Podocyte and Glomerular Filtration Biology: Molecular Architecture and Pathophysiology

Andrew Bland, MD, FACP, FAAP

March 2026

Podocyte and Glomerular Filtration Biology: Molecular Architecture and Pathophysiology

Learning Objectives

By the end of this module, you should be able to:

1. **Describe podocyte architecture** including the slit diaphragm complex and foot process structure
2. **Explain slit diaphragm protein interactions** (nephrin, podocin, CD2AP, claudins) and their molecular assembly
3. **Understand podocyte cytoskeleton dynamics** and regulation of foot process movement
4. **Analyze mechanisms of proteinuria** at the molecular level (foot process effacement, slit pore disruption, charge selectivity loss)
5. **Integrate endothelial, basement membrane, and podocyte contributions** to the glomerular filtration barrier
6. **Relate podocyte injury mechanisms** to clinical glomerular diseases (minimal change, membranous, FSGS, diabetic nephropathy)

I. Overview of Podocyte Biology and the Filtration Barrier

A. Podocytes: Specialized Epithelial Cells

General Definition: Podocytes are specialized visceral epithelial cells that form the outermost layer of the glomerular filtration barrier. They are among the largest cells in the kidney and contain extensive cytoplasmic processes that cover the entire glomerular capillary surface.

Embryologic Origin: - Derived from metanephric mesenchyme (intermediate mesoderm) - Specified by WT1 (Wilms tumor 1) transcription factor during nephrogenesis - Mitotic activity present during fetal development; postnatally become postmitotic (cannot divide in healthy adults)

Key Characteristics: - **Postmitotic in adults:** Mature podocytes do not divide under normal conditions - **Minimal protein turnover:** Stable population; injury leads to permanent loss -

Extreme cell size: Single podocyte covers ~600 μm² of glomerular surface - **Six per glomerulus:** Approximately 6-10 podocytes per glomerulus; loss of even a few has functional consequences - **Long lifespan:** Under normal conditions, podocytes persist for decades

Functional Significance: The postmitotic nature and minimal regenerative capacity make podocytes uniquely vulnerable to injury. Once damaged or lost, replacement is very limited, making podocyte preservation critical for long-term renal function.

B. Three-Layer Filtration Barrier Organization

The glomerular filtration barrier functions as three integrated layers:

Layer 1: Fenestrated Endothelium - Size selectivity: Excludes RBCs, platelets; allows plasma proteins to approach GBM - Charge selectivity: Glycocalyx sialic acids repel anionic proteins (albumin) - Highly permeable to water and small solutes

Layer 2: Glomerular Basement Membrane (GBM) - Size selectivity: ~5-10 nm pore diameter; excludes proteins >50-60 kDa - Charge selectivity: Heparan sulfate negatively charged; repels anions - Structural support: Anchors endothelium above and podocytes below

Layer 3: Podocytes (Visceral Epithelium) - Size selectivity: Slit pores ~30 nm wide; exclude molecules >20-30 kDa - Mechanical integrity: Foot process interdigitation stabilizes filtration apparatus - Slit diaphragm: Molecular "gatekeeper" between podocyte foot processes

Integration: - All three layers together determine final filtration properties - Damage to any layer ☐ proteinuria - Complete barrier function requires integrity of all components

---

II. Podocyte Architecture and Cell Organization

A. Gross Morphology: Cell Body to Foot Processes

Podocyte Cell Body: - Large nucleus (8-10 μm diameter) - Abundant rough endoplasmic reticulum (RER) and ribosomes - Golgi apparatus (extensive; protein synthesis and trafficking) - Numerous mitochondria (high ATP demand for cytoskeletal maintenance) - Located on Bowman's capsule (distal to glomerular capillaries)

Primary (Major) Processes: - Thick extensions (~1-3 μm diameter) arising from cell body - Extend over glomerular capillary surface - Contain cytoplasmic contents (mitochondria, ribosomes, rough ER) - Provide structural scaffold for secondary processes - Anchored to GBM via integrin-focal adhesion complexes

Secondary (Foot) Processes: - Thin extensions (~0.5-1.0 μm diameter) arising from major processes - Highly specialized architecture - Interdigitate with foot processes of adjacent podocytes - Form slit diaphragms at their junctions

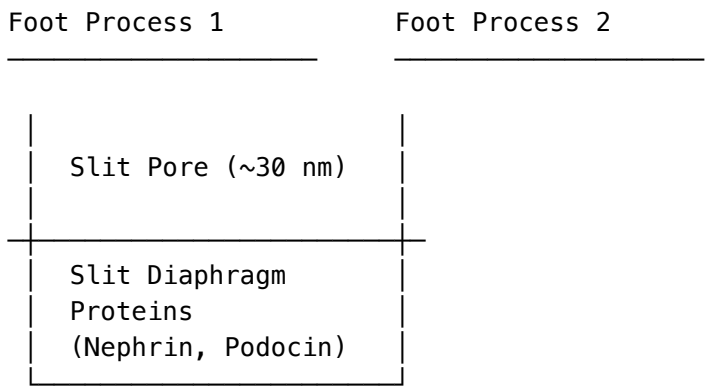
Slit Pore Zone: - 30 nm-wide space between adjacent foot processes - Filled with slit diaphragm proteins - Anchored to actin cytoskeleton - Acts as final size/charge-selective barrier

---

B. Detailed Slit Diaphragm Architecture

Definition: The slit diaphragm is a modified cell-cell junction between adjacent podocyte foot processes. It is structurally and functionally distinct from classical tight junctions (like those in the intestinal epithelium) and represents a specialized "slit pore architecture."

