Focal Segmental Glomerulosclerosis (FSGS)

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DISCLOSURES:

The speaker has the following potential conflicts

- TerumoBCT, Inc. – Honoraria, Consulting
- Therakos, Inc. – Honoraria
- Alexion Pharmaceuticals – Advisory Board
- Aethlon Medical Inc. – Consulting

Institutional support in the form of unrestricted educational grants from TerumoBCT, Alexion, Fresenius-Kabi (formerly Fenwal), Therakos.
Therapeutic Plasmapheresis

Aminco Celltrifuge, Glasgow, circa 1973

IBM 2997 centrifuge, UCSD, 1982

Membrane plasmafiltration, UCSD, 1984
UC San Diego Therapeutic Apheresis Program

Plasma exchange (TPE/PLEX) - 1982-1988

RBC exchange (RBCX-A)

WBC / platelet depletion

Research

Membrane TPE (mTPE/mPLEX) - started 1983
UC San Diego Therapeutic Apheresis Program


RBC exchange (RBCX-A) started 2014

WBC / platelet depletion

Research

IBM 2997 Cobe Spectra

Stem cell harvest (HPC-A) started 1989 started 2012

Membrane TPE (mTPE/mPLEX) 1983-2004

Photopheresis (ECP) started 2003

LDL-apheresis (LDL-A) started 2011 started 2012

Terumo Optia

Kaneka Liposorber
Number of procedures per year by modality

- **Plasmapheresis (TPE) - outpatient**
- **Plasmapheresis (TPE) - inpatient**
- **Stem cell harvest (HPC-A)**
- **Photopheresis (ECP)**
- **Cytapheresis (WBC, Plt., RBCX)**
- **LDL-apheresis (since March 2012)**
- **Research**

(Academic years run from July 1st to June 30th)
SAVE THE DATE
March 3-5, 2016
2½ day conference. Co-directors: David M. Ward MD
Amber P. Sanchez MD
Isagani I. Marquez, Jr, BSN RN
cme.ucsd.edu/apheresis

Apheresis Physicians’ College at UCSD
5-day immersion in the Apheresis Unit, with mentorship by experts.
Round on 70+ procedures; one-on-one discussions; lectures and workshops.
Limited to 3-4 participants. Offered 4 times per year.
Contact dmward@ucsd.edu
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| Faculty: | David M. Ward, MD | Amber P. Sanchez, MD | Nadine Benador, MD | Isagani I. Marquez, Jr., RN, BSN (“Jhun”) |

(Instructor is David except where shown otherwise)

<table>
<thead>
<tr>
<th>Monday 14th</th>
<th>Tuesday 15th</th>
<th>Wednesday 16th</th>
<th>Thursday 17th</th>
<th>Friday 18th</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:30 - 9:30</td>
<td>Badges (Teri), Introductions &amp; Orientation. Pre-test</td>
<td>Classroom – Photopheresis (ECP); Case discussions</td>
<td>Classroom – Medical Director duties &amp; qualifications. Program management and QA.</td>
<td>Classroom – Case discussion session. Remaining issues.</td>
</tr>
<tr>
<td>9:45 - 11:15</td>
<td>Patient rounds – TPE, ECP</td>
<td>Patient rounds – TPE, ECP, LDL</td>
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<td>Patient rounds (Amber) - TPE, ECP, RBCX</td>
</tr>
<tr>
<td>12:15</td>
<td>Lunch break</td>
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<tr>
<td>1:00 - 2:00</td>
<td>Classroom – Anticoagulation, WBC-depletion, RBC-exchange.</td>
<td>Classroom – Adverse events (Amber)</td>
<td>Classroom – LDL-apheresis (Amber)</td>
<td>Weekly Apheresis Patient Care Meeting</td>
</tr>
<tr>
<td>2:00 - 3:30</td>
<td>Patient rounds – TPE, ECP</td>
<td>Patient rounds (Amber) TPE, ECP</td>
<td>Patient rounds – TPE, ECP, RBCX</td>
<td>1:00: Lunch break. 1:30: “Doc Talk” weekly case review with faculty and fellows.</td>
</tr>
<tr>
<td>3:45 - 4:45</td>
<td>Classroom – Case analysis. Case discussion</td>
<td>Machine hands-on and Q&amp;A (Jhun)</td>
<td>RBCX-apheresis hands-on demonstration (Jhun)</td>
<td>2:30: Patient rounds – TPE, ECP, citrate ECP</td>
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OUTLINE:

1. Seminal cases.
2. Glomerular anatomy and pathology.
3. FSGS is a group of diseases with different etiologies.
4. The molecular machinery that regulates podocyte morphology.
5. Candidate glomerular permeability factors that may cause the recurrent type of FSGS.
6. Indications for TPE treatment for FSGS.
7. Use of Immunoadsorption, DFPP, etc.
8. Use of dextran-sulfate plasma adsorption (LDL-apheresis).
FSGS (Focal Segmental Glomerulosclerosis)

Case reports (3) published 1972:

RECURRENT OF IDIOPATHIC
NEPHROTIC SYNDROME AFTER
RENA L TRANSPLANTATION

JOHN R. HOYER
ROBERT L. VERNIER
JOHN S. NAJARIAN

LEOPOLDO RAJ
RICHARD L. SIMMONS
ALFRED F. MICHAEL

Departments of Pediatrics, Internal Medicine, and Surgery,
University of Minnesota Medical School,
Minneapolis, Minnesota 55455, U.S.A.

