Hereditary Nephropathy with Focal Segmental Glomerulosclerosis

Joseph P. Gaut, MD, PhD
Disclosures

• None
Outline

• Background
• Clinical Aspects of Hereditary FSGS
• Pathologic Aspects of Hereditary FSGS
• Genetic Testing
• Summary
A 2 year old presents with proteinuria

- Caucasian boy transferred from an outside hospital with severe nephrotic syndrome and pneumonia
- No extra-renal syndromic manifestations
• Focal segmental glomerulosclerosis
• Diffuse podocyte foot process effacement
Focal Segmental Glomerulosclerosis

- A morphologic lesion composed of focal segmental accumulation of extracellular matrix
- 20% of nephrotic syndrome cases in children
- 40% of nephrotic syndrome cases in adults
- Incidence 7/million
- Prevalence of 4%
## Morphologic Variants of FSGS

<table>
<thead>
<tr>
<th>Histologic Subtype</th>
<th>Glomerular Lesion</th>
<th>Defining Features</th>
<th>Associations</th>
<th>Clinical Features</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mesangial</strong></td>
<td></td>
<td>The usual generic form of FSGS. FSGS/HNS does not meet defining criteria for any other variant. Foot process effacement is variable.</td>
<td>Primary or secondary (including genetic forms and other diverse secondary causes). Cross-sectional studies suggest this is the most common subtype. Other variants can evolve into FSGS (HNS) over time.</td>
<td>May present with the nephritic syndrome or subnephritic proteinuria.</td>
</tr>
<tr>
<td><strong>Perihilar</strong></td>
<td></td>
<td>Predominantly mesangial and subendothelial. The majority of glomeruli with segmental lesions. Perihilar lesions are located at the glomerular vascular pole. In segmental FSGS, there is usually mesangial hypertrophy (glomerulomegaly). Foot process effacement is relatively mild and focal, which probably reflects the heterogeneous adaptive responses of glomeruli.</td>
<td>Common in idiopathic FSGS associated with obesity, elevated blood pressure, hypertension, and renal function. Most patients have proteinuria at the proximal mesangial end of glomerular capillary bed, which is heightened under conditions of compensatory demand and vasodilatation of the effaced capillary.</td>
<td>In idiopathic FSGS, patients are more likely to present with subnephritic proteinuria and normal serum albumin levels.</td>
</tr>
<tr>
<td><strong>Cellular</strong></td>
<td></td>
<td>Usually primary, but also seen in a variety of secondary forms. This is the least common variant.</td>
<td>Usually presents with the nephritic syndrome.</td>
<td></td>
</tr>
<tr>
<td><strong>Tip</strong></td>
<td></td>
<td>Usually primary. Usually involves the tubulo-interstitial component.</td>
<td>Usually presents with abrupt onset of the nephritic syndrome. More common in white race. Best prognosis, with high rate of remission to glomerulonephritis and low rate of progression.</td>
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</tr>
<tr>
<td><strong>Collapse</strong></td>
<td></td>
<td>Primary or secondary to VZV, HIV, and hepatitis B virus.</td>
<td>Most aggressive variant of primary FSGS with black社会(?) and severe nephritis, synchiae, and fibrosis. Poor response to glucocorticoids and rapid course to renal failure.</td>
<td></td>
</tr>
</tbody>
</table>

A Lesion with Varied Etiologies

- Familial/Genetic
- Virus-Associated
- Drug-Induced
- Adaptive
- Idiopathic
Positionally Cloned Gene for a Novel Glomerular Protein—Nephrin—Is Mutated in Congenital Nephrotic Syndrome

NPHS2, encoding the glomerular protein podocin, is mutated in autosomal recessive steroid-resistant nephrotic syndrome

Nicolas Boute¹, Olivier Grouval¹, Séverine Roselli¹, France Benessy¹, Hyunjoo Lee¹, Arno Fuchshuber¹, Karin Dahan³, Marie-Claire Gubler¹, Patrick Niaudet² & Corinne Antignac¹
Search for Gene and FSGS in PubMed

