Membranes basales glomérulaires minces: une lésion courante.

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Thin glomerular basement membrane

1973: Thin GBM
> Familial Benign Essential Hematuria

PM Rogers

1972-1973: Thick and split GBM
➢ Alport Syndrome

N Hinglais, GS Spear, J Churg
The nonspecificity of the ultrastructural alterations in hereditary nephritis and benign familial hematuria (G Hill, Lab Invest 1974)

- No single morphologic parameter (GBM splitting or attenuation) or group of parameters is diagnostic of either hereditary nephritis or benign familial hematuria.
Thin glomerular basement membrane

- Normal GBM thickness
- Thin GBM and familial (or sporadic) benign hematuria
- Thin GBM, always a benign condition?
- Thin GBM and associated glomerular diseases
Thin basement membrane nephropathy / Thin GBM nephropathy/ Thin GBM disease......

- Thin-basement-membrane nephropathy, also called benign recurrent hematuria
  \( (ATMG\ Tiebosch\ et\ al,\ NEJM\ 1989) \)

- Thin GBM nephropathy, so-called benign familial hematuria
  \( (CMG\ Nieuwhof\ et\ al,\ Kidney\ Int\ 1997) \)

- Benign familial hematuria (Thin GBM disease)
  \( (CE\ Kashtan,\ JASN\ 1998) \)

- Thin-basement-membrane nephropathy = Thin BM disease = familial benign hematuria = benign hereditary nephritis = familial benign essential hematuria = ... 
  \( (K\ Tryggvason,\ JASN\ 2006) \)
Normal GBM Thickness

- **Birth:** 100 nm
- **Childhood:** 100 > 300 nm (3-5 ans)
- **Adulthood:**
  - 315 +/- 98 nm (Osawa, 1966) (Normal kidneys)
  - 356 - 464 nm (FE Dische, 1990) (IgA Glomerulopathies)
  - 418 +/- 48 nm (S Saxena, 1990) (Normal renal biopsies)

According to sex:

<table>
<thead>
<tr>
<th>Gender</th>
<th>Male</th>
<th>Female</th>
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<tbody>
<tr>
<td>Male</td>
<td>330 +/- 50 nm</td>
<td>305 +/- 45 nm</td>
</tr>
<tr>
<td>Female</td>
<td>343 +/- 39 nm</td>
<td>300 +/- 12 nm</td>
</tr>
<tr>
<td>Male</td>
<td>364 +/- 56 nm</td>
<td>350 +/- 45 nm</td>
</tr>
<tr>
<td>Female</td>
<td>373 +/- 42 nm</td>
<td>326 +/- 45 nm</td>
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(M Haas, 2006) (Nephrosis, AIN)
(CS Rayat, 2005) (Normal kidneys)
(TMG Tiebosch, 1989) (Normal renal biopsies)
(MW Steffes, 1983) (Kidneys from donors 118 cases)
Normal GBM Thickness
Why so different?

• Differences in the processing of specimens (fixation, inclusion) for electron microscopy.

• Differences in the number of measures, and the methods for measuring GBM thickness (distance between the endothelial and epithelial cell membranes apposed to the GBM)
  - Measures performed at regular intervals along the GBM without any selection
  - Measures avoiding tangential sections of the GBM
  - Measures made where the lines of an overlaid grid cut the endothelium, with or without exclusion of the tangential sections.

• Each laboratory has to establish its own standard.

• At least two glomeruli have to be systematically examined.
Methods for measuring GBM thickness

Measures at regular intervals

At least 100 measures have to be made on at least 2 glomeruli.

