Protecting Podocytes: A Key Target for Therapy of Focal Segmental Glomerulosclerosis

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Keywords

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Abstract

Background: Focal segmental glomerulosclerosis (FSGS) is a histologic pattern of injury demonstrated by renal biopsy that can arise from a diverse range of causes and mechanisms. It has an estimated incidence of 7 per 1 million and is the most common primary glomerular disorder leading to end-stage renal disease in the United States. This review focuses on damage to the podocyte and the consequences of this injury in patients with FSGS, the genetics of FSGS, and approaches to treatment with a focus on the effects on podocytes. **Summary:** The podocyte is central to the glomerular filtration barrier and is particularly vulnerable because of its highly differentiated post-mitotic phenotype. The progressive structural changes involved in the pathology of FSGS include podocyte foot process effacement, death of podocytes and exposure of the glomerular basement membrane, filtration of nonspecific plasma proteins, expansion of capillaries, misdirected filtration at points of synechiae, and mesangial matrix proliferation. Although damage to and death of podocytes can result from single-gene disorders, evidence also suggests a role for soluble fac-

tors, such as soluble urokinase-type plasminogen activator receptor, cardiotrophin-like cytokine-1, and anti-CD40 antibodies, that promote FSGS recurrence post transplant. Several classes of medications, including corticosteroids, calcineurin inhibitors, endothelin receptor antagonists, adrenocorticotrophic hormone, and rituximab, have been shown to be effective for the treatment of FSGS and have been demonstrated to have significant protective effects on podocytes. **Key Messages:** Greater understanding of podocyte biology is essential to the identification of new treatment targets and medications for the management of patients with FSGS.

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Introduction

Focal segmental glomerulosclerosis (FSGS) is a histologic pattern of injury characterized by sclerosis, hyalinosis, foam-cell infiltration, vacuolization of podocytes, and podocyte precursor proliferation. It is “focal” in that only some glomeruli are affected and “segmental” in that only a portion of the affected glomerulus is sclerosed [1]. Idiopathic FSGS is the most common primary glomerular histology in the United States, and one of the leading causes of idiopathic nephrotic syndrome in adults, a key

Table 1. Causes of focal segmental glomerulosclerosis [12]

Type	Cause
<i>Primary</i>	
Idiopathic	Specific cause unknown; some data support mediation by circulating permeability factors
<i>Secondary</i>	
Familial or genetic	Mutations in specific podocyte genes (see Table 3)
Virus-associated	Human immunodeficiency virus type 1, parvovirus B19, simian virus 40, cytomegalovirus, Epstein-Barr virus
Drug-induced	Heroin; interferons alfa, beta, and gamma; lithium; pamidronate; sirolimus; calcineurin-inhibitor nephrotoxicity; anabolic steroids
Adaptive*	<p><i>Conditions with reduced renal mass:</i> oligomeganephronia, very low birth weight, unilateral renal agenesis, renal dysplasia, reflux nephropathy, sequela to cortical necrosis, surgical renal ablation, renal allograft, aging kidney, any advanced renal disease with reduced functioning nephrons</p> <p><i>Conditions with initially normal renal mass:</i> systemic hypertension, acute or chronic vaso-occlusive processes (atheroembolization, thrombotic microangiopathy, renal-artery stenosis), elevated body-mass index (obesity, increased lean body mass [e.g., bodybuilding]), cyanotic congenital heart disease, sickle cell anemia</p>

* The adaptive form is mediated by adaptive structural-functional responses to glomerular hypertension caused by elevated glomerular capillary pressures and flows. Adapted with permission of the Massachusetts Medical Society, from D'Agati et al. [12]; permission conveyed through Copyright Clearance Center, Inc.

prognostic factor in the progression to end-stage renal disease (ESRD) [2]. It has been estimated that FSGS accounts for about 35% of cases of nephrotic syndrome in US adults and 20–55% of cases in US children, with an estimated incidence of 7 per 1 million [3]. A study that included histologic results from 710 patients with glomerulonephritis in Arizona indicated that FSGS accounted for 22.5% of cases [4]. Results from the Olmstead County study indicated that FSGS has an incidence of 1.8 per 100,000 per year and that it increased 13-fold between 1974–1983 and 1994–2003 [5]. This increase in the incidence of FSGS in adults has been noted by other researchers [6, 7]; an increasing incidence of FSGS has also been confirmed in children with idiopathic nephrotic syndrome [8].

African Americans have an increased risk of developing chronic and ESRD, an association that may be related to the presence of 2 common genetic variants (G1 and G2) in the apolipoprotein L1 (*APOL1*) gene [2, 9]. Appreciating the role of *APOL1* was a major step toward an improved understanding of non-diabetic nephropathy in African Americans. The scenario that leads to progression of various forms of kidney disease, including FSGS and progressive chronic kidney disease (CKD) of other etiologies, probably involves *APOL1* susceptibility, modifier loci, and a modifiable environmental “second hit” [10, 11].

It is increasingly recognized that FSGS is not a disease itself; rather, it is a label applied to a specific pattern of

renal injury that may have idiopathic, genetic, or secondary causes (Table 1) [12]. A morphologic classification, commonly referred to as the Columbia classification, is based on light microscopic patterns and describes 5 distinct FSGS variants: not otherwise specified, perihilar, cellular, tip, and collapsing [12, 13]. This classification has been used both as a diagnostic and prognostic clinical tool in studies of FSGS. However, its use may change as we learn more about the pathologies underlying this disorder.

As discussed below, podocytes play a central role in the maintenance of the glomerular filtration barrier. Podocyte loss or damage is a common factor in the pathogenesis of FSGS. This review focuses on damage to the podocyte and the consequences of this injury in patients with FSGS, the genetics of FSGS, and approaches to treatment of this disorder. It is one of a series of articles published in this supplement that summarize the presentations and discussions from a roundtable discussion focused on management of proteinuria in nephrotic syndrome [14] (see Introduction for details).

