Lund, Sweden) includes antibodies to the $\alpha1$, $\alpha3$, and $\alpha5$ chains of collagen IV. Antibodies to the subunits $\alpha1$ and $\alpha2$ (which comprise the major collagen IV network) normally stain all renal basement membranes including the mesangial matrix. Antibodies to subunits $\alpha3$, $\alpha4$, and $\alpha5$ (which comprise the minor collagen IV network) normally stain the entire thickness of the GBM as well as distal tubular membranes. Normal Bowman's capsule and normal epidermal membrane have immunoreactivity for the $\alpha1$, $\alpha2$, $\alpha5$, and $\alpha6$, but not the $\alpha3$ or $\alpha4$ chains.[50,51]

In TBMN, the glomeruli retain their normal immunoreactivity for the $\alpha1, 2, 3, 4$, and 5 subunits of collagen IV, as
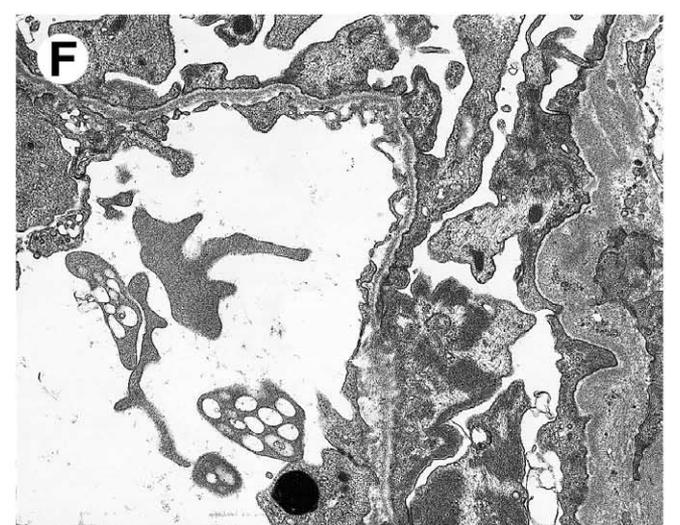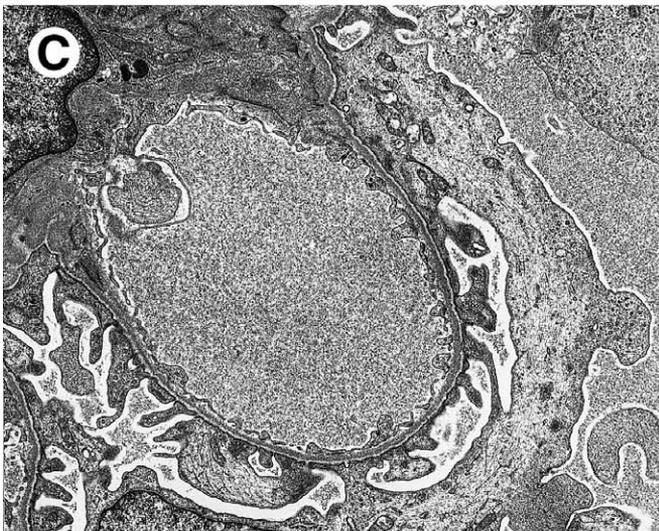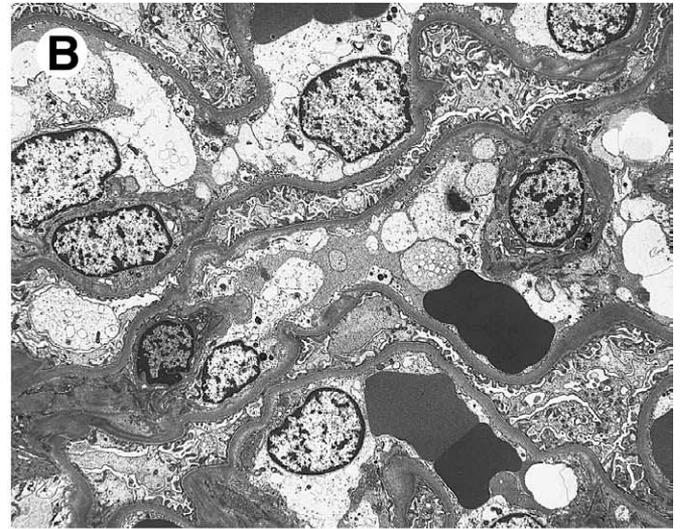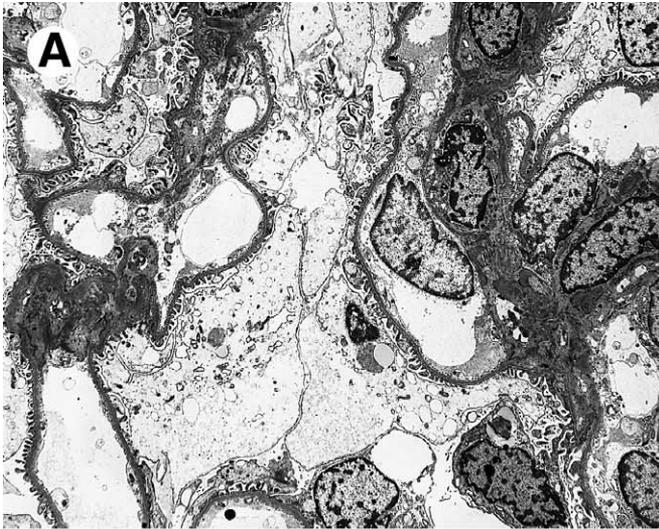
Table 1 Expression Patterns of Type IV Collagen Chains in Kidney and Skin