Structural Organization:



Key Protein Components:

C. Nephrin (NPHS1): The Primary Adhesion Molecule

Gene and Protein: - Gene: NPHS1 (chromosome 19q13.12) - Protein: 1241 amino acids; ~180 kDa transmembrane protein - Homophilic adhesion molecule (nephrin-to-nephrin interactions across the slit)

Structural Domains:

Extracellular Region: - Signal peptide (residues 1-27; cleaved) - 8 immunoglobulin-like (Ig) domains (residues 28-755) - Variable (V) and constant (C) domain architecture - Homophilic binding occurs via Ig domains - Trans-dimerization: One nephrin molecule binds to nephrin on adjacent foot process - Fibronectin type III-like domain (Ig-C2 fold) - Forms linear zipper-like adhesion along slit diaphragm

Transmembrane Region: - Single transmembrane domain (residues 756-778) - Spans cell membrane once

Intracellular (Cytoplasmic) Tail: - ~500 amino acids - Contains FERM domain-binding motifs - PDZ domain-binding sequences - Src kinase phosphorylation sites (Y□pY) - Constitutive binding sites for: - Podocin (adaptor protein) - CD2AP (adaptor protein) - Phospholipase C-γ (PLCγ; signaling) - SH3 domain proteins

Nephrin Function:

Adhesion: - Homophilic trans-interactions form zipper-like structure - Each nephrin molecule contacts ~4-6 nephrin molecules on adjacent foot process - Creates linear adhesion line along slit

Signaling: - Src kinase phosphorylation of nephrin tail tyrosines - Recruitment of signaling proteins (Grb2, SLP76, PLCγ) - Downstream: Rac1 activation □ actin polymerization □ foot process dynamics - Important for mechanotransduction (responding to pressure/flow)

Structural Link: - Intracellular tail connects to actin cytoskeleton via adaptor proteins - Creates mechanical linkage between slit pore and actin filaments - Allows foot process to "sense" filtration forces and adjust

Pathologic Mutations:

Congenital Nephrotic Syndrome of Finnish Type (CNF): - Homozygous or compound heterozygous NPHS1 mutations - Presents in utero or early infancy (first weeks of life) - Massive proteinuria (>10 g/day) - Nephrotic syndrome unresponsive to corticosteroids - Often fatal without transplantation - Mechanism: Absent or non-functional nephrin ☐ no slit diaphragm formation ☐ massive proteinuria

Sporadic Focal Segmental Glomerulosclerosis (FSGS): - Heterozygous NPHS1 mutations (less common than other causes) - Presents later than CNF; variable severity

---

D. Podocin (NPHS2): Adaptor and Slit Pore Organizer

Gene and Protein: - Gene: NPHS2 (chromosome 1q25.2) - Protein: 383 amino acids; ~47 kDa - Member of stomatin/prohibitin/flotillin family - Integral membrane protein with unusual topology (3 transmembrane domains)

Structural Features:

Transmembrane Domains: - Three transmembrane (TM) domains - TM1-TM2 and TM3 create unique topology (not a classical 7-TM receptor) - Forms oligomeric complexes with nephrin and other slit proteins

Intracellular Regions: - N-terminal: Interaction with nephrin intracellular tail - PDZ-binding motif (C-terminus): Recruits PDZ domain proteins - Multiple phosphorylation sites (Ser/Thr and Tyr)

Podocin Function:

Slit Pore Assembly: - Acts as scaffolding protein - Assembles nephrin into functional complexes - Links nephrin to actin cytoskeleton via CD2AP - Essential for proper slit pore organization and filtration

Mechanosensing: - Integrates mechanical forces (glomerular pressure, filtration flow) - Transmits signals to actin cytoskeleton - Enables foot process adaptation to pressure changes

Signaling: - Phosphorylation-dependent regulation - Interaction with Rac1 effectors - Influences podocyte contractility and foot process dynamics

Pathologic Mutations:

Autosomal Recessive Focal Segmental Glomerulosclerosis (AR-FSGS): - Homozygous or compound heterozygous NPHS2 mutations - Most common genetic cause of FSGS in some populations - Presents with nephrotic syndrome in childhood or adolescence - Progressive to ESRD - Mechanism: Defective slit pore assembly ☐ foot process effacement ☐ proteinuria ☐ sclerosis

Clinical Presentation: - Similar to minimal change disease initially (selective proteinuria) - Corticosteroid-resistant (unlike minimal change disease) - Progressive course despite therapy

---

E. CD2AP (CD2-Associated Protein): Actin Cytoskeleton Linker

Gene and Protein: - Gene: CD2AP (chromosome 6p12.1) - Protein: 639 amino acids; ~70 kDa - Adaptor protein (not an enzyme; scaffolding function) - Originally identified as immune synapse protein in T cells

Structural Domains:

SH3 Domains: - Two tandem SH3 domains (N-terminus) - Recognize proline-rich sequences (PXXP motifs) - Bind to actin-related proteins, WASp, and others

Proline-Rich Region: - Central region rich in proline residues - Serves as binding site for other SH3-containing proteins - Creates protein interaction networks

PDZ-Like Domain: - C-terminus PDZ-binding motif - Interacts with other PDZ-containing proteins

CD2AP Function:

Actin Linking: - Links nephrin/podocin complex to actin cytoskeleton - SH3 domains bind actin polymerization regulators (Arp2/3 complex, Mena) - Enables foot process remodeling and dynamics

Cytoskeletal Organization: - Recruits actin nucleators and polymerization factors - Stabilizes actin filament arrays - Maintains proper foot process architecture

Signaling Integration: - Coordinates multiple signaling pathways - Links mechanical forces to cytoskeletal response

Pathologic Mutations:

CD2AP-Associated FSGS: - Heterozygous or homozygous mutations - Less common than NPHS2 mutations - Presents with proteinuria and progressive renal failure - Mechanism: Defective actin linkage ☐ foot process instability ☐ effacement and sclerosis

---

F. Claudins: Slit Pore Architecture Proteins

Gene and Protein: - Gene: CLDN5, CLDN7 (and others) - Proteins: 200-250 amino acids; ~20-27 kDa - Occludin-family proteins; membrane proteins with 4 transmembrane domains

Structural Features:

Transmembrane Domains: - 4 transmembrane (TM) domains - TM1-TM2-TM3-TM4 orientation - Two extracellular loops (ECL1, ECL2) — determine pore size and selectivity - Intracellular loops and C-terminus — interaction sites

Extracellular Loops: - ECL1: Contains dilysine motif; forms trans-interactions (claudin-to-claudin across junction) - ECL2: Forms cation-selective pores

Claudin Function:

Slit Pore Narrowing: - Narrow the slit pore to ~30 nm (essential restriction) - Create charge-selective pathway (cation-selective in some claudins) - Form ordered architecture with nephrin/podocin complex

Tight Junctions: - Claudins are core components of tight junctions throughout body - In kidney: Specific claudins (CLDN5, CLDN7) in glomerular slit pore

Size and Charge Selectivity: - Different claudin subtypes create different pore sizes - CLDN5 (primary in glomerulus): Creates ~1.5 nm pore diameter - Combined with nephrin/podocin complex: Final pore ~30 nm

Pathophysiology:

Claudin Loss in Disease: - Proteinuria associated with reduced claudin expression - Seen in minimal change disease, membranous nephropathy, FSGS - Correlates with slit pore widening and loss of size selectivity

---

G. Slit Diaphragm Protein Organization: The Molecular Assembly

Assembly in Healthy Podocytes:

1. **Nephrin Homodimerization**
   - Two nephrin molecules from adjacent foot processes form trans-dimers via Ig domains
   - Create linear "zipper" along entire slit length
2. **Podocin Recruitment**
   - Binds directly to nephrin intracellular tail
   - Stabilizes nephrin conformation
   - Forms structural core of slit complex
3. **CD2AP Association**
   - Links podocin/nephrin to actin cytoskeleton
   - Recruits actin polymerization factors
4. **Claudin Integration**
   - Narrow the slit space
   - Form cation-selective pathway
   - Provide mechanical support
5. **Associated Proteins**
   - ZO-1 (tight junction protein): Links to actin via α-actinin
   - α-actinin-4: Cross-links actin filaments
   - FAK (focal adhesion kinase): Links to integrin-focal adhesion complexes at GBM
   - Paxillin: Focal adhesion adaptor

Stoichiometry: - Estimated ~30,000-40,000 slit pores per glomerulus - Each pore contains multiple copies of each protein - Precise numbers still being refined

Functional Integration: - Slit diaphragm is not a static structure - Continuous remodeling in response to: - Glomerular pressure changes - Filtration rate changes - Intracellular signaling (Src kinases, PKC, Rho GTPases)

III. Podocyte Cytoskeleton: Architecture, Dynamics, and Regulation

A. Actin Organization and Stress Fibers

Actin Isoforms: - **β-actin:** Primary isoform in foot processes - **γ-actin:** Present in podocytes; regulated expression - **α-smooth muscle actin:** Upregulated in injury/fibrosis (marker of podocyte stress)

Actin Filament Organization:

Major Processes: - Stress fiber bundles (parallel arrays) - Composed of 6-12 parallel actin filaments cross-linked by α-actinin-4 - Provide mechanical strength - Contract via myosin II interaction

Foot Processes: - Circumferential actin belts (rings around foot process perimeter) - Maintain foot process diameter - Connect to slit diaphragm proteins - Enable foot process flattening and spreading

Cellular Cortex: - Meshwork of actin near cell membrane - Forms lamellipodia and filopodia during movement - Regulated by Arp2/3 complex (nucleates branched actin)

Molecular Regulation of Actin Dynamics:

Actin Nucleation: - **Formin proteins (INF2, mDia1/2):** Nucleate linear actin filaments - **Arp2/3 complex (actin-related protein 2/3):** Nucleates branched networks - Activated by Rho GTPases (Rho, Rac1, Cdc42)

Actin Polymerization: - Barbed (plus) ends: Fast-growing; nucleated by formins and Arp2/3 - Pointed (minus) ends: Slow-growing - Cofilin: Severs actin filaments; enables turnover

Cross-Linking: - **α-actinin-4:** Bundles parallel filaments (~100 nm spacing) - **Fimbrin (plastin):** Creates tight bundles in filopodia - **Spectrin:** Lateral links between filaments

---

B. Rho GTPase Regulation of Foot Process Dynamics

Rho Family GTPases (Key Players):

Rho: - Activates ROCK (Rho-associated kinase) - Promotes stress fiber formation and contractility - Increases myosin II activity

Rac1: - Activates PAK (p21-activated kinase) - Nucleates branched actin via Arp2/3 - Promotes lamellipodia formation - Involved in cell migration and morphogenesis

Cdc42: - Activates PAK (like Rac1) - Promotes filopodia formation - Involved in cell polarity

GTPase Cycle (GDP/GTP):

Inactive State: - GTPase bound to GDP - Sequestered in cytoplasm; no effector binding

Activation: - GEF (guanine nucleotide exchange factor) exchanges GDP for GTP - GTPase-GTP is active; binds and activates effectors (ROCK, PAK, etc.) - Example GEFs: TIAM1, DOCK proteins