Podocyte Injury and Loss in FSGS

The podocyte is a highly specialized cell that is an essential component of the glomerular filtration barrier (Fig. 1). A more-detailed description of its morphology

Fig. 1. Schematic representation of the glomerular filtration barrier. The inner surface of glomerular capillaries is decorated by a fenestrated endothelium. The glomerular basement membrane (GBM) is formed by the underlying endothelial cells and overlying visceral epithelial cells (podocytes). Reprinted with permission of the American Society of Nephrology from Möller et al. [102].

Fig. 2. Taxonomy of podocytopathies [17]. Three distinct pathways of injury and repair characterize the podocytopathies. First, in minimal change nephropathy (MCN), podocyte injury is limited to foot-process effacement, and podocyte number remains normal. Second, a more severe form of podocyte injury may occur, leading to podocyte detachment and death. This event initiates an injury cascade that results in the segmental scar characteristic of focal segmental glomerulosclerosis (FSGS). Third, podocyte injury may lead to delayed cellular maturation (DMS), dedifferentiation (CG), and proliferation, with either low rates of cellular proliferation (manifesting as diffuse mesangial sclerosis [DMS]) or high rates of proliferation of parietal cells, some of which can serve as podocyte progenitors [19] (manifesting as collapsing glomerulopathy [CG]). Adapted with permission of the American Society of Nephrology from Barisoni et al. [17]; permission conveyed through Copyright Clearance Center, Inc.

and function is included in the contribution to this supplement by Puneet Garg. The barrier is maintained by the foot process of the podocyte and its associated slit membrane, and foot process effacement is viewed as an accurate indicator of proteinuric glomerular disease [15]. Damage to podocytes is not the sole cause of glomerular diseases, but normal podocyte structure and function are essential for the maintenance of normal glomerular filtration; injury to these cells plays a central role in both the initiation and progression of glomerular diseases [16]. The central role of the podocyte in proteinuric renal disease can be represented as a taxonomy of podocytopathies, which includes minimal change nephropathy, FSGS, diffuse mesangial sclerosis, and collapsing glomerulopathy (Fig. 2) [17]. This scheme recognizes that podocyte injury can be employed to integrate etiology and different podocyte responses to damage (e.g., effacement, apoptosis, arrested differentiation, or dedifferentiation) that give rise to distinct clinical and histologic disease variants [18]. Of note, proliferating cells in collapsing glomerulopathy are no longer thought to be podocytes, but rather parietal cells, some of which can serve as podocyte progenitors [19].

Podocyte damage is the first morphologic characteristic of FSGS that can be detected [20], and it is seen prior to the development of overt sclerosis [21]. Damaged podocytes may undergo a wide range of morphologic changes, including hypertrophy, effacement of the foot process, reduction in the size of the soma, formation of pseudocysts, overload of the cytoplasm, and detachment from the glomerular basement membrane (GBM). Denuding of the GBM results in contact with and attachment of parietal epithelial cells (PECs) and scar formation. Damage-associated changes in podocytes may be reversed, but the scarring that results from denuding of the GBM cannot [22]. Wharram et al. [23] have identified 3 stages of glomerular injury caused by podocyte depletion in transgenic rats.

Stage 1 (0–20% podocyte depletion): mesangial expansion, transient proteinuria, and normal renal function.

Stage 2 (21–40% podocyte depletion): mesangial expansion, capsular adhesions (synechiae), focal segmental glomerulosclerosis, mild persistent proteinuria, and normal renal function.

Stage 3 (>40% podocyte depletion): segmental to global glomerulosclerosis with sustained high-grade proteinuria and reduced renal function.

The authors suggest that this model provides support for the theory that podocyte depletion is a major driver of glomerulosclerosis and progressive renal failure [23]. Al-

though this correlation of the degree of podocyte loss with defined stages of glomerular damage suggests a sequence of structural alterations, the question remains: is there a critical threshold of podocyte depletion in humans below which the development of renal failure is inevitable [24]?

There are about 500–600 podocytes for each glomerular tuft in the adult human kidney [25]. Adult podocytes are terminally differentiated epithelial cells with a very limited ability to proliferate, and podocyte loss following injury can result in a reduction in podocyte number. Podocytes can undergo apoptosis, detachment, or fail to proliferate after damage, and these events may result in a reduction in the number of podocytes (podocytopenia). This decrease contributes to the development and progression of glomerulosclerosis [26]. Importantly, podocytes do not typically proliferate after damage, and those that are lost are not replaced [26]. In addition to disruption of normal filtration, the loss of podocytes may have multiple downstream consequences that contribute to the development and progression of FSGS. For example, results from multiple studies have suggested that vascular endothelial growth factor synthesized by podocytes is necessary for the maintenance of glomerular capillaries and the glomerular endothelium [15]. Podocyte-generated endothelin-1 has also been shown to cause endothelial mitochondrial oxidative damage via endothelin receptor type A, which is required for podocyte loss and glomerulosclerosis. Such podocyte-endothelial crosstalk may represent a new therapeutic target in the prevention of glomerulosclerosis [27].

In reviewing the consequences of podocyte loss, it is also important to consider the limited replacement of these cells. Podocytes might be replaced by PECs that serve as podocyte progenitors. Thus, PECs might be considered as serving a protective role in glomerular diseases by responding to podocyte death with proliferation [28, 29]. However, there is also evidence that PECs contribute to sclerotic lesions. Results from 3 different rodent models as well as findings in biopsies taken from patients with FSGS have indicated that activated PECs invade the glomerular tuft and deposit extracellular matrix. This is associated with a reduction in podocyte number and the development of mesangial sclerosis within the endocapillary compartment [30]. Thus, PEC proliferation secondary to podocyte cell death may have both protective actions (replacement of lost podocytes) and deleterious effects (matrix deposition and contribution to scarring) [25]. It has also been suggested that proliferated PECs might release cytokines and chemokines that could adversely affect glomerular function and perhaps also obstruct urine flow [31].

