Thin GBM disease and Alport syndrome

Alenka Vizjak, Damjan Glavač
Institute of Pathology,
Faculty of Medicine University of Ljubljana,
Ljubljana, Slovenia
# Thin GBM disease and Alport syndrome

<table>
<thead>
<tr>
<th></th>
<th>Locus (protein)</th>
<th>ESRD</th>
<th>GBM</th>
<th>Hearing loss</th>
<th>Ocular abnor m</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Collagen IV disorders</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thin GBM disease</td>
<td>COL4A3 COL4A4</td>
<td>-</td>
<td>Thinning</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Alport syndrome</td>
<td>COL4A5 COL4A3</td>
<td>+</td>
<td>Thickening/</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>COL4A4</td>
<td></td>
<td>splitting</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Non-collagen disorders</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nail patella syndrom</td>
<td>LMX1B (transcription factor)</td>
<td>+</td>
<td>Thickening</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>FSGS with MYO1E mutation</td>
<td>MYO1E (myosin 1E)</td>
<td>+/-</td>
<td>Thickening/</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>splitting</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MYH9-related disorders</td>
<td>MYH9 (myosin HC IIA)</td>
<td>+/-</td>
<td>Thickening/</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>splitting</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Galloway-Mowat syndrome</td>
<td>genetic basis unknown</td>
<td></td>
<td>fatal in childhood</td>
<td>Thickening/splitting</td>
<td>+</td>
</tr>
</tbody>
</table>
Type IV collagen network
From: Jennette JC et al. Heptinstall’s Pathology of the Kidney, 2006, pp 406
Clinical picture:
• Persistent familial hematuria (proteinuria ±)
• No progression of renal disease

Genetics:
• Mutations of COL4A3/A4 in ~40%
Renal pathology in thin GBM disease

- **Light microscopy** unremarkable
- **Characteristic EM changes:**
  Diffuse (>50%) thinning of the GBM
  (<200 nm in children, depending on the age; <250 nm in adults – WHO monograph; <200-330 nm in some studies)
- **Methods of measuring GBM thickness on EM**
  - Harmonic mean thickness
    *(Dische FE. Arch Pathol Lab Med 1992; 116: 43-49)*
  - Arithmetic mean of direct measurements
    *(Das AK et al. Nephrol Dial Transpl 1996; 11: 1256-1260)*
Alport syndrome

Clinical picture:

• Familial hematuria with varying degrees of proteinuria
• Progressive renal disease
• Progressive sensorineural hearing loss
• Ocular abnormalities (dot-an-fleck retinopathy, anterior lenticulus and posterior polymorphous corneal dystrophy)
## Alport syndrome – genetic forms

<table>
<thead>
<tr>
<th></th>
<th>X-linked</th>
<th>Autosomal recessive</th>
<th>Autosomal dominant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutated collagen IV α   chain</td>
<td>α chain 5</td>
<td>α chain 3</td>
<td>α chain 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>α chain 4</td>
<td>α chain 4</td>
</tr>
<tr>
<td>Frequency</td>
<td>85%</td>
<td>10-15%</td>
<td>very rare</td>
</tr>
<tr>
<td>Gender effect</td>
<td>yes</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>ESRD (in 50% of pts)</td>
<td>25 yrs</td>
<td>25 yrs(?)</td>
<td>50 yrs</td>
</tr>
<tr>
<td>Hearing loss</td>
<td>80-90%</td>
<td>100%</td>
<td>?</td>
</tr>
<tr>
<td>Ocular abnormalities</td>
<td>30-40%</td>
<td>30-40%</td>
<td>?</td>
</tr>
</tbody>
</table>
COLA3 and COLA4 mutation associated spectrum of diseases

From: Kashtan CE. Curr Opin Pediatr 2004; 16: 177-181

Mutations in both alleles of COL4A3 or COL4A4

ARAS

Mutations in one allele of COL4A3 or COL4A4

ADAS

TBMN

ASYMPTOMATIC

Hematuria

Proteinuria

Renal Failure

Deafness

ARAS, autosomal recessive Alport syndrome; ADAS, autosomal dominant Alport syndrome; TBMN, thin basement membrane nephropathy.
Renal pathology in Alport syndrome