Summary

Three patients with steroid-resistant idiopathic nephrotic syndrome were studied at onset and during recurrent nephrotic syndrome after renal transplantation. Renal biopsies at the onset of the nephrotic syndrome showed typical

urine does not clear of protein and these patients progress to renal failure. We have studied four such patients at the onset of their disease and after renal transplantation. The nephrotic syndrome recurred in three of them shortly after renal transplantation.

Case-reports

FIRST CASE

This boy developed intermittent periorbital oedema at 7½ years of age. 6 months later the nephrotic syndrome was diagnosed (fig. 1). Prednisone 80 mg. per day for 21 days did not decrease proteinuria. 6 weeks later anasarca was present and laboratory studies demonstrated a nephrotic syndrome (table 1). 7 months later, laboratory studies were unchanged and prednisone 60 mg. per day was given for 20 days without decrease in proteinuria. 10 months later, when renal function was decreasing, azathioprine ('Imu-

FSGS (Focal Segmental Glomerulosclerosis)

Case report published 2012:

- 27 year old man, ESRD due to primary FSGS.
- Kidney transplant from sister.
- Day 2: recurrence of nephrotic syndrome (heavy proteinuria).
- Day 6: Biopsy - recurrence of FSGS (podocyte foot-process fusion).
- Rapid loss of renal function, severe depletion of serum albumin.
- Day 14: Kidney removed and re-transplanted into a 66 year old man with ESRD (diabetic nephropathy).
- Immediate graft function with rapid reduction of proteinuria.
- Biopsies at day 8 & day 25 after re-transplantation
  - glomerular lesions returning to normal.

Kidney

- Renal pyramid (renal medulla)
- Renal cortex
- Renal papilla
- Renal sinus
- Hilum
- Major calyx
- Minor calyx
- Renal column of Bertin
- Renal artery
- Renal vein
- Renal pelvis
- Ureter

Nephron

- Glomerulus
- Efferent arteriole
- Afferent arteriole
- Tubule
Normal Glomeruli

Focal = some glomeruli not affected
Segmental = some parts not affected
FSGS (Focal Segmental Glomerulosclerosis)

Plasma exchange (TPE) for recurrent FSGS:

- Post-transplant recurrence of FSGS known for >40 years (1).
- Recurs post-transplant in ~ 23% of adults with primary FSGS (3).
- Recurrence rates higher in children.
- Recurrence rates higher if previous transplant loss to recurrence.
- Successful treatment of recurrence by TPE is well established (4-11).
- Opinion is in favor of TPE pre-transplant for severe cases.

(7) Zimmerman SW: Nephron 40:241-245, 1985
Clinical features:
- Proteinuria, microscopic hematuria, hypertension.
- Nephrotic syndrome in 30 – 50%.
- Progressive renal failure: 70% reach end stage in 10 years.

Treatment:
- 20 - 40% of nephrotic cases may be helped by corticosteroids.
- Data also support use of cyclosporine, mycophenolate, cyclophosphamide, etc.
- Use ACE-inhibitors or ARB’s (non-specific treatment for heavy proteinuria and/or progressive glomerular impairment).
FSGS is a group of diseases:

- Actually FSGS is a pattern of response to injury that has multiple etiologies.
- How to distinguish the recurrent type before transplant is done?
- Estimates of post-transplant recurrence rates vary because of this denominator problem.
- Conflicting classifications of the different types of FSGS.
- Recent strides in defining different types based on pathogenesis.
- Attempts at standardization of classification beginning to gain acceptance.
FSGS is a group of diseases

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These 4 have in common:
- Primary damage to the podocyte foot-processes of the visceral epithelial cells of the glomerulus.
- When podocyte injury progresses to podocyte cell death, there is consequent sclerosis of the glomerular capillary tuft.
Normal Glomerulus

- Parietal epithelium
- Bowman's capsule
- Bowman's space (urinary space)
- Visceral epithelial (podocyte) cell body
- Glomerular basement membrane (GBM)
- Capillary lumen
- Endothelial cell body
- Mesangium
- Mesangial cell body

© david m ward, 2003
Electron Microscopy - Normal Glomerular Capillary Loop
Outside = epithelium ("podocyte")
Inside = endothelium

