Selective Genetic Diseases of the Kidney

Barry I. Freedman, MD, FACP
John H. Felts III Professor (Internal Medicine)
Professor of Urology
Chief, Section on Nephrology

Molecular genetic techniques have clarified the pathogenesis of many forms of glomerulosclerosis and inherited nephropathies ...... more will follow

Overview

1. GBM disorders (Type IV Collagen)
   - Alport's Syndrome
   - Thin basement membrane nephropathy [TBMN]
   - HANAC [hereditary angiopathy, nephropathy, aneurysms and muscle cramps]
2. Non-GBM disorders (mainly podocyte)
   - Focal Segmental Glomerulosclerosis (FSGS)
   - Focal Global Glomerulosclerosis
   - Diffuse Mesangial Sclerosis
   - Nail-Patella Syndrome, Denys-Drash and Frasier's Syndromes
3. Discussed elsewhere
   - Polycystic kidney disease: PKD1/PKD2 (AD), PKHD1 (AR)
   - Fabry's Disease: α-Gal A (X-linked)
   - Medullary cystic kidney disease: UMOD

The GBM: Type IV collagen gene, α-chains and glomerular isoforms

Type IV collagen gene mutations

- The GBM is composed of type IV collagen, laminin, and proteoglycans (perlecan & agrin)
- Triple helical type IV collagen molecules composed of 3 α-chains that assemble extracellularly to form the normal GBM
- Defects in α1: α4: α5 trimers produce either TBMN or Alport's Syndrome, depending on the genetics involved
- A single-allele mutation in COL4A1 (α1-chain) causes HANAC (hereditary angiopathy, nephropathy, aneurysms and muscle cramps) – AD, hematuria with cystic kidney disease, intracranial aneurysms and muscle cramps. Normal renal bx and normal immunofluorescence of GBM (underproduction of α1-chain?)
- AR mutations in laminin β2 chain gene (LAMR2) cause Pierson's syndrome (ketoacidosis, proteinuria, mesangial sclerosis, small narrow pupils or microcoria)
Alport’s Syndrome (AS)

- Inherited disorder of type IV (basement membrane) collagen
- 85% X-linked, most of the remainder autosomal recessive
- Most patients are male
- Primarily affects glomeruli, with sensorineural hearing loss and ocular lesions (lenticonus +/- maculopathy, occasional cataracts) in X-linked forms among males
- Children / young adults present with hematuria and low level proteinuria
- Absence of a functional type IV collagen isoform leads to disintegration and breakdown of the GBM with resulting escape of red blood cells (hematuria)
- Autosomal dominant COL4A3 and COL4A4 mutations are rare, but may signify milder disease with slower progression

Genetics of Alport’s Syndrome

- X-linked: males are hemizygous for one allele in COL4A5 (hundreds of mutations reported: missense & nonsense frame-shift, insertions, deletions, inversions, and splice site)
- Heterozygous female carriers usually have hematuria, but rarely progressive CKD/ESRD
- AR forms: compound heterozygous or homozygous COL4A3 and COL4A4 mutations on chromosome 2q (single-allele mutations in these genes do not cause Alport’s, but TBMN)
- 22% lack family history, de novo mutations common

Pathology and diagnosis of Alport’s Syndrome

- Light microscopy – not pathognomonic
  - Age <5 yrs: NL
  - Age 5-10 yrs: segmental & diffuse mesangial cell prolif, matrix accum & capillary wall thickening; tubulointerstitial damage ensues
- Electron microscopy – very helpful
  - Early: thinning of GBM (appears as in TBMN)
  - Late: irregular thinning/thickening of GBM, lamellation and “basket weave” pattern
- Immunofluorescence microscopy – often diagnostic
- DNA analysis for COL4A3, COL4A4, COL4A5 commercially available – most accurate

Immunofluorescence findings in Alport’s Syndrome and TBMN

| α3, α4, α5 – comprise adult GBM |
| α5, α6 – comprise Bowman’s capsule BM |

Indirect immunofluorescence studies for type IV collagen α₁(IV) chain

TBMN – diffuse linear staining of GBM and Bowman’s capsule. Occ. linear distal tubule staining (same for α₃(IV) collagen). This pattern is indistinguishable from normal kidney tissue.

