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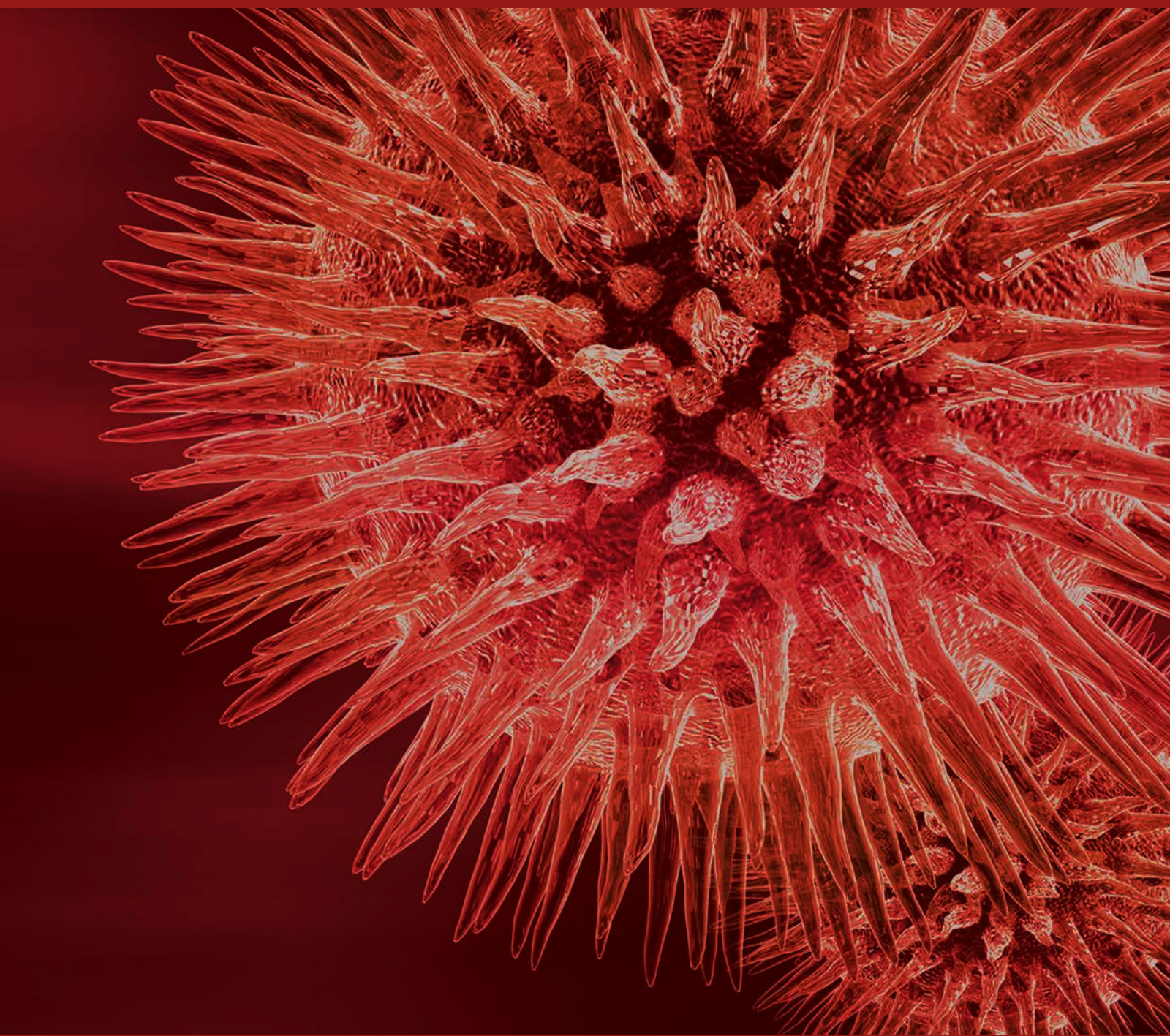
Focal Segmental Glomerulosclerosis: Genetics, Mechanism, and Therapies

Guest Editors: Andreas Kronbichler, Jun Oh, Björn Meijers, and Jae Il Shin



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Editorial

Focal Segmental Glomerulosclerosis: Genetics, Mechanism, and Therapies

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Focal segmental glomerulosclerosis (FSGS) is one of the primary glomerular disorders in both children and adults which can progress to end-stage renal failure. Recent advances in cell biology and genetics have found new molecules and signaling mechanisms in podocytes for the pathogenesis of FSGS. In addition, evidence-based approaches will guide to the best and appropriate therapeutic options both in children and in adults with FSGS in this special issue.

The exact steps in the etiopathogenesis of FSGS are incompletely understood. In an attempt to summarize findings related to the immunologic changes, A. Kronbichler et al. provide an overview of T-cell, CD80 (B7-1), complement, chemokines/cytokines, macrophages, and B-cells in FSGS. Moreover, the authors highlight a potential transition into current and future potential therapeutic options. E. Königshausen and L. Sellin reviewed potential circulating permeability factors in primary FSGS that have been implicated in the pathogenesis due to the potential recurrence in renal allografts after kidney transplantation (KT), focusing on the soluble urokinase plasminogen activator receptor (suPAR), cardiotrophin-like cytokine factor-1 (CLCF-1), and CD40 antibodies. Secondary FSGS is an emerging cause of progressive kidney function decline. With the increased understanding of podocyte biology, several steps have been identified leading to podocyte injury and eventually glomerulosclerosis. In an elegant overview, J. S. Kim et al. provide an overview of common causes leading to secondary FSGS, including adaptive (with reduced or normal renal mass), drug-induced, genetic, or infection-related disease forms.

Regarding pathologic classifications of FSGS, M.-H. Han and Y.-J. Kim reviewed the Columbia classification, which distinguishes five variants (collapsing, tip, cellular, perihilar, and not otherwise specified) and has been widely used over the past 10 years, and pointed out the confusion about terminology of variants and difficulty in its application and the interpretation of lesions with mixed features in the same tissue specimen or evolution of the lesions. For the diagnosis of FSGS, B. Sprangers et al. reviewed on the diagnostic approaches and work-up to the patients using the potential biomarkers of FSGS, genetic testing, and histology.

Treatment of primary FSGS remains a challenge for the treating physicians, since repeated doses of long-term steroid use and steroid-dependence and steroid-resistance are leading to significant morbidities in both children and adults. K. H. Han and S. H. Kim reviewed on the current updated strategies for treatment of primary FSGS in children, including traditional therapies consisting of corticosteroids and calcineurin inhibitors and novel therapies such as rituximab, abatacept, adalimumab, and fresolimumab. A. Beer et al. focused on studies reporting on treatment outcome published during the last two decades. Unfortunately, there has not been

a major progress in the treatment of adults and novel substances, such as sirolimus; adalimumab or galactose should not be considered as effective immunosuppressive measures. While rituximab, mycophenolate mofetil, or calcineurin inhibitors may be used in steroid-dependence, evidence is limited for the former two substances in resistant cases and other strategies such as extracorporeal treatment (immunoadsorption or plasma exchange) and alkylating agents may be used.