Measures at the cutting points.
Normal GBM Thickness (G Osawa, 1966)

- Nine renal biopsies from healthy volunteers and 14 autopsy specimens
- Individuals aged 22 to 92 years. No relationship between age and sex and GBM thickness
- “There was great irregularity in width”
Thin GBM. Definition criteria, prevalence

• **Definition of thin GBM.**
  - **Thickness:**
    - In adults: < 200 nm
    - < 250 nm (most studies)
    - < 300 nm
    - In children: < 180 nm
  - **Extension:**
    - Diffuse
    - > 50%
    - between 10 and 50%

• **Prevalence**
  - In the general population: 1-10%
  - Among renal biopsies: 0.8-11%
  - In hematuric patients: 10-50%
Ultrastuctural study of the GBM

GBM in “TBMD” is remarkably thin  (G Basta-Jovanovic, J Churg.1990)
Measuring GBM thickness
Necessity of looking at several glomeruli

In the same patient

Thin GBM in one glomerulus

Typical AS lesions in the second one.
Thin glomerular basement membrane

- Normal GBM thickness
- Thin GBM and familial (or sporadic) benign hematuria
- Thin GBM, always a benign condition?
- Thin GBM and associated glomerular diseases
Familial benign essential hematuria


Fig 1.—Pedigree of the kindred with familial benign essential hematuria.
Familial benign essential hematuria

- Isolated microscopic hematuria in patients between 2 and 86 yrs
- Diffuse thin GBM: 150 nm in the 5 biopsied patients aged 19, 21, 24, 49 and 51 years.
- Focal effacement of podocyte foot processes.
Thin glomerular basement membrane in familial benign hematuria

- **GBM thickness in 50 children with familial benign hematuria:**
  - Diffusely thin GBM: 19 patients
  - Irregular alternation of normal and thin GBM segments: 22 patients
  - Normal GBM: 9 patients

  *N Yoshikawa, 1988*

Analysis of 65 hematuric children:

Thin GBM in 33 patients with sporadic (10) or familial (23) benign hematuria:

- Diffusely thin GBM (>50% of the GBM length): 23 patients
- Focal thinning affecting 10 to 50% of the GBM length: 10 patients

*CH Schröder, 1990*
Benign familial hematuria with thin GBM
Thin glomerular basement membrane
Prevalence of thin GBM in the general population (FE Dische, 1990)

• According to their definition of normal GBM thickness (330-460 nm), the incidence of thin GBM in the general population lies between 5.2 and 9.2%.

- Patients (8-63 yrs) presenting with hematuria and/or proteinuria were studied during a seven-year period.

- 110 patients (11%) (81F, 30M) with GBM attenuation
  - Mild in 39 pts 241 nm
  - Moderate/severe in 71 pts 214 nm
  - Controls 320 nm

Proteinuria (45%), familial kidney disease (40%), deafness in the family (10%)

“Long-term prospective follow-up is necessary to evaluate the nature and effects of this entity”
Incidence of “thin-membrane nephropathy” in hematuric patients (GJ Perry, 1989)

“Thin-membrane nephropathy” diagnosis in 26 (28%) hematuric patients (Mean age: 42 yrs)

“Limited follow-up has confirmed the good pronostic of the condition”

But
4 patients developed Pu
4 patients have a family history of nephritis.

*NB: controls: MCD or AIN*
Thin glomerular basement membrane. 
The Italian experience in children (R Coppo, 1998)

Analysis of renal biopsies performed in 432 children ≤ 15 years
>>> Thin GBM in 5% of cases

Among the 83 children with isolated hematuria:
>>>> thin GBM in 25,3% of cases

NB: IgA nephritis in 34,9% of cases.
Benign familial hematuria is due to mutation of the type IV collagen $\alpha 4$ gene.

Figure 1. Segregation analysis of type IV collagen $\alpha 3$ and $\alpha 4$ markers in the BFH family. Haplotypes were constructed for the type IV collagen $\alpha 3/\alpha 4$ CA repeat and the type IV collagen $\alpha 4$ HaeIII RFLP. Type IV collagen $\alpha 3/\alpha 4$ CA repeat fragments are indicated as the length of the PCR fragment in basepairs (76, 78, and 80 bp). The HaeIII RFLP alleles are represented by 1 (HaeIII site absent) and 2 (HaeIII site present). The haplotype [78, 2], shown as black bars, cosegregates with BFH. Filled symbols indicate individuals with hematuria. Arrow indicates index patient (III-10).

