Therapeutic Targets in Alport Syndrome: Insights from the Big Picture

Jeffrey H. Miner, Ph.D.
Professor of Medicine
Division of Nephrology
Washington University School of Medicine
St. Louis, Missouri

Disclosure of Relevant Financial Relationships

USCAP requires that all planners (Education Committee) in a position to influence or control the content of CME disclose any relevant financial relationship WITH COMMERCIAL INTERESTS which they or their spouse/partner have, or have had, within the past 12 months, which relates to the content of this educational activity and creates a conflict of interest.

The Nephron

The Glomerulus

Endothelial Cell
Podocyte
Glomerular Basement Membrane

Basement Membrane

The Glomerular Filtration Barrier

Epithelial Basement Membrane

The Glomerular Basement Membrane (GBM)
The Major Glomerular Basement Membrane Proteins

- Laminin-521
  - α5β2γ1 heterotrimer
- Collagen α3α4α5(IV)
- Nidogens-1,2
- Agrin (heparan sulfate proteoglycan)

Human mutations in 4 of these 9 proteins cause kidney disease

Alport Syndrome

- A hereditary glomerular disease usually accompanied by hearing and characteristic lens and retina defects; incidence of ~1 in 10,000
- Often diagnosed in children based on the presence of hematuria and stereotypical defects in the glomerular basement membrane (GBM) seen in biopsy by TEM
- Almost invariably progresses to ESRD by late adolescence or thereafter; progression is signaled by the onset of proteinuria
- Treatment with ACE inhibitors delays proteinuria and ESRD, but is not a cure

Split, Thickened, “Basket Weave” GBM

Pierson Syndrome

Alport Syndrome
Alport Syndrome

- Caused by mutations in glomerular basement membrane type IV collagen genes
  - (COL4A3, COL4A4, or COL4A5)
- The COL4A5 gene is X-linked, so most patients are males
  - COL4A5+- females can manifest various aspects of the syndrome, from hematuria to ESRD
- 15% of cases are autosomal recessive homozygous COL4A3 or COL4A4 mutations
  - Heterozygous COL4A3 or COL4A4 carriers can manifest "Thin Basement Membrane Nephropathy"

Type IV Collagen Chains, Genes, and Trimers

- α1 COL4A1
- α2 COL4A2
- α3 COL4A3
- α4 COL4A4
- α5 COL4A5
- α6 COL4A6

There Are Great Animal Models for Alport Syndrome

- Both mouse and dog models recapitulate most aspects of the human kidney disease

Loss of Collagen α345(IV) and Increased α112(IV) in Alport GBM

Alport Syndrome: Three Genes & A Spectrum of Manifestations

Stereotypical GBM Abnormalities in Alport Mice

Miner, Kidney Int, 2014

Miner, et al., 1996
GBM Abnormalities in “3-D”

A combination of compositional and likely biophysical changes impact the overlying podocytes

3D-EM defines GBM defects


Podocyte-GBM invasion

Invasive phenotype of podocytes in Alport syndrome may be induced by GBM compositional or structural changes


There are Great Animal Models for Alport Syndrome

• Both mouse and dog models recapitulate most aspects of the human kidney disease
• Genetic background influences the rate of progression to ESRD in mice
  – 129’s progress quickly (ESRD at 80 to 90 days)
  – C57BL/6J’s progress slowly (ESRD at 8 to 9 months)
  – 129/B6 hybrids progress moderately (ESRD at ~4 months)
• Identification of modifier loci could provide opportunities for logical, targeted therapeutics

Alport Syndrome

• GBM structural defects eventually lead to:
  – infiltration of immune cells
  – increased expression of extracellular matrix proteins and proteases
  – glomerular scarring
  – obstruction of glomerular capillaries
  – reduced renal blood flow/GFR

Alport Mice: A Good Model for CKD

• Amenable to testing hypotheses about disease pathogenesis and potential therapeutics
  – Generating double mutant mice
  – Treating mice with candidate therapeutics
• In general, reducing inflammatory cell infiltration or interstitial fibrosis (genetically or pharmacologically) has had only modest impact on slowing progression to ESRD
A Notable Pre-Clinical Success

Albumin contributes to kidney disease progression in Alport syndrome

Generation and Characterization of Alb-/- Mice

- Normal lifespan
- Alb-/- mice:
  - No albumin
  - Reduced total serum protein concentration
  - Hypertriglyceridemia
  - Normal blood pressure
  - Normal kidneys
- Alb+/-/ mice:
  - Reduced albumin concentration (1/2 of normal)
  - Hypertriglyceridemia

The Absence of Albumin Improves Kidney Pathology in Alport Mice

The Absence of Albumin Reduces Proximal Tubular Injury in Alport Mice

Diabetic Nephropathy

Mesangial Sclerosis
(Kimmelstiel-Wilson Nodules)
Massive GBM Thickening

Hyperfiltration, Microalbuminuria,
Progressive and Chronic Kidney Disease
Is Albumin itself injurious to the nephron?
Alport Mice (B6) Survive Longer in the Absence of Albumin

Conclusions
- Filtered albumin is injurious to the nephron in Alport syndrome and perhaps in other proteinuric diseases
- Defining pathways activated by filtered albumin (in podocytes) and resorbed albumin (in tubular cells) could reveal novel targets for therapy.

