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The Many Faces of Thin Basement Membrane Nephropathy; A Population Based Study

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1. Introduction

Thin basement membrane nephropathy (TBMN) or benign familial hematuria, is one of the commonest kidney disorders, characterized by recurrent benign hematuria, which is often associated with a family history (Gregory, 2005). It is generally considered to be a non-progressive, life long disorder with a rather benign course (Tryggvason & Patrakka, 2006), although data from some studies show that a proportion of patients manifest more severe symptoms, than originally described and eventually develop endstage renal disease (ESRD) (Dische, Weston, & Parsons, 1985; Frasca et al., 2005; Pierides et al., 2009; Tiebosch et al., 1989). TBMN was first described in 1926 (Baer, 1926) and at presentation is usually characterized by microscopic painless hematuria, by little or negligible proteinuria and normal renal function. TBMN is an autosomal dominant disorder and more than 50% of the cases have a family history of hematuria. In about 40% of families with a confirmed diagnosis of TBMN the condition co-segregates with heterozygous COL4A3/COL4A4 mutations (Lemmink et al., 1996). Mutations in the type IV collagen gene family which also includes the COL4A5 gene, also cause Alport syndrome (Hostikka et al., 1990). It is believed that these disorders are the result of defective, synthesis and/or assembly, of the critical glycoprotein components, that form the glomerular basement membranes (GBMs), among which type IV collagen, is the major constituent.

Clinically TBMN must be differentially diagnosed, between IgA nephropathy and Alport syndrome which are the other two main causes of hematuria, particularly in children. The need for differential diagnosis, often necessitates the examination of a kidney biopsy at the electron microscopical level, which also enables measurement of the thickness of the GBMs. Indeed Rogers et al (Rogers, Kurtzman, Bunn, & White, 1973) were the first to associate recurrent benign hematuria with the presence of thin GBMs. Currently the most widely...
accepted pathognomonic hallmark for diagnosing TBMN is the presence of diffuse thinning of the GBM, in at least 50% of glomeruli examined. Accurate evaluation of the thickness of the GBM is best performed with the use of morphometry and requires the prior establishment of a normal range of GBM thickness, at each diagnostic centre. It is well documented that the thickness of the normal GBM varies with age, gender and method of tissue preparation; thickness is also influenced by the method of measurement. According to general agreement, the GBM thickness is determined from samples fixed in glutaraldehyde. Recently it has been shown that even the choice of intermediate solvent and type of embedding resin are variables that also affect the thickness of the GBM (Edwards, Griffiths, Morgan, Pitman, & von Ruhland, 2009). Normal GBM thickness has been estimated in several reports (Coleman, Haynes, Dimopoulos, Barratt, & Jarvis, 1986; Das, Pickett, & Tungekar, 1996; Dische et al., 1985; Haas, 2009; Jovanovic GB, 1990). Since there is a lack of a general consensus on diagnostic criteria, it is recommended that a GBM normal range should be established for each laboratory (Dische et al., 1985; Tiebosch et al., 1989). This normal range is a necessary pre-requisite that facilitates the subsequent accurate diagnosis of TBMN. In our department we developed initially a detailed morphometric technique (Marquez et al., 1999) which was subsequently simplified to a more direct method, for measuring GBM thickness in kidney biopsies (Marquez et al., 2003). In this context we are one of a few electron microscopy departments which have been applying a standardized "in house" morphometric method, to diagnose TBMN cases, prospectively for more than 15 years. This method involves surveying the glomerulus and selecting the thinnest 4-5 peripheral glomerular capillary loops. Then the thickness of the GBM is measured in at least 4 different points per loop. If the average (arithmetic mean) of the measurements is less than 300nm, then the case is diagnosed as TBMN. We believe that this approach and practice have contributed to a more accurate estimate of the incidence of TBMN in our population (Zouvani et al., 2008).