Podocyte foot-processes
Endothelial fenestrae
Capillary lumen

Scanning Electron Microscopy – Glomerular Capillary Loop

Micrograph © The McGraw-Hill Companies Inc, 2011
Scanning Electron Microscopy – Podocyte Foot Processes
Scanning Electron Microscopy – Glomerular Capillary Loop

Outside = epithelium ("podocyte")

Inside = endothelium

Podocyte foot-processes

Endothelial fenestrae

Capillary lumen

Micrograph © The McGraw-Hill Companies Inc, 2011
Normal Glomerular Capillary Loop

- Capillary lumen
- Mesangial Matrix
- Mesangial Cell
- Glomerular Basement Membrane
- Part of Epithelial Cell (Podocyte)

© David M Ward, 2012
Normal Glomerular Capillary Loop

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© David M Ward, 2012
Normal Glomerular Capillary Loop

Podocyte Foot Processes

“Slit diaphragms” span between podocyte foot processes

Outside = Epithelium (Podocytes)

Formation of glomerular filtrate

Mesangial Matrix
Mesangial Cell

Glomerular Basement Membrane

© David M Ward, 2012
Podocyte Foot Process Architecture

“Slit diaphragms” span between podocyte foot processes.

Podocyte Foot Process Architecture

“Slit diaphragms” span between podocyte foot processes.

Podocyte Foot Process Architecture

Healthy:

Urinary filtrate

Actin cytoskeleton

Filtration slit

Podocyte foot process

SD

GBM

Endothelial cell

Endothelial glycocalyx

Flow of molecules

Glomerular capillary lumen

Podocyte Foot Process Architecture

Healthy:

- Actin cytoskeleton
- Filtration slit
- Urinary filtrate
- Flow of molecules
- Glomerular capillary lumen

Collapsed / “effaced”:

- Podocyte fusion and collapse
- Albumin
- Reorganization of actin cytoskeleton
- Urinary filtrate
- Glomerular capillary lumen

## FSGS is a group of diseases

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FSGS (Focal Segmental Glomerulosclerosis)

Increasing incidence; greater if African genetic background.

- Prominent cause of ESRD. (1)
- The incidence is increasing. (1)
- Most common cause of non-diabetic nephrotic syndrome in USA.
- Global incidence estimated at 8 cases per million per year. (2)
- Lifetime risk in USA is: ~0.2% for European Americans ~0.7% for African Americans. (3)

FSGS (Focal Segmental Glomerulosclerosis)

FSGS is more common in African Americans.

- African-American ancestry confers high risk for FSGS and hypertension-attributed ESRD (end-stage renal disease); (also of HIV-nephropathy and diabetes-associated ESRD).
- Early hypotheses included speculation regarding a one-time selection event for salt-conserving genes.
- Then genome-wide analysis (GWAS) showed association with a locus on chromosome 22, in or near the gene for MYH9 (myosin heavy chain 9). (1)
- However, causal mutations in MYH9 could not be found. (2)
- Linkage disequilibrium with a nearby site was suspected; mutations in the APOL1 gene were found. (3)

(2) Freedman BI et al., Kidney Int 75:736, 2009
GENETICS

Kidney Disease Is Parasite-Slaying Protein’s Downside

Kidney disease could be the price of resistance to a virulent parasite. Researchers describe two Jekyll-and-Hyde genetic variations online in Science this week (www.sciencemag.org/cgi/content/abstract/1193032) that can lead to kidney shutdown but may also fend off a microorganism that causes sleeping sickness in thousands of people in Africa.

“This is perhaps the best example, except for sickle cell anemia, of a common disease being caused by genetic variants that also play a role in resistance to infectious disease,” says human geneticist Sarah Tishkoff of the University of Pennsylvania. Similar findings may soon follow, researchers predict. The study “offers a lot of encouragement that we are going to find more cases where there are genetic bases for human adaptations,” says evolutionary biologist Gregory Wray of Duke University in Durham, North Carolina.

For a long time, the prime example of how natural selection can favor “harmful” mutations if they also confer pathogen protection has been sickle cell disease. A mutation in the gene for hemoglobin produces deformed red blood cells and can lead to an early death in severe cases. But it also enhances resistance to the most serious onus of the malaria parasite. Now scientists are looking for genetic factors that may offer protection against other infections, including those caused by Trypanosoma brucei, the parasites that cause sleeping sickness.

The researchers describe two genetic variants in the APOLI gene, which codes for the blood protein apolipoprotein L-1. The researchers used data from the 1000 Genomes Project—which is sequencing DNA of people from around the world—and scoured this chromosome region for mutations that were much more common in Africans than in Europeans. Then by statistically analyzing the gene variants in African Americans who had either of the past 10,000 years. “The variants must have positive effects in order to balance out kidney disease,” Pollak says.

