Hereditary Nephropathy with Focal Segmental Glomerulosclerosis

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Disclosures

• No financial disclosures

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
- Columbia Classification
- NOS
- Perihilar
- Cellular
- Tip
- Collapsing

A Lesion with Varied Etiologies
- Familial/Genetic
- Virus-Associated
- Adaptive
- Idiopathic

Search for Gene and FSGS in PubMed

Genes Associated with Hereditary FSGS
- NPHS1
- NPHS2
- PLCE1
- CD2AP
- TRPC6
- ANLN
- CRB2
- FAT1
- MAGI2
- Slit Diaphragm
- Mitochondrial – CoQ10 Biosynthesis
- Actin Cytoskeleton
- Lysosome
- Other
- 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
- < 1 year old - 60% with molecular diagnosis
- >12 years old - ~10% molecular diagnosis
- Genetic diagnosis identified in 29.5% of families


Five Genes Account for Majority of Positive Genetic Diagnoses

<table>
<thead>
<tr>
<th>Gene</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPHS1</td>
<td>34%</td>
</tr>
<tr>
<td>NPHS2</td>
<td>25%</td>
</tr>
<tr>
<td>LAMB2</td>
<td>16%</td>
</tr>
<tr>
<td>WT1</td>
<td>7%</td>
</tr>
<tr>
<td>PLCE1</td>
<td>4%</td>
</tr>
</tbody>
</table>

Genetic Etiology by Age

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

SRNS = steroid resistant nephrotic syndrome
SDNS = steroid-dependent nephrotic syndrome

Generalized Features of Inheritance Patterns

<table>
<thead>
<tr>
<th>Autosomal Recessive</th>
<th>Autosomal Dominant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early onset</td>
<td>Late onset</td>
</tr>
<tr>
<td>Loss of function</td>
<td>Gain of function</td>
</tr>
<tr>
<td>Infancy/early childhood</td>
<td>Late-onset/slowly progressive</td>
</tr>
<tr>
<td></td>
<td>Varied penetrance</td>
</tr>
<tr>
<td></td>
<td>Varied disease severity</td>
</tr>
</tbody>
</table>
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Pathologic Clues to Hereditary Etiology
• Collapsing glomerulopathy
  • APOL1
• Glomerular basement thinning/multilayering
  • COL4A3, COL4A4, COL4A5
• FSGS with diffuse mesangial sclerosis
  • WT1, LAMB2, NPHS2, PLCE1, NUP93, NUP205, XPO5
• Abnormal mitochondria
  • COQ2, COQ6, tRNALeu (MELAS)

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

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

Rare hereditary COL4A3/COL4A4 variants may be mistaken for familial focal segmental glomerulosclerosis
• Seven families with FSGS, nephrotic-range proteinuria & hematuria
• One family with thin GBM – same variant as seen in previous case (COL4A3 p.G695R)
• No basement membrane splitting, lamellation, or basket weaving present
• Subset of cases without morphologic clues to diagnosis

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Genetic Testing in FSGS

- Why test?
  - Aid diagnosis
  - Identify appropriate treatment
  - Discontinue steroid e.g. PLCE1
  - CoQ10 supplements
  - Start calcineurin inhibitors – TRPC6
- 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

Single Locus vs. Multiple Gene Testing

- **Locus specific testing**
  - Analyze single gene/locus
  - Narrowly targeted
  - Result may trigger additional gene testing

- **Multiple gene testing**
  - Analyze multiple relevant genes
  - Cost effective
  - Efficient/time-saving
  - Yields unexpected findings

Massively Parallel or ‘Next-Generation’ Sequencing

- **Sanger sequencing – 2x read (Bidirectional)**
  - Narrowly targeted
  - Result may trigger additional gene testing

- **Next-generation – 100-1000x reads at single position**

Next-Generation Sequencing

- **Sanger c. 2000**
  - 0.01 Gigabase/week
  - $100,000,000/Gigabase

- **NGS c. 2016**
  - 10,000 Gigabase/week
  - $1/Gigabase

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

- 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**

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Negative: 17%
P/LP: 10%
APOL1: 13%
VUS: 60%

Diagnostic Yield = 23%
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**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**
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>nonsense</td>
<td>0.A</td>
<td>4.79</td>
</tr>
<tr>
<td></td>
<td>missense</td>
<td>0.C-T</td>
<td>0.000676</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

Podocin Immunofluorescence

Control
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


Thank you