Fig. 3. Causes of podocytopenia [26]. Following damage, podocytes may die via apoptosis, detach, or fail to proliferate. All of these outcomes result in a reduced podocyte number (podocytopenia) that may ultimately result in glomerulosclerosis. Apoptosis of podocytes results from elevated transforming growth factor- β (TGF- β), angiotensin II, reactive oxygen species (ROS), and reduced levels of cyclin-dependent kinase (CDK) inhibitors p21 and p27. $\alpha 3\beta 1$ is believed to play a critical role in podocyte detachment from the glomerular basement membrane. Adapted with permission of the American Society of Nephrology, from Mundel and Shankland [26]; permission conveyed through Copyright Clearance Center, Inc.

Podocyte Apoptosis

Yes-associated protein (encoded by YAP) is a prosurvival signaling molecule that has been shown to be a physiologic inhibitor of podocyte apoptosis [32]. In a recent study, Cre-mediated recombination controlled by the podocin promoter was used to selectively silence YAP in podocytes. This led to podocyte loss, proteinuria, and elevated serum creatinine. Histology characteristics of FSGS (e.g., mesangial sclerosis, podocyte foot process effacement, tubular atrophy, interstitial fibrosis, and casts) were observed in treated animals [33]. It has also been noted that there is decreased glomerular expression of YAP in patients with primary FSGS [33]. All of these results support the view that YAP plays an important role in the maintenance of normal podocyte structure and function.

Mechanisms Underlying Podocyte Injury

Multiple mechanisms may ultimately lead to podocyte loss or injury (Fig. 3; Table 2) [26, 34]. Critical factors that contribute to initiation of these destructive pathways are summarized in the following sections.

Table 2. Mitotic catastrophe and other types of podocyte death in human and experimental glomerular disease [34]

Cell death type	Definition	Morphologic features	Disease	Experimental glomerular disease
Mitotic catastrophe	Aberrant mitosis	Binucleation, micronuclei, aberrant mitotic spindles	HIVAN, FSGS, MCD, other	Adriamycin nephropathy
Apoptosis	Nuclear death	Nuclear condensation, blebbing, nuclear fragmentation, apoptotic bodies	Uncertain	TGF- β overexpression in cultured podocytes
Autophagy	Nutrient-starvation-induced death	Autophagosomes, autophagolysosomes (transient vacuoles and RER stress)	Lysosomal storage diseases	Puromycin aminonucleoside-induced nephrosis
Anoikis	Absence of cell-matrix interactions	Apoptosis induced by lack of correct cell/ECM attachment	Unknown	
Entosis	Cell cannibalism	Cell-in-cell	Unknown	
Necrosis	Cell lysis	Early: cytoplasmic and nuclear edema Late: plasma membrane rupture, nuclear and cytoplasmic disintegration	Toxic ischemic and necrotizing glomerular injury	
Necroptosis	Regulated necrosis	Cell membrane rupture, oncosis, but no nuclear fragmentation into apoptotic bodies	Unknown	

ECM, extracellular matrix; FSGS, focal segmental glomerulosclerosis; HIVAN, human immunodeficiency virus-associated nephropathy; MCD, minimal change disease; RER, respiratory exchange ratio; TGF- β , transforming growth factor- β . Adapted with permission of the American Society for Investigative Pathology, from Liapis et al. [34]; permission conveyed through Copyright Clearance Center, Inc.

Mutations

Familial and sporadic forms of FSGS have both been linked to mutations in genes encoding key podocyte molecules (Table 3) [13]. Many of the genes associated with podocyte defects are related to the structure of the slit diaphragm, the cytoskeleton of the podocyte (e.g., actin genes), or the foot process, which plays a critical role in interactions between the podocyte and the GBM [13, 20].

Several genes appear to play particularly important roles in the development of FSGS. Studies carried out by Kato et al. [35] have indicated that the Wnt/ β -catenin pathway plays a key role in podocyte adhesion, motility, differentiation, and survival, and that balanced expression of β -catenin is essential for maintenance of the glomerular filtration barrier. Their results also suggested that the downregulation of the Wnt/ β -catenin pathway may increase susceptibility for apoptosis in podocytes [35]. The importance of this pathway in podocyte function/survival is further supported by results showing that *WT1* silencing achieved with transgenic expression of the microRNA miR-193a rapidly induces FSGS in mice with extensive podocyte foot process effacement. Decreased

expression levels of *WT1* result in downregulation of the genes encoding podocalyxin and nephrin, and this significantly impairs the podocyte stabilizing system [36].

There is a lack of direct evidence for the pathogenic role of *APOL1* risk variants in the increased risk for CKD, [11] and further understanding of *APOL1*-mediated kidney injury is required to aid development of therapeutic options. Recently, Beckerman et al. [37] have shown that mice with podocyte-specific expression of either *APOL1* risk allele develop functional (albuminuria and azotemia), structural (foot-process effacement and glomerulosclerosis) or molecular (gene-expression) changes that resemble human kidney disease. This is the first demonstration that the *APOL1* expression causes altered podocyte function and glomerular disease in vivo. Sampson et al. [38] have shown that individuals with the high-risk *APOL1* genotype present with more advanced disease and have less remission of proteinuria over time. Furthermore, transcriptomic data suggest that genotype-associated expression differences reflect an aggravated inflammatory response to a challenge rather than activation of a de novo *APOL1*-specific program.

