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Association of thin glomerular basement membrane with other glomerulopathies

FERNANDO G. COSIO, MICHAEL E. FALKENHAIN, and DANIEL D. SEDMAK

Division of Nephrology, Department of Internal Medicine and Department of Pathology, The Ohio State University, Columbus, Ohio, USA

Association of thin glomerular basement membrane with other glomerulopathies. In the present study we assessed the prevalence of thin glomerular basement membrane (TGBM) in a large group of native kidney biopsies done for evaluation of renal disease. TGBM was present in 54 of 1078 biopsies (5%). In 12 of 54 biopsies (24%), TGBM was the only abnormality present. In the remaining biopsies TGBM was associated with other pathologic diagnoses. The overall prevalence of TGBM in this series is comparable to previous population studies. TGBM is significantly more common in patients with IgA nephropathy and mesangial proliferative glomerulonephritis. Compared to control patients, individuals with TGBM were more likely to have a history of familial hematuria (14% vs. 43%, $P = 0.02$). Furthermore, examination of urinary sediments in first degree relatives of patients with TGBM demonstrated microscopic hematuria in 92% of families and, in those families, hematuria was present in 47 ± 6% of relatives. In contrast, hematuria was discovered in 38% of families of control patients, affecting 25 ± 5% of relatives. In conclusion, the presence of TGBM in a kidney biopsy is highly predictable for the presence of familial microscopic hematuria, even in patients in whom TGBM is associated with another glomerulopathy. The present data also indicate that patients with TGBM nephropathy often have concomitant IgA nephropathy and mesangial proliferative glomerulonephritis.

Thin glomerular basement membrane (TGBM) nephropathy is an autosomal dominant disease characterized anatomically by the presence of abnormal thinning of GBM and clinically by the presence of familial benign hematuria [1]. TGBM nephropathy is now recognized as a leading cause of microscopic hematuria in both children and adults [2, 3] and is generally regarded as a benign condition. TGBM is common, having been demonstrated in 5 to 10% of normal kidneys from transplant donors [4].

The diagnosis of TGBM nephropathy is confounded by the fact that TGBM can be demonstrated in other hereditary renal diseases [5, 6] and in patients with acquired glomerular diseases [7]. An additional issue, in regards to the diagnosis of TGBM nephropathy, is the extent of GBM thinning. In TGBM nephropathy GBM thinning is more often segmental than diffuse [1, 6, 8, 9]. Thus, it has been suggested that the diagnosis of TGBM nephropathy should be based on the demonstration of an average GBM width of less than 200 to 250 nm, based on multiple (up to 400) random measurements of GBM width [3, 4, 10, 11]. Unfortunately, this diagnostic approach is not practical in a routine clinical setting. Given all of these considerations, TGBM nephropathy, despite its frequency, is most likely an underdiagnosed disease.

In the present study, we assessed a large number of native kidney biopsies for the presence of TGBM. In our institution, all native kidney biopsies are examined by electron microscopy, and GBM width is routinely measured in selective photographs representative of the glomerular morphology. The aim of the present study was to: first, determine the prevalence of TGBM in patients with renal pathology; second, to determine whether TGBM is associated with other glomerular diseases; and third, to determine whether the presence of TGBM in a biopsy, even when associated with another glomerulopathy, predicts the presence of familial hematuria.