HH Lemmink, 1996
Benign familial hematuria, 
a type IV collagen disorder

- Heterozygous *COL4A3* or *COL4A4* mutations are associated with familial benign hematuria in autosomal recessive Alport syndrome families (Lamprecht, 1996; Boye, 1998; Heidet, 2000, Buzza, 2001, Longo, 2002).

- Heterozygous *COL4A3* or *COL4A4* mutations are associated with familial benign hematuria in families without autosomal recessive Alport syndrome (Badenas, 2002; Buzza, 2003; Gross, 2003).

- So, a certain proportion of patients with BFM and TGBM have a type IV collagen disease, and are carriers of autosomal recessive Alport syndrome.
Benign familial hematuria with thin GBM not linked to the COL4A3/COL4A4 locus.
Yamazaki, 1995; Piccini 1999

8 unrelated kindreds from eastern Sicilia.

Hematuric pts: 4.5-61 years.

Exclusion of COL4A3, COL4A4, COL4A5.
Autosomal recessive Alport syndrome
Phenotype of heterozygous carriers

- No symptom
- Persistent or intermittent microscopic hematuria:
  > Clinical features of familial benign hematuria.

Rare cases of slowly progressive nephropathy.

Thin GBM in all studied carriers

*COL4A4* mutation
Thin glomerular basement membrane

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- Thin GBM and associated glomerular diseases
Thin basement membrane nephropathy / Thin GBM nephropathy/ Thin GBM disease......

Always a benign condition?

<table>
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<tr>
<th>Condition</th>
<th>Patients</th>
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<tbody>
<tr>
<td>Proteinuria &gt; 500mg/24h</td>
<td>6/18 patients</td>
</tr>
<tr>
<td>180-500mg/24h</td>
<td>5/18 patients</td>
</tr>
<tr>
<td></td>
<td>Tiebosch, 1989</td>
</tr>
<tr>
<td>Proteinuria 200-500mg/24h</td>
<td>50/110 patients</td>
</tr>
<tr>
<td>RF (2 pts), HTA (21 pts)</td>
<td></td>
</tr>
<tr>
<td>Family history of nephropathy or deafness</td>
<td>44/110 patients</td>
</tr>
<tr>
<td></td>
<td>Aarons, 1989</td>
</tr>
<tr>
<td>Proteinuria and/or RF and/or</td>
<td>7/14 patients</td>
</tr>
<tr>
<td>family history of progressive nephropathy</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dische 1985</td>
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</tbody>
</table>
Thin GBM disease......
Always a benign condition?

• Thin GBM was observed in 31 children (2-18 years) presenting with isolated hematuria.
  Clinical evaluation and family investigation led to the following diagnosis:
  - Alport syndrome : 7 patients
  - Progressive nephritis without hearing loss 6 patients
  - Familial benign hematuria 7 patients
  - Sporadic isolated hematuria 11 patients

  (MC Gubler, 1990)

• More recently, from a prospective regional study in the Netherland, P. van Paassen et al. concluded that, TBMN is genetically heterogeneous and does not correspond to a benign disease in a substantial number of adult patients (40-50 years): 15% of them develop proteinuria (of the nephrotic range in 5%), associated in most cases with FSGS.

  (P. van Paassen, 2004)
Thin GBM disease......
Always a benign condition?

- Thin GBM is not a specific lesion.
- It may be observed in progressive hereditary nephritis
  - X-linked Alport syndrome
  - Autosomal recessive Alport syndrome
  - Autosomal dominant Alport syndrome
  - Progressive hematuric nephritis without extra-renal symptoms.

- In these conditions, the finding of thin GBM is more frequent in children than in adults but may be the only GBM lesion observed all along the life.
Thin GBM in X-linked Alport syndrome.

One 4 year-old hematuric boy without extrarenal symptoms or family history

De novo COL4A5 mutation
Thin GBM and X-linked Alport syndrome.