- **Light microscopy** – normal → nonspecific: mesangial widening, glomerular capillary wall thickening, collapse of glomerular loops, global and segmental glomerular sclerosis; interstitial fibrosis, interstitial foam cells

- **Immunofluorescence** – negative or nonspecific

- **Immunohistology** for α3, α4, α5 (IV) chain – frequently abnormal pattern of expression or complete absence

- **Electron microscopy** – in early stages diffuse attenuation of GBM (100-200 nm) → irregular thinning and thickening of GBM (800-1200 nm) with multilamellae, fragmentation and reticulation of lamina densa into several strands – “basket-weave” pattern; small electron dense granules
Kidney biopsy study in patients with familial hematuria
(Institute of Pathology, Ljubljana, 1973-2007)

- **AS – 76 patients from 49 families**
  - 36 male (3-43 yrs; 15.9)
  - 40 female (3-53 yrs; 21.3)

- **TBMN – 20 patients from 18 families**
  - 12 male (7-41 yrs; 20.8)
  - 8 female (8-42 yrs; 22.1)

- **AS? TBMN? – 5 patients from 5 families**
  - 2 male (16, 40 yrs)
  - 3 female (12, 13, 31 yrs)
Alport syndrome – pathologic changes in 76 patients from 49 families

(Institute of Pathology, Ljubljana)
LM pathologic changes in AS
EM changes in TBMN and AS
4-year-old male patient with AS
(α chain 5 coll V missense mutation)
Expression of $\alpha_5$ chain (IV) in normal, XLAS and ARAS in kidney and skin

<table>
<thead>
<tr>
<th>Protomer</th>
<th>Kidney</th>
<th>Skin</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha_3$- $\alpha_4$- $\alpha_5$</td>
<td>GBM, BC, dTBM</td>
<td>-</td>
</tr>
<tr>
<td>$\alpha_5$- $\alpha_5$- $\alpha_6$</td>
<td>BC, dTBM</td>
<td>EBM</td>
</tr>
</tbody>
</table>
Pathogenesis of GBM changes in Alport syndrome

- Normal
  $\alpha_1-\alpha_1-\alpha_2$(IV) network $\rightarrow \alpha_3-\alpha_4-\alpha_5$(IV) network

- Alport syndrome
  - GBM initially thin susceptible to digestion
  - reconstruction of GBM attempted by accumulation of $\alpha_1-\alpha_1-\alpha_2$(IV)
  - overexpression of type V and VI collagen, laminin $\alpha_2$ chain, fibronectin
  - collagen receptor on podocyte in contact with abnormal GBM $\rightarrow$ expression of TGF-\(\beta\)1, CTGF, MMPs and chemokines leading to chronic renal lesions
Potential mechanisms underlying chronic renal disease in Alport syndrome

Genetic analysis in hematuric syndromes

- Linkage analysis in informative families
- *COL4A3, COL4A4, COL4A5* – huge genes, 48-53 exons (no “hot spot” of mutations)
- Single-strand conformation polymorphism (SSCP) analysis after PCR amplification of each exon – expensive, time-consuming, sensitivity 65%

- Data from the literature:
  *COL4A5* ~ 400 mutations
  *COL4A3, COL4A4* ~ 300 mutations
Genotype-phenotype correlations in males of 195 families with XLAS
Classified male patients with XLAS into 3 cohorts:

1. Severe AS, ESRD ~20yrs – large rearrangements, frame shift, nonsense, splice donor mutations

2. Moderate-severe AS, ESRD ~26 yrs – non-glycine or glycine-missense (exons 21-47), in-frame deletions/insertions, splice acceptor mutations

3. Moderate AS, ESRD ~30 yrs – glycine substitutions (exons 1-20)
EFFECT OF A "SEVERE" COL4A5 MUTATION:
deletion, frameshift, premature stop