He and his colleagues hypothesized that the G1 and G2 versions of ApolLI better protect against Trypanosoma brucei, a microscopic parasite spread in Africa by tsetse flies. The standard version of ApolLI slays one subspecies of the parasite, T. brucei brucei, but not another subspecies, T. brucei rhodesiense, which makes a protein called SRA that neutralizes the blood defender.

But G1 and G2 reconfigure ApolLI, restoring its potency. Blood plasma from people who carried G1 or G2 killed the rhodesiense version of the parasite, as did lab-made copies of the altered proteins. “The effect was really dramatic,” Pollak says.

Parasitologist Jayne Raper of New York University says the new study illustrates the ongoing “molecular arms race between host and pathogen.” However, the study isn’t conclusive, the authors and outside experts agree. The Yoruba people hail from West Africa, and the new variants were found, along with their red blood cell alteration, in two Yoruba populations in the U.S. But the researchers were only able to examine about 160 individuals total. They hope to verify the findings in additional populations.
FSGS (Focal Segmental Glomerulosclerosis)

FSGS is more common in African Americans.

Good gene, bad gene. The same gene variants that promote destruction of the kidney’s filtration units (above) also combat *Trypanosoma brucei rhodesiense* parasites.

Leslie M. Science 329:263, 2010 (Editorial)
FSGS is more common in African Americans.

- In African-Americans, FSGS and hypertension-attributed ESRD are associated with two independent sequence variants in the APOL1 gene on chromosome 22.
- FSGS odds ratio = 10.5 (95% confidence interval 6.0-18.4); H-ESRD odds ratio = 7.3 (95% confidence interval 5.6-9.5).
- The two APOL1 variants are common in West African chromosomes but absent in chromosomes of other origin. Both reside within haplotypes that harbor signatures of positive selection. ApoL1 (apolipoprotein L-1) is a serum factor that lyses trypanosomes.
- In vitro assays revealed that only the kidney disease-associated ApoL1 variants lysed Trypanosoma brucei rhodesiense.

# FSGS is a group of diseases

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Genetic abnormalities of podocyte proteins cause FSGS-type lesions

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<th>Gene/Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Finnish type</td>
<td>Nephrin</td>
</tr>
<tr>
<td>NPHS2 mutation</td>
<td>Podocin</td>
</tr>
<tr>
<td>Familial FSGS 1</td>
<td>α actinin 4</td>
</tr>
<tr>
<td>Familial FSGS 2</td>
<td>TRP C6</td>
</tr>
</tbody>
</table>


Other genetic pathways in production of glomerular sclerosis

Mitochondrial gene abnormalities can cause podocyte damage.

- Primary coenzyme Q10 deficiency secondary to genetic defects in the COQ2 gene – “COQ2 nephropathy”. (1)
- Multiple others. (2)

## Hereditary podocytopathies

<table>
<thead>
<tr>
<th>DISEASE</th>
<th>LOCUS</th>
<th>GENE</th>
<th>PROTEIN</th>
<th>CLINICAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Congenital nephrotic syndrome (Finnish type)</td>
<td>19q13.1</td>
<td>NPHS1</td>
<td>Nephrin</td>
<td>AR, in utero</td>
</tr>
<tr>
<td>Steroid-resistant nephrotic syndrome</td>
<td>1q25-32</td>
<td>NPHS2</td>
<td>Podocin</td>
<td>AR, young adult</td>
</tr>
<tr>
<td>Pierson’s type nephrosis</td>
<td></td>
<td></td>
<td>Laminin beta 2</td>
<td>AR, post birth</td>
</tr>
<tr>
<td>Nail-Patella syndrome</td>
<td></td>
<td>LMX1B</td>
<td></td>
<td>AD, children</td>
</tr>
<tr>
<td>Denys-Drash syndrome</td>
<td></td>
<td>WT1</td>
<td></td>
<td>AD, ESRD by 3</td>
</tr>
<tr>
<td>Familial FSGS type 1</td>
<td>19q13</td>
<td>ACTN4</td>
<td>alpha Actinin 4</td>
<td>AD, adolescents</td>
</tr>
<tr>
<td>Familial FSGS type 2</td>
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<td>TRP C6</td>
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<th>Etiology/mecchanism</th>
<th>Predict</th>
</tr>
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</table>
| **Primary FSGS**    | - recurrence in transplant  
| Circulating factors toxic to podocyte integrity. | - response to TPE  |
| **Secondary FSGS**  | - **no** recurrence in transplant  
| Adaptive injury (hyperfiltration damage). | - **no** response to TPE  |
| **Familial FSGS**   | -    |
| Genetic defects of podocyte and slit-pore proteins. | -    |
| **“Collapsing” form of FSGS** | -    |
| Toxins & viruses (HIV, parvo B19, pamidronate, etc.) | -    |
| **FSGS due to scarring from other GN** | -    |
| Non-specific scarring after inflammatory type of glomerulonephritis. | -    |
Predicting post-transplant recurrence: genetic markers

**Study patients:**
- 83 children with primary FSGS who received at least one renal allograft. (mean age 6.7 years at diagnosis; 13 years at first transplantation).
- 53 of these were analyzed for *NPHS2* mutations (gene for Podocin).

