“The evolution of the classification of nephrotic syndrome”

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Classification schemes:

Proteinuria and nephrotic syndrome

- MCD
  - Good prognosis and Response to steroid Therapy

- FSGS
  - Poor prognosis and Poor Response to Steroid therapy
• While the definition of minimal change disease did not change over the years, in the mid 80’s other patterns of glomerular damage have became part of the FSGS spectrum.

• **Collapsing glomerulopathy:**
  - first description in 1978 as “malignant FSGS” (Brown Clin Nephrol 1978)
  - 1980’s frequent diagnosis during HIV pandemic (HIV-AN)
  - first described in non-HIV pts in 1986 (Weiss et al AJKD 1986) i.e. “collapsing glomerulopathy” new clinical-pathologic entity.
  - in mid 90’s became “idiopathic collapsing FSGS” (Detwiler et al KI 1994 & Valeri et al JASN 1996)

• **Cellular lesion:**
  - Term used first by Schwarz and colleagues to indicate a group of lesions with endocapillary and/or extracapillary increased cellularity.
  - Other authors used the term cellular to indicate intracapillary cellularity only.

• **Tip lesion:**
  - Howie et al described tip lesion as a well-defined and specific pathological entity with clinical similarity to MCD. (J Pathol 1984)
  - Tip lesions are also seen in associations with other glomerular diseases such as diabetic nephropathy or membranous glomerulopathy.
Relatively recent classification schemes:

- Columbia classification - FSGS variants

Perihilar  NOS  Tip

Cellular  Collapsing
Limitations of the morphologic classification

- Various histopathologic lesions are listed under “focal segmental glomerulosclerosis” regardless the presence or absence of segmental sclerosis.

- Lack of correlation with pathogenetic mechanisms and etiology.

- Lack of correlation with treatment
The attention of scientists, nephrologists and pathologists has been recently focused on the role of podocytes as cause of proteinuria.

In the last 10 years lot of progress has been made in the understanding the biology of podocytes, how they function and how they are injured.

"Taxonomy of the podocytopathies" where morphologic diagnosis are integrated with etiology

*(Barisoni, Schnaper, Kopp, CJASN 2007)*
A taxonomy is organized into multiple levels, each of which represents a taxon with one or more elements (taxa), which are mutually exclusive, unambiguous, and all-encompassing categories.

Taxonomies provide classification and conceptual framework for analysis, discussion, and hypothesis generation.

**Taxonomy of Podocytopathies**

<table>
<thead>
<tr>
<th>ETHIOLOGY</th>
<th>idiopathic</th>
<th>genetic</th>
<th>reactive</th>
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<tbody>
<tr>
<td>HISTOPATHOLOGY</td>
<td></td>
<td></td>
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<tr>
<td>- pattern of glom injury</td>
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<tr>
<td>- podocyte number</td>
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**Taξιον**
**Podocytopathies**

**DEFINITION:** Proteinuric diseases in which pathologic processes arise from intrinsic or extrinsic “primary” podocyte injury and where the podocyte genotype/phenotype is altered.
Podocytopathies: 4 morphologic patterns of glomerular injury

- Normal Histology
- Segmental Sclerosis
- Mesangial Sclerosis
- Collapse of the GBM

MCN, FSGS, DMS, CG
Common denominator of podocytopathies:
Podocyte injury = foot process effacement

(normal)

(a) effacement

GBM

(*)
Causes of foot process effacement

1. Impaired formation of the slit diaphragm complex
2. Abnormalities of the adhesive interaction between podocytes and GBM
3. Alterations of transcription factors
4. Abnormalities of the actin-based cytoskeleton
5. Alterations of the apical domain of podocytes
6. Mitochondria abnormalities
7. Abnormalities of cell metabolism
8. Mechanical stress
9. Viral infection
10. Acute ischemic injury
11. Toxic / metabolic effect
12. Immunologic stimuli
How do we translate this large variety of insults into four morphologic patterns of glomerular injury?
Hypothesis #1: Injured podocytes can take different pathways

Podocyte injury

- Altered phenotype → No change in podocyte number → No change
  - MCN
- Engagement of apoptotic pathways → Cell death
  - Segmental sclerosis
  - FSGS
- Developmental arrest
  - Proliferation (low)
  - Mesangial sclerosis
  - DMS
- De-differentiation
  - Proliferation (high)
  - Collapse
  - CG
Hypothesis #2: The role of the renopoietic system

Hierarchical distribution of CD133+CD24+PDX- and CD133+CD24+PDX+ cells within human glomeruli

Hypothesis #2
The role of CD24+CD133+ renal progenitors in FSGS & CG.