In terms of histopathology, TBMN cases have normal glomeruli but some cases are often associated with mild and often premature glomerular changes. In some reports, the coexistence of thin GBMs with lesions compatible with focal segmental glomerulosclerosis (FSGS), affecting a variable number of glomeruli (up to 25%) was noted (Nieuwhof, de Heer, de Leeuw, & van Breda Vriesman, 1997; van Paassen, van Breda Vriesman, van Rie, & Tervaert, 2004). However, in our experience the occurrence of FSGS lesions in TBMN cases is more frequent and most of the glomeruli in TBMN cases show focal glomerulosclerosis. At the same time the importance of applying ultrastructural morphometry will be highlighted, as this is an essential component of accurate diagnosis. Our department operates as a referral diagnostic centre for the whole of Cyprus so the results are based on 1200 renal biopsies, from an unselected population sample, which is considered to be representative of our population. In this cohort of patients, a total of 75 renal biopsies showed thinning of GBMs, average thickness being less than 300nm and these cases were diagnosed as TBMN. The majority of glomeruli in these patients showed FSGS of different stages (different phases as described in the title), ranging from a mild segmental phenotype to more advance lesions of glomerulosclerosis. Another important aspect of TBMN is its association with other glomerulopathies, as shown by the histological examination of renal biopsy. This simultaneous presentation of TBMN with other glomerulopathies was present in seven of the 75 cases included in our study and gave rise to the selection of the title for this chapter. Consequently this title was chosen to reflect the progressive lesions of FSGS that are associated with TBMN, as well as the
simultaneous occurrence of other glomerulopathies in the background of thin GBMs. In this review, the spectrum of histopathological and ultrastructural phenotypes detected in TBMN patients in our population will be described. We also illustrate the value of the direct GBM measurement method in detecting thin GBMs, in cases of TBMN presenting with more advanced glomerulosclerosis. In such cases the presence of thin GBMs may be overlooked, a factor which contributes to an underdiagnosis of this, rather common genetic disorder.

2. Aims

The main aims of the chapter are:

a. To present the histopathological features of TBMN in a population based study.

b. To highlight the fact that TBMN nephropathy is a more frequent disease and has a much higher incidence, than the 1-2% that is usually quoted in the literature.

c. To emphasize the need to use a standardized direct morphometric method for the accurate and systematic diagnosis of TBMN.

d. To highlight the observation that TBMN as a disease entity, may have different faces (phases), as histologically it is usually associated with different stages of glomerulosclerosis. Another factor that contributes to the different histological faces of TBMN is the phenomenon that TBMN is often associated with other glomerulopathies.

3. Materials and methods

3.1 Patients

In the last eighteen years, 1200 renal biopsies from Cypriot patients were investigated in the department of electron microscopy and 75 of these were diagnosed as TBMN. The TBMN group included 35 males and 40 females who at the time of biopsy had microscopic hematuria. Twenty-two patients had hematuria with proteinuria and of these eight had proteinuria in the nephrotic range. The majority of patients had normal renal function (Table 1). Detailed clinical data for some of these patients were described previously (Marquez et al., 2003; Zouvani et al., 2008). In this group of 75 patients, seven patients were diagnosed with TBMN which was associated with other glomerulopathies (Table 2). All patients included in the study were adults, above 18 years old.

3.2 Renal biopsies

Renal biopsies were obtained using a standard percutaneous technique under local anesthesia. The biopsies were examined and sectioned under an ordinary light microscope to select tissue containing glomeruli for light microscopy, immunofluorescence, and electron microscopy. All biopsies containing at least one glomerulus in the tissue submitted for electron microscopy were included.

3.3 Light microscopy

Tissue was fixed in 10% phosphate-buffered formalin and embedded in paraffin wax. Paraffin sections were cut at 4μm and stained with hematoxylin and eosin, periodic acid-Schiff (PAS), silver methanamine, Masson's trichrome and Congo red according to standard protocols.
3.4 Immunofluorescence
A small piece from each biopsy was embedded in optimal cutting temperature (OCT) compound and frozen directly in liquid nitrogen. Cryostat sections were cut at 4μm and incubated with a series of fluorescein isothiocyanate (FITC)-linked mouse antibodies against human immunoglobulin A (IgA), IgG, IgM, C1q, C3 and fibrin, for direct immunofluorescence.

3.5 Routine electron microscopy
Specimens were fixed for a minimum of 4 h in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2, postfixed for 1 h in 1% osmium tetroxide, dehydrated in a series of graded ethanol, cleared in propylene oxide, and embedded in an Epon/Araldite mixture. Blocks were sectioned with a Recheir Jung Ultracut, ultramicrotome (Vienna, Austria). Semithin sections, 1μm thick, were stained with toluidine blue to locate glomeruli. Ultrathin sections of gold interference color were mounted on 200-mesh copper grids and stained with uranyl acetate and lead citrate. Electron microscopy was carried out in a JEM-1010 (JEOL-Tokyo) transmission electron microscope.