Large duplication in COL4A5 (Arrondel C et al. KI 2004)

Thin GBM in the 5 biopsies (11-29 yrs).

Large X-linked Alport syndrome family:
Progressive nephritis, deafness, lenticous
Thin GBM in X-linked Alport syndrome.
Thin GBM in autosomal recessive Alport syndrome.

2 yrs: hematuria + Pu 100 mg
   no extrarenal symptoms
   no family history
15 yrs: nephrotic syndrome
20 yrs: CRI
22 yrs: ESRD
Mutations COL4A3
Autosomal recessive Alport syndrome
Expression of type IV collagen chains

Control

Patient
Autosomal dominant Alport syndrome
Heterozygous \textit{COL4A3} mutation:

Large family with autosomal dominant Alport syndrome

Jefferson et al. 1997: Linkage to \textit{COL4A3-COL4A4}.
van der Loop et al. 2000: Heterozygous splice mutation in \textit{COL4A3}
Autosomal dominant Alport syndrome

Spectrum of phenotypes associated with heterozygous COL4A3-COL4A4 mutation:

- Analysis of 25 individuals in 4 families with heterozygous mutation in COL4A3 (2 families) or COL4A4 (2 families)

Phenotypic heterogeneity:
- Renal involvement:
  - ESRD between 41 and 70 years in 5 patients
  - Chronic renal failure between 46 and 65 years in 4 patients
  - Isolated hematuria between 22 and 51 in 12 patients
  - No symptom in 3 patients 11, 22 and 90 year-old
- Hearing involvement:
  - Hearing defect in 4 patients after 45 years
- No ocular involvement
- Normal expression of type IV collagen chains

GBM: thick and split in three families
Thin in one family

Pescucci et al. KI 2004
Thin GBM in autosomal dominant Alport syndrome (J Cledes)

Breton family with autosomal dominant Alport syndrome and COL4A4 splice-site mutation >>>>> stop

ESRD at 47 yrs, CRF at 36 and 48 yrs, persistent H +/- proteinuria in others

Thick and split GBM in one patient; Thin GBM in 3 patients
COL4A3/COL4A4 mutations producing focal segmental glomerulosclerosis and renal failure in thin basement membrane nephropathy

K Voskarides et al, JASN 2007

• 13 Cypriot families (82 patients).
• **Microscopic hematuria** in adolescence and early adult life
• Follow-up of 3 decades.
• Progressive development of proteinuria, nephrotic syndrome and CRF (37.8%) and ESRD (19.5%).
• Absence of extrarenal symptoms.
• Mutations **COL4A3: G1334E** (7 families) and **G871C** (3 families)
  **COL4A4: 2854delG** (1 family)

• Renal biopsies >> dual diagnosis of
  “Thin basement membrane nephropathy”
  and “focal segmental glomerular sclerosis”
COL4A3/COL4A4 mutations producing focal segmental glomerulosclerosis and renal failure in thin basement membrane nephropathy

K Voskarides et al, JASN 2008
COL4A3/COL4A4 mutations producing focal segmental glomerulosclerosis and renal failure in thin basement membrane nephropathy

K Voskarides et al, JASN 2008
Thin GBM in Col4a3 +/- mice
(B Bierowski et al JASN 2006)

Development of progressive hematuric nephritis in heterozygous mice:
8 weeks: Hematuria
12 weeks: Mild but increasing proteinuria
33 weeks: Thin GBM (167nm vs 200 nm) + FSGS
18 months: Renal insufficiency

Death: 21.7 vs 30.3 months in wild type mice
Possible type IV collagen mutations associated with thin GBM in patients with isolated hematuria

*COL4A5* mutations >>>> **X-linked Alport syndrome**: the prognosis is always severe at least in men

Col4A3 or COL4A4 homozygous or compound heterozygous mutations

>>>>> **Autosomal recessive Alport syndrome**

Severe prognosis in both sexes

*COL4A3* or *COL4A4* heterozygous mutations

>>>>> **Large clinical spectrum of clinical expression:**

- No symptoms
- Familial benign hematuria
- Progressive nephritis without extrarenal symptoms
- **Autosomal dominant Alport syndrome**
Clinical phenotypes in hematuric patients with thin GBM and COL4A3 or COL4A4 heterozygous mutations

• Why so diverse phenotypes may be associated with heterozygous COL4A3/COL4A4 mutations?