**Results:**
- FSGS recurred in 30 patients (36%) (median 13 days; range 1.5 to 152 days).
- 23 patients received a second kidney transplant, and FSGS recurred in 11 (48%) (median 16 days; range 2.7 to 66 days).
- Recurrence of FSGS: 0% (0 of 11) in patients with homozygous or compound heterozygous *NPHS2* mutations versus 45% in patients without mutations.

**Conclusion:**
- Genetic testing for pathogenic mutations may be important for prognosis and treatment of FSGS both before and after transplantation.

At what anatomic sites could “glomerular permeability factors” act to cause reversible damage to the podocyte cytoskeleton?
Foot Process Effacement (FPE): role of Dynamin and Cathepsin L

Critical elements in maintaining podocyte foot-process integrity:
- Dynamin
- Cathepsin L
- Actin
Foot Process Effacement (FPE): role of Dynamin and Cathepsin L

Evidence from a murine model

<table>
<thead>
<tr>
<th>Dynamin maintains FP structure by regulating Actin.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cathepsin L (Cat L) induces proteinuria by switching off the active, GTP-bound form of Dynamin. Is increased in Hu proteinuric diseases. Is increased in a murine model.</td>
</tr>
<tr>
<td>Cat L-deficient mice resist foot process effacement (FPE)</td>
</tr>
<tr>
<td>Gene delivery into normal mice:</td>
</tr>
<tr>
<td>• of a mutant Dynamin (that does not bind GTP) → induces FPE and proteinuria.</td>
</tr>
<tr>
<td>• of the Cat L-cleaved product of Dynamin → induces FPE and proteinuria.</td>
</tr>
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<td>Gene delivery into proteinuric mice:</td>
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<td>• of 2 different Cat L-resistant Dynamin mutants → reverses proteinuria and FPE.</td>
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Foot Process Effacement (FPE): role of Dynamin and Cathepsin L

Evidence from a murine model

Further Recent Insights into the Machinery of Podocyte Integrity

Puromycin Aminonucleoside Nephropathy (PAN) is a standard model of podocytopathy in animals.

Neph1 protein has an intracellular domain (Neph1CD) that is involved in podocyte response to injury.

Transduction of TAT-Neph1CD, which inhibits phosphorylation of Neph1 by PAN, was shown to protect cultured human podocytes from PAN-induced damage.

Further Recent Insights into the Machinery of Podocyte Integrity

| Control: Neph1 on cell membranes with intact actin cytoskeleton | PAN induces Neph1 loss from cell membranes and disrupts actin cytoskeleton | Transduced TAT-Neph1 on cell membranes with intact actin cytoskeleton | Transduced TAT-Neph1 resists PAN-induced damage to Neph1 & actin cytoskeleton |

Control of actin cytoskeleton:
- Neph1
- Synaptospondin
- Nephrin signalling endosome
- Cathepsin L / Dynamin interaction
- etc.

Effect of “Glomerular Permeability Factors” on foot-process morphology

Plasma from cases of recurrent FSGS has a similar effect

- Proteinuria in experimental animals given FSGS plasma.(1)
- Shrinking of cultured glomeruli in vitro if FSGS plasma added.(2)
- This “Glomerular Volume Variation” (GVV) test has been standardized as a semi-quantitative research assay of permeability factor activity.(3)

Candidate molecules:

- Small, highly glycosylated, hydrophobic protein(s)/peptide(s) 30 to 50 kDa, poorly characterized. (1)
- Permeability activity is decreased by plasmapheresis. (2)
- Normal plasma contains substances that block or inactivate the FSGS permeability factor.
- In vitro, blocking by cyclosporine, indomethacin, etc.
- Proteinuric effect inhibited by galactose. (3)
- Clinical benefit in FSGS patients given oral galactose (4, 5) now disproven.

**Glomerular Permeability Candidate - CLC1**

**Candidate molecule:**

**CLC1** (Cardiotrophin-like cytokine 1)

- CLC1 is a member of the interleukin-6 family (approx. 220 AA, 24kDa).
- Decreases nephrin expression in cultured podocytes.
- CLC1 inhibitors reverse the permeability effect of plasma from FSGS patients.
- Data are preliminary.