Current
>40 genes

Nephrin Identified
Podocin Identified

# Publications

Year


0 10 20 30 40 50 60 70
Genes Associated with Hereditary FSGS

**Slit Diaphragm**
- NPHS1
- NPHS2
- PLCE1
- CD2AP
- TRPC6
- ANLN
- CRB2
- FAT1
- MAGI2

**Actin Cytoskeleton**
- ACTN4
- INF2
- MYO1E
- MYH9

**Nuclear**
- WT1
- WDR73
- SMARCAL1
- XPO5
- NUP93
- NUP107
- NUP205
- LMX1B
- PAX2

**Mitochondrial – CoQ10 Biosynthesis**
- COQ2
- COQ6
- ADCK4
- MTTL1 – mitochondrial DNA
- PDS52
- XPNPEP3

**Actin Regulation Rho/Rac/Cdc42**
- KANK1
- KANK2
- KANK4
- ARHGAP24
- ARHGDI A

**Integrin/Laminin**
- LAMB2
- ITGA3
- ITGB4

**Lysosome**
- SCARB2

**Other**
- PTPRO
- EMP2
- DGKE
- NEIL1

Red = Autosomal Dominant
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Clinical Aspects of Hereditary FSGS

- Age of disease onset
  - Typically younger patients (infants & young children)
  - Depends on genetic etiology
- Steroid resistance
- Steroid dependence
- Nephrotic syndrome
- Positive family history
- Syndromic manifestations
Age of Onset and Hereditary FSGS

1589 families with SRNS
Onset before 25 years
27 genes evaluated

Genetic Etiology by Age

Five Genes Account for Majority of Positive Genetic Diagnoses

## Genetic Etiology by Age

<table>
<thead>
<tr>
<th>Age of Onset/Steroid Response</th>
<th>Most Common Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infant, &lt;1 year of age</td>
<td><em>NPHS1, NPHS2, LAMB2, WT1, PLCE1</em></td>
</tr>
<tr>
<td>Age 1-10 years, SRNS</td>
<td><em>NPHS2, LAMB2, WT1, PLCE1</em></td>
</tr>
<tr>
<td>Age 1-10 years, SDNS</td>
<td><em>MAGI2, TENC1, DLC1, CDK20, ITSN1</em></td>
</tr>
<tr>
<td>Age 10-18 or adult w/ +FH</td>
<td><em>INF2, ACTN4, TRPC6, WT1</em></td>
</tr>
</tbody>
</table>

SRNS – steroid resistant nephrotic syndrome
SDNS – steroid dependent nephrotic syndrome
• Gene causing mutation identified in 29.5% of families

• Likelihood of genetic etiology decreases with age to ~10% in patients over 12

• Genetic etiology varies with age of disease onset
Generalized Features of Inheritance Patterns

**Autosomal Recessive**
- Early onset
- Loss of function
- Infancy/early childhood

**Autosomal Dominant**
- Late onset
- Gain of function
- Late-onset/slowly progressive
- Varied penetrance
- Varied disease severity
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Pathologic Clues to Hereditary Etiology

- Collapsing glomerulopathy
  - *APOL1*

- Abnormal mitochondria
  - *COQ2, COQ6, tRNALeu* (MELAS)

- Glomerular basement thinning/multilayering
  - *COL4A3, COL4A4, COL4A5*

- FSGS with diffuse mesangial sclerosis
  - *WT1, LAMB2, NPHS2, PLCE1, NUP93, NUP205, XPO5*

Collagen type IV variants and FSGS

- COL4A3 and COL4A4 associated with Alport syndrome
- Patients may present with TBMD and/or FSGS

Presented with proteinuria and hematuria

Positive family history

FSGS on light microscopy

Thin basement membranes on EM

COL4A3 p.G695R variant
Rare hereditary COL4A3/COL4A4 variants may be mistaken for familial focal segmental glomerulosclerosis

Andrew F. Malone\textsuperscript{1,2}, Paul J. Phelan\textsuperscript{1,2}, Gentzon Hall\textsuperscript{1,2}, Umran Cetincelik\textsuperscript{3}, Alison Homstad\textsuperscript{1,4}, Andrea S. Alonso\textsuperscript{1,4}, Ruiji Jiang\textsuperscript{1,4}, Thomas B. Lindsey\textsuperscript{1}, Guanghong Wu\textsuperscript{1}, Matthew A. Sparks\textsuperscript{2}, Stephen R. Smith\textsuperscript{2}, Nicholas J.A. Webb\textsuperscript{5}, Philip A. Kalra\textsuperscript{6}, Adebowale A. Adeyemo\textsuperscript{7}, Andrey S. Shaw\textsuperscript{8}, Peter J. Conlon\textsuperscript{9}, J. Charles Jennette\textsuperscript{10}, David N. Howell\textsuperscript{11}, Michelle P. Winn\textsuperscript{1,2} and Rasheed A. Gbadegesin\textsuperscript{1,4}