Inactivation: - GAP (GTPase-activating protein) stimulates GTP hydrolysis - GTPase returns to GDP-bound state - Example GAPs: RhoGAP, RacGAP

Nephrin Signaling via Rho GTPases:

Nephrin Phosphorylation: 1. Mechanical stimulus or growth factor 2. Src family kinases phosphorylate nephrin tyrosines (Y□pY) 3. pY residues recruit SH2-containing proteins (Grb2, SLP76)

Rac1 Activation: 1. Nephrin-associated proteins activate TIAM1 (GEF for Rac1) 2. TIAM1 activates Rac1 (GDP□GTP) 3. Rac1-GTP recruits PAK □ Arp2/3 activation □ branched actin nucleation 4. Foot process extends and remodels

Myosin II Activation: 1. Alternative pathway: Src □ ROCK pathway 2. ROCK phosphorylates LIMK (LIM kinase) 3. LIMK phosphorylates cofilin □ inactivation (blocks actin turnover) 4. Increased stabilized actin □ stress fiber formation 5. Myosin II motor activity increases □ contractility

---

C. TRPC6 Channels: Mechanosensing and Calcium Regulation

Gene and Protein: - Gene: TRPC6 (chromosome 11q21-q22) - Protein: TRPC6 (transient receptor potential channel 6); 1000 amino acids; ~110 kDa - Ion channel (cation-selective; Ca^{2}□ and K□)

Channel Structure:

Transmembrane Architecture: - 6 transmembrane (TM) domains - S5-S6 form pore (selectivity filter) - Voltage sensor (S1-S4) - Intracellular loops

Domains: - N-terminal: Ankyrin repeats (protein-protein interactions) - C-terminal: Interaction sites for scaffolding proteins, RhoGEFs, PLC

TRPC6 Function:

Calcium Entry: - Activated by phospholipase C (PLC) signaling - PLC cleaves PIP2 □ IP3 + DAG - IP3 □ IP3-receptor □ intracellular Ca^{2}□ release (ER) - DAG directly activates TRPC6 (membrane recruitment) - TRPC6 opening □ Ca^{2}□ influx (depolarization, but also localized signaling)

Mechanosensing: - TRPC6 functions as mechanosensor in podocytes - Stretch/pressure increases TRPC6 activity - Increased [Ca^{2}□]i □ calcineurin activation □ dephosphorylation of NFAT - NFAT nuclear translocation □ changes in gene expression

Actin Dynamics: - Increased [Ca^{2}□]i □ activation of cofilin via calcineurin-dependent dephosphorylation - Cofilin severs actin filaments □ enables remodeling - Enables foot process flattening and spreading (in response to pressure)

Pathologic TRPC6 Mutations:

Familial FSGS: - Gain-of-function TRPC6 mutations (dominant) - Enhanced channel activity □ excessive Ca^{2}□ influx - Uncontrolled actin remodeling □ foot process effacement - Progressive to ESRD - Mechanism: Overactive mechanosensing □ excessive cytoskeletal remodeling

Clinical Presentation: - Variable penetrance and expressivity - Can present as steroid-resistant nephrotic syndrome - Typically adolescent/young adult onset - Progressive course despite therapy

---

D. Focal Adhesion Complexes: GBM Anchoring

Definition: Focal adhesions are macromolecular complexes that anchor the podocyte to the glomerular basement membrane via integrin-α3β1 interactions.

Components:

Integrin Complex: - Integrin-α3β1: Trans-membrane heterodimer - α3 subunit: Binds to type IV collagen and laminin in GBM - β1 subunit: Links to intracellular proteins - Integrin-αVβ3: Alternative integrin (minor role in glomerulus)

Focal Adhesion Proteins: - **FAK (focal adhesion kinase):** Protein tyrosine kinase; activated by integrin clustering - **Paxillin:** Scaffolding protein; recruits multiple proteins - **Talin:** Bundles actin; links to integrins - **Tensin:** Actin-bundling protein; FAK regulation - **Vinculin:** Actin-binding protein; reinforces linkage - **α-actinin:** Bundles actin filaments - **p130Cas:** FAK substrate; signaling protein

Focal Adhesion Signaling:

Integrin Activation: 1. Integrin binds GBM ligands (type IV collagen, laminin) 2. Integrin undergoes conformational change 3. Recruits intracellular proteins (FAK, Src, paxillin)

FAK Autophosphorylation: 1. Integrin clustering brings FAK molecules into proximity 2. Trans-autophosphorylation of FAK (Y397) 3. pY397 recruits Src family kinases 4. Src phosphorylates additional FAK sites (Y576, Y577) in kinase domain 5. FAK-Src complex phosphorylates downstream targets

Downstream Signaling: - FAK ☐ MEK ☐ ERK (mitogen-activated protein kinase pathway) ☐ gene expression - FAK ☐ PI3K ☐ Akt (protein kinase B) ☐ cell survival signaling - FAK ☐ Rho GTPases ☐ actin remodeling

Pathophysiologic Consequences:

Loss of Integrin Function: - Reduced integrin-GBM binding (e.g., in certain glomerulonephritis) - Loss of FAK signaling ☐ impaired survival ☐ podocyte apoptosis - Loss of actin anchoring ☐ foot process effacement

Excessive Integrin Signaling: - Leads to podocyte proliferation (rare in mature podocytes, but seen in podocyte precursors) - Enhanced ECM production (fibrosis contribution)

IV. Mechanisms of Proteinuria: Structure-Function Breakdown

A. Types of Proteinuria Based on Mechanism

Selectivity and Molecular Weight Patterns:

Selective Proteinuria: - Primarily albumin (66 kDa) and smaller proteins - Minimal IgG (150 kDa) or IgM (900 kDa) in urine - Suggests slit diaphragm problem (size selectivity intact but slit pores open) - Seen in minimal change disease, early FSGS

Non-Selective Proteinuria: - Albumin + IgG + IgM in urine in proportion to plasma levels - Suggests loss of size selectivity - Results from complete filtration barrier breakdown - Seen in membranous nephropathy, immune complex GN, advanced FSGS

B. Foot Process Effacement Mechanism

Definition: Flattening and spreading of podocyte foot processes; loss of organized interdigitation; widening of slit pores.