FSGS is known to recur after KT and is an important cause of graft loss early after transplantation. H. G. Kang et al. reviewed on the risk factors of FSGS recurrence after KT in children, potential biomarkers for predicting recurrence, treatment of FSGS recurrence after KT, and the strategies to prevent recurrences. M. Rudnicki reviewed on FSGS recurrence in adults after KT, emphasizing prophylactic and perioperative treatment with plasmapheresis and high-dose (intravenous) cyclosporine as the main cornerstones of immunosuppressive therapy and recent promising results of rituximab therapy.

Articles published in this special issue covered the various fields of FSGS such as pathophysiologic mechanisms of primary and secondary FSGS, histopathologic considerations, potential biomarkers and diagnostic work-up, evidence-based therapeutic strategies, and recurrence of FSGS after KT. Understanding theses various faces of FSGS will lead to better patient treatment and outcome.

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Review Article

FSGS: Diagnosis and Diagnostic Work-Up

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Focal segmental glomerulosclerosis is a histologic lesion, rather than a clinical disease. FSGS is common cause of nephrotic syndrome in both adults and children worldwide. In the United States it is the most common primary glomerular disease resulting in end-stage renal disease and recent reports have suggested that its incidence might be on the rise. Currently the incidence is estimated to be 7 per million. The podocyte is the cellular target cell in FSGS and in recent years substantial insight in the pathogenesis and genetics of FSGS have accumulated. Furthermore the discovery of potential novel biomarkers to diagnose FSGS and monitor disease activity has renewed interest in this disease. In this review article we will focus on the clinical presentation and diagnosis of FSGS.

1. Introduction

FSGS is estimated to be responsible for 40% of adult nephrotic syndromes and 20% of pediatric nephrotic syndromes and has an incidence of 7 per million [1]. FSGS has an estimated prevalence of 4% and is the most common primary glomerular disease resulting in end-stage renal disease in the United States [2]. FSGS is a histologic lesion resulting from glomerular injury primarily affecting the podocytes and characterized by the presence of sclerosis in parts (segmental) of some (focal) glomeruli. Initially lesions are confined to a limited number of glomeruli and are segmental in nature. FSGS is commonly termed primary or secondary. Primary FSGS is caused by a primary podocytopathy while secondary FSGS denotes the development of FSGS lesions as an adaptive phenomenon following a reduction in nephron mass, direct toxicity by drugs or viral infections, or healing from endothelial injury. Upon identification of FSGS in a renal biopsy a thorough examination should be initiated to identify the underlying cause. The distinction between primary and secondary FSGS is important for both prognostic and therapeutic reasons.

2. Epidemiology

In the United States FSGS is the most common histologic lesion found in patients with adult nephrotic syndrome accounting for 35% of all cases and even 50% of cases among individuals from African American descent [3]. The frequency of FSGS as cause of adult nephrotic syndrome in African Americans is 2-3 times higher compared to Caucasian individuals [3]. In the time period 1975 to 1994, FSGS accounted for 57% of cases in blacks (versus 23% in whites), and its prevalence in blacks increased from 39% to 64% in 1975–1984 and 1985–1994, respectively [4]. Furthermore, African Americans with FSGS have a higher likelihood of a family history of ESRD [5]. The high incidence of FSGS in African Americans with the nephrotic syndrome and the genetic predisposition to this lesion in blacks (vide infra) explains in part why reported incidences in other countries are considerable lower. For example, in a 2004 Spanish study, membranous nephropathy (24%) was the most common cause of nephrotic syndrome in patients between 15 and 65 years with FSGS only being the 4th most common cause (12%) [6]. However, a number of studies show the incidence of FSGS is increasing in non-blacks in both Canada and the

United States [7] (Dan Cattran, personal communication). A study of the United Renal Database System demonstrated that idiopathic FSGS is the most common primary glomerular disease resulting in end-stage renal disease in both black and white people in the United States [2]. Data suggest that FSGS as cause of ESRD is on the rise from 0.2% in 1980 to 2.3% in 2000 with African Americans at an increased risk [2].

3. Clinical Presentation

Proteinuria is the most common presenting feature of FSGS. In patients with primary FSGS the full nephrotic syndrome is very common and often associated with hypertension, microscopic hematuria, and some degree of renal insufficiency [8, 9]. In secondary FSGS, patients tend to have subnephrotic-range proteinuria at presentation (although nephrotic range proteinuria will develop in the majority over time) in the absence of oedema, hypoproteinaemia, or hypoalbuminaemia [10, 11].

FSGS can be classified according to etiology into the following (see Table 1).

Primary or idiopathic FSGS are undefined circulating factors that mediate abnormal glomerular permeability with podocyte injury and dedifferentiation.

Secondary FSGS are as follows:

(1) Familial/genetic:

cfr (Table 1).

(2) Virus-associated:

HIV, parvo B19, CMV, EBV, hepatitis C, and Simian virus 40.

(3) Drug-induced:

heroin, interferon, lithium, pamidronate, sirolimus, and anabolic steroid.

(4) Mediated by adaptive structural-functional responses to the following:

✓ reduced renal mass with hyperfiltration and stretch on podocytes:

oligomeganephronia,
unilateral renal agenesis or hypoplasia,
renal dysplasia,
reflux interstitial nephropathy,
surgical renal ablation,
obesity,
low birth weight,
chronic allograft nephropathy,
extensive loss of functional nephrons secondary to any advanced renal disease.

✓ Ischemia:

(malignant) hypertension,
cholesterol crystal emboli,
renal artery stenosis,
atheroemboli or other acute vasoocclusive processes,
hypertensive nephrosclerosis,
cyanotic congenital heart disease,
renal transplant rejection,
calcineurin-inhibitor toxicity.

FSGS can histologically be subdivided according to the Columbia classification into the following:

(i) Classical FSGS or FSGS NOS (not otherwise specified).

(ii) Collapsing variant (although there is discussion whether this is truly FSGS or rather a distinct pathology).

(iii) Tip variant.

(iv) Perihilar variant.

(v) Cellular variant.

About 80% of cases are primary or idiopathic. FSGS is by some authors believed to be closely related to minimal change disease and both diseases are postulated to be part of the same spectrum of diseases (podocytopathies) [12]. Injury of the podocytes gives rise to foot process effacement and proteinuria. The distinction between primary and secondary FSGS can be made on both clinical and histologic bases. Nephrotic range proteinuria without the full nephrotic syndrome is suggestive for secondary FSGS. Secondary FSGS is usually associated with slowly increasing proteinuria (initially subnephrotic) [10, 11], lower prevalence of nephrotic syndrome, higher serum albumin, lower serum cholesterol, lower rate of edema, and progressive renal insufficiency over time [10, 13, 14]. Interestingly, despite massive proteinuria most patients with secondary FSGS (due to obesity, reflux nephropathy, or renal mass reduction) usually do not develop full blown nephrotic syndrome [13]. The precise reason for this dichotomy is not clear but has been suggested to be related to the development of secondary compensatory mechanism during a more gradual appearance of proteinuria in secondary FSGS [13]. Another histologic variant where massive proteinuria may not be associated with the development of edema is the collapsing FSGS variant. This is, however, believed to be related to accompanying rapid loss of GFR [15–17]. The distinction between primary and secondary FSGS has important therapeutic implications as primary FSGS often requires immunomodulatory treatment whereas treatment in secondary FSGS should be focused on the reduction of intraglomerular hypertension using RAAS blockade.

