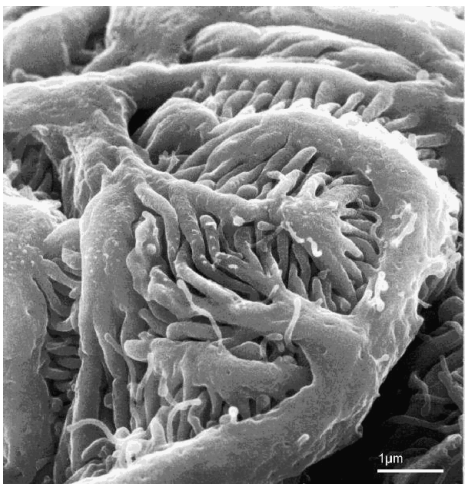

DNA and Destiny: Heritable and Nonheritable causes of Focal Segmental Glomerulosclerosis

Denise K. Marciano, MD, PhD
Assistant Professor

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Biographical Information

Dr. Marciano is an Assistant Professor in the Division of Nephrology since 2011. She graduated with a B.A. in Chemistry from Dartmouth College and then obtained her M.D.-Ph.D. training at the Tri-Institutional Program of Cornell University Medical College, The Rockefeller University, and Memorial Sloan Kettering Cancer Center. She completed her Internal Medicine residency and Nephrology fellowship at the University of California, San Francisco.

Dr. Marciano has an independent laboratory with research focusing on the development and repair of tubules and glomeruli in animal models. Her lab studies the role of cellular adhesion and polarity in these processes. She is currently the Director of the O'Brien Kidney Center seminar series and the Director of the O'Brien Kidney Center Cell Biology and Imaging Core.

Purpose and Overview

To provide a synopsis of the causes and pathogenesis of Focal Segmental Glomerulosclerosis, placing special emphasis on the underlying genetic etiologies and predispositions.

Educational Objectives

1) Recognize that Focal Segmental Glomerulosclerosis (FSGS) is the most common cause of primary nephrotic syndrome in the Unites States. It is also the most common cause of ESRD due to a primary glomerular disease.
2) Understand that FSGS is a clinical pathologic syndrome that has multiple etiologies.
3) Understand how rare human mutations that cause FSGS expand our understanding of the disease.
4) Recognize the central role of the podocyte in FSGS pathobiology.

Introduction

Focal Segmental Glomerulosclerosis (FSGS) refers to a clinical syndrome and histological pattern of kidney injury with many etiologies. The clinical syndrome is characterized by proteinuria, ranging from mild proteinuria to full-blown nephrotic syndrome, and glomerular filtration rate (GFR) is variably reduced. The histological findings are characterized by sclerotic lesions on glomeruli that are focal (<50% of all glomeruli affected) and segmental (<50% of the glomerular tuft affected). Although not part of the definition, FSGS is frequently steroid-resistant, with 30-50% of adults failing to respond to steroid therapy (3, 4). Additionally, it is often associated with rapid progression to End Stage Renal Disease (ESRD) (3, 4).

Epidemiology of FSGS

Recent data demonstrates that the incidence of FSGS is increasing (1). In a large study from the University of Chicago, Mark Haas and his colleagues compiled data of renal biopsies from patients with unknown nephrotic syndrome. Patients with a systemic disease or known condition associated with nephrotic syndrome, such as diabetes, lupus, hepatitis B, and HIV, were excluded from the study. Biopsies from the 1970s were compared with those from the 1990s, revealing that there is an increase in the incidence of FSGS during the two decades (Fig. 1). FSGS has become the most common cause of idiopathic nephrotic syndrome, exceeding the incidence of membranous nephropathy. Similar results have been obtained in several additional studies from Chicago, New York, and Massachusetts (2, 5, 6). One limitation of this particular study is that there were few Hispanics and Asians included. This is reflected in the low incidence of IgA nephropathy, which is the most common cause of primary glomerular disease in Asians.

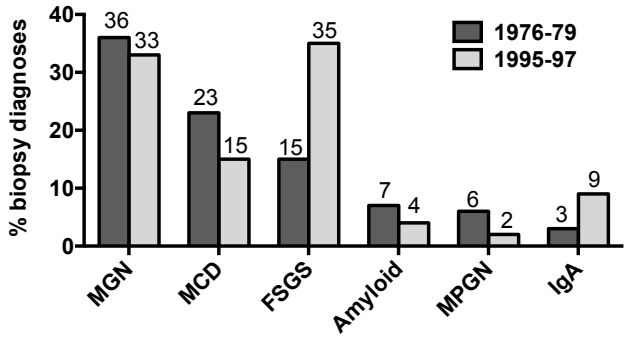


Figure 1, Etiologies of idiopathic nephrotic syndrome in adults. Modified from Haas *et al.* (1). MGN: membranous glomerulonephritis; MCD: minimal change disease; MPGN: membranoproliferative glomerulonephritis.