- Seven families with FSGS, nephrotic-range proteinuria & hematuria
- One family with thin GBM – same variant as seen in previous case (\textit{COL4A3} p.G695R)
- No basement membrane splitting, lamellation, or basket weaving present
- **Subset of cases without morphologic clues to diagnosis**
Pathology is Often Non-Specific in Hereditary FSGS

- Typically NOS pattern
- Diffuse podocyte foot process effacement in non-segmentally sclerotic glomeruli
- Looks identical to primary FSGS
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Genetic Testing in FSGS

- Why test?
  - Aid diagnosis
  - Identify appropriate treatment
  - Determine risk of disease recurrence post-transplantation
  - Assess risk of kidney donors
  - Prenatal diagnosis
  - Family screening

Brown EJ, Pollak MR, and Barua M. Kidney Int. 2014;85:1030-1038
Clinical Genomic Testing

The process of using medical tests to look for *clinically relevant changes* in a person’s chromosomes or genes.

<table>
<thead>
<tr>
<th>Clinical Utility</th>
<th>Constitutional ‘Germline’ vs Somatic</th>
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<tbody>
<tr>
<td><strong>Clinical settings:</strong></td>
<td></td>
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<tr>
<td>Inherited disease (constitutional)</td>
<td>Diagnosis: mutation present results in disease (Huntington disease)</td>
</tr>
<tr>
<td>Cancer (somatic mutations)</td>
<td>Prognosis: mutation present results in mild or aggressive disease course</td>
</tr>
<tr>
<td>Mitochondrial disease (affecting vision, hearing, muscles)</td>
<td>Therapy: genetic differences influence drug choice and dose (PGx)</td>
</tr>
<tr>
<td>Prenatal diagnosis - may replace riskier procedures (CVS)</td>
<td>Carrier status/disease risk: mainly for autosomal recessive diseases (Cystic fibrosis)</td>
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*Pathology and Immunology*

*Anatomic and Molecular Pathology*

*Washington University School of Medicine in St. Louis*
Single Locus vs. Multiple Gene Testing

**Locus specific testing**
- Analyze single gene/locus
- Determine mutation status of limited region

**Multiple gene testing**
- Analyze multiple relevant genes
- Determine mutation status of all relevant genes simultaneously

**Advantages of Multiple Gene Testing**
- Narrowly targeted
- Result may trigger additional gene testing
- Cost effective
- Efficient/time-saving
- Yields unexpected findings

**Genes**
- NPHS1
- NPHS2
- WT1
- PLCE1
- LAMB2
- ACTN4
- INF2

**Sequence**
- CTATGCTCG
Massively Parallel or ‘Next-Generation’ Sequencing

- Sanger sequencing – 2x read (Bidirectional)

- Next-generation – 100-1000x reads at single position
### Next-Generation Sequencing

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<thead>
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<tbody>
<tr>
<td>• 0.01 Gigabase/week</td>
<td>• 10,000 Gigabase/week</td>
</tr>
<tr>
<td>• $100,000,000/Gigabase</td>
<td>• $1/Gigabase</td>
</tr>
</tbody>
</table>
Next Generation Sequencing Publications

- Next-generation sequencing citations in PubMed
- Total = 15,715
- 4,655 in 2016
- 1 in 2005
Application of NGS Technology

- Patient develops SRNS
- Biopsy reveals FSGS
- A genetic cause is suspected
- Case is referred for genetic sequencing of genes known to be involved in FSGS
A multitude of genetic variants are identified

The greater number of genes sequenced, the greater number of variants identified

How to determine if a genetic variant is causing disease?
Genetic Variant Interpretation

Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology

Sue Richards, PhD\textsuperscript{1}, Nazneen Aziz, PhD\textsuperscript{2,16}, Sherri Bale, PhD\textsuperscript{3}, David Bick, MD\textsuperscript{4}, Soma Das, PhD\textsuperscript{5}, Julie Gastier-Foster, PhD\textsuperscript{6,7,8}, Wayne W. Grody, MD, PhD\textsuperscript{9,10,11}, Madhuri Hegde, PhD\textsuperscript{12}, Elaine Lyon, PhD\textsuperscript{13}, Elaine Spector, PhD\textsuperscript{14}, Karl Voelkerding, MD\textsuperscript{13} and Heidi L. Rehm, PhD\textsuperscript{15}; on behalf of the ACMG Laboratory Quality Assurance Committee
Variant Interpretation