	Alpha-1 in Kidney	Alpha-3 in Kidney	Alpha-5 in Kidney	Alpha-1 in Skin	Alpha-3 in Skin	Alpha-5 in Skin
Normal	All renal BMs	GBM, D-TBM	GBM, D-TBM, BC	EBM	Negative	EBM
TBMN	All renal BM	GBM, D-TBM	GBM, D-TBM, BC	EBM	Negative	EBM
X-linked Alport syndrome (male)	All renal BM	Negative	Negative	EBM	Negative	Negative
X-linked Alport syndrome (female)	All renal BM	Mosaic*	Mosaic*	EBM	Negative	Mosaic*
Autosomal-recessive Alport syndrome	All renal BM	Negative	D-TBM, BC	EBM	Negative	EBM

Abbreviations: BM, basement membrane; GBM, glomerular basement membrane; D-TBM, distal tubular basement membrane; EBM, epidermal basement membrane; BC, Bowman's capsule
*Mosaic pattern, segmental loss of positivity.

well as for Goodpasture antisera (directed to the α3 subunit) (Figs 1D-1F).[48,52,53] However, because of the GBM thinning, the staining often appears attenuated, giving an impression of weaker positivity than normal.[48]

By contrast, males with X-linked Alport syndrome who have mutations in the *COL4A5* gene typically lack immunoreactivity for the α5 subunit of collagen IV in their GBM and epidermal membranes. Because mutations in the gene encoding the α5 subunit cause defective incorporation of α3 and α4 subunits into the supramolecular collagen IV network, lack of reactivity for α3 and α4 usually accompanies the loss of α5 from the GBM.[54] However, because the α3 and α4 chains are not expressed normally in the epidermal basement membrane, immunostaining for α3 and α4 in skin biopsy specimens has no diagnostic utility. In males with X-linked disease, Bowman's capsule and the distal tubular membranes do not stain for the α5 and α6 chains. Immunoreactivity for the α1 chain in the glomerular and epidermal membranes is normal or even increased because this major collagen IV chain is part of a collagen IV network that does not form heterotrimers with the α5 subunit.[55,56] Females with X-linked Alport syndrome usually show mosaicism with segmental or patchy loss of the α3, α4 and α5 chains from the GBM and segmental loss of the α5 chain from their epidermal membrane.