EFFECTS OF A COL4A5 MISSENSE MUTATION

From: Kashtan CE. Curr Diagn Pathol 2002; 8: 349-360
Genetic analysis of Slovenian families with AS and BFH

AS 17 families: 12 COL4A5 mutations (71%)
BFH 40 families: 5 COL4A5 mutations*
  4 COL4A4 mutations
  3 COL4A3 mutations

*COL4A5 mutation G624D;G>A2073 found in:
  1 AS family (characteristic EM in a 45-year-old female pt, her brother ESRD at the age of 40)
  2 BFH families (thin GBM lesion in EM)
  3 BFH families (male pts, > 40 yrs old with isolated hematuria and normal renal function, no renal biopsy)

Male patient, 16 yrs old (isolated hematuria; *COL4A5* missense mutation p.G624D)
Our finding of the same missense mutation of *COL4A5* in a family with progressive form of AS and 5 families affected with BFH suggest the possibility of significantly different phenotypes associated with the same gene mutation.

The five families could be affected by a very mild AS. It might be also possible that AS and BFH with TBMN may represent two opposite poles of a spectrum of hereditary *COL4A5* nephropathies as it is well known for COLA3 and COLA4 nephropathies.
Kidney in nail-patella syndrome

- Autosomal dominant disorder due to mutation of *LMX1B* (promoter transcription factor)
- Protein LMX1B in kidney expressed in podocytes; underlie protein-protein reactions with other transcription factors. Among target genes for LMX1B are *COL4A3* and *COL4A4*, *COL III* and several podocyte genes (for nephrin, podocin, CD2AP, GLEPP-1 and synaptopodin)
- Clinical picture: nail displasia, hypoplastic or absent patella, elbow displasia, illiac horns, open-angle glaucoma and glomerular involvement in 30-40% with hematuria and proteinuria, progression to NS and ESRD in ~10% patients.
Kidney in nail-patella syndrome

LM: glomeruli unremarkable, variable degree of glomerulosclerosis
EM: irregular thickening of the GBM, bundles of type III collagen, patchy lucent ("moth-eaten") areas, focal effacement of podocyte food processes

FSGS with MYO1E mutation

- A rare autosomal recessive FSGS due to mutation in \textit{MYO1E} (membrane-associated nonmuscle class I myosin expressed in podocytes)
- Mutation greatly alters Myo1E subcellular localization, its interaction with the podocyte cytoskeleton (with central role of the actin in podocyte structure and function), its ability to promote podocyte motility and homeostasis and consequent integrity of glomerular filtration barrier
- Clinical picture: childhood-onset, glucocorticoid resistant nephrotic syndrome

FSGS with MYO1E mutation

From: Leh S et al. Virchows Arch; 2013; 463: 131

LM: mesangial changes, FSGS, interstitial foam cells
EM: thickening, lamellation and splitting of the GBM
MYH9-related disorders

• A rare autosomal dominant disorder due to mutation in MYH9 (nonmuscle myosin heavy chain IIA). Nonmuscle myosin expressed in most cells and tissues
• Mutations associated with Fechtner syndrome, and Epstein syndrome (Alport variants in past)
• Clinical picture: macrothrombocytopenia, basophilic cytoplasmic inclusion bodies in leukocytes, sensorineural hearing loss, cataract. Renal involvement in 30-70% patients, proteinuria and/or hematuria, nephrotic proteinuria with progressive renal failure.

Kyoung HH et al. Pediatr Nephrol 2011; 26: 549-555
MYH9-related disorders

From: Kyoung HH et al. Pediatr Nephrol 2011; 26: 549-555
EM differential diagnosis:
AS vs. IgA nephropathy
Conclusions

• In patients with familial hematuric syndrome diagnosis and determination of the mode of transmission are important for prognosis and genetic counselling.

• Mutation screening would be theoretically the best approach, but there are still some important limitations of this technology.

• Particularly in Alport syndrome evaluation of kidney biopsy including immunohistology for the type IV collagen α chains remains a useful diagnostic tool.