• Role of
  - type / localization of the mutation?
  - additional variants in COL4A3/COL4A4 sequences?
  - other genes?
  - environmental factors?

• Prognosis? IHC: expression of type collagen chains
  Gene screening? at this time, no genotype-phenotype correlation
  +++ Importance of clinical and family investigation and long-term follow-up

+++ Importance of clinical and family investigation and long-term follow-up
Thin GBM and kidney donation in families with progressive hematuric nephritis

- Difficult ethical question when heterozygous relatives (asymptomatic or hematuric) are willing to serve as kidney donors, usually for their children.

- They should not be accepted if they have proteinuria, HTA or sensorineural deafness.

- They may be accepted if they are strongly motivated, well informed and asymptomatic.

- Or if they have isolated hematuria over a certain age (for ex 40-50 years)
Thin glomerular basement membrane

• Normal GBM thickness
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Thin GBM observed in other glomerulopathies

- Thin GBM has been observed in patients with:
  - Steroid sensitive nephrotic syndrome with minimal glomerular changes
  - Nephrotic syndrome with FSGS
  - Diabetic nephropathy
  - Membranous nephropathy
  - Mesangioproliferative glomerulopathy

In patients with this association, microscopic hematuria is more frequent than in controls. But no difference in the clinical course of the associated disease has been observed.

“Thin GBM is a developmental abnormality of little or no significance”.
(Sue, Nephrology 2004)
Thin GBM observed in patients with sporadic or familial IgA nephropathy

- Thin GBM is a common finding in IgA nephritis and has been observed in about 1/3 of all IgA nephritis (Berthoux et al, 1996)

- The association is more frequent in familial than in sporadic IgA (Frasca et al, 2004)

- This association should characterize a subgroup of IgA nephritis, as suggested by the recent finding of a difference in the glycosylation profile of serum IgA according to the GBM thickness (Linossier et al, 2003)
Conclusion (1)

• Diffuse thinning of the GBM is a frequent anomaly observed in 1 to 10% of all renal biopsies

• Thin GBM is usually associated with microscopic hematuria and has been regarded as a marker of a benign entity, sporadic or familial benign hematuria transmitted as an autosomal dominant disorder.

• Heterozygous mutations in COL4A3 or COL4A4 have been detected in 40% of families. BFH patients can be carriers of autosomal recessive Alport syndrome.

• Exclusion of linkage to the COL4A3/COL4A4 locus has been shown in some BFH families.
Conclusion (2)

- However, thin GBM is not a specific lesion. It may be observed in progressive hematuric diseases, in children, but also in adults affected with:
  - X-linked Alport syndrome due to COL4A5 mutations
  - Autosomal dominant or recessive Alport syndromes due to COL4A3 or COL4A4 mutations
  - Progressive hematuric nephritis without extrarenal symptoms linked to COL4A3 or COL4A4 mutations

The main clinical problem is to differentiate between FBH and progressive hematuric nephritis
Conclusion (3)

Hematuria + thin GBM
Clinical examination + family evaluation

Isolated sporadic or familial hematuria

Mode of transmission?

IHC (skin or kidney)

IHC Normal
SA or FBH
Regular F.U. examination

IHC Abnormal
Molecular diagnosis

Mode of transmission +
Genetic counselling

Linkage analysis
Conclusion (4)

• The unsolved questions:

  - What is the meaning of the effacement of foot processes along the thin GBM, in non proteinuric patients?

  - And the classical dilemma: how to explain the contrast between the presence of persistent hematuria and the absence of proteinuria?
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