In infants with steroid-resistant nephrotic syndrome, a genetic defect can be identified in up to two-thirds of patients [18]. Furthermore, genetic testing is more likely to identify a genetic basis of FSGS in young children, patients with syndromic disease, or a positive family history. In a Spanish study in patients with steroid-resistant nephrotic syndrome, the percentage of patients in whom a genetic basis could be identified in congenital-onset, infantile-onset, early and late childhood onset, adolescent-onset, and adult-onset was

Table 1: Genetic defects associated with FSGS.

	Gene	Inheritance	Location	Function of the encoded protein
Slit diaphragm proteins				
(i) Nephrin	NPHS1	AR	19q13.1	Member of the immunoglobulin family, cell adhesion molecules
(ii) Podocin	NPHS2	AR	1q25 31	Regulation of glomerular permeability
(iii) CD2 associated protein	CD2AP	AD (AR)	6p12	Scaffolding molecule that regulates the actin cytoskeleton
Cell membrane associated proteins				
(i) Transient receptor potential cation channel 6	TRPC6	AD	11q21 22	Receptor-activated calcium channel in the cell membrane
(ii) Protein tyrosine phosphatase receptor type O	PTPRO	AR	12p22	Member of the R3 subtype family of protein tyrosine phosphatases at the apical surface of polarized cells
(iii) Laminin 2	LAMB2	AR	3p21	Family of extracellular matrix glycoproteins in the basement membranes
(iv) 4 integrin	ITGB4	AR	17q11	Transmembrane glycoprotein receptors
(v) Tetraspanin CD151	CD151	AR	11p15	Member of the transmembrane 4 superfamily, cell-surface proteins
(vi) LIM homeobox transcription factor 1β	LMX1B	AD	9q33.3	Member of LIM-homeodomain family, transcription factor
Cytosolic or cytoskeletal proteins				
(i) Actinin 4	ACTN4	AD	19q13	Member of the spectrin gene superfamily, cytoskeletal proteins
(ii) Phospholipase C1	PLCE1	AR	10q23 24	Member of the apolipoprotein C1 family, role in HDL and VLDL metabolism
(iii) Myosin heavy chain 9	MYH9	AD	22q12.3	Nonmuscle myosin, involved in cytokinesis, cell motility, and maintenance of cell shape
(iv) Inverted formin 2	INF2	AD	14q32	Member of the formin family, function in de- and polymerization of actin filaments
(v) Myosin 1E	MYO1E	AR	15q21 26	Member of the nonmuscle class I myosins, involved in intracellular movement and membrane trafficking
(vi) Rho GDP-dissociation inhibitor 1	ARHGDIA	AR	17q25	Key role in the regulation of signaling through Rho GTPases
Nuclear proteins				
(i) Wilms tumor 1	WT1	AD	11p13	Transcription factor, role in the normal development of the urogenital system
(ii) SMARCA-like protein	SMARCAL1	AR	2q34 36	Member of the SWI/SNF family, transcription factor
Mitochondrial components				
(i) tRNAleu	mtDNA A3243G	Maternal	mtDNQ	Function unknown
(ii) Parahydroxybenzoate polyprenyltransferase	COQ2	AR	4q21 22	Functions in the final steps in the biosynthesis of CoQ
(iii) Coenzyme Q10 biosynthesis monooxygenase 6	COQ6	AR	14q24.3	Member of the ubiH/COQ6 family, required for the biosynthesis of coenzyme Q10
(iv) Decaprenyl diphosphate synthase subunit 2	PDSS2	AR	6q21	Enzyme that synthesizes the prenyl side chain of coenzyme Q or ubiquinone
(v) AarF domain-containing protein kinase 4	ADCK4	AR	19q13.2	Precise function unknown (possible protein kinase activity)
Lysosomal proteins				
(i) Lysosomal integral membrane protein type 2	SCARB2	AR	4q13 21	Type III glycoprotein located in limiting membranes of lysosomes and endosomes
Unknown cellular location				
(i) Apolipoprotein L1	APOL1	AR	22q12	Secreted high density lipoprotein, involved in the formation of cholesteryl esters and efflux of cholesterol from cells

100, 57, 24 and 36, 25, and 14%, respectively [19]. A study of the PodoNet Consortium found a 11% overall percentage of disease-causing abnormalities in adolescent with steroid-resistant nephrotic syndrome [20].

Histologically FSGS is classified in 5 different subtypes: perihilar, tip, collapsing, cellular, and not otherwise specified [21]. A recent study evaluated renal biopsies of 138 patients included in the FSGS Clinical Trial to study the association between histologic subtype and clinical features and outcome [22]. The histologic subtype was associated with clinical features: patients with NOS FSGS were more likely to present with subnephrotic proteinuria whereas patients with tip or collapsing variants tended to be older and have higher degrees of proteinuria and hypoalbuminemia at presentation [22]. Tip variant in this study was associated with Caucasian race, lower baseline creatinine, and rate of progression. In contrast, the collapsing variant was associated with African American descent, elevated baseline creatinine, and higher rate of progression [22]. The perihilar form is common in secondary FSGS in patients with obesity, hypertension, reflux nephropathy, or renal agenesis, and patients usually have subnephrotic proteinuria. The tip form is usually primary presenting with sudden onset of nephrotic syndrome and has a good prognosis (high response rate to glucocorticoids and low risk of progression). The cellular variant can be both primary or secondary and is the least common variant usually presenting with a nephrotic syndrome. The NOS can also be primary or secondary and is the most common variant. The collapsing subtype can be both primary or secondary and has an infaust prognosis (severe nephrotic syndrome resistant to immunosuppressive treatment and associated with rapid progression to renal failure).

Both clinical and histologic features have been reported to be predictive towards outcome. African American descent, degree of proteinuria, and renal insufficiency have been associated with poor outcome. Chun et al. have reported that the attainment of partial of complete remission is associated with better long-term outcome in primary adult FSGS [8]. As histology is concerned, increased degrees of interstitial fibrosis and tubular atrophy are a predictor of poor outcome.

4. Diagnostic Work-Up

When confronted with a patient with FSGS a careful medical history and clinical examination should be performed. The presentation (sudden onset of nephrotic syndrome or more subtle gradual changes) and associated medical conditions (infections, obesity, hypertension, etc.) help to make a distinction between primary and secondary FSGS. Also careful attention should be paid to the medications and drugs the patient is using. Lab testing including viral serology, kidney function, serum albumin, and lipids should be performed. A biopsy is necessary to establish the diagnosis of FSGS and determine the subtype of FSGS. Genetic screening of patients with steroid-resistant nephrotic syndrome should be performed when FSGS occurs early in life as the likelihood of identifying a genetic basis for FSGS is high. Genetic screening of adolescent/adult patients with FSGS can be done relatively quickly these days but its place in clinical practice is not clear at this moment. The interpretation of negative results especially remains problematic as these patients may have mutation in noncoding regions of candidate genes or mutations in novel genes not yet reported. Undoubtedly at this time there are many more genetic causes FSGS to be discovered. Exome sequencing is expected to dramatically improve our knowledge in this respect; however, exome sequencing requires specialized bioinformatics support for analysis. It has been suggested by some authors to screen also adolescents and adults with steroid-resistant nephrotic syndrome to avoid unnecessary exposure to second-line immunosuppressive therapies [20]. However, the therapeutic implications of results from genetic screening as some patients with WT1-mutation associated steroid-resistant nephrotic syndrome have been reported to have a favorable response to cyclosporine [23]. In the setting of transplantation, genetic testing could be useful as it has been demonstrated that patients with inherited forms of FSGS have a low risk of recurrence after transplantation [24, 25].