Table 3. Genes related to FSGS or nephrotic syndrome [13]

Gene	Protein	Function	Phenotype
<i>NPHS1</i>	Nephrin	Podocyte slit diaphragm	Congenital nephrotic syndrome Finnish type, sporadic FSGS, or nephrotic syndrome
<i>CD2AP</i>	CD2-associated protein	Podocyte slit diaphragm	Autosomal-dominant or autosomal-recessive sporadic adult-onset FSGS
<i>NPHS2</i>	Podocin	Podocyte slit diaphragm	Early-onset autosomal-recessive FSGS
<i>ACTN4</i>	α -Actinin-4	Podocyte cytoskeleton	Adult-onset autosomal-dominant FSGS
<i>MYO1E</i>	Unconventional myosin 1E	Actin function	Early-onset autosomal-recessive FSGS
<i>INF2</i>	Inverted formin-2	Actin regulation	Adult-onset FSGS
<i>PTPRO</i>	Receptor-type tyrosine-protein phosphatase 0*	Podocyte signaling	Autosomal-recessive childhood FSGS
<i>ARHGDIA</i>	Rho GDP-dissociation inhibitor 1	Rho GTPase signaling, actin dynamics	Early-onset nephrotic syndrome or FSGS
<i>TRPC6</i>	Transient receptor potential channel 6	Calcium channel, podocyte mechanosensing	Autosomal-dominant or autosomal-recessive sporadic adult-onset FSGS
<i>WT1</i>	Wilms' tumor protein	Podocyte development	Autosomal-dominant sporadic FSGS, diffuse mesangial sclerosis
<i>PLCE1</i>	Phospholipase C ϵ 1	Podocyte differentiation, signaling	Early onset autosomal-recessive FSGS or diffuse mesangial sclerosis
<i>LMX1B</i>	LIM homeobox transcription factor 1- β	Podocyte and GBM development	Nail-patella syndrome, rare FSGS
<i>CD151</i>	CD151 antigen	Podocyte and GBM, laminin-integrin interactions	Early FSGS, deafness, β -thalassemia
<i>LAMB2</i>	Laminin B2 chain	Interacts with integrin α 3 β 1, links GBM to actin cytoskeleton	Autosomal-recessive Pierson syndrome or FSGS
<i>ITGB4</i>	Integrin β 4	Cell-matrix adhesion	Rare FSGS
<i>SMARCA1</i>	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A-like protein 1	Chromatin bundling and gene transcription	Autosomal-recessive Schimke immunosseous dysplasia, childhood FSGS
<i>COQ2</i>	Polyprenyltransferase	Mitochondrial function, deficient coenzyme Q10	Autosomal-recessive early onset nephrotic syndrome or FSGS
<i>COQ6</i>	Ubiquinone biosynthesis monooxygenase COQ6	Ubiquinone biosynthesis	Autosomal-recessive nephrotic syndrome, FSGS, deafness
<i>PDSS2</i>	Decaprenyl diphosphate synthase subunit 2	Coenzyme Q10 synthesis, mitochondrial function	FSGS or collapsing FSGS
<i>ADCK4</i>	AarF domain-containing protein kinase 4	Coenzyme Q10 modulation	FSGS
<i>MTTL1</i>	Mitochondrially encoded tRNA leucine 1	Mitochondrial tRNA	Autosomal recessive MELAS or FSGS
<i>SCARB2</i>	Scavenger receptor class B member 2	Putative lysosomal receptor	FSGS or collapsing FSGS
<i>APOL1</i>	Apolipoprotein L1	Function unknown	Risk of FSGS, collapsing FSGS or HIVAN

* Also known as glomerular epithelial protein 1.

FSGS, focal segmental glomerulosclerosis; GBM, glomerular basement membrane; HIVAN, human immunodeficiency virus-associated nephropathy; MELAS, mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes. Adapted by permission from Macmillan Publishers Ltd, Fogo [13].

Table 4. Circulating permeability factors in primary FSGS: summary of proposed candidates [44]

	Experimental results	Clinical data for FSGS and CKD
suPAR	<ul style="list-style-type: none"> – Administration of suPAR caused albuminuria in uPAR^{-/-} mice; however, not in WT mice – Activation of podocytic αvβ3-integrin leading to cytoskeletal rearrangement – Decrease of nephrin expression via suppression of WT1 	<ul style="list-style-type: none"> – suPAR levels are inversely correlated with eGFR, no discrimination of primary FSGS to other proteinuric diseases – suPAR seems to be a microinflammatory marker in FSGS – suPAR predicts CKD in a cardiovascular cohort – Significance of suPAR levels as a biomarker for FSGS in patients with preserved renal function unclear
CLCF1	<ul style="list-style-type: none"> – Binds to galactose columns and galactose blocked increase in glomerular permeability by FSGS sera – Administration of CLCF1 increases glomerular permeability and proteinuria in mice – Decreases nephrin expression and disrupts the podocytic cytoskeleton – Inhibitors of the Jak/Stat3 pathway abolish CLCF1 and FSGS sera effects 	<ul style="list-style-type: none"> – Concentration of CLCF1 in FSGS patients up to 100-fold higher than in controls; however, available assay too insensitive at the moment – Current data do not support therapy of FSGS patients with galactose – Not yet tested in FSGS cohorts due to measurement difficulties
CD40 autoantibodies	<ul style="list-style-type: none"> – Expressed in glomeruli from FSGS patients – Disrupt podocyte actin cytoskeleton – Injection of CD40 autoantibodies leads to albuminuria only if recombinant suPAR is co-administered – Administration of CD40 autoantibodies does not increase glomerular permeability in CD40^{-/-} mice 	<ul style="list-style-type: none"> – Identified in autoantibody panel from sera of patients with recurrent FSGS

CKD, chronic kidney disease; CLCF1, cardiotrophin-like cytokine factor-1; eGFR, estimated glomerular filtration rate; FSGS, focal segmental glomerulosclerosis; Jak, Janus-kinase; Stat3, signal transducer and activator of transcription 3; suPAR, soluble urokinase plasminogen activator receptor; uPAR, urokinase-type plasminogen activator receptor; WT, wild-type; WT1, Wilms' tumor-1.

Adapted from Königshausen and Sellin [44].