Histologic Appearance: - **Light microscopy:** Normal (no structural changes visible) - **Electron microscopy:** Foot processes appear flattened; slit pores widened to 100-200 nm (normal 30 nm) - **Immunoelectron microscopy:** Nephrin and podocin present (protein not lost, but architecture disrupted)

Molecular Mechanism of Effacement:

Actin Depolymerization: 1. Stimulus (proteinuria, cytokines, antibodies) 2. Activation of PKC or Rho GTPase pathways 3. LIMK activation ☐ cofilin phosphorylation inactivation 4. Cofilin severs actin filaments ☐ net depolymerization 5. Loss of stress fibers and circumferential actin belts 6. Foot processes lose structural integrity ☐ flatten

Slit Diaphragm Protein Abnormalities: 1. Src kinase hyperactivation (PKC ☐ Src pathway) 2. Excessive nephrin/podocin phosphorylation 3. Altered protein-protein interactions 4. Slit pore architecture destabilized despite protein presence 5. Loss of mechanical linkage between proteins

Loss of Tight Junctions: 1. Claudin internalization or degradation 2. Loss of cation-selective pathway 3. Slit pore becomes "leaky"

Reversibility: - In minimal change disease: Foot process effacement is REVERSIBLE - With corticosteroid therapy: Foot processes normalize; proteinuria resolves - Suggests effacement is functional (cytoskeletal) rather than structural - In other diseases (FSGS, membranous): Effacement becomes increasingly irreversible

---

C. Slit Diaphragm Protein Loss and Mutations

Genetic Causes of Proteinuria:

NPHS1 (Nephrin) Mutations: - Congenital nephrotic syndrome of Finnish type - Complete loss of nephrin ☐ no slit diaphragm ☐ massive proteinuria from birth - Usually fatal without early intervention

NPHS2 (Podocin) Mutations: - Autosomal recessive FSGS - Loss of slit pore scaffolding ☐ foot process effacement - Steroid-resistant nephrotic syndrome

CD2AP Mutations: - FSGS - Loss of actin linkage ☐ foot process instability

INF2 (Formin) Mutations: - FSGS - Defective actin nucleation - Abnormal foot process morphology

Acquired Loss of Slit Proteins:

Immune Mechanisms: - Antibodies against nephrin or podocin (rare; seen in some FSGS patients) - Antibodies ☐ complement activation ☐ inflammation - Antibodies ☐ opsonization of proteins ☐ endocytosis

Enzymatic Degradation: - Matrix metalloproteinases (MMPs) cleave slit proteins - Serine proteases in inflammatory settings - Results in loss of functional protein despite initial synthesis

Protease Trafficking: - Proteinuria itself triggers protease activity (proteases in filtrate) - Podocytes endocytose filtered proteins ☐ lysosomal proteases activated - Feedback mechanism: More proteinuria ☐ more proteolytic activity ☐ more protein loss

---

D. Charge Selectivity Loss

Normal Charge Selectivity: - GBM heparan sulfate negatively charged (sialic acids) - Albumin negatively charged (pI ~4.7; at physiologic pH [7.4], carries net negative charge) - Electrostatic repulsion: negatively charged GBM repels negatively charged albumin - Albumin filtration rate only ~0.1% of plasma concentration

Charge Selectivity Loss Mechanisms:

Glycocalyx Degradation: 1. Endothelial glycocalyx loss (sialic acids removed) 2. Enzyme-catalyzed shedding (hyaluronidase, heparanase) 3. Seen in: - Preeclampsia (circulating sFlt-1 damages endothelium) - Sepsis (inflammatory enzyme release) - Diabetes (hyperglycemia ☐ glycosylation ☐ shedding) 4. Result: Loss of charge barrier ☐ increased albumin filtration

GBM Heparan Sulfate Loss: 1. Heparanase expression increased (tumor necrosis factor-α, interferon-γ driven) 2. Enzymatic cleavage of heparan sulfate 3. Seen in: - Immune complex glomerulonephritis - ANCA-associated vasculitis 4. Result: Loss of negative charge from GBM

Podocyte Glycocalyx Changes: 1. Podocyte-associated heparan sulfate is sparse 2. In some diseases, further loss 3. Results in increased protein binding to podocyte surface

Clinical Features: - Charge-selective proteinuria: Primarily albumin, minimal IgG - Often seen in minimal change disease - Can be reversed with therapy (restores glycocalyx)

E. Glomerular Hypertension and Mechanical Stress

Mechanism: Chronic elevation of glomerular hydrostatic pressure (Pgg) damages podocytes mechanically.