5. Histology

Initially FSGS lesions are concentrated in the corticomedullary region and are therefore easily missed on biopsy. Cellular changes within the podocyte precede scarring as the initial event will affect the podocyte attachment to the glomerular basement membrane. Denudation of the glomerular basement membrane will result in sticking of capillary loops and collapse. Subsequently, sclerosis, deposition of hyaline material, adhesions to Bowman's capsule, and synechiae formation will develop. Even segmental lesions will result in glomerular dysfunction due to misdirected filtration and filtrate spreading on the remaining part of the nephron [26]. FSGS is probable not as focal and segmental as suggested by its name. Some subtypes of FSGS the histologic lesions even are not focal, segmental, or sclerotic, that is, glomerular tip lesion and collapsing glomerulopathy. In animal models of FSGS almost all glomeruli show sclerotic lesions on three-dimensional morphometric analysis [27]. As the volume of the sclerotic lesion is on average only 12.5% of the total glomerular volume, the number of glomeruli affected by sclerosis is grossly underestimated on conventional single section kidney biopsy evaluation [28]. As a consequence, a kidney biopsy containing only few glomeruli cannot exclude FSGS. It is to be advised that consecutive sections are evaluated from 12 to 15 serial sections and at least 8 glomeruli are analyzed [21, 28]. Furthermore, initial changes of FSGS can be limited and only detectable by electron microscopic examination. Therefore, it is not uncommon that after an initial biopsy showing no clear FSGS lesion a subsequent biopsy taken months or years later shows clear FSGS lesions [29]. With progression of the disease more widespread and global glomerulosclerosis develops together with tubulointerstitial and vascular lesions.

Histologically FSGS is classified in 5 different subtypes: perihilar, tip, collapsing, cellular, and not otherwise specified

[21]. A study from the Columbia group demonstrated that the most common FSGS variant in all FSGS patients was NOS or perihilar variant (62.3%) followed by collapsing (23.7%), tip (9.4%), and cellular (3%) variants [30]. Another study reported similar data: NOS, 42%; perihilar, 26%; collapsing, 11%; tip, 17%; and cellular, 3% [31]. In the FSGS Clinical Trial the incidences in children and young adults with steroid-resistant FSGS were 68% in FSGS not otherwise specified, 12% in collapsing, 10% in tip, 7% in perihilar, and 3% in cellular variants [22, 32]. The rates of complete and partial remission have been shown to be related to the histologic subtype: with the highest remission rates for tip variant, intermediate remission rate for cellular, perihilar, and NOS variants, and the lowest remission rates for the collapsing variant. Moreover, several reports have demonstrated that the histologic variant of FSGS is independently associated with outcome [30, 31]; in an analysis of the FSGS Clinical Trial in which patients between 2 and 40 years old with steroid-refractory primary FSGS were randomly assigned to receive either cyclosporine or dexamethasone plus MMF the risk of ESRD at 3-year follow-up was 47% in collapsing, 20% in not otherwise specified, and 7% in tip variant patients [22]. In general, the outcome for secondary FSGS is better than that for primary forms as a consequence of increased likelihood to obtain remission with RAAS blockade and lower serum creatinine at presentation [11].

Histologically secondary FSGS is predominantly associated with the perihilar variant and limited foot process effacement confined to sclerotic areas, whereas foot process effacement is diffuse in primary FSGS [10, 12]. Foot process effacement is most severe in cases of primary FSGS while being relatively limited in secondary forms of FSGS and there is little overlap between them [33]. As the variants of primary FSGS are concerned: foot process effacement is more pronounced in tip, cellular, and collapsing variants, while it is variable in the not otherwise specified variant and limited in the perihilar variant [12]. Some forms of secondary FSGS are associated with widespread foot process effacement: HIV-associated FSGS [16] and FSGS associated with use of interferon [34] and pamidronate [35].

6. Biomarkers

SuPAR has been proposed to be a new marker to diagnose and predict FSGS recurrence after transplantation and monitor treatment response in patients with primary FSGS [36–38]. The findings of the initial paper by Chicago group could not be confirmed by other groups and an important inverse relationship between suPAR and eGFR has become evident [39–45]. Moreover, urinary suPAR has been proposed as a marker as well [46]. Thus, although suPAR may be a marker for progressive renal damage [47], it cannot be considered a biomarker for FSGS. More recently, it has been suggested that podocyte expression of B7-1 (CD80) may help to differentiate between primary and secondary FSGS. CD80 is under normal condition not expressed on human podocytes and has been shown to be upregulated in patients with MCD and primary FSGS [48]. A recent report suggested that CD80 expression on podocytes could differentiate primary from secondary FSGS and predict response to abatacept [49]. Urinary CD80, however, appears to be elevated in minimal change disease (especially minimal change disease in relapse) and not in FSGS [50–52]. Moreover, other groups have either not been able to stain FSGS podocytes for B7-1 or, when able to stain, not been able to show a response to costimulatory blockade as seen with abatacept [53, 54]. Recently positive B7-1 staining of podocytes has been found in one group in both animal models and patients with diabetes mellitus [55]. Again other groups have not been able to confirm these results [56]. Thus, until there is consistent and reproducible staining techniques for this biomarker, there will be no clinical test for FSGS using it.

The detection of activated parietal epithelial cells immunohistochemically has been shown to be able to make a distinction between early FSGS and minimal change disease [57] and show similar patterns in both primary and secondary FSGS [58]. FSGS lesions can also be associated with rare genetic diseases (Dent's disease) [59] and tubulopathies [60]. Concerning tubulopathies causing FSGS lesions it was suggested by Sethi and colleagues to include a comparison of urinary protein/creatinine ratio to a urinary albumin/creatinine ratio in the diagnostic work-up of FSGS [61]. If <40–50% of total proteinuria is the result of albuminuria the possibility of tubular proteinuria or the presence of light chains should be excluded.

7. Genetic Testing

Several genes, instrumental in podocyte homeostasis, have been reported to be associated with genetic forms of FSGS and these findings have propelled the field of podocyte biology in the last decade (Table 1) [62]. Numerous genetic defects have been associated with FSGS [63] and these genetic forms account for a significant proportion of patient with steroid-resistant disease in young children [64, 65]. Most of the genetic defects are located in genes coding for proteins involved in glomerular basement membrane formation and/or podocyte biology. Both autosomal dominant and autosomal recessive inheritance have been described. As penetrance is not 100% the detection of familial or genetic FSGS can be difficult. A family history and onset in early life are suggestive for genetic forms of FSGS. Histology does not allow for the differentiation between genetic and nongenetic forms of FSGS except in NPHS1 and alpha-actinin-4 mutations. The usefulness of genetic testing in the setting is disputed and is dependent on the presence of a familial history and onset in early life [19, 66]. In the first year of life the most common causes of genetic FSGS are mutations in nephrin and podocin genes which present in an autosomal recessive manner and with a full blown nephrotic syndrome. In contrast, during adolescence and early adulthood most cases of genetic FSGS are caused by autosomal dominant forms caused by mutations in TRPC6 and alpha-actinin-4 [18, 19]. Genetic abnormalities in the inverted formin 2 gene may be one of the more common forms of hereditary FSGS [67]. FSGS in adulthood is rarely

caused by a genetic abnormality and often proteinuria is not massive [19]. Commercial tests are currently available to detect NPHS1 and NPHS2 mutations but not the alpha-actinin-4, TRPC6, or CD2AP genes.