3.6 Morphometry
For measuring the thickness of the GBMs two approaches were used as our electron microscope was recently fitted with a digital camera. Consequently for the years spanning 1991-2008 the following methodology was used.
Each available block from a biopsy was serially sectioned to obtain the maximum number of glomeruli. In each glomerulus, each patent peripheral loop was photographed and this sometimes required a maximum of three separate micrographs per loop. Micrographs were taken at a magnification of x4,000 on 6.5 x 9cm Kodak electron microscope film. A grating replica with 2160 cross lines/mm was used for calibration (Electron Microscope Sciences, Washington, PA 19034). Prints were made to a final magnification of x12,000 and morphometric measurements were taken as described previously, using the simplified method (Marquez et al., 2003).
Since 2008 the software tool of the MEGAVIEM, Olympus Soft Imaging, system is being used to measure GBM thickness. In each biopsy every available glomerulus is scanned and at least four thin glomerular loops that are situated peripherally, are measured at a magnification of ×8000. For measurement, a square grid, size 2000x2000 nm, is superimposed over the glomerular capillaries and point measurements are taken at regions where the grid intersects the capillary loops exactly at right angles. Each point measures the distance from the cell membrane of the epithelial cell, as it attaches on the lamina rara externa, to the inner aspect of the endothelial cell membrane. In this manner a minimum of 16-20 separate points were measured in each peripheral glomerulus which represented 4-5 points per individual loop (Figs. 8 and 9b). In each patient the arithmetic mean of the GBM thickness was calculated, averaging measurements at 16 points, which is the minimum number of points measured per glomerulus. This approach was very similar to the direct method that was used previously (Marquez et al., 2003).

4. Results
4.1 Inclusion criteria
The selection criteria of biopsies for this study included:
a. Normal histology or minimal to moderate histological glomerular abnormalities.
b. Presence of thin GBMs having an arithmetic mean of less than 300 nm, or having more than 50% of the points measured below 300 nm upon direct measurement.

c. Presence of diffuse GBM thinning in a minimum number of four peripheral capillaries per glomerulus.

d. Absence of splitting or lamellation of the GBM.

e. Absence of diffuse fusion of podocytes in order to exclude primary FSGS.

4.2 Light microscopy

Seventy-five renal biopsies were selected for the present morphometric analysis based on the light microscopical observations and inclusion criteria outlined above (Table 1). On histology, 14 cases exhibited normal glomeruli. In 26 cases, up to 10% of glomeruli were affected by glomerulosclerosis (Figs. 1a and 1b) and in 27 cases glomerular sclerosis was present in between 10-50% of glomeruli (Figs. 2a and 2b). Focal glomerulosclerosis was characterized most often by an increase in mesangial matrix and very rarely this also included an increase in mesangial cells. In the remaining eight cases, glomerulosclerosis was evident in more than 50% of the total glomeruli identified in each biopsy (Fig. 3 and Table 1). These 75 patients included seven cases with the diagnosis of incidental TBMN which was associated with other glomerulopathies (Table 2).

4.3 Immunofluorescence

In all cases the immunofluorescence results were essentially negative except in the three cases that had a diagnosis of IgA nephropathy (IgAN), where moderate mesangial staining for IgA was present. In ten cases, there was mild and focal mesangial staining for IgM and two cases showed staining for C3.

4.4 Electron microscopy

All biopsy specimens, especially the ones obtained from patients with proteinurin showed a variable degree of epithelial cell foot process fusion. In all 75 cases foot process effacement was segmental and never global. In the selection process, renal biopsies showing global foot process fusion were excluded from the TBMN group, in order to eliminate inclusion of cases with primary FSGS. In terms of GBM thickness, 14 out of 75 patients who exhibited histologically normal glomeruli showed widespread thinning of the GBM. In the remaining patients, that showed glomerulosclerosis affecting between 10-50% of glomeruli, thinning of the GBM was more obvious in the glomerular segments that were unaffected by increased mesangial matrix and glomerulosclerosis. In all patients examined attenuation of GBM was the predominant finding. Thin GBMs were present in normal-looking capillary loops (Fig. 4) as well as in areas showing mild expansion of the mesangial matrix (Fig. 5). In some biopsies that exhibited a more marked increase in mesangial matrix, thin GBMs could still be distinguished even in severely affected areas, especially in peripheral glomerular capillaries (Fig. 6). In some patients, the thickness of the GBM was not uniform, and this was not related to orientation or differences in sectioning (Fig. 7). In such cases morphometry (see below) provided more accurate estimates of the GBM thickness.