The reasons for the increase in FSGS incidence over the past several decades are unclear. While a true increase is one possibility, changes in biopsy practices and changes in the racial makeup could be contributors. Indeed the racial makeup of the two time periods in the Haas study indicate that in the 1976-79 study period only 28% of the

patients were African American, while in the 1995-1997 study period, 35% of patients were African American. When the biopsy results were examined according to race, it became clear there is a much higher incidence of FSGS among African Americans in both time periods (Fig. 2a). These results in African American populations have been validated in several other studies, including one reproduced as Fig. 2b (2), with approximately two-thirds of African Americans with nephrotic syndrome having a biopsy result consistent with FSGS when secondary causes are excluded. Importantly, the Haas study shows that the incidence of FSGS increased in both Caucasians and African Americans, suggesting that African race alone cannot account for the differences.

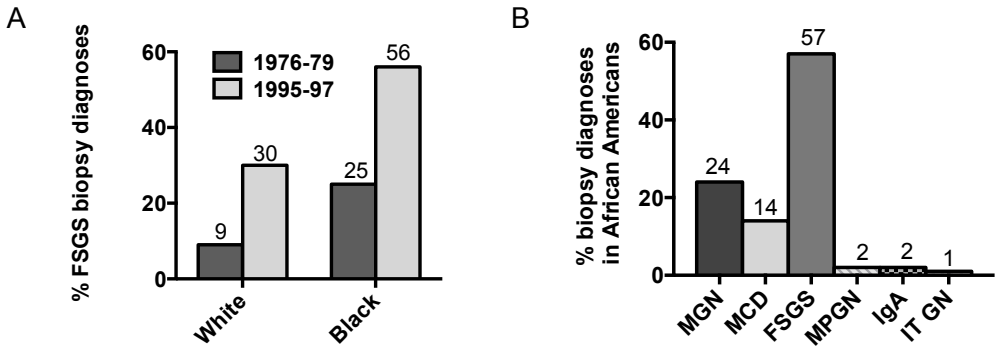


Figure 2. Racial differences in frequency of FSGS. Fig. 2a is modified from Haas *et al.* (1). Fig 2b modified from Korbet *et al*. (2).
MGN: membranous glomerulonephritis; MCD: minimal change disease; MPGN: membranoproliferative glomerulonephritis; ITGN: immunotactoid glomerulonephritis.

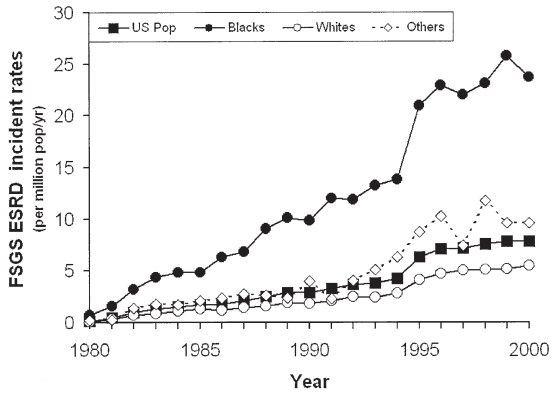
One additional interesting finding from these studies is that African American patients presenting with FSGS tend to be young, with a higher incidence of FSGS among those less than 45 years old. This suggests the possibility of a genetic contribution (See section on *APOL1* alleles in African Americans). Overall, these studies concluded that FSGS is currently a common (if not the most common) cause of primary glomerular disease in the US, and it has a higher incidence among African Americans.

The clinical course of primary FSGS is typically one of progressive decline in renal function, leading to ESRD. Rydel et al. (3) reported 57% of patients with persistent nephrotic proteinuria reach ESRD after 10 years of follow-up. Other cohort studies have reported a range from 50-70% (4), although one study showed a rate of 30% (7). The degree of proteinuria has prognostic significance, with those having >10 g of proteinuria/day having the most unfavorable prognosis (8).

The incidence of ESRD due to FSGS is increasing in the United States (Fig. 3) (9), mirroring the increase in FSGS incidence. The data is obtained from the United States Renal Data System (USRDS), which is mandated by federal law, so data is collected for all patients receiving therapy for ESRD in the United States. The abrupt change in the trend in 1995 (Fig. 3) likely represents changes in data acquisition

methods. Before 1995 only Medicare-entitled patients were included in the USRDS database; after 1995, additional patients (~8% of the total) that were not Medicare-entitled were included (9). In 2000, the incidence of FSGS ESRD was ~2.3%, increasing 11-fold since 1980 (Fig. 3). The proportion of incident ESRD cases due to FSGS, Membranous glomerulonephritis, and IgA nephropathy (the three most common causes of primary glomerular disease) reveal that FSGS is the most common cause of ESRD by a primary glomerular disease in the United States. It is the most common cause in both Caucasian and African American patients.

A



B

Age Group (y)	FSGS ESRD, 1980 (%)	FSGS ESRD, 2000 (%)
0-24	0.9	10.8
25-44	0.4	5.2
45-64	0.1	2.1
≥65	0.0	1.2
Total	0.2	2.3
Total, age adjusted	0.2	3.1

Figure 3. Incident rates of FSGS ESRD in the US. (A) USRDS data is expressed as million population/year and is adjusted for age and sex. (B) Percentage of incident ESRD cases caused by FSGS. Data from Kitiyakara *et al*. (9).

