Alport Syndrome and Thin Membrane Disease

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The clinical challenge

Alport syndrome: A disease progressing to ESRD

Disease Progression

Birth  Late childhood  Early adolescence  Adulthood

Phase I
Hematuria
Alport syndrome (AS)

- First description by A.C. Alport in 1927 (Alport AC, Br Med J 1927)
  - Progressive hereditary nephropathy with
  - Characteristic changes of glomerular basement membrane, sensorineural hearing loss and ocular abnormalities

- Prevalence of X-linked AS 1:10,000; autosomal-recessive AS 1:50,000

- >700 mutations described today

- 1% of dialysis patients

- In 1990, localization of collagen IV α5 chain to chromosome Xq22 (Hostikka et al Proc Natl Acad Sci USA 1990)

- In 1990, identification of first three collagen IV α5 chain mutations (Barker et al, Science 1990)
Collagen IV

- Six collagen IV α chains (α1-6) (monomer) encoded by six genes (COL4A1-6)
- Three collagen IV α chains assemble to form three protomers (heterotrimers: 1/1/2; 3/4/5; 5/5/6) with specific distribution pattern (development / tissue)
- Protomers (heterotrimers) consist of:
  - 7S triple helical domain (N-terminus)
  - Triple helical collagenous domain (middle)
  - Noncollagenous trimer (C-terminus)

Collagen IV network

Organ-specific distribution of collagen IV protomers

<table>
<thead>
<tr>
<th>α1α1α2</th>
<th>α3α4α5</th>
<th>α5α5α6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ubiquitously present during embryogenesis in</td>
<td>Replaces α1α1α2 (partially) in</td>
<td>Replaces α1α1α2 (partially) in</td>
</tr>
<tr>
<td>- All basement membranes</td>
<td>- GBM</td>
<td>- Bowman’s capsule</td>
</tr>
<tr>
<td></td>
<td>- Cochlea</td>
<td>- Distal renal tubule</td>
</tr>
<tr>
<td></td>
<td>- Eyes</td>
<td>- Skin</td>
</tr>
<tr>
<td></td>
<td>- Testes</td>
<td>- Esophagus</td>
</tr>
<tr>
<td></td>
<td>- Lung</td>
<td>- Smooth muscle</td>
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</table>

Organ-specific distribution of collagen IV 
$\alpha_3/\alpha_4/\alpha_5$ protomer

# Genetics of AS

<table>
<thead>
<tr>
<th>Mode of inheritance</th>
<th>Gene-locus</th>
<th>Collagen chain / gene</th>
<th>Mutation</th>
<th>Affected individuals</th>
<th>Relative frequency</th>
</tr>
</thead>
</table>
| X-linked AS (XLAS)      | Xq22       | COL4A5                | Hemizygous                     | Male – AS  
Female – carrier                                                                    | 80-85%             |
| Autosomal-recessive AS (ARAS) | 2q35 | COL4A3 COL4A4 | Homzygous or compound heterozygous | Male = female  
Parents and/or siblings likely to have TBMN  
Father may have hematuria                                                               | 15%                |
| Autosomal-dominant AS (ADAS) | 2q35 | COL4A3 COL4A4 | Hemizygous | Male = female                                                                 | Rare               |
| *Thin basement membrane nephropathy (TBMN)* | 2q35 | COL4A3 COL4A4 | Heterozygous | *Male = female*                                                                | Majority of benign hematuria, i.e. 1% of population |
X-linked Alport syndrome (XLAS)

- 80-85% of AS cases – mutations in COL4A5
- Family history of AS likely; cases tend to skip generations
- All males develop AS; females are hemizygous carriers
- Affected males early progression to ESRD (50% at 25 years)
- Extra-renal manifestation likely
- Sensorineural hearing loss:
  - Occurs in up to 80% of patients
  - Begins during first or second decade of life and can progress to deafness
  - Can become aggravated by environmental factors
- Ophthalmological symptoms:
  - Can be severe and result in blindness
  - Are in 40% the first symptom of AS
  - Dot-and-fleck retinopathy (80%); anterior lenticonus; posterior polymorphous corneal dystrophy
Progressive GBM damage in AS

The Type IV collagen α3/α4/α5 network:
- is not essential for normal glomerular development
- is essential for long-term maintenance of glomerular function
Sensorineural hearing loss (SNHL)

Due to abnormal organ of Corti basement membrane?