4.5 Morphometry

For classifying our own patients, the normal adult mean GBM thickness range of 300–400nm was adopted. This is our “in-house” range of normal adult GBM thickness that was
Number of patients

| Characteristics                  | Total = 75 | TBMN = 68 | TBMN + associated GN=7 |
|----------------------------------|------------|-----------|------------------------|
| Male/Female                      | 35/40      | 32/36     | 3/4                    |
| Age-mean                         | 46         | 44        | 50                     |
| Age range                        | 20-71      | 20-71     | 24-67                  |
| Family history                   | 20         | 20        | 0                      |
| Hematuria                        | 75         | 68        | 7                      |
| Proteinuria                      | 22         | 15        | 7                      |
| Normal renal function (serum creatinine <1.0 mg/dL) | 46 | 46 | 0 |
| Normal glomeruli                 | 14         | 14        | 0                      |
| Glomerular sclerosis in <10% of glomeruli | 26 | 26 | 0 |
| Glomerular sclerosis in >10% - <50% of glomeruli | 27 | 20 | 7 |
| Glomerular sclerosis in >50% of glomeruli | 8 | 8 | 0 |
| Mean GBM thickness <200nm       | 17         | 17        | 0                      |
| Mean GBM thickness >200nm - <300nm | 58 | 51 | 7 |

Table 1. Demographic and clinicopathological findings in TBMN patients.

| Number of cases | 7 |
|-----------------|---|
| IgA nephropathy (IgAN) | 3 |
| Mesangial proliferative GN (MesPGN) | 1 |
| Minimal change disease (MCD) | 2 |
| Transplant glomerulopathy (TG) | 1 |

Table 2. TBMN cases associated with other glomerulopathies.

established previously (Marquez et al., 1999). For the morphometric measurements of the thickness of GBM, the simplified method was used for biopsies obtained between 1991-2008; details of which were previously published (Marquez et al., 2003). For the renal biopsies investigated after 2008, the morphometric method using the iTEM software of the MEGAVIEW digital camera was applied as described in section 3.6 (Figs. 8 and 9b). Out of the 75 patients investigated 58 had an average GBM thickness between 200 and 300 nm, whereas for the remaining 17 patients the mean GBM thickness was below 200 nm. None of the 75 patients had a mean GBM thickness below 100nm.

In some cases showing progressive glomerulosclerosis, some glomerular capillaries exhibited uniformly thin GBMs (Fig. 10a), but other capillaries in adjacent areas, within the same glomerulus, required morphometry as there was a big variation in the thickness of the GBM (Fig. 10b). In many biopsies RBCs were frequently seen in the Bowman’s space, as evidenced by the hematuria observed in this cohort of patients. Seven patients had TBMN associated with other glomerulopathies among which the commonest was IgA nephropathy. In these patients electron microscopy revealed the presence of isolated mesangial and subendothelial, electron dense deposits as well as thin GBMs (Figs. 11a and 11b).
Fig. 1. a. Renal biopsy from a 35 year old male patient with hematuria. Light microscopy of renal biopsy stained with hematoxylin and eosin showing glomerulus with an almost normal histology. Early lesions of focal segmental glomerulosclerosis are shown (arrows) but most capillary loops are patent (L) (magnification x400).

Fig. 1. b. Semithin araldite section stained with toluidine blue showing a glomerulus from the same patient as fig. 1a with patent peripheral capillary loops (L) (x400).
Fig. 2. a. Renal biopsy from a 45 year old male patient with hematuria. Light microscopy showing two adjacent glomeruli, with different stages of FSGS. On the left a mild lesion is present, whereas on the glomerulus on the right the lesions are more advanced (arrows). In this patient the GBM measured 230nm (Hematoxylin and eosin stained section) (x400).