Olabisi et al. [39] reported observations using tetracycline-inducible *APOL1* transgenic T-REx-293 cells as a model that add to the understanding of *APOL1*-mediated toxicity. In particular, they found that the expression of G1 or G2 *APOL1* results in net loss of intracellular potassium and subsequent induction of stress-activated protein kinase pathways, which ultimately results in cytotoxicity. A greater knowledge of the mechanisms of *APOL1*-mediated kidney injury will be required for eventual treatment and improved clinical management.

Circulating Factors

The podocyte is exposed to components of the plasma that penetrate the endothelial cell and GBM layers. As a result, molecules in the plasma are likely to have significant effects on podocyte function and survival [40]. Experimental findings supporting the theory of a circulating factor as the cause of primary FSGS include the first detailed description of the time course and dose-dependence of proteinuria caused by FSGS factor in an animal model

[41] and evidence that circulating factors in the serum of collapsing glomerulopathy patients produce podocyte damage [42]. The concept that circulating factors may be a cause of primary FSGS is supported by a report of the rapid recovery of allograft function after retransplantation of a kidney that was failing in the first recipient due to recurrent primary FSGS. This finding, and the accompanying histopathologic resolution, implies that podocyte injury before scar formation may be reversible [43]. The characteristics of 3 proposed permeability factors in patients with FSGS are summarized in Table 4 [44].

There is evidence from a study in urokinase-type plasminogen activator receptor (uPAR)-knockout (Plaur^{-/-}) mice that soluble uPAR (suPAR) is a pathogenic mediator in FSGS [44]. suPAR is able to activate a specific repressor-signaling pathway that leads to decreased expression of *WT1* and nephrin genes [45]. Wei and colleagues provided information that links suPAR to podocyte dysfunction and FSGS. They showed that suPAR levels are increased in the majority of patients with FSGS when compared with

healthy subjects; this is not the case in patients with other glomerular diseases, for example, those with minimal change disease (MCD), membranous nephropathy, or pre-eclampsia. Using 3 mouse models of renal disease, they also demonstrated that circulating suPAR activates podocyte $\beta 3$ integrin and that this results in podocyte foot process effacement, glomerulopathy resembling FSGS, and proteinuria [46]. Furthermore, studies in proteinuric animal models have demonstrated that bone marrow-derived immature myeloid cells are a source for suPAR in FSGS [47]. While these studies indicate a role for suPAR in the podocytopathy that characterizes FSGS, clinical studies are needed to define its contribution to the development of FSGS and its possible use as a biomarker of disease.

The CD40 axis is important in immunity and inflammation, and it has been shown that the presence of anti-CD40 antibodies is highly predictive for the occurrence of recurrent FSGS after renal transplantation. In addition, anti-CD40 antibodies purified from patients with recurrent FSGS result in significant toxicity in human podocyte cultures. It has been suggested that anti-CD40 antibodies may enhance the pathogenicity of suPAR by binding to CD40 on podocytes and facilitating or prolonging suPAR-mediated integrin activation. This may potentiate increases in enhanced podocyte motility and disruption of the slit diaphragm [48].

Cardiotrophin-like cytokine factor 1 (CLCF1 or cardiotrophin-like cytokine-1) is another candidate for circulating permeability factor in the development of primary FSGS [44]. CLCF1 is a member of the interleukin-6 family and has been isolated from the serum of patients with active FSGS [49]. It has also been demonstrated that CLCF1 activates podocytes and that this results in the formation of lamellipodia and a reduction in basal stress fibers. CLCF1 increases glomerular permeability and the urine albumin:creatinine ratio [50].

These proposed permeability factor candidates have been reviewed by Königshausen and Sellin [44] who note that both CLCF1 and CD40 antibodies require validation by independent research groups. They further note that some studies have questioned the validity of suPAR as a biomarker to distinguish primary FSGS from other proteinuric kidney diseases, as well as suPAR's pathogenic role in podocyte damage. However, more recently, research has shown that decline in kidney function associated with *APOL1* risk variants is dependent on plasma suPAR levels: *APOL1*-related risk was attenuated in patients with lower suPAR, and strengthened in those with higher suPAR levels in 2 large, unrelated cohorts [51]. Furthermore, in children with CKD, elevated

suPAR levels were associated with renal disease progression [52].

Nevertheless, further studies are required to determine whether suPAR levels can identify risk for renal disease progression. Maas et al. [53] suggest that this research should be collaborative to ascertain the validity of findings and define disease causality, and they emphasize the need for appropriate patient phenotyping, the use of appropriate controls, and validation studies. The authors also note that research should recognize that the presence of multiple candidate proteins may indicate that more than one permeability factor is responsible or that increased permeability may not be caused by the presence of a causative permeability factor, but may be related to the absence of an inhibitor.

Finally, plasmapheresis often serves as adjunctive therapy to immunosuppression in patients with recurrent FSGS after transplantation. The mechanism is presumed to be related to the removal of factors that alter glomerular permeability [54]. Successful response to plasma exchange appears to be associated with early initiation of treatment after recurrence, and possibly an early recurrence of disease [54–56]. However, in primary FSGS, plasmapheresis is not effective. The early effectiveness of plasmapheresis post transplant, and the lack of a relationship between plasmapheresis and remission in primary FSGS patients (who are probably at a late stage of disease), suggest that local factors associated with advanced renal injury, or systemic factors unrelated to glomerular permeability, play a significant role in determining proteinuria progression of FSGS during the later stages.