Heterozygous female carrier of X-linked Alport – discontinuous linear staining for α₅(IV) collagen, same for α₃(IV) collagen.

Male with X-linked Alport Syndrome (son of patient [B], above) – No GBM, BC or tubule staining for α₅(IV) collagen, same for α₃(IV) collagen.

Autosomal recessive Alport syndrome – no GBM staining, But BC and distal TBM staining preserved (note: α₃(IV) collagen staining was completely negative).

Haas M; Arch Pathol Lab Med 2009; 133:224-232

Clinical Course Alport’s Syndrome

• Persistent hematuria (boys) with hearing loss
• Occ. supharyngitic macroscopic hematuria
• Proteinuria increasing with age, late HTN
• Females - milder course w/ persistent hematuria
• 90% of males with X-linked AS develop ESRD; 82% hearing loss, 44% ocular lesions in 40 yrs
• 10% of female carriers with X-linked AS develop ESRD, 95% hematuria, 75% proteinuria, 30% hearing loss and 15% ocular defects in 40 yrs
• Supportive Rx – ACEi, calcineurin inhibitors (?)
• Anti-GBM disease up to 10% post-transplant

Thin basement membrane nephropathy

• Leading cause of hematuria in children and adults – up to 1% of the population, typically not a progressive disease
• Dominant inheritance, 2 of 3 pts have (+) FH of hematuria
• Heterozygous mutations in a type IV collagen α₃-, α₄-, or α₅-chain (would cause AS… if homozygous or a compound heterozygote)
• Typically COL4A3 or COL4A4 mutation (COL4A5 in women)
• Dose effect on collagen IV in GBM:
  – 1 allele = low levels of α₃:α₄:α₅ trimer
  – 2 alleles = absence (or malfunction) of α₃:α₄:α₅ trimer
• See a similar carrier status as in female relatives of men with X-linked AS
• Same mutation can have different severity of symptoms

Clinical Course TBMN

• Benign course, typically lacks proteinuria. No Rx needed
• Macroscopic hematuria more commonly seen w/ AS
• Diff Dx: AS, IgAN, lupus nephritis, mesangiocapillary GN
• Need to differentiate from early AS to determine prognosis
• Renal fxn normal in children, low prevalence of CKD in adults
• Biopsy: LM: normal (occ. mild ↑ mesangial cells and matrix; FSGS and tubulointerstitial changes in 5-25% with aging)
• Immunostaining for α₃-, α₄-, α₅-chains normally reveals all 3; whereas usually absent in AS. Rare false(-) w/ low protein level
• EM is not diagnostic for TBMN (vs Alport’s Syndrome)
• Genetic testing diagnostic for COL4A3/4A4 (AR form and X-linked female carriers), particularly w/ immunofluorescence
Major etiologies of FSGS

- Idiopathic (… until genes discovered), ? SuPAR
- Hereditary
- Infection: HIV, Parvo B19, Hep C, CMV, EBV
- Medication/toxin: pamidronate, heroin, interferon-α, Li²⁺, calcineurin inhibitor toxicity
- Hyperfiltration: obesity, reflux, nephron loss, aging
- Hypertension-attributed: focal global GS with interstitial & vascular changes in those of African ancestry
- Sickle cell nephropathy

Treatment of FSGS/SRNS

- Steroids, calcineurin inhibitors, cytotoxic agents
- Is there a role for MMF or Rituximab?
- Plasmapharesis if recurs after kidney txp (>30%)
- Idiopathic forms:
  - Best Prognosis: tip lesion (origin prox tubule)
  - Worst Prognosis: collapsing variant
- Do causative genes impact diagnosis & therapy?