Fig. 2. b. Renal biopsy from a 50 year old female patient with hematuria and mild proteinuria. Light microscopy showing two adjacent glomeruli, with a different degree of glomerulosclerosis. Left glomerulus shows more advanced sclerosis (arrows) compared to the right. This patient had a mean GBM thickness, in unaffected glomeruli of 200 nm (Hematoxylin and eosin stained section) (x400).
5. Discussion

In the discussion that follows, the main aims of this chapter as outlined in section 2 will be discussed.

5.1 Histopathological features of TBMN

In this study we present the ultrastructural and histopathological features of a large series of TBMN cases diagnosed in the Cypriot population. TBMN is generally characterized by attenuation of GBMs, a finding that can be documented only by ultrastructural examination of kidney biopsies. This key ultrastructural feature was initially described by Rogers et al., in 1973; they were the first to associate the clinical symptoms of hematuria with the presence of thin GBMs. The above observations provided the basic criteria for distinguishing the more severe, Alport-type progressive nephritis, which ultrastructurally is characterized by thinning, thickening, and GBM lamellation from the more benign form of TBMN. It is well established that electron microscopy is absolutely essential for the accurate diagnosis of TBMN since the light microscopic alterations are usually nonspecific and include a spectrum of findings. Histology may show the presence of normal glomeruli or a range of pathological lesions such as focal glomerular sclerosis and mesangial matrix expansion. Direct immunofluorescence staining is usually negative, but sometimes traces of segmental mesangial staining for IgM and C3 may be present in some TBMN cases, as was observed in this study. In some renal biopsies, GBM attenuation can be observed histologically in Jones methenamine silver or PAS stains, but these findings are only suggestive of TBMN. Consequently, electron microscopy remains the only modality that can confirm the presence of thin GBMs. Our results show that the majority of TBMN cases on renal biopsy show early glomerular changes such as mesangial matrix increase that are compatible with FSGS.
association of TBMN with FSGS has been previously noted in other studies (Foster, Markowitz, & D’Agati, 2005; van Paassen et al., 2004). The presence of glomerular changes that inadvertently lead to increased mesangial matrix and thickening of GBMs, should be taken into consideration as this will affect the accurate diagnosis of TBMN. Indeed in our study, eight cases contained more than 50% glomerulosclerosis but on morphometry had GBM thickness of less than 300 nm. These eight cases, as well as the seven cases of TBMN associated with other glomerulopathies were included in our study as they had clinicopathological features resembling TBMN and fulfilled the inclusion criteria defined. In recent years it has become increasingly evident that within the TBMN group, the degree of thinning is quite variable, not only between patients but also within individual glomeruli from the same patient. Indeed, in some of our cases, we observed variations in the thickness of adjacent GBMs and even within the same capillary loop, GBM thinning was not uniform. Therefore, ultrastructural examination of renal biopsies has to include a detailed morphometric analysis in order to clearly define such cases accurately, and for confirming that the GBM attenuation is the major lesion.

5.2 Incidence of TBMN
TBMN is the commonest cause of recurrent glomerular hematuria that affects both children and adults (Tryggvason & Patrakka, 2006). Although a common nephropathy, the incidence of TBMN is poorly defined and estimates vary widely in the literature from 1% to 9% (Tryggvason & Patrakka, 2006), (Dische et al., 1990). Using electron microscopy in conjunction with a direct quantitative technique for measuring GBM thickness we recently estimated the incidence of TBMN to be 5.4% in the Cypriot population (Zouvani et al., 2008). These results are in agreement with those obtained by other studies (Cosio, Falkenhain, & Sedmak, 1994), where it was estimated that persistent hematuria occurs consistently in as much as 6% of both children and adults (Wang & Savige, 2005). Since some cases of Alport syndrome particularly the autosomal recessive type, can resemble TBMN ultrastructurally, children were excluded from this study as well as cases showing splitting or lamellation of the lamina densa (Haas, 2006). The exact incidence of TBMN is difficult to assess, since the diagnosis is mostly made on the basis of persistent hematuria combined with minimal proteinuria. Indeed, the prevalence of hematuria in adults is not well known (Gregory, 2005). In addition, when estimating the prevalence of TBMN by analyzing the incidence of hematuria it should be remembered that not all patients who have TBMN have hematuria. Some patients with persistent hematuria, have other signs of renal dysfunction that exclude presence of TBMN, and most importantly, hematuria is not always of glomerular origin (Nieuwhof et al., 1997). In our case, the incidence was estimated on the basis of electron microscopic diagnosis and the presence of thin GBMs, having an arithmetic mean of less than 300 nm. However, the number of electron microscopic analyses of renal biopsies showing thin GBMs are few (Marquez et al., 1999), so many cases remain undiagnosed. It is likely that our figure of 5.4% is accurate, since it is based on the presence of thin GBMs as documented by electron microscopical examination, in a large series of consecutive kidney biopsies. These biopsies were unselected for a family history of hematuria or other familial renal disorder, so they represent an unbiased pool of samples on which our estimate is based. Therefore, it is not surprising that the incidence rate for TBMN in our population was estimated to 5.4%, (Zouvani et al., 2008) since our results are based on the actual presence of thin GBMs, which
Fig. 4. Electron micrograph from the kidney biopsy of a 48-year old male patient presenting with hematuria showing uniform thinning of GBM of a peripheral glomerular capillary loop. The average thickness of the GBM was 220 nm. Note a widely patent capillary lumen (L) containing several RBCs. The foot processes are well preserved (arrowheads) (x15,000).