Impaired Autophagy

Autophagy plays a central role in protein and organelle degradation. It is primarily a protective process for the cell, but it can also play a role in cell death [57]. It has been shown that the level of autophagy is high in podocytes under both stressed and non-stressed conditions [58]. Autophagy plays a key role in glomerular maintenance, and induced autophagy in glomerular injury increases the risk factor for glomerular disease and ESRD [59]. Disruption of normal autophagic pathways in the nephrons of mice results in dramatic glomerular and tubular abnormalities by 4 months of age that closely resemble those observed in human disease; organ failure follows by 6 months. Changes in podocytes and tubular cells include vacuolization, abnormal mitochondria, and evidence of endoplasmic reticulum stress. Pathology similar to that demonstrated in these mice is seen in kidney biopsy specimens from patients with FSGS. Kawakami et al. [60] concluded that mitochondrial dysfunction and endoplasmic reticulum

stress resulting from impaired autophagic organelle turnover in podocytes and tubular epithelium are sufficient to cause many of the manifestations of FSGS in mice.

Alteration in Glomerular Structure and Function in FSGS

FSGS is characterized by alterations in normal glomerular structure and function [61]. The normal function of the glomerulus requires that endothelial cells, podocytes, and the GBM, which compose the glomerular filter, are intact and provide a selective barrier for filtration [62]. Failure within this system (e.g., podocyte loss or injury) leads to structural glomerular lesions that represent the starting points for irreversible glomerular injury [63].

It is well established that the podocyte is the major culprit accounting for the progression of chronic renal disease [64]. The term FSGS refers to a disease of primary podocyte injury or a lesion caused by secondary scarring processes in any type of CKD [13]. Glomerular visceral epithelial cells undergo profound morphological changes in collapsing glomerulopathy, such as multinucleation and detachment from the basement membrane, and there is a loss of normal podocytic phenotypes [65]. Furthermore, the loss of specific podocyte markers in collapsing FSGS defines a novel dysregulated podocyte phenotype [66]. In addition, in primary FSGS, some podocytes, occasionally some PECs, and possibly some tubular epithelial cells, undergo a process of transdifferentiation with acquisition of epitopes that are characteristic of activated macrophages [67]. The phenotypic and morphological changes observed in diseased podocytes have been described as an epithelial-mesenchymal transition. However, there is a growing appreciation that this term does not accurately describe the changes. May et al. [68] have suggested the term podocyte disease transformation to describe podocyte dedifferentiation in disease rather than epithelial-mesenchymal transition, as the use of “transformation” suggests a less transient change than “transition”.

The progressive structural changes involved in the pathology of FSGS include podocyte foot process effacement, death of podocytes and exposure of the GBM, filtration of nonspecific plasma proteins, expansion of capillaries, formation of synechiae, misdirected filtration at points of synechiae, and mesangial matrix expansion [62]. Results from a study that correlated changes in structure and function in a cohort of 64 patients with primary FSGS indicated that interstitial fibrosis, synechiae in the Bowman capsule, and global scars were correlated with laboratory measures of

Fig. 4. Survival from renal failure in patients with complete (CR), partial (PR), and no (NR) remission. Republished with permission of the American Society of Nephrology, from Troyanov et al. [74]; permission conveyed through Copyright Clearance Center, Inc.

serum creatinine, plasma albumin, and glomerular filtration rate [69]. Several studies have documented a favorable clinical prognosis based on the histologic subclassification of FSGS [70]. For example, patients with tip variant FSGS have high complete remission rates with therapies that incorporate steroids [71, 72] compared with rates observed in patients with cellular lesions [73].

Treatment of FSGS: Linking Therapy to Pathology

Importance of Achieving Partial or Complete Remission

Achievement of complete (proteinuria value ≤ 0.3 g/day) or partial ($>50\%$ reduction in peak proteinuria and proteinuria value <3.5 g/day) remission is an important predictor of long-term outcomes for patients with FSGS. Results from a cohort of 281 nephrotic patients with FSGS who were followed for a minimum of 12 months indicated significantly slower declines in renal function for patients who achieved these goals versus those who did not (Fig. 4) [74].

Current Guidelines

Initial Treatment

The current Kidney Disease: Improving Global Outcomes (KDIGO) practice guidelines for the initial treatment of patients with FSGS include treatment with in-

Fig. 5. Efficacy of prolonged steroid therapy in idiopathic adult focal segmental glomerulosclerosis [76].

hibitors of the renin-angiotensin system, that is, either an angiotensin-converting enzyme (ACE) inhibitor or angiotensin receptor blocker (ARB) [75]. In addition, corticosteroid and immunosuppressive therapy should be considered only in idiopathic FSGS associated with clinical features of nephrotic syndrome. Calcineurin inhibitors (CNIs) are considered as first-line therapy for patients who failed 16 weeks of corticosteroid therapy or developed intolerance or complications including uncontrolled diabetes, psychiatric conditions, and severe osteoporosis [75]. The efficacy of CNIs in FSGS is considered in the following section.

The benefit of long-term treatment with corticosteroids in patients with FSGS is supported by results from a study of 53 patients that showed that, with administration of corticosteroids, 58% of nephrotic adults with FSGS can achieve partial or complete remission as a first event (Fig. 5) and maintenance of stable renal function for approximately 10 years [76]. The clinical benefit of prednisone treatment in patients with FSGS may be related to the ability of glucocorticoids to decrease podocyte apoptosis and reduce glomerular sclerosis in an animal model of the disease [77]. A direct beneficial effect of steroids on podocytes has been observed in humans. Dexamethasone regulates glucocorticoid receptors, cell maturation and survival, cytoskeleton, and expression of key proteins nephrin, vascular endothelial growth factor, and interleukin-6. Such effects suggest that the effectiveness of glucocorticoids may be a consequence of podocyte modulation and augmented podocyte repair mechanisms [78].