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Candidate molecule: 

**suPAR** (= soluble urokinase-type Plasminogen Activator Receptor)

In 2011, research implicated “suPAR”, the soluble form of the urokinase receptor present on podocytes:

- suPAR levels (22 to 45 kDa fragments) are elevated in 70% of patients with FSGS, but not in other glomerular diseases.
- In animal models, suPAR causes podocyte injury by activation of β3 integrin.
- In kidney biopsies, β3 integrin is found on podocytes in patients with FSGS (but not other diseases).

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suPAR removal by plasmapheresis in recurrent FSGS (post-transplant)

- Initial studies of plasmapheresis (TPE):
  - clinical remission if suPAR levels <2,000 pg/ml.
  - serum no longer induces podocyte β3 integrin.

- In 2 patients:
  - TPE failed to reduce suPAR levels <2,000 pg/ml.
  - did not achieve clinical remission.
  - serum still strongly activated β3 integrin.

Further evidence of pathogenic role of suPAR

Study patients: Two cohorts with biopsy-proven primary FSGS:
- 70 patients from the North America–based FSGS clinical trial (CT).
- 94 patients from European PodoNet study of steroid-resistant nephrotic syndrome.

Results:
- Elevated suPAR in 84.3% (CT) and 55.3% (PodoNet), versus 6% of controls (P=0.0001); inflammation did not account for this difference.
- Reduction of suPAR correlates with treatment and with reduction of proteinuria, with higher odds for complete remission (P=0.04).

Conclusions:
- suPAR levels elevated in geographically and ethnically diverse patients with FSGS.
- Reductions in suPAR levels correlate with different therapeutic regimens and with remission; this supports the role of suPAR in pathogenesis.

Unexpected finding:
- In the PodoNet cohort, patients with an NPHS2 mutation had higher suPAR levels than those without a mutation. (NPHS2 codes for Podocin.)

**Contradictory evidence for a pathogenic role of suPAR in FSGS**


Cathelin D, et al. Administration of recombinant soluble urokinase receptor per se is not sufficient to induce podocyte alterations and proteinuria in mice. *JASN* 25:1662-1668, 2014

“After adjusting for baseline suPAR concentration, age, gender, proteinuria, and time, the change in suPAR from baseline was associated with eGFR, but this association was not different for patients with FSGS as compared with other diagnoses. Thus these results do not support a pathological role for suPAR in FSGS.”

Data from 3683 patients

**Figure 3. Levels of suPAR and Incident Chronic Kidney Disease.**

Other candidate systems for intervention in FSGS

Corticotrophin and Melanocortin receptors

- ACTH (corticotrophin) is cleaved to α-MSH (melanocyte stimulating hormone) which binds to the receptor MC1R on the podocyte. (1)
- Thus ACTH gel (Acthar) may work by stimulating corticosteroids or directly by the above mechanism

Other candidate systems for intervention in FSGS

Angiotensin System receptors

- Angiotensin II regulates and enhances the expression of transient receptor potential cation channel 6 (TRPC6) on podocytes. (1)
- Antibodies to angiotensin (AT1) receptors on podocytes can cause proteinuria. (2)
- Therefore ACE-inhibitors and ARBs perhaps not just non-specific treatments for nephrotic diseases.

Indications for plasmapheresis for FSGS

- TPE for post-transplant recurrence of FSGS
- TPE for peri-transplant prophylaxis of FSGS
- TPE for primary FSGS in native kidneys
TPE for post-transplant recurrence (slide 1 of 2):

- TPE is established first-line therapy (plus immunosuppression with mycophenolate, cyclophosphamide or rituximab).
- ASFA (2013) recommends initial regimen of TPE daily for 3 days, then at least 3 times per week for the next 2 weeks. Thereafter, TPE can be continued 2 - 3/week until remission occurs, as judged by serial quantitation of urine protein and serum creatinine, which can take weeks to months. (1, 2)
- One series performed 17 TPE treatments in each of 7 adults, all of whom had functioning grafts 10 months later. (3)
- Other series claim remission rates up to 80% in adults (4), and 88% in children. (5)

TPE for post-transplant recurrence (slide 2 of 2):

- One large retrospective series concluded that:
  - Modern post-transplant immunosuppressive drug regimens do not reduce the recurrence rate of FSGS in adults.
  - However, TPE achieved remission in 75% of cases. (1)

- Patients receiving treatment for recurrent FSGS or preemptively (for high-risk profile):
  - Of the different treatment approaches, TPE combined with rituximab (anti-CD20) was most associated with prolonged remission of proteinuria. (2)


TPE for peri-transplant prophylaxis:

- 10 patients at high risk because of rapid progression (4) or prior recurrence in a transplant (6) received 8 TPE treatments in the peri-operative period.
  - 3 had recurrence within 3 months (all had prior graft loss to recurrence); 2 developed ESRD, 3\textsuperscript{rd} with significant renal dysfunction.
  - 7 (including 3 with prior graft loss to recurrence) were free of recurrence at follow-up (238–1258 days), mean creatinine 1.53 mg/dL. (1)