Methods

Materials

The present study is based on a retrospective review of 1,250 native kidney biopsies performed at the Ohio State University from 1983 to the beginning of 1993. All patients included in this study were adults (>15 years old). All biopsies were examined by light, immunofluorescence (IF) and electron microscopy (EM). One hundred seventy-two biopsies were not considered for further analysis because the tissue available for electron microscopy was not adequate, either due to lack of glomeruli or the presence of sclerotic glomeruli only. All biopsies containing at least one glomerulus in the EM block were included in the present study. As part of the routine EM evaluation of tissues, all glomeruli were visualized under the microscope and all those glomerular areas suspicious for pathology, including possible abnormalities in GBM width, were photographed. Subsequently, measurements of GBM width were done by described methods [3, 11] in those selected photographs. The results of those GBM measurements are routinely included in the final pathology report. For the present studies, all biopsies containing areas of GBM less than 200 nm in width [11] were re-examined by light microscopy and EM, and new EM photographs were obtained in four cases for measurement of additional GBM segments and confirmation of diagnosis. Biopsies that met the following criteria were included in this study: (1) the presence of diffuse or segmental thin GBM with a width of less than 200 nm; (2) the areas of thin GBM occupied at least 50% of the filtration surface of particular capillaries; (3) GBM thinning was demonstrable in more than 1 capillary; (4) to exclude Alport's syndrome, we did not include biopsies with any

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evidence of GBM splitting or the presence of intramembranous dense bodies [5].

Clinical parameters at presentation and follow-up clinical data were obtained by chart review. In addition, we attempted to contact all patients and controls to obtain additional historical data and request urine samples. All patients with a family history of deafness affecting more than one relative, and/or a family history of renal failure, were excluded. All patients were contacted and we requested sterile urine samples from patients and their first degree relatives. Those midstream urine samples were collected at home and mailed to us in sealed receptacles containing a tablet of the preservative Stabilur (Cargille Laboratories, Cedar Grove, New Jersey, USA). Upon arrival, all urine samples were examined by Chemstrip (Boehringer Mannehaim Diagnostics, Indianapolis, Indiana, USA) for the presence of protein, blood, glucose, and white blood cells. Urine samples were centrifuged to separate the urine sediment. Results of the Chemstrip were recorded and each sample was given an identification number which was used for labeling the microscopic slide containing the urine sediment. Those slides were examined independently by two investigators (FGS and MEF) who had no previous knowledge of the individual's name or the results of Chemstrip test. Two groups of control subjects are included in the study: (1) control patients ($N = 17$), that is, individuals included in the biopsy series who demonstrated glomerulonephritis, but had no GBM thinning in the kidney biopsy. Control patients demonstrated, by kidney biopsy, IgA nephropathy ($N = 10$), focal glomerulosclerosis (FGS) ($N = 5$), or minimal change nephrotic syndrome ($N = 2$). (2) Healthy controls ($N = 20$) were individuals with no history or family history of kidney problems. Urine samples from patients, relatives of patients, control patients, relatives of control patients, and healthy controls were collected simultaneously. A total of 281 urine samples were evaluated. Those samples with evidence of urinary tract infection (polymorphonuclear leukocytes and bacteria in the urine sediment) were not included in the study. The number of RBC encountered in each high power microscopic field (×400) varies in each sediment and some fields contain 0 RBC even in patients with microscopic hematuria. In the present study, urine sediments containing a range of RBC/microscopic fields greater that 0 to 2 were considered abnormal. This level of hematuria is also used in our routine clinical practice, where all urinary sediments are examined by a nephrologist. There was a 95% agreement in the interpretation of urinary sediment findings between the two investigators examining urine sediments independently.

Analysis of data

Results were analyzed by chi-square with a Fisher's exact test. Results throughout the manuscript are expressed as means ± standard error of the mean.

Results

Of 1,078 biopsies studied, 54 (5%) met the diagnostic criteria of TGBM nephropathy described above. These patients had a mean age of 47 years (range from 14 to 85) and included 37 females and 17 males. The results of the light and IF microscopic evaluation of these 54 biopsies are summarized in Table 1. Thirteen of the 54 biopsies (24%) demonstrated normal glomerular morphology by light microscopy. The remaining biopsies demonstrated a variety of pathologic abnormalities, as indicated on Table 1. When the prevalence of TGBM was considered for each disease category, there was a significantly higher than predicted prevalence of TGBM in patients with normal glomerular morphology by light microscopy, patients with IgA nephropathy and patients with mesangial proliferative glomerulonephritis. These differences in the prevalence of TGBM among biopsies with glomerular diseases remained significant even when those biopsies demonstrating GBM abnormalities which could have obscured the presence of TGBM were excluded from the analysis such as membranous nephropathy and membranoproliferative glomerulonephritis. The group of patients with mesangial proliferative glomerulonephritis included five biopsies with negative immunofluorescence, three with mesangial IgM, and three with both IgM and C3 staining of the glomerular mesangium. Figure 1 demonstrates representative EM photographs of normal and abnormally thin GBM in patients with normal glomerular morphology and in a patient with IgA nephropathy.