Fig. 5. Electron micrograph from the kidney biopsy of a 37-year old female patient presenting with hematuria showing variability in the thickness of the GBMs. The capillary lumina (L) are widely patent but areas of mesangial matrix increase are evident (MM). The foot processes (arrowheads) are well preserved. This patient had a mean GBM diameter of 250 nm (x9,000).
is the gold standard for diagnosing this disorder. Application of such morphometric methods at the ultrastructural level, are bound to increase the sensitivity and accuracy of the diagnosis, so it is not surprising that our incidence rate is much higher than the 1%, that is usually quoted. The accurate diagnosis of these TBMN cases by electron microscopy provided the basis and impetus for the subsequent molecular genetic studies that were recently published in this cohort of patients (Pierides et al., 2009; Voskarides, Patsias, Pierides, & Deltas, 2008). Another factor that could contribute to an increased incidence of TBMN in the Cypriot population is the recent characterization of founder mutations in our population (Voskarides et al., 2008).

5.3 Use of morphometry

It should be noted that the thickness of the GBM varies with age, gender, and method of tissue preservation. Considering the different results obtained by the various studies and the fact that there is no gold standard, as regards the method or cut off values for distinguishing between normal and thin GBMs, we established our “in house” normal range of GBM thickness as recommended (Dische et al., 1985). In this context, previous morphometric work in our department at the ultrastructural level resulted in establishing the normal adult GBM thickness in our kidney biopsies to be between 300 and 400 nm (Marquez et al., 1999; Marquez et al., 2003). Using the above “in-house” GBM normal range values, our results show that the incidence of TBMN in Cyprus is 5.4%, which is much higher than the 1% usually quoted in the literature (Tryggvason & Patrakka, 2006). However, recent estimates put the incidence of TBMN to be not less than 1% and not greater than 10%. Indeed, our results agree with those of Wang and Savige (2005) who estimated that persistent hematuria occurs consistently in as much as 6% of both children and adults.

Indeed in such cases, if one applies the gold standard technique for estimating GBM thickness, from orthogonal intercepts recommended by others (McLay, Jackson, Meyboom, & Jones, 1992; Ramage et al., 2002), the presence of thin GBMs will almost certainly be missed. For this reason we recommend the use of a much simpler and direct measurement technique (Das et al., 1996 Marquez et al., 2003), which is performed in the thinnest GBM loops after surveying the glomeruli to be examined in each case. This approach contributed to the correct diagnosis of eight of the 75 cases presented in this report in which the presence of thin GBMs would have been overlooked. It is important to note that some of the cases show a progressive glomerulosclerosis and at the time of diagnosis, present with only isolated segments showing capillary loops with thin GBMs. These segments could be easily overlooked during the examination of the renal biopsy in the electron microscope and this could be another contributing factor to the wide variation that exists in the literature, regarding estimates of the incidence of TBMN.