Based on USRDS data from 2000 indicating that ~ 3.1% of all Americans have ESRD, the lifetime risk for FSGS ESRD is estimated to be 0.06% in the United States (9). For comparison, the lifetime risk of ESRD due to diabetic nephropathy is 1.5%. Kopp and colleagues made further estimates based on the assumption that 50% of patients with FSGS undergo biopsy, and 50% progress to ESRD. This estimates the lifetime risk of FSGS to be 0.24% overall, and 0.72% in African Americans. This corresponds to an absolute lifetime risk of ~1:400 for all Americans and ~1:130 for African Americans (9). The risk of ESRD due to FSGS is higher in pediatric populations than in adults. Children with steroid-resistant nephrotic syndrome predominantly have FSGS as their underlying pathology (10, 11), and steroid-, nephrotic syndrome accounts for 5-20% of all pediatric ESRD cases (9).

Etiologies of FSGS

It is common to discuss FSGS in terms of the multiple etiologies that lead to the histologic pattern of FSGS. This has important therapeutic implications because secondary causes of FSGS are not typically treated with immunosuppression. Genetic causes of FSGS and primary/idiopathic FSGS, which is thought to be due to a circulating permeability factor, will be discussed in more detail below. The secondary causes are quite varied. Reduced or low nephron mass, as occurs in unilateral renal

agenesis or reflux nephropathy, is a cause of FSGS in which the loss of nephrons induces a compensatory hyperfiltration in the remaining nephrons, leading to intraglomerular hypertension and hypertrophy in the remaining glomeruli (12). This compensatory glomerular HTN leads to epithelial and endothelial cell injury, as well as mesangial cell changes that lead to progressive FSGS (12). Other conditions with normal renal mass, such as obesity, also predisposes one to FSGS. Obesity causes hyperfiltration and glomerulomegaly, which may lead to FSGS, although metabolic and inflammatory changes may also contribute (13). FSGS can also be caused by infections, notably HIV which causes a collapsing FSGS (14), and various medications, as listed in Table 1.

Table 1. FSGS Etiologies

CLASSIFICATION	ETIOLOGY	CAUSE
GENETIC	Mutations in podocyte genes	• Highly penetrant Mendelian mutations: Nephrin, podocin, etc. • Genetic variations
PRIMARY/IDIOPATHIC	Circulating permeability factor	• Unknown
SECONDARY	Adaptive response mediated by glomerular hyperfiltration and hypertrophy	• Reduced nephron mass/number Congenital (i.e. low birth weight), unilateral agenesis, reflux nephropathy, etc. • Normal kidney mass (obesity, SSD, hypoxemic states)
	Viral infection	• HIV, parvovirus, CMV
	Drugs (Partial list only)	• Pamidronate/alendronate, lithium, sirolimus, IFNa

FSGS: a disease of podocyte dysfunction

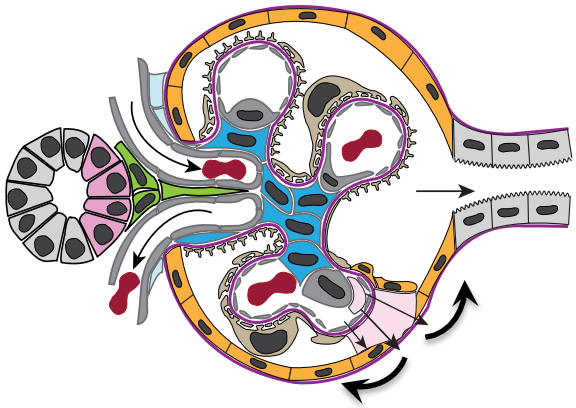
Our general view of FSGS pathogenesis implicates dysfunction of several glomerular cells types. However, there is strong data to support the current dogma that podocyte injury or malfunction is the primary defect in FSGS. This is based largely on evidence from histological studies, animal models, and the identification of genes mutated in human hereditary FSGS.

Seminal histological studies in animal models by Wilhelm Kriz laid the groundwork for our current understanding of the step-by-step sequence of events, starting with the initial glomerular injury and culminating in FSGS (15, 16). By examining the injury pattern of FSGS over a time course, Dr. Kriz determined that histologic evidence of FSGS starts with podocyte injury, but thereafter involves multiple cell types.

Podocytes respond to injury by undergoing tremendous changes in cell shape. One of the first cellular changes appears to be the flattening of podocytes so that the individual feet are no longer present, referred to as foot process effacement. How and why this process occurs is somewhat enigmatic, but it appears to involve the replacement of slit diaphragms by occlusive junctions and the broadening of foot processes (17). They continue to broaden in areas, while other areas become devoid of podocyte coverage. This stage can be repaired, but may become the basis for further injury. The loss of podocytes leaves glomerular basement membrane uncovered,

promoting matrix deposition by PECs and the formation of a tuft adhesion (also called a crescent). At the tuft adhesion, there is misdirection of glomerular filtrate into the surrounding interstitium (Fig. 4) (15). There also is migration of PECs into the affected capillary loop (18). Eventually hyalinosis and mesangial expansion, as well as the presence of foam cells, obliterate the capillary lumen. This injury may progress to global sclerosis, and the misdirection of fluid is thought to promote the degeneration of surrounding tubules and interstitial fibrosis.