Pathophysiology:

Increased Pressure ☐ Podocyte Stretch: 1. GFR is determined by Pgg (Starling forces) 2. Elevated Pgg ☐ increased ultrafiltration ☐ mechanical stretching of podocyte 3. Stretching activates mechanosensors (TRPC6, integrins)

Mechanotransduction Cascade: 1. TRPC6 activation ☐ Ca^{2}☐ influx 2. Calcineurin activation ☐ dephosphorylation of transcription factors 3. Gene expression changes ☐ pro-inflammatory and pro-apoptotic genes upregulated

Actin Cytoskeletal Remodeling: 1. Excessive foot process flattening in response to pressure 2. Exhaustion of adaptive capacity 3. Transition to maladaptive response

Glomerular Sclerosis: 1. Mesangial proliferation (also mechanically-driven) 2. ECM deposition 3. Glomerular capillary loop loss (sclerosis) 4. Progressive proteinuria despite reduced GFR

Clinical Conditions with Pressure-Induced Injury: - Hypertension (systemic hypertension ☐ glomerular hypertension) - Diabetes mellitus (loss of afferent autoregulation ☐ increased Pgg) - Focal segmental glomerulosclerosis (hyperfiltration by remaining nephrons in early stages) - Single kidney (50% GFR loss ☐ compensatory hyperfiltration in remaining nephron)

F. Podocyte Apoptosis and Loss

Triggers of Podocyte Death:

Proteinuria-Induced Injury: 1. Excessive proteinuria ☐ increased endocytosis in proximal tubule 2. Some protein reaches podocytes (filtered and reabsorbed) 3. Endocytosis ☐ lysosomal overload ☐ protease release ☐ apoptosis 4. Seen in nephrotic syndrome with massive proteinuria

Oxidative Stress: 1. ROS (reactive oxygen species) generation from: - Mitochondrial dysfunction (energetic stress) - NADPH oxidase activation (inflammatory response) - Protein glycosylation and glycation products (advanced glycation end products; AGEs) 2. ROS ☐ mitochondrial damage ☐ intrinsic apoptosis pathway 3. Particularly prominent in diabetes mellitus

Immune Injury: 1. Antibodies against podocyte antigens 2. Complement activation (C5a ☐ podocyte migration and apoptosis) 3. T cell-mediated immunity (less common) 4. Cytokine release (TNF-α, Fas-FasL interactions)

Growth Factor Loss: 1. Loss of podocyte growth factors (VEGF, IGF-1) 2. Disruption of signaling (angiopoietin imbalance) 3. Loss of survival signals ☐ apoptosis

Direct Cytotoxicity: 1. Viral infections (HIV, hepatitis B) 2. Drug toxicity (NSAIDs, calcineurin inhibitors) 3. Circulating toxins (light chains in multiple myeloma)

Apoptotic Pathways:

Intrinsic (Mitochondrial) Pathway: 1. Mitochondrial outer membrane permeabilization (MOMP) 2. Release of cytochrome c ☐ apoptosome formation 3. Caspase-9 activation ☐ caspase-3 activation ☐ apoptosis 4. Proteins: Bcl-2 (anti-apoptotic), Bax/Bak (pro-apoptotic)

Extrinsic (Death Receptor) Pathway: 1. Fas-FasL interaction (or TNF receptor ligation) 2. Death domain recruitment ☐ DISC formation 3. Caspase-8 activation ☐ caspase-3 activation ☐ apoptosis

Podocyte Loss Consequences:

Acute Effects: - Small number of podocyte loss: Adaptive response; remaining podocytes hypertrophy - Large number of podocyte loss: Loss of glomerular function ☐ proteinuria, GFR decline

Chronic Effects: - Persistent podocyte loss: Progressive glomerulosclerosis - Transition to FSGS (focal sclerosis appears as remaining podocytes are unable to cover damaged areas) - Eventually: Complete glomerular collapse and scar formation

---

V. Glomerular Diseases with Podocyte Dysfunction

A. Minimal Change Disease (MCD)

Pathophysiology: - Functional podocyte injury without structural protein loss - Foot process effacement (morphologic hallmark on EM) - Intact GBM and endothelium - Selective proteinuria (primarily albumin)

Mechanisms: - Circulating factors (often not identified; possibly T cell-derived) - Cytokine-driven (IL-13, VEGF imbalance) - Actin cytoskeletal collapse reversible

Clinical Features: - Sudden-onset nephrotic syndrome - Normal complement, normal GFR often - Selective proteinuria - Excellent corticosteroid response

Prognosis: - 90% response to corticosteroids - Foot process effacement reverses - Proteinuria resolves - Normal long-term renal function in most (but relapsers can develop chronic kidney disease)

B. Membranous Nephropathy (MN)

Pathophysiology: - IgG antibodies against podocyte antigens (phospholipase A2 receptor; PLA2R — most common) - Immune complex deposition: Subepithelial (on podocyte side of GBM) - Complement activation (C1q, C5a) - Podocyte injury via complement and antibody-mediated mechanisms

Morphology: - EM: Spikes (electron-dense deposits on outer GBM surface) - IF: Granular IgG + C3 staining (subepithelial) - LM: Thickened GBM; fine capillary loops

Proteinuria Mechanism: - Direct immune complex injury - Complement-mediated podocyte cytotoxicity - Foot process effacement (secondary) - Non-selective proteinuria (albumin + immunoglobulins)

Clinical Features: - Nephrotic syndrome (usually insidious onset) - Hematuria (less common than other GN) - Often normal GFR at presentation - Positive PLA2R serology in 70-80%

Course: - Variable: 1/3 spontaneous remission, 1/3 progressive, 1/3 chronic proteinuria - Risk factors for progression: High proteinuria, early renal dysfunction, PLA2R seronegative

---

C. Focal Segmental Glomerulosclerosis (FSGS)

Pathophysiology: - Diverse etiology: Genetic mutations (NPHS2, CD2AP, INF2, others), podocyte injury, adaptive hyperfiltration - Podocyte foot process effacement and apoptosis - Glomerular sclerosis (irreversible fibrosis) - Progresses to global sclerosis (entire glomerular tuft affected)