5.4 TBMN, a disease with many faces (phases)

In our study, the majority of TBMN cases showed signs of FSGS, ranging from early lesions characterized by a mesangial matrix increase to the appearance of more progressive sclerosis, affecting more than 50% of the glomeruli in some cases. In this context, when we first started to perform quantitative morphometric measurements for estimating GBM width in the mid 1990’s we carefully selected cases that on histology showed a maximum of 10% sclerosed glomeruli (Das et al., 1996). However it is evident from our results and those of other studies (Foster et al., 2005; van Paassen et al., 2004) that
the most frequent morphologic entity associated with TBMN is FSGS. The extent and severity of glomerulosclerosis seen in each TBMN case is variable and ranges from mild, premature glomerular sclerotic changes, to more advanced stages presenting with glomerulosclerotic lesions that may affect up to 50% of the glomeruli. Although as a diagnostic entity TBMN may contain a heterogeneous group of conditions with different aetiologies, molecular genetic studies show that more than 40% of cases are known to be associated with heterozygous mutations in COL4A3 and COL4A4 genes (Tryggvason & Patrakka, 2006) (Lemmink et al., 1996). Recent genetic studies in Cyprus have revealed the presence of founder mutations in the COL4A3 gene in some Cypriot kindreds manifesting TBMN and FSGS (Voskarides et al., 2008). It is noted that the Cypriot population presents an interesting and rather isolated genetic pool, as novel founder mutations have also been characterized in other susceptibility genes, by our group such as the BRCA2 gene, that predisposes to the breast ovarian cancer syndrome (Hadjisavvas et al., 2004).

Patients with TBMN, or benign familial hematuria as the name implies, are believed to have a non-progressive, stable outcome, although some studies report that 30-40% of patients progress to ESRD (Dische et al., 1985; Frasca et al., 2005; Pierides et al., 2009; Tiebosch et al., 1989). At present very little is known about the mechanisms or the involvement of other genes in influencing the severity of the TBMN phenotype. As observed in the present as well as in other studies the extent of FSGS seen in patients, even from the same families is variable and does not depend on age of diagnosis (Voskarides et al., 2008). In some studies TBMN was divided into diffuse and segmental types (Ivanyi, Pap, & Ondrik, 2006), but it is possible that what was observed is different stages of FSGS. How mild or how severe, the glomerular sclerosis lesions are in each renal biopsy, depends on the interplay of other factors, genetic or not, of which little is known at present. Consequently the diagnosis of TBMN has clinical implications for the prognosis of patients, as now it is believed that it may not be as benign a lesion as was originally thought of. In addition, TBMN poses...
scientific challenges as there is a need to understand how mutations in GBM proteins, including the collagen type IV genes, produce thinning which alters glomerular physiology and leads to glomerulosclerosis in some patients. In the study by Voskarides et al. (2007) nearly 20% of patients, with adequate follow-up information, developed ESRD. Consequently the mechanisms that determine the outcome of TBMN need to be elucidated and research is in progress in our department towards achieving this aim. In this context, there is also increasing evidence that TBMN predisposes to the development of other nephropathies (Berthoux, Laurent, Alamantine, & Diab, 1996; Costo et al., 1994).

Fig. 7. Electron micrograph from the kidney biopsy of a 45-year old female patient, showing a marked increase in mesangial matrix (MM) and very thin GBMs (arrows), with an average GBM thickness of less than 200 nm. Note well preserved foot processes (arrowhead) (x10,000).

TBMN may occur in a familial setting and up to 50% of cases have a family history or it may be sporadic. The molecular defects underlying TBMN were characterized in the early 1990s, with the identification of type IV collagen genes, found to be mutated in Alport syndrome (Barker et al., 1990; Hostikka et al., 1990). Subsequently Lemmink et al (1996) noted that some carriers of the autosomal forms of Alport syndrome had thin GBMs, so they analyzed the COL4A3 and COL4A4 genes in patients with TBMN. They were the first to associate the presence of heterozygous mutations in these two genes with TBMN and thus provided the underlying genetic defect that explains about 40% of cases. The remaining cases may be sporadic and caused by de novo mutations or by defects in other genes that are as yet to be identified. It should be noted that such genetic tests are not widely available, because the presently known candidate genes COL4A3 and COL4A4 are quite large, consist of about 50 exons each, making genetic analysis expensive and rather insensitive. Furthermore, the occurrence of frequent polymorphisms in the COL4A3 and COL4A4 genes makes it difficult to confirm the pathogenicity of sequence variants with certainty. In addition, recent genetic studies in TBMN patients in Cyprus (Pierides et al., 2009; Voskarides et al., 2007; Voskarides et al., 2008), show that heterozygous carriers of mutations in the COL4A3 and COL4A4
manifest TBMN with many different clinical and histological features. Indeed, very little is known at present about the pathophysiologic mechanisms involved in the manifestation of thin GBMs, the development of which may lead to FSGS under the influence of as yet unknown modifier genes. In this context, the examination of a renal biopsy in the electron microscope and use of ultrastructural morphometry, remains the most accurate modality for correct diagnosis and prognosis of patients presenting with recurrent hematuria and proteinuria. An additional reason for the wider application of electron microscopy is the fact that several cases of TBMN are associated with other glomerulopathies, see section 5.5 below, which further necessitates the examination of a renal biopsy for correct diagnosis.