It is well established that FSGS is more prevalent and the course of disease is more severe as well in African American individuals. Besides socioeconomic factors, genetic factors such as variants in the apolipoprotein 1 gene are closely related to the development of nondiabetic kidney diseases in African Americans. Initial studies pointed to variants in the MYH9 gene (which is located closely to the APOL1 gene) as risk factor for kidney disease in African Americans [68, 69]. Subsequent studies showed that, however, a strong linkage disequilibrium exists in the chromosomal region of APOL1 and MYH9, and it is now accepted that the MYH9 haplotype simply reflects APOL1 variation [70]. APOL1 risk variants are associated with 17-fold higher odds for FSGS and 29-fold higher odds for HIV-associated nephropathy [71]. In recent studies, APOL1 risk genotype was determined in a subset of patients included in the FSGS Clinical Trial [72]. The 2 risk alleles were predominantly present in African American patients and were associated with collapsing variant and an increased risk of progression to ESRD. Interestingly, APOL1 risk alleles did not influence the response to treatment [72]. These APOL1 risk alleles for kidney disease have been associated with resistance to African trypanosomiasis and are geographically restricted [70, 73, 74]. There is an ongoing discussion whether African American individuals with subnephrotic-range proteinuria, with an FSGS lesion on biopsy associated with only segmental foot process effacement, and with the APOL1 risk alleles should receive a diagnosis of FSGS or should alternatively by diagnosed with APOL1-associated nephropathy.

8. Conclusion

Focal segmental glomerulosclerosis is a histologic lesion, rather than a clinical disease. FSGS is common cause of nephrotic syndrome in both adults and children worldwide. FSGS is divided into primary and secondary FSGS. This distinction is mainly important for therapeutic reasons. The podocyte is the cellular target cell in FSGS and in recent years substantial insight in the pathogenesis and genetics of FSGS have accumulated. Furthermore the discovery of potential novel biomarkers to diagnose FSGS and monitor disease activity has renewed interest in this disease.

Competing Interests

The authors declare that they have no competing interests.

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Review Article

Practical Application of Columbia Classification for Focal Segmental Glomerulosclerosis

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Focal segmental glomerulosclerosis (FSGS) is a heterogeneous clinicopathological entity. Two frameworks for the classification of FSGS have been described: etiologic and morphologic. The etiologic classification is distinguished among genetic, adaptive, virus-associated, drug-induced, and idiopathic types. Morphologic classification is commonly referred to as the Columbia classification published in 2004, which distinguishes five variants: collapsing, tip, cellular, perihilar, and not otherwise specified (NOS). This classification is based on light microscopic patterns with rigorously defined specific criteria, which can be applied to primary and secondary forms of FSGS, and has been widely used over the past 10 years both as a diagnostic and as a prognostic clinical tool. This paper defines common histopathological features of FSGS, distinguished characters among five variants, and points out the confusion about terminology of variants, because most were proposed in the past with different definitions. Despite good interobserver reproducibility of this classification system, difficulty in its application may arise in the interpretation of lesions with mixed features of more than one variant in the same tissue specimen and with late lesions, because other variants may evolve into the NOS variant over time.

1. Introduction

Focal segmental glomerulosclerosis (FSGS) is the name of the primary glomerular disease as well as the terminology describing the secondary scar phenomena by injury of other glomerular diseases. Since the first descriptions by Fahr and Rich, several different histologic variants of FSGS have been described [1]. Histologically, it is characterized by sclerosis, hyalinosis, foam cell infiltration, vacuolization of podocytes, and podocyte proliferation. Mixed use of the term FSGS and heterogeneous morphologic features cause confusion both in making a diagnosis and in correlating with underlying pathogenesis. We attempted to clarify the morphologic terminologies for featuring FSGS and described practical application of the Columbia classification [2] and discussed confusion points of previous subtypes: tip, cellular, and collapsing variants.

2. Common Histologic Features

2.1. Focal and Segmental Lesions (Figure 1). "Focal" is defined as a focal lesion that affects some glomeruli. Thus, occasionally, only one sclerotic glomerulus can be found despite a diligent search in a renal biopsy specimen [3]. "Segmental" is defined as a lesion partially involving a single glomerulus. The unaffected glomeruli show normal finding. In case of absence of sclerotic glomeruli in a renal biopsy, differentiation of FSGS from "minimal change disease" (MCD) is difficult.

2.2. Sclerosis and Hyalinosis (Figure 2). Sclerosis, the representative and typical change of FSGS, is a vascular change showing stiffness and obstruction similar to arteriosclerosis. As sclerosis progresses, proteinaceous material resulting from plasmatic insudation may be found in sclerotic glomerulus. The proteinaceous material shows a glassy pink appearance

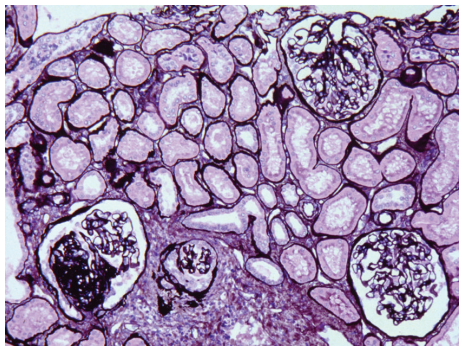


Figure 1: Segmental sclerotic glomerulus is at left lower and right two glomeruli look normal. Interstitial fibrosis and tubular atrophy are observed focally. Artery shows intimal fibrosis. Jones methenamine silver stain (PAM), ×200. (Permitted by the Journal of Korean Society of Pediatric Nephrology [3].)

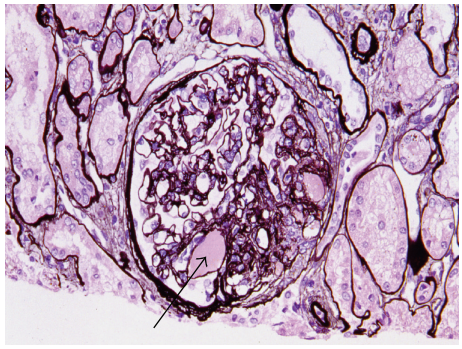


Figure 2: Perihilar variant of FSGS. Sclerosis is observed at the glomerular vascular pole. Hyaline (arrow) is the amorphous material in the middle of sclerosis, PAM, ×200. (Permitted by the Journal of Korean Society of Pediatric Nephrology [3].)

in H&E stain and is thus called "hyalinosis." This lesion turns pale pink in periodic acid-Schiff (PAS) stain and dark red in trichrome stain. Hyalinosis was considered a characteristic lesion of FSGS in the past; thus, the term "focal segmental hyalinosis" was used. It is still used together with FSGS, occasionally [1]. Because they have morphologic similarity, differentiation of a scar from sclerosis sometimes can be difficult. Therefore, it is necessary to examine the nonsclerotic glomeruli and check the clinical features for differential diagnosis from a scar.