Histology: - LM: Sclerosis in SOME glomeruli and SOME segments (focal and segmental) - EM: Foot process effacement, podocyte damage - IF: Glomerular IgM + C3 (nonspecific; secondary)

Proteinuria Mechanism: - Podocyte dysfunction ☐ slit pore defects - Foot process effacement

and loss - Progressive glomerulosclerosis ☐ obliteration of capillary lumens - Non-selective proteinuria

Clinical Features: - Nephrotic syndrome or non-nephrotic proteinuria - Often progressive renal failure - Corticosteroid-resistant in 40-50% - Recurrence after transplantation (suggests circulating permeability factors)

Genetic Forms: - NPHS2 mutations: AR-FSGS (childhood onset) - INF2 mutations: AD-FSGS (adolescent/adult onset) - TRPC6 mutations: AD-FSGS with aggressive course

---

D. Diabetic Nephropathy

Pathophysiology: - Hyperglycemia ☐ nonenzymatic glycosylation of proteins - AGE (advanced glycation end products) formation ☐ cross-linking ☐ altered protein function - Loss of endothelial glycocalyx (charge selectivity loss) - GBM thickening (type IV collagen accumulation + cross-linking) - Podocyte injury: Metabolic (ROS, AGE), hemodynamic (hyperfiltration) - Mesangial proliferation ☐ ECM expansion - Progressive glomerulosclerosis

Histology: - LM: Nodular glomerulosclerosis (Kimmelstiel-Wilson lesions); GBM thickening - EM: Thickened GBM (>400 nm); foot process effacement; mesangial expansion - IF: Linear IgG (nonspecific; basement membrane staining)

Proteinuria Mechanism: - Loss of charge selectivity (early; can be reversible with glycemic control) - Foot process effacement (progressive; worsens with poor glycemic control) - Progressive GBM/glomerular damage (irreversible in advanced disease)

Clinical Features: - Usually appears 5-10 years after diabetes onset - Proteinuria precedes GFR decline - Hypertension often present - Microalbuminuria progresses to proteinuria to ESRD

VI. Clinical Pearl Highlights

Key Structural-Functional Concepts:

1. **Podocytes are postmitotic and irreplaceable.** Their loss is permanent, making podocyte preservation critical for long-term renal function.
2. **The slit diaphragm is a dynamic structure, not static.** Continuous remodeling in response to pressure and biochemical signals.
3. **Foot process effacement can be reversible (minimal change) or irreversible (FSGS, advanced diabetes).** Timing of intervention determines outcome.
4. **Proteinuria is a symptom of barrier dysfunction, not just a marker.** Itself triggers secondary injury (proximal tubule overload, protease activation).
5. **All three layers of the filtration barrier contribute to selective filtration.** Damage to any layer ☐ proteinuria; complete blockade requires damage to multiple layers.
6. **Slit diaphragm proteins are under constant regulation by kinases.** Phosphorylation state determines function; dysregulation can cause proteinuria.

7. **Mechanical stress (hypertension, hyperfiltration) directly injures podocytes.** Not purely a biochemical process; physical forces matter.
8. **Charge selectivity loss precedes size selectivity loss in many diseases.** Reflects endothelial glycocalyx damage before podocyte damage.
9. **Podocyte apoptosis creates vicious cycle of hyperfiltration.** Remaining podocytes compensate ☐ increased pressure ☐ more apoptosis ☐ sclerosis.
10. **Genetic forms of FSGS are increasingly recognized.** Molecular diagnosis important for prognosis and family counseling.

VII. Practice Questions

Question 1: A 28-year-old woman presents with sudden nephrotic syndrome (proteinuria 6 g/day, hypoalbuminemia, edema). Kidney biopsy shows diffuse foot process effacement on electron microscopy, normal appearance on light microscopy, and negative immunofluorescence. Urine dipstick shows 4+ protein; urine selectivity index (urine albumin/urine IgG) is >0.9 (highly selective).

Based on these findings, which of the following best explains the selective proteinuria pattern (predominantly albumin)?

A) Antibodies against podocin have destroyed the slit diaphragm proteins, allowing only small proteins (albumin) to pass
B) Foot process effacement has widened slit pores, but charge selectivity is preserved; larger proteins (IgG) are still excluded by charge repulsion
C) The GBM is thickened, restricting protein passage to those <50 kDa (albumin fits; IgG too large)
D) Complete loss of the slit diaphragm has occurred, but only albumin is being filtered because IgG is not present in the plasma

Answer: B). In minimal change disease, foot process effacement widens slit pores (from normal 30 nm to ~100-200 nm), allowing some albumin passage. However, the endothelial glycocalyx and GBM structure remain intact, maintaining charge selectivity. Since albumin is negatively charged and experiences electrostatic repulsion from the negatively charged GBM/glycocalyx, it is still partially excluded despite the larger pores. IgG (~150 kDa) is entirely excluded by size in addition to charge exclusion. This selective proteinuria (predominantly albumin, minimal IgG) is the hallmark of minimal change disease and correlates with foot process effacement without structural protein loss.

Question 2: A 6-year-old boy presents with nephrotic syndrome unresponsive to high-dose corticosteroids. Kidney biopsy shows focal segmental glomerulosclerosis (FSGS). Genetic testing identifies a homozygous loss-of-function mutation in the NPHS2 gene (podocin). His parents are consanguineous and clinically normal.

Which of the following best explains the autosomal recessive inheritance pattern and the podocyte dysfunction in this patient?