Fig. 8. Electron micrograph from a 48-year old male patient showing application of the morphometric method used for measuring the thickness of the GBM. The iTEM software of the MEGAVIEW digital camera was used to overlay a grid on top of the glomerular capillary. As shown 4-5 measurements were taken at points of the GBM, where the grid intersected the GBM exactly at right angles (arrows) (x15,000).

5.5 Association of TBMN with other diseases
TBMN is inherited as an autosomal dominant disease and is caused by the presence of heterozygous mutations in the COL4A3 and COL4A4 genes. Carriers of such pathogenic mutations, therefore have a pre-existing genetic condition, which predisposes them to the development of TBMN and thin GBMs. It is not unreasonable to expect that other glomerulopathies will develop in this setting as noted by other investigators (Cosio et al., 1994; Lanteri, Wilson, & Savige, 1996; Matsumae, et al., 1994). In our series of 1200 kidney biopsies and based on the selection criteria employed in our study, seventy-five biopsies were diagnosed as TBMN. Seven of these biopsies had additional glomerular changes that were characteristic of other glomerulopathies. As shown in table 2, three patients had IgAN, one had MesPGN, one had a TG, and two had MCD. The existence of TBMN with other glomerulonephropathies has been previously described (Cosio et al., 1994) and it was
suggested that this disease entity may predispose to other glomerulopathies. In our study the concurrence of TBMN with other glomerulopathies was nearly 10%.

Fig. 9. a. Electron micrograph from the kidney biopsy of a 39 year old female presenting with hematuria showing a mild increase in mesangial matrix (MM) and patent capillary loops (x12,000).

Fig. 9. b. Higher power electron micrograph from the kidney biopsy of the same patient showing application of the morphometric method used as described in the text and fig. 8, for measuring the thickness of the BM. The GBM measured 100nm (x15,000).
Fig. 10. a. Electron micrograph from the kidney biopsy of a 41 year old male presenting with hematuria, showing patent capillary loops with uniformly thin GBMs measuring 230nm (x12,000).

Fig. 10. b. Higher power electron micrograph from the same patient, showing increased mesangial matrix (MM) in the capillary on the left and thin GBM in the capillary on the right. An RBC is seen in the Bowman’s space (x20,000).
Fig. 11. a. Electron micrograph from the kidney biopsy of a 46 year old female patient presenting with hematuria showing the presence of subendothelial deposits (arrows) and increased mesangial matrix (MM). The patient had positive immunofluorescence of IgA and a mean GBM diameter of 200nm (x25,000).

Fig. 11. b. Electron micrograph from the kidney biopsy of the same patient showing patent glomerular capillary loops with mesangial deposits (arrow) (x15,000).
6. Conclusion

When Rogers et al., (1973) first recognized and described TBMN, there was little expectation that he was actually describing one of the commonest genetic disorders, affecting glomerular structure and function. Generally, TBMN is characterized by recurrent hematuria and a non-progressive course; although recent data alert to the development of more progressive renal symptoms in 20-30% of patients. Accurate diagnosis relies on rigorous ultrastructural morphometric measurements and on the presence of thin GBMs even in cases that show glomerulosclerosis in more than 50% of glomeruli, as alluded to in this study. Our results demonstrate that the majority of TBMN cases are associated with glomerular histopathology that resembles FSGS; this finding should be taken into consideration when morphometry is applied for accurate diagnosis. Recent advances in genetics, particularly in relation to the collagen IV family of genes have greatly improved our understanding of the underlying genetic causes that lead to TBMN.

However, our knowledge of the pathophysiologic mechanisms involved in the manifestation of TBMN is limited. There is a pressing need to identify markers that can aid in the better diagnosis and prognosis of patients diagnosed with this heterogeneous disease, in order to provide more effective patient management strategies and prevent progression to ESRD.

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