Figure 4. Schematic of a glomerulus in FSGS. This illustrates the segmental lesion and breach of the parietal epithelial cell layer, allowing filtrate to pass into the surrounding tissue.

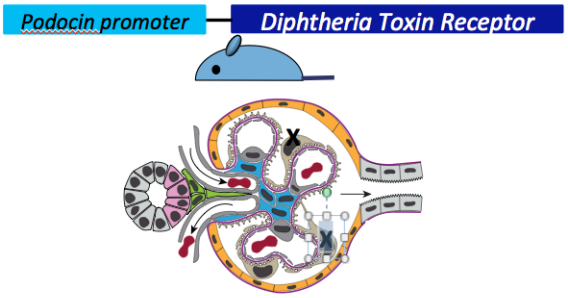


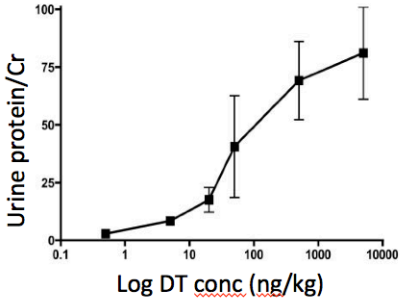
Evidence supporting the idea that filtrate becomes misdirected comes from studies in which ferritin (which is anionic) was administered to rats subjected to podocyte injury (15). In healthy rats, ferritin is filtered by the glomerulus and passes into the nephron tubules. In rats with FSGS, there is evidence of ferritin deposition over the entire glomerular circumference, at the basal side of the tubular epithelium, and in the interstitium, indicating a breach in the PEC barrier (15).

Animal models:

In light of the histological evidence identifying podocyte injury as an early defect in FSGS, it was vital to directly test the hypothesis that podocyte injury is central to disease pathogenesis. Data from rodents has shown that podocyte depletion is central to the initiation and progression of the glomerular lesion of FSGS. In 2005, Roger Wiggins and colleagues published a study using a diphtheria toxin depletion strategy to address this hypothesis (Fig. 5) (19). They engineered rats to express human diphtheria toxin receptor under the control of a podocyte-specific promoter, restricting its expression to podocytes. They administered varying doses of diphtheria toxin to the rats to specifically deplete podocytes. Diphtheria toxin causes an increase in the amount of urinary protein and podocyte cell death. The dose response curve seven days after the toxin was administered is shown (Fig. 5), which indicates the proteinuria is dose-dependent. The degree of podocyte cell death is also dose-dependent. With mild podocyte loss, the remaining podocytes undergo hypertrophy to cover the glomerular basement membrane and develop mesangial expansion. In these rats, the proteinuria is transient. With more severe depletion, there is progressive development of FSGS and ultimately global sclerosis ensues. In addition to the Wiggins study, Matsusaka et al.

used a similar approach to deplete podocytes in mice (20). Transgenic mice expressing human CD25 from podocytes were administered an immunotoxin, LMB2, that binds CD25, causing podocyte depletion. As with the former study, mice with significant podocyte depletion develop FSGS.





Podocyte Depletion	Pathology	Proteinuria	Renal Function
0-20%	Mesangial expansion	Low-level, transient	Normal
21-40%	Mesangial expansion, adhesion, FSGS	Low-level, sustained	Normal
>40%	Mesangial expansion, adhesion, FSGS, global sclerosis	High-level, sustained	Decreased

Figure 5. Podocyte depletion can cause proteinuria and FSGS. Mice expressing diphtheria toxin (DT) receptor under the control of a podocyte specific (*Nphs2*) promoter have a dose-dependent increase in proteinuria and podocyte depletion with DT administration. FSGS develops with podocyte depletion >20%. Graph and table are from Wharram *et al*. (19).

Human mutations in genes encoding podocyte proteins:

In 1998, Karl Tryggvason and colleagues from Finland were the first to identify a gene mutated in Congenital Nephrotic Syndrome of Finnish children (21). These children are characterized by steroid-resistant nephrotic syndrome that often reaches ESRD by 3-5 years of age (22). Clinically, the children are often premature and the onset of massive proteinuria occurrs prenatally. Kidney biopsies demonstrate foot process effacement and absence of slit diaphragms (23). Positional cloning revealed mutations in a gene called *Nphs1*, encoding the protein nephrin. In the Finnish population there are two classes of *Nphs1* mutations, one that causes frameshift mutations in exon 2, and the other causes a nonsense mutation in exon 26 (21). Since 1998, more than 60 mutations in *Nphs1* have been found in patients with nephrotic syndrome (24-27). Nephrin mutations are also causes of childhood- and adult-onset FSGS (28). Nephrin is a 180 kD protein with 6 immunoglobulin motifs (21). In glomeruli, nephrin is exclusively produced by podocytes, as determined by in situ hybridization. Subsequent studies have revealed that it localizes predominantly to the slit diaphragm (29, 30).