Table 1. Prevalence of TGBM in the population of patients undergoing diagnostic native kidney biopsies

Pathologic diagnosis	N[a]	Biopsies with TGBM	Prevalence %
All biopsies	1,078	54	5
No glomerular abnormalities	42	13	31[b]
Mesangial proliferative GN	95	12	13[b]
IgA nephropathy	83	10	12[b]
FGS	190	10	5
Other[c]		9	

Patients are grouped according to light and IF microscopic diagnosis.
[a] N = number of biopsies
[b] $P < 0.01$ compared to the prevalence of TGBM in all biopsies in this series
[c] This group includes 3 patients with SLE, 1 with ATN, 2 with crescentic glomerulonephritis, 1 with TTP, 1 patient with interstitial nephritis, and 1 biopsy containing insufficient tissue for light microscopic examination

At the time of biopsy, all patients had microscopic hematuria but none had a history of macroscopic hematuria. Table 2 displays additional renal-related clinical parameters present at the time of kidney biopsy in patients with TGBM. In general, patients with normal glomerular morphology had a benign clinical presentation and only one patient demonstrated an abnormally elevated serum creatinine. Of interest, three patients with TGBM and normal glomeruli were biopsied because microscopic hematuria was discovered during their work up as potential kidney donors for their children, all of whom suffered from congenital renal malformations.

Patients with TGBM and abnormal glomerular morphology frequently presented with nephrotic range proteinuria, hypertension and/or renal insufficiency (Table 2). Seven patients with TGBM progressed to end-stage renal failure. Among these, one patient had initially normal glomerular morphology, two patients had FGS, and four patients had IgA nephropathy.

Four patients had complaints of episodic severe flank pain and carried the diagnosis of loin pain-hematuria syndrome. The prevalence of other symptoms was not different between patients with TGBM and controls.

Family history of hematuria was positive in 15 of 35 (43%) patients with TGBM, and in 2 of 14 (14%) control patients ($P = 0.02$ by Fisher's exact test). Urine samples were collected from 13 families of control patients (50%), and 26 families of patients with