2.3. Vacuolization of Podocytes. Vacuoles may be observed in cytoplasm of podocytes as a result of damage. This lesion is considered evidence that FSGS is attributable to damage of podocytes. Vacuolization is more clearly observed using the electron microscope.

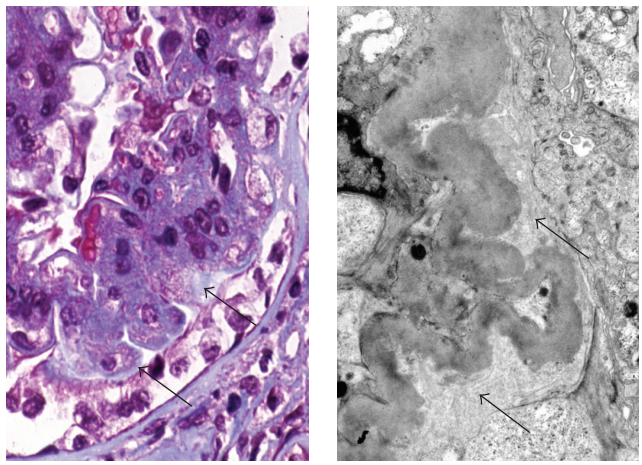
2.4. Halo Formation (Figure 3). Podocytes in involved glomeruli may be detached from the glomerular capillary basement membrane. The space between the podocyte and the glomerular capillary basement membrane is filled with new collagen fiber. In trichrome stain, the collagen fiber shows a paler blue than other capillaries. This seems like the appearance of the "halo" of the moon and is observed better using the electron microscope.

2.5. Distribution and Location of Sclerosis. In early FSGS, only a few glomeruli are involved, and these show small sclerotic lesions. Sclerosis initially occurs in the juxtamedullary area [4]. This is believed to be due to the fact that the juxtamedullary area shows high blood pressure and high blood flow [5]. Glomeruli in upper cortex are involved last. If the corticomedullary junction is not included in a biopsy specimen, the diagnosis of FSGS would likely be missed. In one glomerulus, sclerosis occurs in a peripheral area rather than center and adhesion between Bowman's capsule and sclerotic lesion is often exhibited. Correlation between distribution of sclerosis and prognosis has been studied for a long time. The tip lesion (Figure 4), which occurs in an adjacent area to the origin of the proximal tubule, is noted for showing the most favorable prognosis [6, 7]. However, as sclerosis progresses, it is difficult to determine the original location of sclerosis.

2.6. Global Sclerosis. As segmental sclerosis is progressed, entire glomerulus becomes involved. Terminal FSGS shows global sclerosis in most glomeruli. However, because global sclerosis may normally occur with age, it should not be concluded that FSGS would be somewhere [8, 9].

2.7. Glomerulus without Sclerosis (Unaffected Glomerulus). In cases of unaffected glomerulus, due to normal findings (Figure 4), it cannot be differentiated from MCD. However, in morphometric study, it was proved that glomerulus showing no sclerosis in FSGS was increased slightly compared to the normal size for the age [10]. Therefore, when the size of glomerulus is larger than normal, FSGS should be considered, even if there is no renal glomerulosclerosis in the biopsy specimen.

2.8. Hypercellularity. FSGS is essentially a nonimmunologic disease; therefore, cell proliferation is not the fundamental lesion in FSGS. Mesangial cell proliferation, believed to be a feature of FSGS in the past, can be confused with other diseases including like IgA nephropathy with FSGS pattern. Proliferation of podocytes may be found in FSGS. In 1985, Schwartz and Lewis classified these lesions as a feature of proliferative FSGS [11]. However, it has since been excluded from the cellular type and recognized as characteristic changes in the collapsing type (Figure 5) [2]. In cases involving severe proliferation of podocytes, it is often confused with the crescent. Endocapillary proliferation can also occur in FSGS. Excessive infiltration of inflammatory cells and many foam cells are recognized as features of cellular type of FSGS (Figure 6). Foam cells are considered as vascular endothelial cells or monocytes including mainly fat of the plasma component.



(a) (b)

Figure 3: Halo formation. In trichrome stain (a), the pale zone (arrow) is between sclerosis and overlying podocytes. Trichrome stain, ×400. Some areas on the electron micrograph (b) are filled with newly formed thin collagen bundles (arrows). ×5000. (Permitted by the Journal of Korean Society of Pediatric Nephrology [3].)

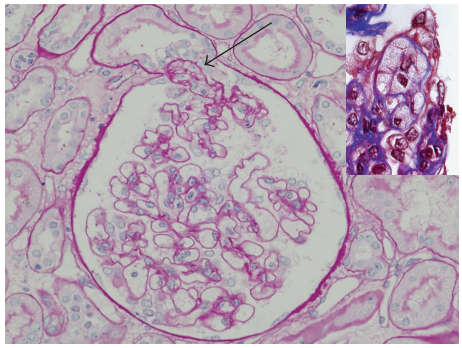


Figure 4: Tip variant of FSGS. Foam cell accumulated segment prolapsed into the tubular pole, the origin of the proximal tubule. The remainder of the glomerular tuft appears normal. PAS stain, ×200. (Courtesy of Professor Mi Sun Choi, DongSan Hospital of Keymyung Medical College, Daegu, Korea.) Inset shows endocapillary foam cells in trichrome stain. ×400.

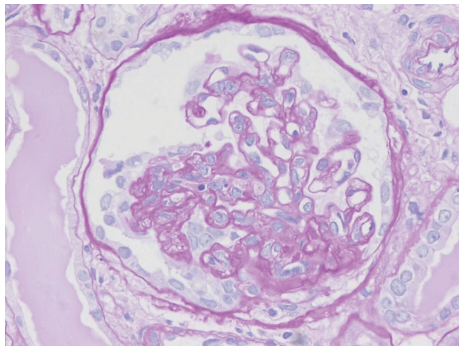


Figure 5: Collapsing variant of FSGS. Segmental collapse of glomerular capillaries is accompanied by proliferation of overlying podocytes. PAS stain, ×200.

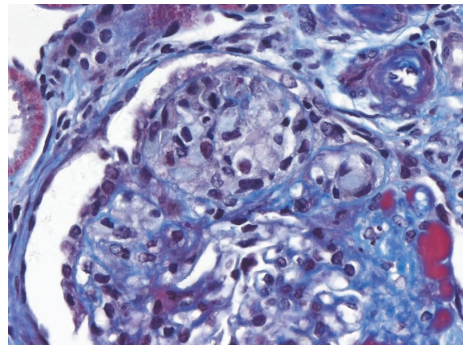


Figure 6: Cellular variant of FSGS. Segment is expanded by endocapillary foam cells. Overlying epithelial cells are also prominent, but capillary collapse is not observed. Trichrome stain, ×200.

2.9. Tubules and Interstitium (Figure 1). Focal tubular atrophy, interstitial fibrosis, and lymphocytic infiltration are features of FSGS. The severity of these lesions is associated with the severity and the number of involved glomeruli, but it is not necessarily proportional to the severity. Most MCD do not show these changes in tubules and interstitium. Therefore, these are important findings that are more consistent with FSGS rather than MCD, particularly in cases of suspicious FSGS clinically but no definite sclerotic lesion was found in a biopsy specimen [8, 9].