Since the original description of *Nphs1* mutations, mouse models of *Nphs1* knockout have been generated (31). Like the glomerular phenotype in children, in which there is loss of distinct foot processes and absence of slit diaphragms, *Nphs1* knockout mice have a similar histologic lesion. Expression of glomerular membrane components,

collagen and laminin, are normal in knockout mice, suggesting the phenotype is not related to defects in the glomerular basement membrane (31).

In 2000, Corinne Antignac and colleagues in France used positional cloning to identify a second gene mutated in steroid-resistant nephrotic syndrome, *Nphs2*, which encodes the protein podocin (32, 33). Patients are characterized by an autosomal recessive pattern of inheritance with variable timing of onset, ranging from 3-months to 5-years of age. Generally, there is rapid progression to end stage renal disease. Renal biopsies reveal the histology pattern reflects Minimal Change Disease early in disease progression, with normal light microscopy and electron microscopy demonstrating foot process effacement. More advanced disease shows histological evidence of FSGS (33). Since then, more than 116 mutations in *Nphs2* have been found to segregate with disease (34-37). Among these familial cases are families with homozygous mutations, compound heterozygous mutations or single heterozygous mutations. In addition to *Nphs2* causing autosomal recessive steroid-resistant nephrotic syndrome, *Nphs2* mutations have also been found in children with sporadic nephrotic syndrome (38) and adult onset FSGS (34, 35, 37). These findings indicate that mutations in *Nphs2* are associated with a wide range of clinical characteristics.

Podocin is specifically produced in podocytes and found to localize to lipid rafts of podocyte foot processes at the insertion site of the slit diaphragms (39). Biochemical studies including immunoprecipitation, GST-pulldown, and sucrose gradients show podocin interacts directly with nephrin and an actin-binding protein, CD2AP (40). Heterozygous and homozygous CD2AP mutations in humans have been shown to cause adult onset FSGS (41-43). In vitro studies also show wild type podocin recruits nephrin to lipid rafts, but disease-causing mutations in podocin do not have this capability (44). These findings suggest that podocin may have a role in the assembly of the slit diaphragm complex and may act as a scaffolding protein (27). Furthermore, they provide biochemical evidence linking two of the most common mutations accounting for steroid-resistant nephrotic syndrome and FSGS.

Subsequent to the original identification of *Nphs1* and *Nphs2* mutations, there have been a large number of genes with expression in podocytes whose mutation causes steroid-resistant nephrotic syndrome and/or FSGS in humans and/or mice. The mutated genes generally involve proteins that are involved in slit diaphragm formation and/or maintenance of the actin cytoskeleton. In general, patients with autosomal recessive inheritance present at a younger age than those with autosomal dominant inheritance. Recently, there have also been patients with mutations in the genes encoding *ADCK4, CoQ6*, and *CoQ2*, all of which are involved in CoQ10 biosynthesis. A summary of the genes involved in congenital nephrotic syndrome or familial FSGS is listed as Table 2, and a summary of the genes involved in syndromes that include steroid-resistant congenital nephrotic syndrome and FSGS is listed as Table 3.

Table 2. Summary of mutations causing congenital steroid-resistant nephrotic syndrome and FSGS.

GENE	PROTEIN	INHERITANCE	FUNCTION	PHENOTYPE	GENE
NPHS1	Nephrin	AR	Slit diaphragm	Finnish Congenital NS (45),	NPHS1

			adhesion protein	Childhood/Adult FSGS (28)	
NPHS2	Podocin	AR	Associates with CD2AP, nephrin	Most common cause SRNS in children (10, 32); Adult FSGS (46, 47)	NPHS2
PLCε1	PLCε1	AR	Phospholipase	Childhood FSGS or DMS (48)	PLCε1
Myo1E	Non-muscle myosin 1E	AR	Interacts with actin	Childhood FSGS (49)	Myo1E
ARHGDIA	Arhgdia	AR	Modulates Rho GTPases	Childhood onset SRNS (50)	ARHGDIA
ADCK4	aarF domain containing kinase 4	AR	CoQ10 biosynthesis	Childhood to young adult FSGS (51)	ADCK4
CD2AP	CD2-associated protein	AD/AR	Actin binding protein	Het/homo mutations cause adult FSGS (48, 52)	CD2AP
TRPC6	TRPC6	AD	Calcium channel	Childhood SRNS (53), Gain of function mutation causes FSGS at 20-40 y, incomplete penetrance (54)	TRPC6
ACTN4	Alpha-actinin 4	AD	Actin binding protein	Adult FSGS (55)	ACTN4
INF2	Formin	AD	Actin dynamics	FSGS in 2^{nd}-3^{rd} decades (56, 57)	INF2
ANLN	Anillin	AD	Binds actin, CD2AP, Rho	Adult FSGS (and 1 childhood FSGS) (58)	ANLN

NS, nephrotic syndrome.

Table 3. Summary of mutations causing *syndromes* that include steroid-resistant nephrotic syndrome and FSGS.