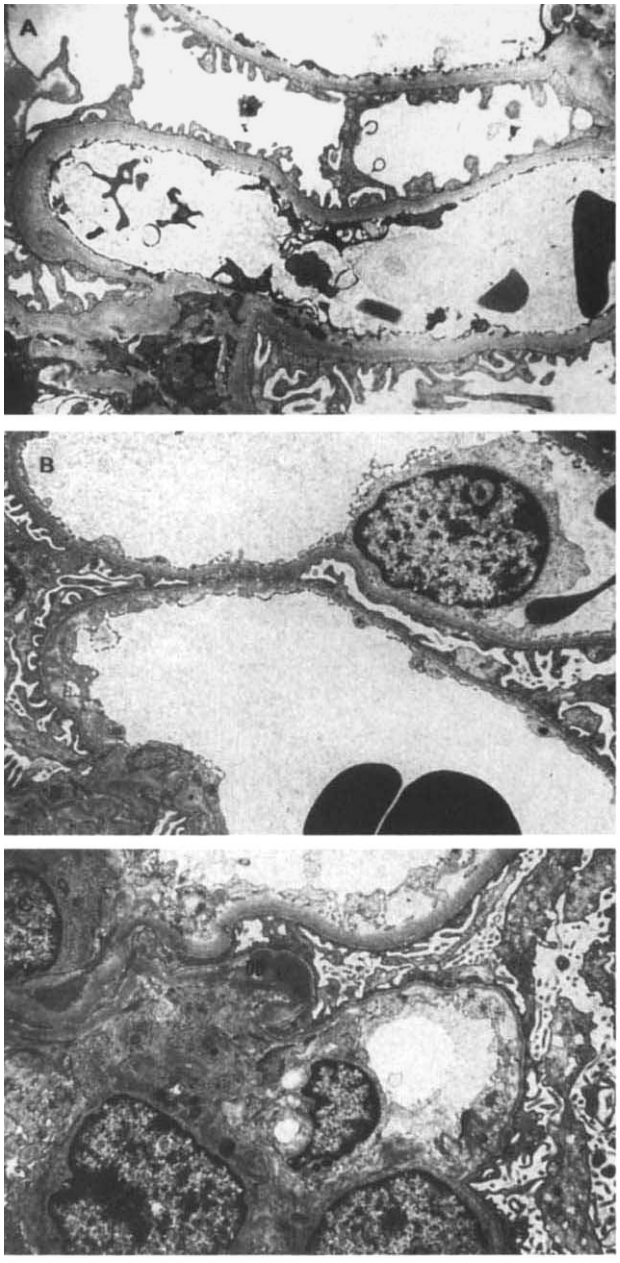

Fig. 1. *Representative electron microscopic photomicrographs (magnification 8,381 for all three pictures).* **(A)** Normal glomerulus demonstrating normal GBM width measuring from 298 to 441 nm; **(B)** TGBM in a patient with normal glomerular morphology by light and IF microscopy. In this particular biopsy GBM width was measured between 131 and 334 nm; **(C)** TGBM in a patient with IgA nephropathy. Note electron dense deposit (DD) and the variability in GBM width from very thin (GBM in lower capillary loop GBM measured an average of 150 nm) to normal width (see upper capillary loop).

TGBM (48% of patients). The distribution of pathologic diagnosis among these 26 TGBM probands was not different from that demonstrated previously for the entire population of patients with TGBM (Table 1). Urine samples from patients, relatives and healthy controls were examined blindly. In urine samples mailed to us by patients (proband), we demonstrated microscopic hematuria in 78% of patients with TGBM, 62% of controls patients,

Table 2. Incidence (%) of renal-related clinical parameters in patients with TGBM grouped according to their renal pathologic diagnosis

Diagnosis	Urine protein >3 g/24 hr	Hypertension	Serum creatinine >1.5 mg/dl
Normal[a]	0%	30%	10%
Mesangial GN	50%	50%	25%
IgA nephropathy	50%	40%	40%
FGS	50%	63%	38%
Other (see Table 1)	75%	50%	86%

[a] Patients in this group had discernible glomerular abnormalities by light and IF microscopy
[b] Values represent percent of patients

Table 3. Prevalence of hematuria in first degree relatives of patients with TGBM and control patients with glomerular disease without TGBM

Parameter	Control patients	TGBM patients	P^a
Percent of patients who had positive family urines	38%	92%	< 0.0001
Percent of first degree relatives with hematuria	25 ± 5%	47 ± 6%	< 0.0001

[a] Statistical comparison, by chi-square (Fisher's exact test), between control patients and TGBM patients

and in none of the urine samples from healthy controls. Results of the examination of family urines is displayed in Table 3. One hundred forty urine samples were collected from first degree relatives of patients with TGBM (mean of 5 relatives per patient, range from 2 to 12). In 92% of those families at least one first grade relative demonstrated microscopic hematuria. In families positive for microscopic hematuria, 47 ± 6% of family members were affected. Seventy-two urine samples were examined from first degree relatives of control patients (mean 6 relatives per patient, range from 3 to 11). In 38% of those families microscopic hematuria was present in at least one relative. In those positive families 25 ± 5% of relatives were affected. Both the familial prevalence of microscopic hematuria (92% vs. 38%), and the percent of family members with hematuria (47% vs. 25%) were significantly higher in patients with TGBM than in control patients (Table 3).