2.10. Vessel (Figure 1). Findings such as arterial and arteriolar nephrosclerosis, accompanied by high blood pressure, appear in the blood vessels of FSGS (i.e, the thickening of arterial wall, intimal fibrosis, and sometimes subendothelial hyaline deposition). As these findings become severe, glomerulosclerosis and the damage of interstitium and tubules

Table 1: Columbia classification of FSGS variants.

Variant	Inclusion criteria	Exclusion criteria
FSGS (NOS)	At least 1 glomerulus with segmental increase in matrix obliterating the capillary lumina. There may be segmental glomerulus capillary wall collapse without overlying podocyte hyperplasia.	Exclude perihilar, cellular, tip, and collapsing variants.
Perihilar variant	At least 1 glomerulus with perihilar hyalinosis, with or without sclerosis. >50% of glomeruli with segmental lesions must have perihilar sclerosis and/or hyalinosis.	Exclude cellular, tip, and collapsing variants.
Cellular variant	At least 1 glomerulus with segmental endocapillary hypercellularity occluding lumina, with or without foam cells and karyorrhexis.	Exclude tip and collapsing variants.
Tip variant	At least 1 segmental lesion involving the tip domain (outer 25% of tuft next to origin of proximal tubule). The tubular pole must be identified in the defining lesion. The lesion must have either an adhesion or confluence of podocytes with parietal or tubular cells at the tubular lumen or neck. The tip lesion may be cellular or sclerosing.	Exclude collapsing variant. Exclude any perihilar sclerosis.
Collapsing variant	At least 1 glomerulus with segmental or global collapse and overlying podocyte hypertrophy and hyperplasia.	None.

FSGS, focal segmental glomerulosclerosis; NOS, not otherwise specified.
Reprinted from [13].

become more severe, resulting in greater reduction of renal function.

2.11. Immunofluorescence Findings. Because FSGS is essentially a nonimmunologic disease, immunologic deposition is not present in FSGS in principle. However, deposition of C3 and IgM is sometimes found in the site of sclerosis, particularly in the site where hyaline material is deposited [6]. That is not attributable to the immunologic reaction and is a nonimmunologic and nonspecific finding as the result of combination with the absorbed or retained plasma protein. Thus, IgM, the most common among immunoglobulin, and C3 are demonstrated in the lesions [3]. In unaffected glomeruli, there is no deposition and, even if there is, it is nearly negligible.

2.12. Electron Microscopic Findings. The sclerotic segment shows wrinkled basement membrane and foamy macrophages containing bubble-shaped protein and lipid droplets. Increased electron density, a result of fusion of wrinkled basement membrane, may sometimes be mistaken for the immune-type deposit. However, it shows no clear shape of electron dense deposits in certain types of immune complex glomerulonephritis and is not present in nonsclerotic area [3]. Effacement of foot processes, mainly observed in MCD, is seen in unaffected areas. Although the severity of effacement of foot processes is variable according to the amount of urinary protein excretion, it is generally less in FSGS than in MCD. This lesion is not discriminated between FSGS and MCD, but in FSGS, unlike MCD, vacuolization and proteinaceous material are frequently found in cytoplasm of podocytes.

3. Application of Columbia Classification (Table 1)

Two frameworks for the classification of FSGS can be described: etiologic and morphologic. The first, etiologic classification, is distinguished among genetic, adaptive (hyperfiltration), virus-associated, drug-induced, and primary or idiopathic types [12]. The second, morphologic classification, is commonly referred to as the Columbia classification published in 2004, which describes five distinct FSGS variants: collapsing, tip, cellular, perihilar, and not otherwise specified (NOS), which is based on light microscopic patterns [13] and has been widely used over the past 10 years both as a diagnostic and as a prognostic clinical tool. This classification system can be applied to both primary and secondary forms of FSGS but should not be confused with pathogenic mechanisms in the development of that defined lesion. On the other hand, in the Columbia classification each variant of FSGS is rigorously defined by specific criteria, tremendously reducing the confusion about the terminology that characterized the last two decades of the 20th century.

3.1. Collapsing Variant (Figure 5). This type is characterized by the presence of at least one glomerulus with collapse and overlying podocyte hypertrophy and hyperplasia, regardless of the presence of other lesions resembling the other four variants of FSGS [13]. Thus, the finding of a single collapsing lesion trumps all other variants. Podocyte proliferation within Bowman's space sometimes has a "pseudocrescent-like" feature. Collapsing lesions are more commonly global than segmental and are often accompanied by severe tubulointerstitial injury with microcysts and hypertrophic tubular

epithelial cells swollen with hyaline protein reabsorption droplets. Foot process effacement is usually diffuse. Most cases are either idiopathic in origin or HIV-associated and are more commonly found in black patients [14, 15].

3.2. Tip Variant (Figure 4). Diagnosis of tip variant, after excluding collapsing and perihilar lesions, requires at least one segmental lesion involving the "tip" domain, the outer 25% portion of the glomerular tuft next to the origin of the proximal tubule with either extracellular matrix adhesion or confluence of podocytes with parietal or tubular epithelial cells at the tubular lumen or neck [13]. Tip lesions are typically cellular (81%) and contain prominent endocapillary foam cells, but they may be sclerosing and contain hyaline [16]. The tip variant typically shows only mild chronic tubulointerstitial injury and arteriosclerosis [2]. Foot process effacement is typically severe and diffuse. Most cases of tip lesion FSGS are idiopathic in etiology and predominate in white adults [17]. Most pure tip lesions have a very good prognosis and response to steroid therapy [2, 18].

In the early description of the glomerular "tip lesion" by Howie and Brewer [6], it was not restricted to FSGS but rather a novel report of a curious glomerular abnormality seen independently in patients with proteinuria and was also found in other heterogeneous renal abnormalities with associated proteinuria, including membranous glomerulopathy and diabetic glomerulosclerosis. However in Columbia classification tip variants contained nontip segmental lesions (75%) involving the periphery of the tuft [10]. Thus, the presence of a nontip lesion of this variant differs from the original description. And, because it may occur in association with other glomerular diseases, the question among renal pathologists is whether a tip lesion simply represents a protrusion of the tip of the glomerulus into the tubular pole and a nonspecific glomerular abnormality in response to proteinuria or a variant of MCD [18, 19].

3.3. Cellular Variant (Figure 6). This is defined by identification of at least one glomerulus with endocapillary hypercellularity (including foam cells, macrophages and other leukocytes, and endothelial cells, occasionally associated with hyalinosis, karyorrhexis, and fibrin) involving more than 25% of the glomerular tuft, leading to occlusion of the capillary lumen [13]. Because foam cells may be seen in other FSGS subtypes, the diagnosis requires exclusion of tip lesion and collapsing variants [6]. Cellular lesions are typically found in the peripheral tuft [2]. This variant may lack any evidence of segmental scars, mimicking a focal proliferative glomerulonephritis. Foot process effacement is usually severe [2]. This is the least common variant but has poor prognosis [20].