A Notable Clinical Success

A Notable Clinical Success

The Nephron

The Glomerulus

Split, Thickened, “Basket Weave” GBM

“Fix it!” (Karl Tryggvason)
Potential Targets for Therapy in Alport Syndrome

- The deranged matrix deposition/matrix degradation in glomeruli associated with GBM splitting and glomerular scarring
- The inflammatory response in glomeruli and elsewhere in the kidney
- The defective GBM itself
  - gene therapy approach
  - cell therapy approach
  - collagen replacement approach

Could these approaches even change the GBM’s composition?

The Major Glomerular Basement Membrane Proteins

| LM-521 (α5β2γ1) | α3α4α5 |

Structure of collagen IV protomers and their assembly into networks

Type IV Collagen Chains, Genes, and Trimers

- α1 COL4A1
- α2 COL4A2
- α3 COL4A3
- α4 COL4A4
- α5 COL4A5
- α6 COL4A6

Can the Defective GBM Be Repaired?

- Express a Doxycycline-inducible Col4a3 transgene in the podocytes of Col4a3−/− mice

The Real Test: Restore the missing Collagen IV after the GBM is mature and functioning.

Restoration of the Collagen IV Network in the GBM (Nephrin-rTfA; Dox at 3 weeks)
Glomerular Therapeutic Targets

- Restore the missing Collagen IV network
  - a.k.a., “Fix the Defect”

- Define aberrant signaling within podocytes and normalize it
  - “neo” collagen IV interactions with podocyte receptors
  - impaired laminin interactions with integrin α3β1
  - pathogenic mechanical strain

Laminin β2 Knockout Mice
*Nephrotic syndrome and neuromuscular defects*
*A model for Pierson Syndrome*

P15

Lamb2 -/-

Lamb2 +/-

Lethal at ~1 month of age
Heavy albuminuria at 3 weeks
Human LAMB2 Mutations Cause Pierson Syndrome

Mostly Null/Nonsense Mutations

Foot Process Effacement in Lamb2-/- Mice (3 weeks of age)

Hypothesis: Laminin β2 in the GBM is a signal that induces podocytes to form foot processes and slit diaphragms.

Proteinuria is Followed by Foot Process Effacement in Lamb2-/- Mice

High Proteinuria and Widespread Foot Process Effacement at 3 weeks

Typical Podocyte Architecture, with Proteinuria at 2 days of age

Protein Therapy: Inject the missing LM-521 i.v. to deliver it to the GBM before widespread foot process effacement.
Treatment with hLAM-521 from day 12 Inhibited Proteinuria up to day P18

Localization of Injected hLM-521 by Super-Resolution Microscopy

Podocyte Injury in Lamb2-/- Kidney at P18 was Ameliorated by hLAM-521 Treatment

“Pierson Syndrome” LAMB2 Null and Missense Mutations

Matejas et al. Hum. Mutat. 2010
What about the LAMB2-S80R mutation?

Ocular abnormalities from infancy
Proteinuria from age 6
Nephrotic range proteinuria by age 11
Diffuse mesangial sclerosis on biopsy

Introduce S80R into the Mouse Lamb2 Gene by CRISPR/Cas9-Mediated Genome Editing

Required
Cas9 Nuclease
Guide RNA (gRNA)

Optional
DNA oligo or dsDNA for HDR

Include a 200 nt oligo to change AGT (Ser) to CGT (Arg) at S80

CRISPR-engineered LAMB2-S80R Allele: No Phenotype

LAMB2-S80R in Alport mice:
LAMB2-S80R Acts as a Modifier Allele

Early and Severe GBM and Podocyte Defects in Lamb2+/S80R Alport Mice

Hypothesis: Strengthening Laminin/Collagen IV Interactions Will Slow Splitting/Thickening

Normal

Alport

Miner et al., 1996
Conclusions

- Modifying the composition of the GBM in Pierson syndrome via the bloodstream can slow the onset of proteinuria.
- Altering or enhancing the interactions of the laminin and collagen IV networks with each other or with linking proteins in Alport syndrome could reduce GBM splitting and thickening and slow the onset of proteinuria and CKD.