Risk (males): 50% by age 15, 75% by age 20

Ocular lesions

- **Posterior polymorphous corneal dystrophy**
  - Descemet’s membrane

- **Recurrent corneal erosions**
  - Corneal BM

- **Anterior lenticonus**
  - Lens capsule

- **Maculopathy**
  - Bruch’s membrane; Internal limiting membrane

http://webvision.med.utah.edu/imageswv
Autosomal-recessive Alport syndrome (ARAS)

- 15% of AS cases
- Homozygous or compound heterozygous mutations in $COL4A3$ or $COL4A4$
- Parents can be carriers (TBMN); siblings can also have ARAS (25%) or TBMN (50%)
- Progression to ESKD in both sexes equal (severity similar to XLAS)
- Hearing impairment likely
- Ocular involvement possible
- On biopsy, absence of collagen IV $\alpha3$, $\alpha4$ and $\alpha5$ chains from the GBM
- Presence of collagen IV $\alpha5$ chain in Bowman’s capsule and the basement membrane of renal tubules and epidermis
Autosomal-dominant Alport syndrome (ADAS)

- 5% of AS cases
- Mutations in \textit{COL4A3} or \textit{COL4A4} (like in ARAS)
- Some uncertainty about distinction from ARAS
- Affects both sexes with similar severity
- Inconstant but rather slow progression to ESKD (>50 years)
- Late hearing impairment
- No ocular involvement
- GBM splitting or diffuse GBM thinning
ADAS progresses less rapidly to ESRD compared to XLAS

**Fig. 3.** Men with Alport’s syndrome. X-linked (n = 56) versus autosomal dominant (n = 20). Median renal survival.

Pochet et al, Kidney Int 1989
Heterozygous mutation in \textit{COL4A3} or \textit{COL4A4}: ADAS or TBMN?

- Features favoring ADAS:
  - CKD in patient or affected relative
  - Proteinuria in patient or affected relative
  - Hearing loss in patient or affected relative
  - GBM thickening and lamellation

- Diagnosis may only become evident after extended monitoring
Genetic testing is the gold standard for the diagnosis of AS

X-linked Alport syndrome

Progression to ESRD

Type of COL4A5 mutation
Age at onset of ESRD in affected males

C- vs. N-terminal COL4A5 mutations
Age at onset of ESRD in affected males

Figure 2. Age at onset of ESRD associates with mutation position (n = 364 with ESRD). Dotted lines represent upper and lower 95% confidence limits. $R = 0.33$, $P < 0.0001$ with mutations positioned at 5’ end of the gene associated with earlier age at ESRD onset.

Bekheirnia et al, J Am Soc Nephrol 2010
Number of side-chain carbon atoms in the substituting amino acid influences age at onset of ESRD in males with *COL4A5* missense mutations.

![Graph showing the correlation between number of carbon atoms in the side chain and age at onset of ESRD.](image)

*Fig. 1. Genotype-phenotype correlation examining the effect of the difference in the number of side-chain carbon atoms carried by the substituting amino acid (X-axis) with age-at-onset of end-stage kidney disease (Y-axis). r²-value: 0.1362; p-value: 0.0017; number of mutations analysed: 70.*
Modified *Flinter* criteria for non-genetic diagnosis of AS

Hematuria, plus one or more of:

- Positive family history of hematuria, chronic renal failure or both
- High frequency sensorineural hearing loss
- Pathognomonic ocular anomalies (e.g. dot-and-fleck retinopathy or anterior lenticonus)
- Characteristic ultrastructural changes in glomerular kidney or skin basement membranes

Flinter et al, J Med Genet 1997
Collagen IV immunohistochemistry:
kidney and skin biopsy

**NORMAL**

**XLAS male**
Hemizygous COL4A5 mutation

**XLAS female**
Heterozygous COL4A5 mutation

**ARAS**
Biallelic mutations in COL4A3 or COL4A4

**TBMN**
Heterozygous mutation in COL4A3 or COL4A4
Thin basement membrane nephropathy (TBMN)

- Most common cause of isolated microscopic hematuria ("benign") in children and adults
- Estimated to effect about 1% of population
- Many cases (non familial) remain undiagnosed
- Structural abnormality of the GBM (i.e. >50% of GBM <LLN, e.g. <150 nm)
- Heterozygous mutations in COL4A3 or COL4A4
- Hematuria only; no/minimal proteinuria
- Low prevalence of renal insufficiency and hypertension in adults
- No extra-renal disease manifestation
- Regular clinical follow up q 1-2 years (?proteinuria; ?hypertension; ?decline in renal function)
AS vs. TMBN – a diagnostic challenge

• Isolated microscopic hematuria is characteristic of both conditions

• Family history is often positive for hematuria in both conditions

• In both, renal biopsy shows:
  – No abnormalities by light microscopy or routine immunofluorescence
  – Thin glomerular basement membranes by electron microscopy
AS vs. TBMN – why does the diagnosis matter?