• Literature

• Clinical Databases
  • HGMD, ClinVar

• Locus Specific Databases
  • Leiden, FH HUS

• Laboratory Specific Databases
  • EmVClass (Emory)
Variant Interpretation

- Frequency Data
  - dbSNP, 1000 genomes
  - NHLBI ESP
  - ExAC Browser

- Effect on Protein
  - Conservation Data
  - Grantham scores
  - *In-silico* predictions
    - Protein function
    - Splicing
  - Functional Studies
Variant Interpretation Classifications

- Pathogenic
- Likely Pathogenic
- Variant of Uncertain Clinical Significance
- Likely Benign
- Benign
Clinical Application

• Washington University Experience

• Using standardized variant interpretation criteria

• Patients referred for nephrotic syndrome/FSGS sequencing

• All cases reviewed by a pathologist with subspecialty boards in Molecular Genetics or a clinical laboratory geneticist certified in Clinical Molecular Genetics

• 30 cases sequenced to date as part of routine practice
Patient Characteristics

- 16 Male
- 14 Female
- 15 Caucasian
- 7 AA
- 3 Hispanic
- 5 Other
Washington University Experience

Diagnostic Yield = 23%

- VUS 60%
- P/LP 10%
- APOL1 13%
- Negative 17%
Differences in Clinical Application

- Clinical population is less well defined compared to a research population with well-characterized families
- Indications for testing vary
- Broader age range tested
- Strict variant interpretations are applied
## Likely Pathogenic Variants

<table>
<thead>
<tr>
<th>Age</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>32</td>
<td>INF2</td>
</tr>
<tr>
<td>71</td>
<td>INF2</td>
</tr>
<tr>
<td>75</td>
<td>COL4A3</td>
</tr>
</tbody>
</table>

- Typically worked up in setting of transplantation
- All have a pathologic diagnosis of FSGS
- All have a positive family history
Variants of Uncertain Clinical Significance

![Bar chart](chart.png)

- **LAMB2**: 4 patients
- **NPHS1**: 3 patients
- **NPHS2**: 1 patient
- **INF2**: 1 patient
- **TRPC6**: 1 patient
- **COL4A3**: 1 patient
- **COL4A4**: 1 patient
- **COL4A5**: 1 patient
- **ACTN4**: 1 patient
- **CD2AP**: 1 patient
- **CRB2**: 1 patient
- **PLCE1**: 1 patient
- **PTPRO**: 1 patient

Pathology and Immunology
Anatomic and Molecular Pathology
Variant Interpretation Challenges

• Incomplete penetrance
• Polygenic inheritance patterns
• Environmental factors
• Lack of centralized database
• Lack of functional studies
Variants of Uncertain Clinical Significance

• Most common classification
• Not clinically actionable

• What to do with these variants?
Case Revisited

• 2 year old with SRNS

• Pathology – FSGS

• Next generation sequencing performed
Genetic Variants Identified

- *NPHS2* – p.R138Q
- *NPHS2* – p.R196X
## Variant Interpretation

<table>
<thead>
<tr>
<th>Variant</th>
<th>MAF (overall)</th>
<th>GERP</th>
<th>SIFT</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPHS2</td>
<td>0</td>
<td>4.79</td>
<td>n/a</td>
</tr>
<tr>
<td>NPHS2</td>
<td>0.000676</td>
<td>5.82</td>
<td>0</td>
</tr>
</tbody>
</table>

- PVS1 – nonsense/truncating mutation
- PS1 – previously described pathogenic variant
- PM2 – low frequency relative to controls
- PP3 – predicted deleterious effect on protein function

**Pathogenic**
Podocin Immunofluorescence

A  Control

B  Patient
Summary

• Hereditary FSGS is more common in a younger population

• However, adults with a positive family history may harbor significant genetic variants

• Pathology is helpful in certain cases
  • COL4 variants, mitochondrial disease, APOL1, DMS

• However, most cases of hereditary FSGS show non-specific findings similar to primary FSGS

• NGS is increasingly utilized, but most commonly yields a diagnosis of VUS

• Need for centralized database, functional studies
References

• Brown EJ, Pollak MR, and Barua M. *Kidney Int*. 2014;85:1030-1038
Thank you