In patients with autosomal-recessive Alport syndrome (caused by homozygous or compound heterozygous mutations in *COL4A3* or *COL4A4*), there is absent GBM staining for the α3, α4, and α5 chains, but in contrast to X-linked cases, normal staining of α5 and α6 in Bowman's capsule, distal tubular basement membranes, and skin.[57]

Unfortunately, immunostaining for the collagen IV sub-

units has only a 60% to 70% sensitivity for detection of Alport syndrome, and positive staining for α5 does not exclude the disease, especially in patients with single nucleotide substitutions.[49,58] To this point, Lajoie[52] tested a group of hematuric women with mean GBM thicknesses ranging from 161 to 239 nm and found that most had diffuse staining for the α3, α4, and α5 chains consistent with TBMN, including 3 women with segmental GBM lamellation who had male family members with end-stage renal failure, suggesting the women were heterozygotes for X-linked Alport syndrome. Liapis et al[53] also found that preserved α3 and α5 immunostaining did not always differentiate thinning caused by TBMN from that caused by X-linked Alport syndrome. Thus, immunostaining is a helpful, but not an absolute means to distinguish TBMN from Alport syndrome.

Differential Diagnosis

The major disease to be differentiated from TBMN is Alport syndrome. The characteristic and predominant ultrastructural feature of Alport syndrome is lamellation of the GBM, often alternating with segments of thinning (Fig 3A).[59,60] However, in some kindreds with Alport syndrome, particularly with autosomal-recessive inheritance, thinning is the only detectable ultrastructural abnormality.[43] Diffuse thinning also is observed commonly in young children with X-linked Alport syndrome and in some females who are heterozygotes for this condition (Figs 3B and 3C) indicating considerable phenotypic overlap between TBMN and Alport syndrome. Such cases can be classified as "Alport syndrome with thin basement membrane phenotype".[53,61] A full clinical history including the mode of inheritance, a family history of