- More recently, in 34 pediatric transplant cases, prophylactic TPE post-transplant appeared not to confer any outcome benefit compared with treatment of actual recurrence. (2)

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TPE for primary FSGS (in native kidneys):  

- TPE (averaging 17 treatments) plus corticosteroids and cyclophosphamide achieved sustained remissions in 8 of 11 previously unresponsive adults. (1)  
- TPE (six treatments) without consistent immunosuppressive drugs reduced proteinuria in only 2 of 8 patients. (2)  
- Expert opinion “based on very limited experience” (3):  
  “Consider TPE for  
  • Severe disease manifestations despite an adequate trial of initial immunosuppressive therapy, in which very high levels of circulating permeability factor have been demonstrated.  
  • Continued massive proteinuria and hypoalbuminemia despite exposure to an adequate course of prednisone, cyclosporine, and mycophenolate.”

(3) Appel GB and Cattran DC. Treatment of primary FSGS. In “UpToDate” ® online.

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Type of plasmapheresis for FSGS

Conventional plasma exchange (plasma removal and replacement):

- Established first-line treatment for recurrent FSGS (1-9)
- Removes macromolecules of all sizes:
  - IgG (140 kDa)
  - suPAR (22 to 45 kDa)
  - Ill-defined permeability factors (30 to 50 kDa)
  - CLC1 (24 kDa), etc., etc.

References:
Fig. 3. Circuit diagrams of (a) primary membrane plasma separation plus secondary plasma fractionation, and (b) primary centrifugal plasma separation plus secondary plasma perfusion column. In the left panel (a), the primary separation of plasma from blood (#1) is in a hollow-fiber membrane plasma filter with a pore size of 0.3 microns and a molecular weight cut-off in excess of 1,000 kDa. The secondary processing of plasma (#2) is in a hollow-fiber membrane plasma fractionator with a pore size of 0.01–0.03 microns and a molecular weight cut-off of approximately 100 kDa. Albumin (67 kDa) passes through the secondary membrane and can be used as replacement fluid for the patient. Immunoglobulins, including IgG (146 kDa), stay within the hollow-fiber lumen which drains to the effluent bag, thus removing most of the autoantibody present in the plasma. Membrane specifications are those of Asahi ® products (Asahi Kasei Kuraray Medical Co., Tokyo 101-8,101, Japan). In the right panel (b), the primary separation of plasma from blood (#1) is by a continuous-flow centrifuge, and the secondary processing of plasma (#2) is in a perfusion column that can contain an immuno-adsorbent or chemical adsorbent (see text). The pathogenic molecule binds to the column, which is replaced when exhausted. Other systems employ pairs of columns that can be regenerated by washing out the bound pathogenic molecule; one column is in active use while the other is being washed clean, and they switch periodically during the procedure. Either type of primary separation (#1) can in principal be coupled to any type of secondary plasma purification (#2). Many secondary devices in use in Europe and Japan, and some primary/secondary combination systems, are not FDA-Approved in the USA. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
Type of plasmapheresis for FSGS

**Immunoadsorption (IA) plasmapheresis:**

**Protein A columns**
- Reported as effective for recurrent FSGS (1)
- Removes IgG, but probably not small proteins like suPAR, etc.

**Anti-IgG columns**
- Reported as effective for recurrent FSGS (2, 3)
- Removes IgG, but probably not small proteins like suPAR, etc.

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Double-filtration (cascade) plasmapheresis:
- Returns albumin (67 kDa) and all smaller molecules to the patient.

#1: Plasma-filter
Pore size: large
Cut-off: >1000 kD

Membrane specifications are those of Asahi products
(Asahi Kasei Kuraray Medical Co., Tokyo 101-8,101, Japan)

Diagram from
Ward DM,
J Clin Apheresis
26:230-238, 2011

#2: Plasma-fractionator
Pore size: medium
Cut-off: ~ 100 kD

IgM ~ 970 kDa
IgG ~ 140 kDa
Albumin ~ 67 kDa
suPAR ~ 22-45 kDa
Type of plasmapheresis for FSGS

Tryptophan adsorption column:

- “Effective for steroid resistant FSGS”. (1)

Update on immunoglobulin-binding IA columns:

- IgG-binding columns are specifically designed to extract only immunoglobulins. Example –
  Globaffin ® columns use peptide ligand PGAM146 (Fresenius, Germany). (1)

- However, there is evidence from one case report that Immunoadsorption (IA) using Globaffin reduced suPAR also. The authors speculate that suPAR may bind to immunoglobulin molecules. (2)


Plasma exchange vs. Immunoadsorption (anti-IgG column)