Steroid-Resistant FSGS

The level of steroid responsiveness among FSGS patients is unclear, primarily due to a lack of prospective, controlled trials. The above-mentioned retrospective study in 53 patients with FSGS found that 16 weeks of prednisone therapy (1.0 mg/kg/day) resulted in a complete or partial response rate of 58%. However, a full 32% of these patients experienced a recurrence of their nephrotic syndrome following steroid tapering. When patients who failed primary therapy were given a second course of steroids, only 33% were able to achieve a complete or partial response [76]. Korbet [79] examined the outcome of steroid therapy in FSGS patients from multiple uncontrolled studies and determined that a 2–3 month course of prednisone (1.0 mg/kg/day) resulted in overall remission rates of 47–66%, with complete remission rates of 32–47% and partial remission rates of 19–29%. Although these response rates are suggestive of a positive treatment effect, prolonged glucocorticoid therapy is associated with significant patient morbidity, including excessive weight gain, steroid-induced diabetes, and osteoporosis [80]. The KDIGO guidelines recommend that patients with steroid-resistant disease (defined as persistence of proteinuria despite prednisone 1 mg/kg/day or 2 mg/kg every other day for >4 months) be treated with cyclosporine (3–5 mg/kg/day in divided doses) for at least 4–6 months. If there is partial or complete remission, cyclosporine should be continued for at least 12 months, followed by a slow taper [75].

The use of cyclosporine in patients with steroid-resistant FSGS is supported by results from a randomized trial with a 26-week treatment period that compared cyclosporine plus low-dose prednisone versus prednisone alone in 49 patients with steroid-resistant FSGS. Patients were followed for an average of 200 weeks and study results indicated that 70% of patients on combination therapy had partial or complete remission of their proteinuria by 26 weeks versus 4% of patients on prednisone alone (Fig. 6). There was also better preservation of renal function with combination treatment versus prednisone monotherapy [81]. While the clinical benefit of cyclosporine in FSGS has been attributed to its immunosuppressive effect, it has also been shown that this drug stabilizes the actin skeleton in podocytes [82]. Cattran et al. [83] assessed the permeability of glomeruli to albumin (P_{alb}) in adults with steroid-resistant nephrotic syndrome secondary to FSGS who were involved in the clinical trial to test the efficacy of cyclosporine. In this follow-up study, no association was identified between P_{alb} and partial or complete remission of proteinuria during treatment. Furthermore, the antiproteinuric effect of cyclosporine was independent of

changes in permeability factor level. The researchers suggested that this was consistent with a direct protective effect of cyclosporine on glomerular barrier function that is independent of P_{alb} . Concerns over the long-term nephrotoxicity of cyclosporine, especially in patients with FSGS, warrant careful consideration and monitoring, especially as treatment progresses beyond 1 year [84].

A multicenter, randomized, controlled trial assessed whether treatment with mycophenolate mofetil (MMF) and oral pulses of dexamethasone (DEXA) was more effective than treatment with cyclosporine alone for steroid-resistant FSGS. The incidence of complete and partial remissions after 52 weeks of treatment was 33% in the MMF and DEXA group compared with 46% in the cyclosporine group [85]. An open-label, 6-month trial of MMF in 18 biopsy-proven, steroid-resistant FSGS patients lowered proteinuria in 44% of patients. No patient had a complete remission and relapses were common [86]. Additionally, in a multicenter study in which 98 patients with biopsy-proven primary glomerulonephritis resistant to other drugs received MMF monotherapy for 1 year, 54% of the patients achieved either complete or partial remission of proteinuria [87]. Further studies are needed to assess the future role of treatment with MMF in patients with FSGS.

Tacrolimus has also been employed for the treatment of steroid-resistant FSGS. Results from a study of 44 patients treated with oral tacrolimus and oral prednisolone indicated that complete and partial remissions were achieved in 38.6 and 13.6% of patients, respectively, and that the time to response was 107 days. Responses to this treatment were observed in 66.7% of patients with the tip subtype of FSGS, 54.5% of those with not otherwise specified FSGS, and 37.5% of those with the cellular subtype (Fig. 7) [88].

Treatments Not Currently Included in KDIGO Guidelines

Endothelin Receptor Antagonists

Sparsentan is a dual-acting ARB and endothelin type A receptor antagonist. The efficacy and safety of sparsentan is being investigated in the DUET trial (NCT01613118), a phase-2, randomized, active-control (irbesartan), dose-escalation study with an 8-week, fixed-dose, double-blind period followed by 136 weeks of open-label sparsentan treatment. The primary efficacy endpoint is decreased in the urinary protein:creatinine ratio over 8 weeks of treatment. [89]. Initial results ($n = 96$) indicated that after 8 weeks of treatment, the mean decrease from baseline in proteinuria for patients treated with sparsentan 200, 400, or 800 mg/day was 45 vs. 19% for patients who received 300 mg/day of irbesartan ($p = 0.006$) [90].

Fig. 6. Efficacy of cyclosporine in patients with steroid-resistant focal segmental glomerulosclerosis. Republished with permission of the International Society of Nephrology, from North America Nephrotic Syndrome Study Group [81]; permission conveyed through Copyright Clearance Center, Inc.

Fig. 7. Response to treatment with tacrolimus and prednisolone in patients with steroid-resistant focal segmental glomerulosclerosis (FSGS). NOS, not otherwise specified [88].

Adrenocorticotrophic Hormone

Adrenocorticotrophic hormone (ACTH), which is able to potentially activate all 5 melanocortin receptors, is a member of the proopiomelanocortin family of proteins [91]. Recent animal model and pre-clinical studies have demonstrated that melanocortin type 1 receptor (MCR-1) is expressed in the podocyte and glomerular endothelial cells, as well as in the tubular epithelium [92]. In a puromycin model of nephrotic syndrome, injury to the podocyte leads to an upregulation of MCR-1 receptor, suggesting that activation of this pathway may exert pro-

Fig. 8. Combined complete, partial, or no response rates for patients with steroid-resistant focal segmental glomerulosclerosis (FSGS) after 6 months of adrenocorticotrophic hormone (ACTH) alone and 6 months of combined ACTH and tacrolimus. Reproduced from Tumlin et al. [96]. Open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

protective effects on the podocyte. When cultured podocytes were incubated with a specific MCR-1 agonist, the level of caspase-3-dependent apoptosis was reduced [93]. It has also been shown that an ACTH gel reduced the severity of tumor necrosis factor-induced acute kidney injury, reversed acute renal dysfunction, and decreased mortality in a rat model. It also improved the viability of tumor necrosis factor-exposed tubular epithelial cells in vitro [91].