Discussion

In the present study we demonstrated that TGBM nephropathy is a common finding in patients undergoing native kidney biopsies for evaluation of renal pathology. The overall prevalence of TGBM in the present study population is not different from that previously reported in a study that used rigorous methods to assess GBM thickness [4]. This finding suggests that the method used to assess GBM width in the present study is sufficiently sensitive to accurately identify the prevalence of TGBM nephropathy. Additional support for the validity of our approach to identify TGBM is that the diagnosis of TGBM in our patients was associated with a high likelihood of discovering hematuria in first degree relatives.

Based on the present observations, and in previous studies, we propose that the diagnosis of TGBM nephropathy should be based on the following criteria: (1) the presence of diffuse or segmental GBM thinning in at least one glomerulus; (2) the

absence of family history of deafness and/or renal failure; and (3) in patients with thin GBM and another glomerulopathy, the diagnosis of TGBM also requires the demonstration of microscopic hematuria in first degree relatives. Indeed, in affected families we have found a prevalence of hematuria of 47%, consistent with an autosomal dominant mode of inheritance of TGBM nephropathy [1].

The importance of the examination of family urines is exemplified by the following observations. Previous studies have demonstrated the presence of areas of TGBM in patients with acquired glomerulopathies, and suggested that TGBM is a nonspecific diagnostic finding [7]. However, because those studies did not include an examination of urine samples from patient's relatives, we cannot exclude the possibility that those patients suffered from two diseases: TGBM nephropathy and an acquired glomerulopathy. Indeed, the findings of the present study will support this possibility, because most patients with TGBM and another glomerulopathy had familial hematuria. This finding may not be unexpected considering the high prevalence of TGBM nephropathy and presuming, as it seems reasonable, that patients with TGBM are not protected from other glomerular diseases. In fact, the prevalence of TGBM in patients with FGS, the most common diagnosis in our biopsy series, was not different from that encountered in the population as a whole. In contrast, the prevalence of TGBM nephropathy was significantly higher in patients with IgA nephropathy and mesangial proliferative glomerulonephritis suggesting that patients with TGBM nephropathy are predisposed to these glomerular diseases. A previous publication has described a patient in whom TGBM nephropathy was associated with IgA nephropathy [12]. It should be clarified that the association of TGBM nephropathy with IgA nephropathy cannot fully explain the familial prevalence of the latter disease [13]. Thus, we have observed patients with familial IgA nephropathy in the absence of TGBM.

In the present series, patients with TGBM nephropathy and other glomerulopathies did not have a benign clinical course. Previous studies have indicated the potential progression of TGBM nephropathy into renal failure [14]. At the least, these observations should discourage the interchangeable use of the terms familial benign hematuria and TGBM nephropathy.

The demonstration of TGBM in a kidney biopsy has several relevant clinical implications: (1) TGBM indicates the presence of familial hematuria; (2) TGBM may predispose to other glomerulopathies; and (3) in patients with TGBM nephropathy and another glomerulopathy, the former disease may confuse the evaluation of the latter. For example, our series includes three patients with SLE and TGBM nephropathy. In one particular patient, the demonstration of red blood cell casts in the urine was interpreted as indicating an active glomerular process and led to the initiation of immunosuppressive therapy. However, a kidney biopsy demonstrated the presence of TGBM nephropathy and the absence of active lupus nephritis. Based on these clinical implications it is our recommendation that a routine evaluation of kidney biopsies should include EM studies. Alternatively, in patients whose biopsies demonstrate normal glomerular morphology, mesangial proliferative glomerulonephritis, or IgA nephropathy we recommend an examination of urine samples in first degree relatives.

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Reprint requests to F.G. Cosio, M.D., The Ohio State University, Division of Nephrology, N210 Means Hall, 1654 Upham Drive, Columbus, Ohio 43210-1228, USA.

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