GENE	PROTEIN	INHERITANCE	ASSOCIATED FINDINGS	RENAL PHENOTYPE
WT1	WT-1	AD	1) Denysh Drash syndrome: male, pseudohermaphroditism, Wilm tumor, nephrotic syndrome 2) Frasier syndrome: male pseudoherma-phroditism, gonadoblastoma	1) Early childhood, DMS, ESRD by 3-4 y 2) Childhood onset, FSGS ESRD by 2^{nd}/3^{rd} decade *Also isolated Adult FSGS
Mitochondrial tRNA leucine 1	tRNA Leu (UUR)	Maternal	Associated with MELAS (3)	Adult FSGS
LAMB2	Laminin b2	AR	Peirson's syndrome: microcoria, CNS, DMS (4)	Early childhood NS, <1 y
ITGB4	β4 integrin	AR	Epidermolysis bullosa (5)	Congenital NS, FSGS
SCARB	SCARB2/LIMP2	AR	Action-myoclonus renal failure synd. (6) at 15-20 y	FSGS
CoQ6	CoQ6	AR	Decr CoQ10 biosyn, deafness (7)	Infant-childhood SRNS
CoQ2	CoQ2	AR	Decr CoQ10 biosyn, +/-sz, dev delay (8)	Infant-childhood SRNS
LMX1b	LIM1	AD	Nail patella syndrome (9)	Microalbuminuria, FSGS
MYH9	Non-muscle myosin IIa	AD	Overlap of Fechtner (Alport-like synd) Epstein synd and May Hegglin anomaly; GN, dohle body inclusions, deafness, macrothrombocytopenia, cataracts (10, 11)	Alports/FSGS

DMS, diffuse mesangial sclerosis; NS, nephrotic syndrome.

Genetic screening for steroid-resistant nephrotic syndrome and familial FSGS:

Careful consideration of the benefits and risks of genetic screening must be considered for each individual patient with steroid-resistant nephrotic syndrome and familial FSGS. Genetic testing can be very expensive, and several factors should be considered in the decision making process. To help with this decision, physicians can ask if the results of genetic screening alter one of the following (59):

1) Treatment
2) Care and counseling of patients
3) Counseling of family members
4) Decisions about kidney transplant

The first question deals with whether or not the results of screening influence treatment decisions. The majority of patients with hereditary FSGS are steroid-resistant and may be less responsive to cyclosporine A, as ascertained in small studies (28, 35, 60). Thus, it is likely, but not definitive, that many steroid-resistant patients would not benefit from further immunosuppression. A second consideration is if knowledge of the screening results will affect care and counseling. Mutations in genes known to cause syndromes would warrant examination for extra-renal disease. For example, if a mutation of *WT1* were identified, one would investigate for extra-renal manifestations that often accompany *WT1* mutations, such pseudohermaphroditism in female patients. A third consideration is if screening results would affect family planning. A finding of a mutation in a child with steroid-resistant nephrotic syndrome may prompt the parents of this child to undergo prenatal genetic testing with future pregnancies. Screening may also be of benefit when the child reaches adulthood and is planning parenthood. This is particularly important for autosomal dominant FSGS, in which the risk of transmission is 50%. A final consideration is if the results of screening would affect transplant decisions. Except in cases of *Nphs1* mutations, the risk of recurrence after transplant is very low (~2.5%) (61, 62). Results from genetic screening could also affect donor selection. There have been examples in which a family member donates a kidney, and is subsequently found to have FSGS. Thus, if a mutation is identified in a patient considering transplant, a family member could be screened for that particular mutation as part of the donor evaluation.

In addition to addressing *if* genetic screening is of benefit in individual patients, there are decisions about *what* genetic mutations should be screened. Jack Wetzels and colleagues compiled data from many studies to assess the prevalence of mutations in SRNS and FSGS (59). Pollak and colleagues have also assessed the prevalence of mutations in autosomal dominant FSGS for *IFN2* (9%), *ACTN4* (3%), and *TrpC6* (2%) (63).

In general, children with familial and sporadic steroid-resistant nephrotic syndrome will undergo screening for mutations after a renal biopsy is performed to confirm FSGS histology. In adults, the decision to perform genetic testing is debatable, and the current Kidney Disease Outcomes Quality Initiative (KDOQI) clinical practice guidelines do not recommend screening. However, if a patient chooses to undergo genetic testing,

screening for *Nphs2* mutations could be considered, based on its high prevalence. One could also consider screening for *INF2*, *Actn4, and TrpC6* mutations in patients with familial autosomal dominant FSGS.

APOL1 variant alleles in African Americans predispose to FSGS

Much of our knowledge of the pathogenetic mechanisms that contribute to FSGS comes from studies of rare monogenic mutations and animal models. More recently, data has emerged indicating that common genetic variants contribute to disease susceptibility in FSGS. An important GWAS study led by Martin Pollack's group found an association between genetic variations of the *APOL1* gene with FSGS in African Americans (64). The authors compared the DNA from a cohort of African Americans with biopsy-proven FSGS to African American controls, identifying two strong associations in the last exon of *APOL1*, the gene encoding the Apolipoprotein L-1 protein. These variants were termed G1 and G2. The G1 variant is a two locus allele in which two separate base pair substitutions lead to amino acid changes at the protein level. The G2 variant has a 6 base pair deletion, leading to a two amino acid deletion. The GWAS study found that African Americans with both risk alleles had an OR for FSGS of 10.5 compared to those with neither or 1 allele.