Monotherapy with ACTH and the combination of ACTH and tacrolimus have both suggested potential effectiveness in patients with steroid-resistant and steroid-dependent FSGS. A retrospective analysis was performed on findings from 24 patients with nephrotic syndrome from idiopathic FSGS. Results indicated 2 complete remissions and 5 partial remissions at the end of treatment (median dose, 80 units subcutaneously twice weekly), with a cumulative remission rate of 29%. Two responders relapsed during the follow-up period [94]. A multicenter retrospective case series included 15 patients with FSGS, most of whom (80%) had previous immunosuppressive or cytotoxic therapy, who were treated with ACTH gel 80 U twice weekly for ≥ 6 months. Twelve patients (80%) attained partial remission ($\geq 50\%$ reduction in proteinuria from baseline and final proteinuria 500–3,500 mg/day) or clinical response ($\geq 30\%$

reduction in proteinuria from baseline that did not meet the criteria for complete or partial remission). An additional patient who had terminated treatment (no reason provided) had attained partial remission prior to termination [95].

A prospective study of combination therapy included 22 patients with steroid-resistant nephrotic syndrome (9 with idiopathic membranous glomerulonephritis, 13 with FSGS), all receiving an ACE inhibitor, ARB, and/or mineralocorticoid receptor antagonist for 4 weeks prior to treatment. Patients were treated with ACTH gel (40–80 units, 2–3 times a week) for 6 months and then had tacrolimus (1–3 mg twice daily) added if they achieved no response or partial remission. After 6 months of ACTH therapy, 1 of the 13 patients with FSGS (7.7%) achieved complete remission while 62% achieved partial remission. The remaining 12 patients went on to receive oral tacrolimus in combination with ACTH therapy. After 6 months of combined ACTH-tacrolimus therapy, 17% achieved a complete remission, with the partial remission rates rising to 66% (Fig. 8) [96].

Although clinical experience has shown reduction in proteinuria in multiple glomerular nephropathies, the dataset is limited because there was no comparison group for interpretation of safety and efficacy findings. Furthermore, the limited pre-clinical data may not reflect the physiology of FSGS in humans, and the exact mechanism of action is unknown.

Rituximab

Rituximab is being used increasingly in patients with steroid-dependent and steroid-sensitive nephrotic syndrome (MCD and FSGS). Results in kidney transplantation recipients with FSGS recurrence in a small multicenter retrospective study ($n = 19$) suggest that rituximab therapy may be useful in patients who have failed either the initial treatment or weaning from plasmapheresis [97]. Rituximab also reduced the incidence of recurrence and the need for maintenance/induction immunosuppression and maintained remission in patients with steroid-dependent or frequently relapsing idiopathic nephrotic syndrome (10 children, 20 adults; including 8 with FSGS) [98]. Rituximab treatment of high-risk FSGS patients has been associated with lower incidence of post-transplant proteinuria and might prevent recurrent FSGS after kidney transplantation, potentially through a direct regulation of podocyte function, as rituximab directly preserves SMPDL-3b and ASMase activity in podocytes [99, 100].

Kronbichler et al. [101] have conducted a systematic review of 14 studies involving 86 adult patients with either steroid-dependent or frequently relapsing MCD or FSGS. Overall, rituximab reduced the number of relapses

and the use of immunosuppressants. After rituximab treatment, there was a statistically significant reduction in the relapse rate per year from a mean of 1.3 before treatment to zero after treatment. Rituximab treatment decreased proteinuria, increased serum albumin, and reduced the need for immunosuppression. Larger controlled trials are needed to evaluate the mechanism of action and the efficacy of rituximab in these patients.

Conclusions

FSGS is the most common primary glomerular disorder causing ESRD. It manifests as proteinuria and hypertension, and, in the worst cases, progresses to kidney failure. Diagnosis is based on histopathological features observed on traditional kidney biopsy. Although the pathogenesis of FSGS is not fully understood, podocyte damage leading to apoptosis and foot-process effacement is a key factor. Podocytes are involved in the formation of the glomerular barrier, and dysfunction or depletion can result in the development of proteinuria. Recent studies have identified the importance of podocyte structural changes, alterations with metabolic signaling pathways, and the role of circulating factors and the contribution of PECs to sclerotic lesions. An increased level of circulating suPAR has been identified as one of the possible causes of podocytopathy in FSGS, and it has been proposed as a potential diagnostic FSGS biomarker. The discovery of noninvasive biomarkers to aid the diagnosis of FSGS is essential.

There is a strong association between *APOL1* and progression of renal disease. *APOL1* G1 and G2 variants confer an increased risk of development of ESRD and a greater rate of decline in renal function. Variants G1 and G2 of *APOL1* are common in populations of African descent who have an increased risk of ESRD compared with those of European descent, and progress more quickly once they develop CKD. Evidence linking *APOL1* to ESRD has promoted a search for *APOL1* inhibitors. The future identification of other FSGS genes may reveal specific targets respon-

sible for the development of FSGS and will hopefully translate into the development of novel therapeutic approaches.

FSGS poses a significant health burden, but treatment options are few and have limited efficacy; only a small proportion of patients will achieve complete remission. Therapies available include corticosteroids, CNIs, endothelin receptor antagonists, ACTH, and rituximab, which have been shown to be effective for the treatment of FSGS and have all been demonstrated to have significant protective effects on podocytes. Immunosuppression therapies are currently not recommended.

Continuing growth in our understanding of podocyte biology may change our future diagnostic strategies and is likely to result in the identification of new treatment targets and medications for the management of patients with FSGS.

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