TPE = conventional plasmapheresis replacing with FFP and 5% albumin (1 to 1.5 x PV).
IA = immunoadsorption plasmapheresis with Globaffin® columns (2 to 2.5 x PV)

Plasma exchange vs. Immunadsorption (anti-IgG column)

*“Podocyte AP5 activity” = bioassay for podocyte β3 integrin activation by AP5 staining quantitated by mean fluorescence intensity (MFI)
Dextran Sulfate Adsorption (LDL-Apheresis) for FSGS

Use of LDL-apheresis (dextran sulfate columns):

- FDA approval for pediatric FSGS in October 2013.
- Humanitarian Use Device application for Kaneka Liposorber.
- Pediatric FSGS indication:
  - children with
  - nephrotic syndrome,
  - proteinuria >3.5g/day,
  - hypoalbuminemia,
  - hyperlipidemia, and
  - progressive renal decline.
LDL Apheresis - systems available worldwide

**LDL removal from separated plasma**

1. Adsorption
   - Liposorber (Dextran sulfate adsorption) *
   - TheraSorb LDL (Anti-ApoB immunoadsorption)

2. Precipitation
   - H.E.L.P. (Heparin-induced precipitation) *

3. Filtration
   - Double Filtration Plasmapheresis (DFPP)

**Direct LDL adsorption from whole blood**

- Liposorber D (Dextran sulfate adsorption)
- Direct Adsorption of Lipoprotein (DALI) (Polyacrylate adsorption)

11 pediatric patients with biopsy proven FSGS, all steroid resistant after 8 weeks (and prior treatment with cyclosporin-A).

- LDL apheresis on Kaneka Liposorber® 2x per week for 3 weeks, then 1x per week for 6 weeks.
- 7 of 11 had marked reduction in proteinuria or achieved remission.
- Appeared to improve response to steroids.


Dextran Sulfate Adsorption (LDL-Apheresis)
Role of Lipids in Glomerulosclerosis


- 7 adults (age 34-62) with hypercholesterolemia due to nephrotic syndrome from diseases other than FSGS (4 Membranous GN, 2 MPGN and 1 IgA GN)
- Treated with LDL-apheresis using Kaneka dextran sulfate columns.
- All benefitted with regard to reduction of LDL and Lp(a).
- Serum albumin increased (p=0.01) and proteinuria tended to decrease (NS) during this time. Confounding factors include immunosuppressive drugs.

Hyperlipidemia contributing to glomerular damage:

The POLARIS Trial
(Prospective Observational Survey on the Long-Term Effects of LDL Apheresis on Drug-Resistant Nephrotic Syndrome)

- 64 courses in 58 patients with steroid +/- cyclosporine resistant nephrotic syndrome, ages 18-84. (17 courses excluded for insufficient data).
- All treated on Kaneka Liposorber system (dextran sulfate adsorption).
- Average 9.6 LDL-A procedures per course.
- 3.5 L. (av.) plasma processed per procedure.
- 55% of courses were in patients with FSGS.
- Proteinuria fell similarly in FSGS cases (from 6.47 ± 2.98 to 3.26 g ± 3.13) and in non-FSGS cases (6.13±3.41 to 3.89±4.01).

Therapeutic results that illuminate pathogenesis:

- There is secure evidence that whole plasma removal (TPE) is of major clinical benefit in FSGS (post-transplant recurrent type).
- Rituximab, corticosteroids and other immunosuppressants can also be effective – what is the role of the immune system?
- Are immunoadsorption (IAPP) or double-filtration (DFPP) truly effective?
  - Do they have mechanisms of action other than removal of glomerular permeability factors?
  - Research needs first to validate claims for their clinical effectiveness.
- Until there is clarification, conventional plasma exchange (TPE) has been recommended as the preferred apheresis modality (1).
- The efficacy of dextran-sulfate plasma adsorption (LDL-apheresis) needs to be confirmed, and its mode of action elucidated.

SUMMARY:

1. **Recurrence of FSGS** after kidney transplant: evidence for endogenous circulating permeability factors.
2. **FSGS is a group of diseases.**
3. **West African genes** promote FSGS-like glomerular lesions.
4. **Genetic abnormalities of podocyte proteins** compromise podocyte foot-process architecture and lead to FSGS-like lesions.
5. **FSGS of the type that recurs post-transplant** is less common than other types.
6. **Circulating permeability factors** can cause podocyte foot-process damage.
7. **Candidate molecules:** 30-50 kDa factors, CLC1, suPAR, others.
8. **TPE for FSGS recurring post-transplant** is established and effective treatment.
9. **Use of TPE for native-kidney FSGS** and peri-transplant prophylaxis is less clear.
10. **Immunoadsorption, DFPP, etc.** may not be equivalent to TPE.
11. **Dextran-sulfate plasma adsorption (LDL-apheresis)** appears effective.
Thank you for your attention

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or dmward@ucsd.edu