Figure 2 Ultrastructural findings in TBMN. (A) On low-power view, a case of TBMN displays uniform diffuse attenuation of the GBM compared with (B) a normal age-matched control photographed at the same magnification. Overall, the foot processes are well preserved (magnification, 2,000×). (C) A case of TBMN with characteristic thinning of the lamina densa. There is relative accentuation of the laminae rarae. Mild segmental effacement of foot processes is seen (magnification, 6,000×). (D) A more extreme example of GBM thinning (mean, 100 nm) is shown. There is mild segmental foot process effacement (magnification, 4,000×). (E) There is uniform thinning of the GBM over the peripheral capillary walls. Where the GBM reflects over the mesangium, it retains its usual folded pattern and appears less attenuated. For this reason, measurement of GBM thickness should be performed only on peripheral capillary wall segments (magnification, 5,000×). (F) On high-power examination, the thinned GBM exhibits mild textural irregularities including slight lamellation of the lamina densa and scalloping of the lamina rara interna (magnification, 8,000×).
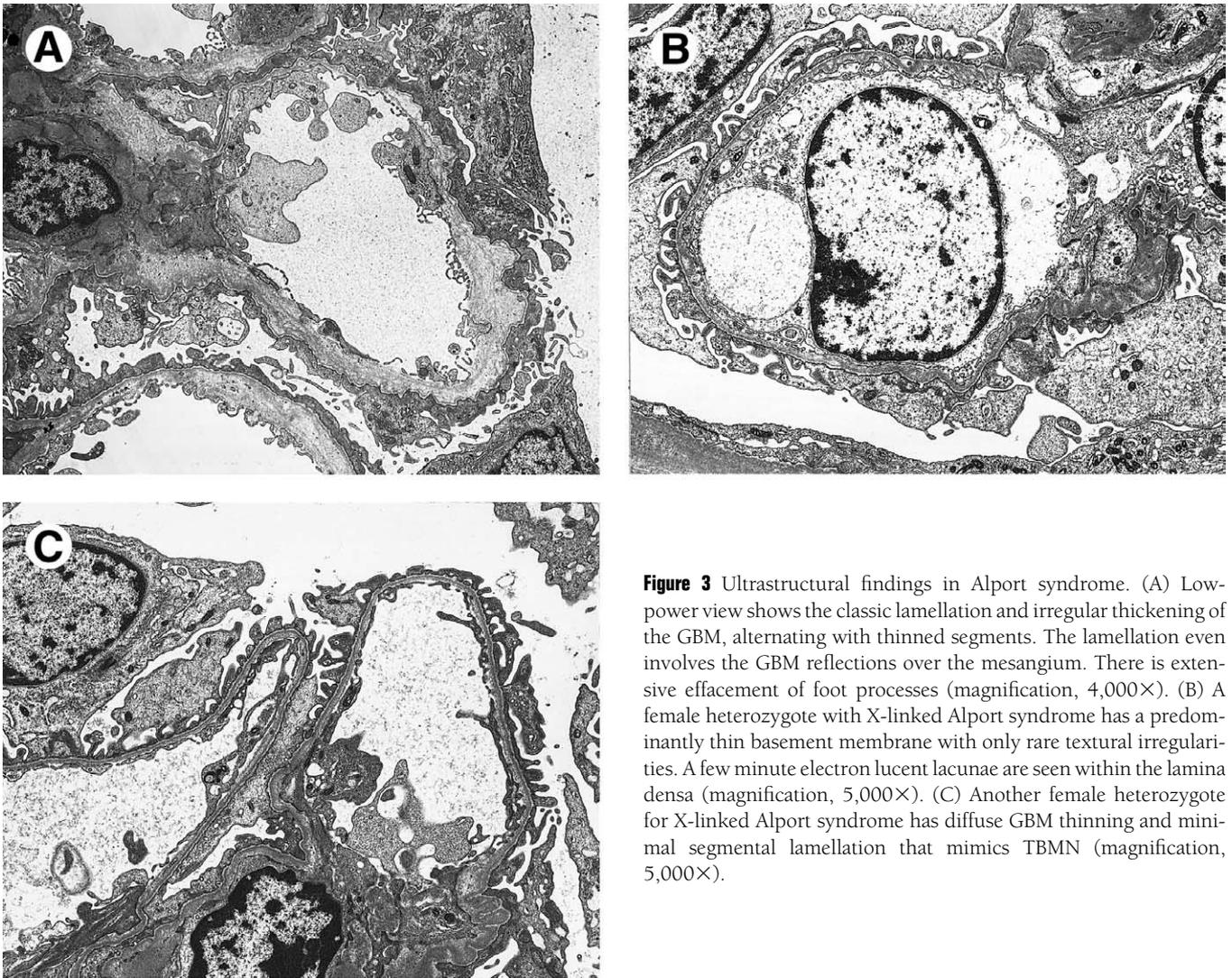
Figure 3 Ultrastructural findings in Alport syndrome. (A) Low-power view shows the classic lamellation and irregular thickening of the GBM, alternating with thinned segments. The lamellation even involves the GBM reflections over the mesangium. There is extensive effacement of foot processes (magnification, 4,000×). (B) A female heterozygote with X-linked Alport syndrome has a predominantly thin basement membrane with only rare textural irregularities. A few minute electron lucent lacunae are seen within the lamina densa (magnification, 5,000×). (C) Another female heterozygote for X-linked Alport syndrome has diffuse GBM thinning and minimal segmental lamellation that mimics TBMN (magnification, 5,000×).

renal failure and deafness, as well as the immunohistochemical examination of the GBM for collagen type IV chains all help differentiate between TBMN and thinning in X-linked Alport syndrome. Furthermore, in Alport syndrome, in contrast to TBMN, progression to secondary focal segmental glomerulosclerosis with prominent interstitial foam cells and progressive chronic tubulointerstitial disease is often found.

GBM thinning also occurs as an acquired non-specific secondary lesion in diverse glomerular diseases because of injury and remodeling. Hill et al[59] reported focal thinning in a variety of glomerulopathies. Thinning may occur because of glomerular capillary distention by thrombosis or endocapillary proliferation. Thus, focal GBM thinning may occur in acute postinfectious glomerulonephritis, lupus nephritis, necrotizing pauci-immune glomerulonephritis, and thrombotic microangiopathies. Localized GBM thinning may be detected in immune complex diseases such as membranous glomerulopathy or resolving postinfectious glomerulonephritis, particularly in foci of resorbed deposits where there has been GBM remodeling.[59] In all of these conditions, an accurate diagnosis can be reached by careful consideration of the observations on electron microscopy together with the clinical,

serologic, light microscopic, and immunofluorescence findings.