Podocyte injury

- Altered phenotype
  - No change in podocyte number
    - No change
      - MCN

- Podocyte death
  - Insufficient CD24+CD133+ repair activity
    - Segmental sclerosis
      - FSGS

- Developmental arrest
  - Proliferation (low)
    - Mesangial sclerosis
      - DMS

- Podocyte death
  - Exuberant CD24+CD133+ activity
    - Pseudocrescents
      - CG
MINIMAL CHANGE NEPHROPATHY
Minimal Change Nephropathy

**DEFINITION**
- Normal histology.
- Extensive foot process effacement, with preserved number of podocytes.

**ETIOLOGY AND CLINICAL ASSOCIATION**
- **Idiopathic**
- **Inherited**
  - Non-Syndromic (NPHS1, NPHS2)
  - Syndromic (DYSF)
- **Reactive**
  - Drug-induced
    - NSAID, pamidronate, interferon, others
  - Dysregulation of the immune system
  - Hematologic malignancy

**CLINICAL PRESENTATION**
- Steroid sensitive
- Steroid resistant
FOCAL SEGMENTAL GLOMERULOSCLEROSIS
DEFINITION

Segmental solidification of the tuft accompanied by sinechiae. Hyalinosis and foam cells can also be present. Low number of podocytes (podocytopenia).

ETIOLOGY AND CLINICAL ASSOCIATION

- **Idiopathic**
- **Inherited**
  - syndromic
  - non-syndromic
- **Reactive**
  - hyperfiltration-mediated
    - normal renal mass
    - reduced renal mass
  - medication-induced
  - permeability factor (?)
Idiopathic FSGS

Is idiopathic really idiopathic?

**APOL1 is a major-effect risk gene for FSGS.**

*(Genovese et al Science 2010)*

APOL1 risk alleles are more frequent in AA. Odd ratios of 10.5 in FSGS and 7.3 in HTN-ESRD
Genetic forms of FSGS

Associated with other organ abnormalities (syndromic):
- Freiser Syndrome (WT-1).
- Nail-patella syndrome (LMX1B).
- Renal-coloboma syndrome with oligomeganephronia (PAX2).
- Alport’s disease (COL4A3, A4, A5).
- Metabolic disorders (GLA – Fabry’s).
- Mitochondriopathies (mtDNA tRNA\textsubscript{Leu} and tRNA\textsubscript{Tyr}, CoQ2 NP, CoQ6 NP).

Limited to the kidney (non-syndromic):
- NPHS1 – nephrin – autosomal recessive.
- NPHS2 – podocin – autosomal recessive.
- NPHS3 – phospholipase C\textsubscript{ε}1 – autosomal recessive.
- ACTN4 – α-actinin-4 - autosomal dominant.
- INF2 – autosomal dominant.
- TRPC6 – Transient Receptor Potential channel 6 - autosomal dominant.
- WT1 – sporadic/isolated FSGS.
- CD2AP – susceptibility to FSGS.
- MYH 9 – susceptibility to FSGS.
- APOL1 – susceptibility to FSGS.
Reactive forms: Hyperfiltration-mediated FSGS

glomerulomegaly in pt with single kidney

Segmental sclerosis

large non-sclerotic glomerulus
DIFFUSE MESANGIAL SCLEROSIS
DEFINITION:
Diffuse increase of mesangial matrix accompanied by mild proliferation of hypertrophic podocytes.