• Very different outcomes: AS frequently causes progressive renal disease, TBMN does not (with exceptions).

• Early intervention with ACEI and/or ARB delays ESRD in affected males and females with AS – thus, early and accurate diagnosis of AS is crucial.

• Diagnosis of AS allows identification of affected relatives who may be candidates for treatment, and who should be excluded as potential kidney donors.

• In the (near?) future therapies for AS may be selected on the basis of the collagen IV genotype.
Persistent glomerular hematuria

**Family history**
- Family history of Alport syndrome and no other cause for haematuria (highly likely)
- No family history of Alport syndrome or renal failure (likely)

**Clinical features**
- Characteristic clinical features, including lenticous or retinopathy (highly likely)
- None of the characteristic clinical features of Alport syndrome, such as renal failure, hearing loss, lenticous, retinopathy (likely)

**GBM appearance**
- Diffuse GBM lamellation (confirmatory)
- Diffuse GBM thinning but no GBM lamellation (likely)

**GBM collagen IV composition**
- GBM lacking collagen IV α3 and α5 chains (highly likely)
- Normal α3 and α5 collagen chain composition (likely)

**Genetic testing**
- COL4A5 mutation or 2 COL4A3 or COL4A4 mutations (confirmatory)
- No mutation in the COL4A5 gene (highly likely)

**Diagnosis**
- Alport syndrome
- Thin basement membrane nephropathy

**Mode of inheritance**
- Genetic testing for X-linked or autosomal recessive inheritance
Is additional work-up required when TBMN is suspected based on clinical findings and pedigree?

Additional work-up (renal biopsy, genetic testing) may not be necessary when

• Pedigree shows multiple individuals with hematuria, but no one with ESRD
• Hematuria is isolated and there is
  - No microalbuminuria or proteinuria
  - Normal blood pressure
  - Normal renal function
  - Normal hearing

Patients given a diagnosis of TBMN should have regular follow-up to detect proteinuria, hypertension or decline in renal function
Clinical History
Pedigree

Hematuria
+/- proteinuria
+/- deafness
+/- eye findings
Pedigree positive for hematuria, ESRD

Hematuria
Negative pedigree

Hematuria (isolated)
Pedigree positive for hematuria, no ESRD

Clinical History
Pedigree

Alport probability

High

Skin biopsy
Molecular diagnosis*

Skin biopsy?
Molecular diagnosis*

Kidney biopsy

Observation

Diagnostic Approach

Probability < other diagnoses

Probability < TBMN

* Where available
If NGS, sequence COL4A3 / 4 / 5
If conventional (Sanger), start with COL4A5
COL4A3 -/- mice

- COL4A3 knock out-mice (ARAS)

- From week 4  Hematuria
  From week 6  Proteinuria
  From week 7  Nephrotic syndrome
  At about 10 weeks  Death from renal failure (fibrosis)

- *In vivo* model to study Alport syndrome
- *In vivo* model to study chronic proteinuric kidney disease in general

Cosgrove et al, Genes Dev 1996
ACE inhibitors

No treatment (placebo) vs.

ACE inhibitor treatment:
- Late: from week 7 (proteinuria)
- Early short: week 4-10 (hematuria)
- Early long: from week 4 (hematuria) until death

Gross et al, Kidney Int 2003
AT1 receptor blockers – *Less efficient than ACE inhibitors*

No treatment (placebo) vs.