ApoL1 is known to have lytic activity against Trypanosomes by forming pores in lysosomes (65, 66), and *Trypanosoma brucei* species cause African sleeping sickness. However some species have evolved a resistance to ApoL1 lytic activity through production of a lysosomal virulence factor called serum-resistance associated (SRA) protein, which binds and inactivates ApoL1 (65). Genovese *et al.* (64) demonstrated that plasma from people with variant alleles G1 and G2 of ApoL1 lyse resistant clones of *Trypanosoma brucei rhodesiense* while the wild type alleles do not. However, whether the G1 and G2 variants provide a selective advantage, akin to the Haldane hypothesis in which sickle cell trait is protective against malaria, is unknown. In fact, *APOL1* G1 and G2 prevalence is not coincident with *Trypanosoma brucei rhodesiense* endemic regions (67). Thus, whether people with these variant alleles have a selective advantage against African sleeping sickness or perhaps another endemic infection is undetermined.

Subsequent to the initial GWAS study, there have been many additional studies examining the role of ApoL1 risk alleles and kidney disease (For review see (67)). Variant alleles are associated with a broad spectrum of kidney disease, including FSGS, HIV nephropathy, lupus nephropathy and hypertensive nephropathy with large effect sizes (Table 4) (67-71).

Table 4. APOL1 variant alleles and risk of kidney disease among African Americans

DISEASE	N	OR or RR	% with 2 alleles	REFERENCES
Primary FSGS	217	17 (11-26)	72	Kopp *et al.*, 2011
HIV nephropathy	54	29 (13-68)	72	Kopp *et al.*, 2011
Lupus nephropathy	26	5.4 (0.4-12.1)	50	Larsen *et al*. 2013
Lupus ESRD	855	2.7 (1.8-4.2)	25	Freedman *et al.,*2014
HTN nephropathy	675	2.6 (1.8-3.6) & 4.6 (3.1-6.8) in progressors;	23	Lipkowitz *et al.,* 2013

The role of other glomerular cells in FSGS pathogenesis and protection

Although the podocyte is considered central to FSGS pathogenesis, recent data has revived interest in the role of parietal epithelial cells (PECs). Using PEC reporter mice, it was observed that PECs move into the glomerular tuft in FSGS and are present in the crescent lesions (18, 72). PECs are known to deposit extracellular matrix proteins into the glomerular tuft in animal models of FSGS, leading to the hypothesis that PECs contribute to FSGS pathogenesis (18, 72). Subtotal ablation of PECs in mice, via inducible expression of diphtheria toxin in PECs, leads to transient proteinuria and foot process effacement, demonstrating that PEC loss/injury can cause secondary podocyte injury (73). The ablation of PECs also leads to increased PEC proliferation and the formation of cellular crescents.

PECs have also been proposed to be protective in FSGS by serving as a reservoir to replenish lost or dying podocytes. Recent lineage tracing experiments have now disputed this claim, indicating that PECs in adult mice do not transdifferentiate into podocytes (74).

In addition to podocytes and PECs, other cells potentially involved in FSGS include mesangial and glomerular endothelial cells. Mesangial cell expansion is a common and early finding in FSGS. However, a causative role of mesangial cell injury in FSGS has been difficult to ascertain. In primary mesangial diseases, such as IgA nephropathy and the experimental anti-Thy1.1 nephritis, FSGS lesions are sometimes present, but whether or not there is a separate podocyte injury that contributes to the FSGS lesions is unknown. Glomerular endothelial cells are not thought to play a major role in FSGS pathogenesis, as injury or loss only becomes evident at later stages of disease.

Primary FSGS: the role of a “permeability factor”

Several clinical observations suggest that idiopathic FSGS might be caused by a circulating serum factor that increases glomerular permeability. In the early 1970’s John Hoyer and colleagues presented the first two cases of steroid- nephrotic syndrome and biopsy proven FSGS that recurred post-transplant (75). The recurrences occurred within hours to days after the transplant. They hypothesized that a systemic circulating factor exists in these patients. This was met with a great deal of skepticism at the time, as many believed the recurrence of the nephrotic syndrome to be graft rejection, despite no evidence of rejection on renal biopsy. Subsequent studies have confirmed that FSGS does recur with high frequency post-transplant, with estimates of recurrence ranging from 20-50% in adults, and higher in children (76-78).

Further evidence for a circulating permeability factor came in 1984, when the serum from patients with recurrent FSGS or controls was injected into rats. Whereas FSGS serum caused proteinuria, serum from healthy controls and patients with other renal disease did not cause proteinuria (79). As a result of these data, physicians began efforts to eliminate the circulating factor from serum. FSGS patients treated with immunoadsorption or plasmapheresis were reported to have decreased proteinuria and FSGS remission (80, 81). Savin and colleagues not only found a strong beneficial effect

of plasmapheresis, but also a strong correlation between the serum permeability activity (which was determined by measuring the glomerular capillary expansion produced by an oncotic gradient) and recurrence of FSGS within 6 months of kidney transplantation (81). Additionally, the permeability activity decreased with plasmapheresis.