ETIOLOGY:
- Idiopathic
- Genetic
  - Non-syndromic
    - WT1
    - NPHS1
    - NPHS2
    - NPHS3
    - COQ6
  - Syndromic
    - LAMB2 (Pierson S.)
    - WT-1 (Denys-Drash S.)
COLLAPSING GLOMERULOPATHY
Definition: GBM collapse and pseudocrescent formation
Etiology and clinical associations

- Idiopathic
- Genetic
  - Syndromic - action myoclonus renal failure (SCARB2)
  - Non-Syndromic - CoQ2 NP
- Reactive
  - Virus associated
    - HIV
    - parvovirus B19
    - CMV
  - Infections
    - filariasis
    - leishmania
    - TB
  - Autoimmune
    - Still’s disease
    - lupus like
    - RA
    - mixed connective tissue
  - Malignancy (myeloma, AML)
  - Medications
    - pamidronate
    - interferon
    - valproic acid
  - Vascular insult - TMA
  - Permeability factor
Differently from other podocytopathies, in HIV-CG podocytes do not express maturity markers.
de-differentiated podocytes re-enter the cell cycle and proliferate

Barisoni et al. KI 2000
idiopathic and HIV-associated CG dedifferentiated podocytes have a dysregulated phenotype
Inherited CG (COQ2-NP) phenotype is dedifferentiated but not dysregulated.
Which is the underlying mechanism of pseudocrescent formation and collapse of the basement membranes?
Pathogenesis of CG: from podocyte injury to pseudocrescent formation

The dysregulated podocyte

Podocytes are injured

They dedifferentiate and dysregulate their phenotype

Dedifferentiated podocytes can re-enter the cell cycle and proliferate

Pseudocrescent formation

The exuberant renopoietic system

Podocytes are injured

They undergo apoptosis/death

CD24+CD133+ cells migrate from the Bowman’s capsule

Exuberant proliferation

Pseudocrescent formation
Collapsing glomerulopathy is a proliferative disease
Amelioration of nephropathy in mice expressing HIV-1 genes by the cyclin-dependent kinase inhibitor flavopiridol
# Taxonomy of Podocytopathies

<table>
<thead>
<tr>
<th></th>
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<th>genetic</th>
<th>reactive</th>
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<tbody>
<tr>
<td>MCN</td>
<td>Idiopathic</td>
<td>Non-syndromic</td>
<td>Clinical association (immunologic stimuli, Tumors)</td>
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<tr>
<td></td>
<td></td>
<td>• Steroid-sensitive</td>
<td>NPHS1, NPHS2</td>
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<tr>
<td></td>
<td></td>
<td>• Steroid-resistant</td>
<td>Syndromic</td>
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<tr>
<td></td>
<td></td>
<td>Non-syndromic</td>
<td>Medications (NSAID, gold, penicillamine, lithium, IF, pamidronate)</td>
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<tr>
<td></td>
<td></td>
<td>• NPHS1, NPHS2</td>
<td>Post-adaptive</td>
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<tr>
<td></td>
<td></td>
<td>Syndromic</td>
<td>Initially normal nephron mass</td>
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<tr>
<td></td>
<td></td>
<td>• DYSF</td>
<td>Medications (tacrolimus, lithium, IF, pamidronate)</td>
</tr>
<tr>
<td>FSGS</td>
<td>Idiopathic</td>
<td>Non-syndromic</td>
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<td>Post-adaptive</td>
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<td>ITGB4, NPSH2, NPHS3, NPHS1 + NPHS2, COQ2, MHY9, ACTN4, CD2AP, TRCP6, WT-1, SYNPO, INF2</td>
<td>Initially normal nephron mass</td>
</tr>
<tr>
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<td>Syndromic</td>
<td>Medications (tacrolimus, lithium, IF, pamidronate)</td>
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<td>MtdNA, WT1, PAX2, COQ6, COL4, GLA, LMBX1</td>
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<tr>
<td>DMS</td>
<td>Idiopathic</td>
<td>Non-syndromic</td>
<td>Infections (viruses, TB, others)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WT1, NPHS1, NPSH2, NPHS3, LAMB2,</td>
<td>Clinical association</td>
</tr>
<tr>
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<td>Syndromic</td>
<td>Medications (IF, pamidronate, valproic acid)</td>
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<tr>
<td></td>
<td></td>
<td>WT1, LAMB2, COQ6,</td>
<td></td>
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<tr>
<td>CG</td>
<td>Idiopathic</td>
<td>Non-syndromic</td>
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<td></td>
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<td>COQ2</td>
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<td>MHY9</td>
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<tr>
<td></td>
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<td>Syndromic</td>
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<td>SCARB</td>
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- **MCN**: Membranous nephropathy
- **FSGS**: Focal segmental glomerulosclerosis
- **DMS**: Diffuse mesangial sclerosis
- **CG**: Cystic glomerulosclerosis
MCN, FSGS, DMS and CG are patterns of glomerular damage where the common denominator is podocyte injury.