Overlap Between TBMN and Other Renal Diseases

Because TBMN occurs in up to 5% of the population,[6] it is not surprising that TBMN often is recognized coexistent with other renal diseases at the time of renal biopsy. The most common of these is IgA nephropathy (Fig 4).[33,62] In this setting, it is not clear whether TBMN occurs as a coincidental finding caused by the overlap of 2 unrelated entities, whether TBMN predisposes to IgA disease, or whether the thinning occurs because of basement membrane remodeling. In one study, 31% of biopsy specimens with IgA nephropathy had segmental GBM thinning defined as less than 250 nm, often accompanied by small foci of lamellation or splitting of the lamina densa.[63] Yoshikawa et al[64] hypothesized that these changes resulted from lysis and attenuation of GBM during resorption of immune deposits. Others have suggested that diffuse GBM thinning in IgA nephropathy represents dual glomerulopathies that are diagnosed coincidentally because of their similar presentations with hematuria.[65]
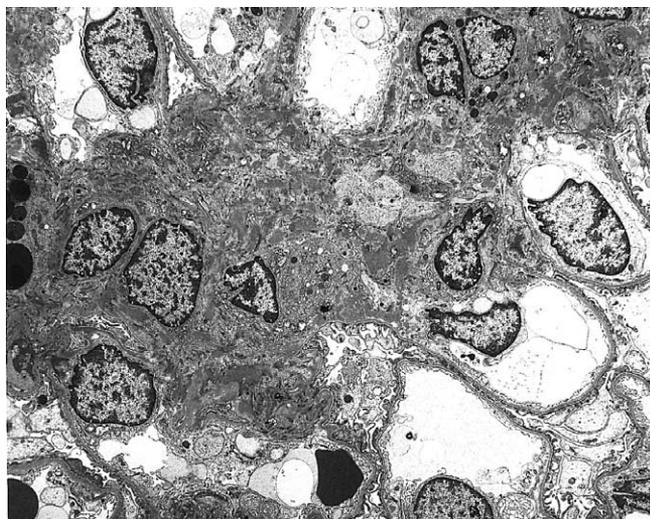
Figure 4 An example of overlap between IgA nephropathy and TBMN. The mesangium is expanded by increased cell number and matrix, which contains abundant mesangial electron dense deposits. The GBM is diffusely and uniformly thinned (magnification, 2,000×).

There are other reports of TBMN occurring as an underlying condition in renal biopsy examinations performed for another glomerular disease. TBMN has been described in association with membranous glomerulopathy.[66] Whereas patients with idiopathic membranous glomerulopathy had a mean GBM thickness of 876 nm, patients with membranous glomerulopathy and thin membranes had an average GBM thickness of 163 nm.[66] Of interest, the patients with underlying TBMN all exhibited stage 1 membranous glomerulopathy, whereas all 4 stages were observed in the group of membranous glomerulopathy controls without TBMN. Thus, the defective GBM synthesis in patients with TBMN appears to inhibit the formation of spikes and overlying neomembranes normally seen in response to the subepithelial deposits in the course of membranous glomerulopathy. Thin membranes also have been reported in patients with membranoproliferative glomerulonephritis and lupus nephritis.[67,68] Some metabolic nonimmune renal diseases show thinned GBM too, including diabetic nephropathy.[69] Interestingly, in a study of type 1 and type 2 diabetic patients, the patients with overlap between diabetic nephropathy and TBMN were reported to have a significantly lower incidence of Kimmelstiel-Wilson nodules, suggesting dysregulated matrix synthesis.[69] Biopsy specimens showing early diabetic nephropathy had a GBM thickness of less than 200 nm, whereas those with advanced disease had progressively more thickened GBM segments greater than 1,000 nm.[69]

In conclusion, the diagnosis of TBMN by renal biopsy challenges the skills of the renal pathologist. The clinicopathologic diagnosis of TBMN is problematic because of the wide range of GBM thickness in the normal population, the lack of uniform criteria for the diagnosis of TBMN, and the observation that GBM thinning also occurs in other glomerulopathies. In the future, pathologic examination of the renal bi-

opsy specimen may be supplemented by molecular or genetic testing that further refines our ability to differentiate TBMN from Alport syndrome, a disease with very different prognosis.

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