Over the years that followed these initial observations, there were numerous attempts to identify the elusive permeability factor. For example, Savin *et al.* (81) found the circulating factor bound not only protein A and hydrophobic interaction columns, but also had an estimated molecular weight of 50 kD. However, different biochemical data and estimates of molecular weight were obtained among different investigators. These inconsistencies were attributed to the use of different experimental models (Reviewed in (82)).

To determine the causality of a soluble factor's role in promoting FSGS, Jack Wetzels and colleagues proposed a list of four criteria that should be met before a permeability factor is considered pathogenic in FSGS (82):

I	The permeability factor (PF) must have biological effects in vitro and in vivo.
II	PF in patients with the specified disease, but not in controls or other glomerular diseases.
III	Relationship of the PF with disease activity and remission.
IV	Removal or inhibition of PF *in vivo* blocks the biological effect.

In recent years, the two most attractive candidates that have been investigated are soluble urokinase receptor (suPAR) and cardiotrophin-like cytokine 1 (CLC-1). suPAR was identified through a candidate approach based on knowledge of the underlying biology (83). suPAR, which was a known receptor of urokinase, was found to bind and activate integrins, specifically integrin β3. Wei *et al.* (83) found 1) suPAR injection or overexpression in mice causes foot process effacement; 2) suPAR increases in two-thirds of patients with idiopathic FSGS, but not other glomerular diseases; and 3) there is a correlation between a decrease in proteinuria and suPAR activity. However, this initial sPAR study has not been validated by others who, using the same construct, have failed to find increased proteinuria with suPAR injection (84). Thus, the first criterion has not been met. The second criterion was validated with high suPAR levels in 84% of patients in the NIH-FSGS Clinical Trial and 55% of patients in PodoNet consortium (85). However, control patients were not matched for eGFR. Subsequent analysis showed suPAR levels increase as GFR decreases, and this inverse correlation is not specific for FSGS (86); thus, criterion II has not been fullfilled. Criterion III awaits validation, and criterion IV has not yet been performed. Thus, the original enthusiasm by the nephrology community for suPAR has dissipated, although additional studies are ongoing.

Unlike the candidate gene approach used to identify suPAR, Savin and colleagues took an unbiased approach to permeability factor identification based on the observation that oral treatment with galactose decreases permeability activity in a patient with FSGS (87). These investigators hypothesized that the permeability factor

binds galactose. The plasma from this patient was purified over a galactose affinity column, and the affinity purified fraction was subjected to mass spectrometry (88). A candidate protein called Cardiotrophin-like cytokine-1 (CLC-1) was identified. The authors reported that CLC-1 is enriched 100-fold in FSGS; purified CLC-1 increases glomerular permeability; and, antibody to CLC-1 reverses the permeability effect of FSGS sera. Unfortunately, while this abstract was published six years ago, no papers have been published to support the findings.

It is clear that determining the etiology of excessive glomerular permeability would be of significant therapeutic benefit. The identification of a causative protein would allow small molecule or biologic inhibitors to be developed. However, to date, it is unclear if such factor(s) exist and what they are. One possibility is there may be multiple permeability factors, and/or that different patients have different permeability factor(s). Alternatively, the absence of an inhibitor of permeability or the failure to degrade a permeability factor could account for high glomerular permeability in FSGS. Finally, though an endogenous protein seems an ideal candidate, the permeability factor may not be a protein altogether.

FSGS: Conclusions and summary

- FSGS is the most common cause of idiopathic NS.
- FSGS is a clinical-pathologic description.
- Multiple etiologies can cause FSGS (see Fig. 6 below).
- The pathogenesis of FSGS centers on podocyte dysfunction.
- Rare genetic mutations have led to a wealth of information on FSGS pathogenesis and other types of CKD.

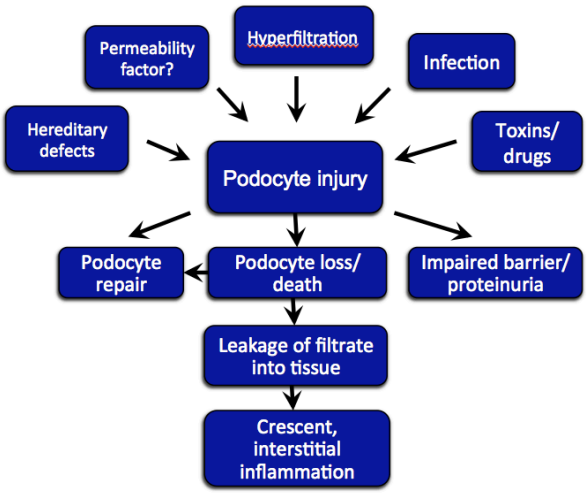


Figure 6: Schematic of etiologies and pathogenesis of FSGS

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