Morphologic classifications alone are insufficient to capture the complexity and heterogeneity of diseases presenting with NS.
- multiple specific disease processes can present with indistinguishable histopathology
- a specific monogenetic disorder can present with more than one form of histopathologic pattern of glomerular damage.

Final diagnosis of the podocytopathies should occur in 3 steps:
- clinical evaluation
- morphologic evaluation
- additional clinical tests, such as genetic or serology for evidence of infections, or others, when indicated.
Nephrotic Syndrome: the future

**NEPTUNE**: The Nephrotic Syndrome Study Network
International effort with the following major goals:

- Determination of rates and predictors of clinical remission or progression in NS
- Identification of gene expression profiles
- Identification of patient specific molecular signatures
- Clinically useful classification based on morphologic & molecular phenotype
NEPTUNE: evolution of the renal biopsy procedure

The renal biopsy: standard procedure (diagnostic purposes)
- Cortex
- Medulla
- Electron microscopy: Glutaraldehyde fixed, Resin embedded
- Immunofluorescence: Frozen
- Light microscopy: Formalin

NEPTUNE renal biopsy: 3rd core procedure
- Cortex
- Medulla
- Electron microscopy: Glutaraldehyde fixed, Resin embedded
- Immunofluorescence: Frozen
- Light microscopy: Formalin
- NEPTUNE: Cryovial RNA-Later (green cup)
Evolution of morphologic analysis methodology

Conventional LM methodology

- UNC-Chapel Hill
- NYU-New York
- C. Sinai - LA
- Consensus meeting
- Data analysis
- Discrepant

Novel VM methodology

- Glass slides are scanned using the Aperio scanner at 100X under oil immersion
- Kidney bx slide
- Mail
- Annotator
- Scoring pathologist #1
- Scoring process on annotated images

- Data analysis
- Scoring pathologist #2
- Scoring process on annotated images
**Pathology Scoring Sheet**

### Light Microscopy

<table>
<thead>
<tr>
<th>Case</th>
<th>Glomerulus #</th>
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#### Membranous
- Membranous
- Membranoproliferative (30%)  
- Minimal change

#### Mesangial
- Segmental mesangial hypercellularity
- Global mesangial hypercellularity

#### Podocytes
- Hyperplasia, segmental (30%)
- Hyperplasia, global (30%)
- Hypertrophy, segmental (50%)
- Hypertrophy, global (20%)

#### Mesangial
- Segmental glomerulosclerosis
- Membranous
- Membranoproliferative
- Minimal

#### Vascular
- Perihilar arteriosclerosis
- Interlobular arteriosclerosis

### EM

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<th>Case</th>
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#### Podocytes
- Foot process effacement (0-3+)
- Loss of primary processes (present or absent)
- Cellular foot process
- Mesangial (present or absent)

#### Basement Membranes
- IgG
- IgA
- IgM
- C3
- C1q

#### Mesangium
- IgG
- IgA
- IgM
- C3
- C1q

#### Tubulo-interstitial features
- Tubules
  - Acute injury
  - Tubular atrophy
  - Interlobular arteriosclerosis
  - Hyalinization

- Interstitium
  - Fibrosis
  - Eosinophils (0-5%)
  - Neutrophils (0-5%)
  - Edema

- Arterioles
  - Intimal fibrosis
  - Hyalinization

#### Predominance of stages

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<th>Stage</th>
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**NEPTUNE**

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The evolution of the classification of NS