**Treatment:**
- Ramipril (ACE inhibitor) from week 4
- Candesartan (AT1 receptor blocker) from week 4

Gross et al, Nephrol Dial Transplant 2004
RAAS inhibition in pediatric & adult AS patients

Webb et al, Nephrol Dial Transpl 2011

- Multicenter, randomized, double-blind trial of losartan vs. placebo or amlodipine
- 30 children, ℞ for 12 weeks
- Significant reduction in proteinuria in losartan vs. palcebo or amlodipine
- No safety concerns, increase in creatinine or hyperkalemia

Gross et al, Kidney Int 2012

- European Alport syndrome registry
- 283 patients followed >20 years
- 109 patients received no ℞ (NoT)
- 3 groups received ACEI ℞:
  - Onset of microhematuria / microalbuminuria (T-I)
  - Proteinuria >0.3 g/day (T-II)
  - CKD stage III and IV (T-III)
RAAS inhibition – age at onset of RRT

Gross et al, Kidney Int 2012
RAAS inhibition – age at onset of RRT in siblings

In 15 sibling pairs where the elder sibling had invariably been started later on ACEI Rx, the median age of ESKD / RRT was 27 years for the elder, and 40 years for the younger sibling.
RAAS inhibition – life expectancy

Gross et al, Kidney Int 2012
Age at onset of RRT initiation

ERA-EDTA registry

Clinical practice recommendations for the treatment of Alport syndrome: a statement of the Alport Syndrome Research Collaborative

Clifford E. Kashtan • Jie Ding • Martin Gregory • Oliver Gross • Laurence Heidet • Bertrand Knebelmann • Michelle Rheault • Christoph Licht
Kashtan et al, Pediatr Nephrol 2012

Clinical: 1. hematuria 2. family history 3. Sensorineural deafness 4. ocular changes
Kidney biopsy with EM, collagen IV IHC*
Skin biopsy with type IV collagen IHC*
Molecular genetics plus genetic counseling*

Diagnosis

Alport Syndrome
Isolated hematuria or hematuria + microalbuminuria
EARLY PRO-TECT Alport trial or consider therapy

Hematuria with thin GBM
Proteinuria
Isolated hematuria
Annual urine protein measurement
Proteinuria
Therapy

! Report to national or international registries!

* Depending upon availability and local practice
Recommendations (continued)

• **Treatment**
  First line: ACE inhibitors (e.g. ramipril)
  Second line: ARB (e.g. losartan) or Aldosterone antagonists (e.g. spironolactone)

• **Proteinuria (>2 years)**
  >0.2 mg protein / mg creatinine
  >4 mg / m² / h

• **Target**
  Baseline proteinuria >1.0 mg/mg aim for 50% reduction
  Baseline proteinuria 0.2-1.0 mg/mg aim for <0.5 mg/mg

Kashtan et al, Pediatr Nephrol 2012
Aldosterone inhibition

• Increasing evidence that aldosterone inhibition has a reliable blood pressure independent antiproteinuric effect

• Remains to be proven to translate to an improvement in GFR

• Spironolactone is known to be relatively safe in the pediatric setting

• May offer renoprotection in CKD patients, especially if exhibiting the phenomenon of “aldosterone breakthrough”

• ESCAPE trial: Proteinuria decreased by 50% but gradually rebounded

Ku et al, Pediatr Nephrol 2009
Aldosterone inhibition in AS

• The only pediatric study of aldosterone inhibition (spironoactone) in kidney disease involved 5 Alport syndrome patients

• Persistent proteinuria despite ACEI or combined ACEI / ARB treatment

• Significant improvement in proteinuria achieved & maintained for ≥18 months

• Risk profile:
  - eGFR remained unchanged
  - Lower BP (no ill effect)
  - Tendency to hyperkalemia (never >5 mmol/L)

Kaito et al, Pediatr Nephrol 2006
Aldosterone inhibition in AS

Fig. 1 Proteinuria in patients treated with SP for 18 months. Proteinuria expressed as morning urinary protein/creatinine ratio (U-P/C). Before the start of spironolactone (SP) therapy, U-P/C showed no significant reduction, but was significantly reduced 3, 6, 12 and 18 months after the start of treatment with SP. *P<0.05

Kaito et al, Pediatr Nephrol 2006
Take home messages

• AS and TBMN are genetic diseases caused \textit{COL4} mutations.
• Patient history, lab tests and kidney biopsy (including IH) are helpful to establish the diagnosis of AS and/or TBMN – genetic testing is the gold standard
• RAAS inhibition (ACEI and ARB) is safe and effective in children with AS
• Early therapeutic intervention seems to be key for
  - delaying or preventing ESKD
  - improving quality of life
  - ensuring survival
• International registries and treatment trials in children are needed focusing on safety, efficacy and cost saving of early therapy (e.g. EARLY PRO-TECT Alport [Gross-Germany]; ASTOR [Kashtan-USA])
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