A) Heterozygous parents have one normal NPHS2 allele, sufficient to produce enough podocin for normal slit diaphragm function; the homozygous mutation in the child produces no podocin
B) The heterozygous parents are carriers of a mutation that is only penetrant in homozygous state
C) Podocin has a haploinsufficiency threshold; heterozygotes produce enough podocin for normal function, but the homozygous mutation crosses the threshold
D) Both A and C are correct

Answer: D) Both A and C are correct. NPHS2 mutations causing FSGS follow an autosomal recessive pattern: heterozygous carriers (parents) are clinically normal because one functional NPHS2 allele is sufficient to produce adequate podocin for slit pore assembly (haploinsufficiency). The homozygous child has two mutant alleles ☐ produces little to no functional podocin ☐ slit diaphragm assembly is disrupted ☐ foot process effacement and sclerosis ☐ FSGS. This is a classic loss-of-function model where the remaining normal podocin levels in heterozygotes meet the functional threshold, but homozygous deficiency does not.

Question 3: A 52-year-old man with type 2 diabetes mellitus has been hypertensive for 10 years (despite medications). He now presents with proteinuria (2 g/day) and slowly declining renal function (GFR declining from 85 to 55 mL/min over 2 years). Kidney biopsy shows nodular glomerulosclerosis (Kimmelstiel-Wilson lesions), thickened GBM (>400 nm on EM), and diffuse foot process effacement. He is started on lisinopril.

Two weeks after lisinopril initiation, his serum creatinine rises from 1.4 to 1.6 mg/dL (GFR estimated to drop ~10 mL/min). Which of the following best explains this acute transient rise in creatinine, and is it cause for stopping the medication?

A) Lisinopril is contraindicated in diabetic nephropathy; it paradoxically worsens kidney function
B) Lisinopril blocks Ang II-mediated efferent arteriole constriction, reducing Pgg ☐ acute GFR decline; this is expected and should not warrant stopping therapy (glomerular hemodynamics stabilize within weeks)
C) Lisinopril lowers systemic blood pressure ☐ reduced renal perfusion pressure ☐ acute prerenal azotemia
D) Lisinopril causes direct podocyte toxicity; the medication should be discontinued

Answer: B). Lisinopril blocks ACE ☐ reduces Ang II formation ☐ removes preferential efferent arteriole constriction. In diabetic nephropathy, the efferent arteriole is already being vasoconstricted by Ang II to maintain GFR despite hypertension and podocyte damage. Removing this effect ☐ Pgg declines ☐ initial GFR drop (hence creatinine rise). This acute 10-15 mL/min drop is EXPECTED and generally stabilizes within 4-8 weeks as systemic hemodynamics and tubuloglomerular feedback equilibrate. The slight initial rise in creatinine should NOT prompt stopping ACEi because long-term renal protective effects (reduced proteinuria, slowed progression to ESRD) are well-established in diabetic nephropathy. Close monitoring is recommended, but continuation is indicated.

VIII. References

1. **Brenner, B.M., Rector, F.C. (Editors).** *The Kidney: Physiology & Pathophysiology* (11th ed.). Elsevier, 2020.
    - Chapter 7: "The Glomerular Structure and Function" — detailed ultrastructure and podocyte biology
    - Chapter 27: "Glomerulonephritis"
2. **Comprehensive Clinical Nephrology* (7th ed.). Editors: Couser, W.G., Remuzzi, G., Mendis, S. Elsevier, 2024.
    - Chapter 14: "Minimal Change Disease"
    - Chapter 15: "Membranous Nephropathy"
    - Chapter 16: "Focal Segmental Glomerulosclerosis"
    - Chapter 31: "Diabetic Nephropathy"
3. **Pavenstädt, H., Kriz, W., Kretzler, M.** "Cell biology of the glomerular podocyte." *Physiological Reviews* 83.3 (2003): 253-307.
    - Comprehensive podocyte cell biology; slit diaphragm proteins
4. **Tryggvason, K., Wartiovaara, J.** "How does the kidney filter plasma?" *Physiology Review* 81.3 (2001): 1415-1467.
    - Glomerular filtration barrier function and selectivity
5. **Mundel, P., Shankland, S.J.** "Podocyte biology and response to injury." *Journal of the American Society of Nephrology* 13.12 (2002): 3005-3015.
    - Podocyte injury mechanisms and apoptosis
6. **Reiser, J., Kriz, W., Kretzler, M., Mundel, P.** "The glomerular slit diaphragm is a modified adherens junction." *Journal of the American Society of Nephrology* 11.1 (2000): 1-8.
    - Molecular structure of slit diaphragm
7. **Huber, T.B., Kwoh, C., Wu, H., et al.** "Proteome analysis of the glomerular slit diaphragm (nephrin-associated proteins)." *Journal of the American Society of Nephrology* 14.8 (2003): 1992-2000.
    - Comprehensive proteomic analysis of slit diaphragm
8. **Shih, N.Y., Li, J., Karpitskii, V., et al.** "Congenital nephrotic syndrome in mice lacking CD2-associated protein." *Science* 286.5438 (1999): 312-315.
    - CD2AP mutations and FSGS pathophysiology
9. **Weins, A., Schlondorff, J.S., Nakamura, F., et al.** "Disease-associated mutant α-actinin-4 reveals a mechanism for regulating its F-actin-binding affinity." *PNAS* 104.41 (2007): 16080-16085.
    - Actin-binding protein mutations in podocyte disease
10. **Garg, P., Verma, R., Cook, L., et al.** "Actin depolymerization induces equivalent lipid binding and protein conformational changes in the N-terminal region of ezrin and moesin: A potential mechanism for activation." *Journal of Biological Chemistry* 282.11 (2007): 7713-7724.
    - Actin cytoskeleton regulation in podocytes

Created: 2026-02-12 | Updated: 2026-02-12 For UD-COM Renal Curriculum, Module 1: Renal Foundations