Podocyte disorders

Mutations in FSGS

- Dependent on:
  - Family history
  - Single generation – favors autosomal recessive
  - first affected child will appear to be sporadic, suspect AR if consanguineous parents
  - Multi-generation – favors autosomal dominant
  - Incomplete penetrance (genetic carriers not manifesting disease) can lead to failure to detect AR or AD inheritance
  - Age at onset
  - Ethnicity
  - Histology

Prevalence of FSGS/SRNS mutations in childhood

- Congenital nephrotic syndrome (CNS)
  - Detectable mutations approach 100% of cases
  - In Finland – 95% relate to nephrin NPHS1
  - In non-Finnish – other gene variants found
- Infants (4-12 months) and early childhood (1-5 years) SRNS - podocin (NPHS2) mutations common (up to 40% of familial and 17% sporadic SRNS in this group)
- WT1 up to 16% of children – consider in females (or males with abnormal genital development)

<table>
<thead>
<tr>
<th>Gene/Genes</th>
<th>Consequences</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPHS1</td>
<td>Nephrotic syndrome</td>
<td>95% in Finnish</td>
</tr>
</tbody>
</table>
**Adult familial FSGS**

- Typically autosomal dominant (except APOL1)
- Penetrance may be incomplete with non-nephrotic proteinuria
  1. Formin gene INF2 - up to 17% of cases
  2. Transient receptor potential cation channel subfamily 6 TRPC6 - up to 12%
  3. Alpha-actinin-4 ACTN4 - up to 3.5%
  4. Apolipoprotein L1 APOL1 (AR) - 70% of FSGS & HIV-associated collapsing FSGS, >50% hypertension-attributed nephropathy in African Americans

---

**Spectrum of APOL1-associated nephropathy**

- Focal Global Glomerulosclerosis ("Hypertensive nephropathy")
- Focal Segmental Glomerulosclerosis
- Collapsing FSGS (HIVAN)

---

**APOL1 clinical implications**

- 2 coding variants in APOL1 fully explain the excess risk of non-diabetic nephropathy in African-Americans vs. Caucasians
- Associate with earlier age at ESRD
- Weak association with mild CKD/proteinuria, strong with ESRD
- Progression factor in FSGS, FGGS or "hypertension-attributed nephropathy", sickle cell nephropathy, lupus-associated ESRD
- APOL1 genotype of kidney donors explains shorter graft survival from African-ancestry donor kidneys
- Kidney disease and progression in AASK participants strongly associate with APOL1 genotype – an effect independent from blood pressure treatment target or medication class used
Clinical Correlates

- Recessive mutations in \textit{NPHS2}, \textit{CD2AP}, & \textit{PLCE1} more often associated with severe disease presenting with early onset proteinuria/ESRD in infancy and childhood
- Dominant mutations in \textit{ACTN4}, \textit{TRPC6}, & \textit{INF2} more often associated with later onset proteinuria in 2nd decade and ESRD in 3rd-4th decades
- \textit{APOL1} accounts for 70% of idiopathic FSGS and HIVAN in African Americans; >50% of “hypertension-attributed CKD and ESRD” – lesion can be focal global glomerulosclerosis
- \textit{NPHS2} accounts for 40% of childhood familial FSGS and 6-17% of sporadic childhood FSGS
- \textit{INF2} accounts for 17% of adult onset familial FSGS
- Remaining mutations are rare in adult sporadic FSGS (do not screen for them)!

Clinical Question

- 60 yo African American female w/ 10 years T2D
- Proteinuria ~4 g/d w/o hematuria, casts, cells
- Diabetic retinopathy: absent
- Family history: brother with ESKD due to chronic glomerular disease (he is not diabetic)
- Genotype: two \textit{APOL1} nephropathy risk variants
A kidney biopsy would most likely reveal:

1. Arteriolar nephrosclerosis w/ ischemic collapse of glomeruli (hypertensive nephropathy)
2. Diabetic nephropathy
3. Idiopathic FSGS
4. Lesions in 1 & 2
5. Lesions in 2 & 3

Podocin NPHS2 in adult FSGS

- Rare in cases of adult sporadic FSGS
- Exception = compound heterozygosity for mutations in the common R229Q polymorphism + one pathogenic NPHS2 variant in Western Europe
  - R229Q in 5-10% of Caucasians
  - R229Q in 1-2.5% of African Americans
  - R229Q alone non-pathologic
  - NS age ~19 yrs in R229Q compound hets
  - Far lower disease freq. outside of Western Europe

Nail Patella Syndrome

- Autosomal dominant loss of function mutation in LMX1B (podocyte expressed)
- LIM homeobox transcription factor 1, beta
- Symmetric nail, skeletal, ocular & kidney disease
- Variable nephropathy risk (ESRD in childhood to no renal disease)
- GBM thickening, splitting and fibrillar collagen deposits
- LMX1B normally regulates nephrin, podocin, CD2AP and α2- and α3 type IV collagen chains; dysregulation of podocyte genes leads to renal involvement

Genetic dissection of a complex trait: 60 yo African American female w/ 10 yrs type 2 DM. FH + for brother w/ non-DM ESRD. She now has ~4 gm/day proteinuria without diabetic retinopathy. APOL1 risk variants: +/+ A – diffuse mesangial expansion and GBM thickening, early nodule formation The classic histologic changes of diabetic glomerulosclerosis
B – idiopathic collapsing FSGS; C - focal global glomerulosclerosis

Gopalakrishnan & Freedman. Human Pathol 2010
Nail-Patella Syndrome

Genetic screening in clinical practice
• Will results influence:
  – treatment of your patient?
  – screening for extra-renal disease?
  – family counseling?
  – decisions on kidney transplantation?

Family counseling
• Gene detection in childhood SRNS is high. This can help parents with family planning.
• Pre-natal genetic testing is possible.
• If a sibling of a patient with a known mutation develops NS – 7 less benefit to steroids.
• Family planning for patients – homozygous or compound het. NPHS2 mutations + partner with R229Q variant (10% Western Europeans) = 50% disease risk, possibly more severe
• Women with isolated WT1 (FSGS) = potential for Denysh-Drash Syndrome or Frasier Syndrome in children

Potential drawbacks of genetic testing
• Expense
• Evaluation of genes one at a time
• Selected populations often studied, where other (more common) genetic etiologies have been excluded – can inflate prevalence rates
• Few studies test for heterozygous mutations in two different genes

Family planning in adult onset FSGS
• If strong family history suggests AD disease (note possible incomplete penetrance) – risk of transmission is high and should be discussed
• Sporadic FSGS without a family history – prevalence of mutations is very low
• There is less value in screening adult sporadic FSGS; except for possible NPHS2 R229Q compound hets. w/ one NPHS2 pathogenic mutation. Half of offspring will have the pathogenic NPHS2 variant w/o disease; but if combined with R229Q it will cause disease. Hence, consider testing for R229Q as up to 10% of population may carry it.

Clinical Screening
• All children with congenital nephrotic syndrome
• Children with familial and sporadic SRNS
• Adults with familial FSGS
• Limited value in adults with sporadic FSGS, except testing young adults for the R229Q NPHS2 variant
Patient Care

- Mitochondrial mutations (maternally inherited; often appear to be dominant inheritance)
  - screen for visual and auditory abnormalities
  - test for diabetes
- WT1
  - investigate the gender in apparent females (XY genotype = pseudohermaphrodite)
  - screen for development of Wilms' tumor
  - screen for gonadoblastoma

Genetic screening and kidney transplantation

- Recurrence after transplantation is lower in familial forms of FSGS
- Detection of homozygous or compound heterozygous risk mutations predict lower risk of recurrence
- Living donors may be more likely to donate knowing recurrence rates are low
- Assist with living related donor selection in familial FSGS (exclude donors with risk variants)
- Exceptions
  - Congenital Nephrotic Syndrome due to NPHS1: recurrence rate ~25% and possibly related to antibodies against nephrin
  - Anti-GBM disease in Alport’s Syndrome