In contrast with the original description of a cellular lesion [21], the Columbia classification restricts the hypercellularity to the endocapillary compartment of the glomerulus and does not (as originally reported) occur with collapse of the glomerular basement membranes. Therefore, podocyte abnormalities are not a defining feature for the cellular variant. Stokes and D'Agati [2] pointed out a problem in recognition of cellular lesions because endocapillary foam cells are not a specific feature, but they may occur to some degree in other variants. What distinguishes the cellular variant is the expansile, purely cellular nature of the endocapillary lesions which typically lack appreciable extracellular matrix.

3.4. Perihilar Variant (Figure 2). The variant is defined by the presence of at least one glomerulus with perihilar hyalinosis with or without sclerosis and sclerotic lesions at the glomerular vascular pole (perihilar) in more than 50% of affected sclerotic glomeruli [13]. Tip lesion, collapsing, and cellular variants must be excluded [13]. This form has been described in both primary FSGS and secondary adaptive forms stemming from nephron loss or glomerular hypertension (i.e., due to obesity, reflux nephropathy, hypertension, and sickle cell disease), usually accompanied by glomerular hypertrophy. In the adaptive conditions, reflex dilatation of the afferent arteriole leading to glomerular hypertension may cause particular stress on the perihilar segment [20]. Foot process effacement is usually focal and relatively mild.

3.5. FSGS NOS Variant. Finally, this applies to a renal biopsy that does not meet the criteria for any other variant with findings of focal and segmental consolidation of the glomerular tuft by increased extracellular matrix, leading to obliteration of glomerular capillary lumen [13]. This is the most common subtype, and, interestingly, it has been observed from repeat biopsies that other variants may evolve into FSGS NOS over time [17, 22].

4. Limitation of Classification

In general, the detection of a FSGS depends on the percentage of glomeruli affected, the size of the segmental lesions, and the number of serial sections studied. Lesions may be lost during histologic preparation as well as due to sampling error, leading to underdiagnosis. Thus, biopsy size is also an important component of assessment of focal lesions. It is estimated that a minimum of 25 glomeruli is necessary to detect a low prevalent lesion [17]. Multiple sectioning may be necessary especially to define tip lesions or cellular variant. Of even further importance, the biopsy must include the juxtamedullary region. Because some FSGS variants, including perihilar type, initially started from juxtamedullary glomeruli, superficial cortical biopsy samples may not contain lesions. Despite good interobserver reproducibility of this classification system [23], difficulties in application of this classification may arise in the interpretation of lesions with mixed features of more than one Columbia type of FSGS in the same tissue specimen. In addition, because other variants may evolve into the NOS variant over time, such variants may be less frequent in biopsies obtained late in the disease course. Thus, the spectrum of FSGS lesions likely includes dynamics related to time of biopsy, as well as divergence of initial pathogenic insults. The histologic phenotype, thus, gives clues to both stage and type of initial injury [24].

Barisoni et al. [25] suggested that collapsing glomerulopathy and glomerular tip lesion should be classified separately from FSGS because of the lack of sclerosis. However,

it is reasonable to include them within the spectrum of FSGS, because both of these entities demonstrate segmental glomerular lesions and may be accompanied by "classic" FSGS lesions [17]. And the findings of different clinical characteristics in both variants suggest that these morphologic variants reflect distinct biological pathways regardless of etiology [2].

This classification is only based on glomerular changes and does not mandate tubulointerstitial injury, global glomerulosclerosis, interstitial inflammatory changes, or degree of effacement of foot processes in electron microscopic changes. All those have been considered as prognostic indices. Further study of the biopsy, including immunohistochemistry and electron microscopy, may help in further understanding the spectrum of segmental lesions. And recent tremendous advances in discovery of genetic and molecular mechanisms of renal diseases are, indeed, the case of FSGS [26–29]. This group of authors recently suggested a new approach to classification of diseases with primary FSGS that integrates conventional histologic features with etiology.

5. Conclusion

Ideally, classification of a disease should be based on pathogenesis and/or etiology, but the Columbia classification is just based on light microscopic morphologic changes. However, when we think about the fact that at the time of biopsy, usually, causes of FSGS in patients were unknown, this morphologic classification has been a useful working proposal to identify subgroups. Using this morphologic classification, significant differences in baseline clinical characteristics and outcomes among the variants of had been demonstrated in several studies, especially tip and collapsing variants [2, 20, 24].

For further identification of clinical significance, morphologic studies including tubulointerstitial changes and findings of immunohistochemistry and electron microscopy would be necessary. And a goal of future investigations should be to correlate morphologic variants with etiology especially genetic factors.

Competing Interests

The authors declare that they have no competing interests.

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Review Article

Recurrence and Treatment after Renal Transplantation in Children with FSGS

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Focal segmental glomerulosclerosis (FSGS) is a common cause of end-stage renal disease and a common pathologic diagnosis of idiopathic nephrotic syndrome (NS), especially in steroid-resistant cases. FSGS is known to recur after kidney transplantation, frequently followed by graft loss. However, not all patients with FSGS suffer from recurrence after kidney transplantation, and genetic and secondary FSGS have a negligible risk of recurrence. Furthermore, many cases of recurrence achieve remission with the current management of recurrence (intensive plasmapheresis/immunosuppression, including rituximab), and other promising agents are being evaluated. Therefore, a pathologic diagnosis of FSGS itself should not cause postponement of allograft kidney transplantation. For patients with a high risk of recurrence who presented with classical symptoms of NS, that is, severe edema, proteinuria, and hypoalbuminemia, close monitoring of proteinuria is necessary, followed by immediate, intensive treatment for recurrence.

1. Introduction

Focal segmental glomerulosclerosis (FSGS) is a common cause of end-stage renal disease (ESRD). In children, the major causes of ESRD are congenital anomalies of the kidney and urinary tract and hereditary nephropathies, followed by FSGS as the most common form of acquired glomerulopathies causing ESRD [1, 2]. FSGS is the second-most common pathologic diagnosis of idiopathic nephrotic syndrome (NS) [3, 4]. Although the majority of pediatric idiopathic NS patients respond to steroid treatment, some are resistant to treatment and eventually progress to ESRD [5], and their renal pathology often reveals FSGS. Because FSGS is known to recur after kidney transplantation, frequently followed by graft loss in up to 60% of the cases [6–9], the diagnosis of idiopathic FSGS requires a thorough discussion of its prognosis with patients and their families. However, not all patients with FSGS suffer from recurrence after kidney transplantation, and many cases of recurrence achieve remission with the current management of recurrence and enjoy life as a kidney allograft recipient for as long as the average kidney recipient [10–12]. In this paper, the current knowledge of the risk factors for recurrence of FSGS and its treatment in children will be reviewed.

2. Who Is at Risk of Recurrence and Who Is Not?

The reported rates of recurrence are quite variable, from 6 to 58%, depending on the characteristics of the population studied [11–14]. The suggested risk factors for recurrence include the age at onset of disease [14–16], a rapid progression to ESRD (<48–72 months) [17–21], and a history of previous recurrence in an allograft [6, 18, 22]. Pathologic characteristics of the native kidney biopsy, such as mesangial hypercellularity [23] and fewer sclerotic glomeruli [19, 20], and a living donor allograft [24] have also been proposed as risk factors but have not been confirmed [25]. Native kidney nephrectomy prior to kidney transplantation has been suggested by some as a preventive measure of recurrence [21, 26, 27], but it has not been effective and has even shown a higher risk

of recurrence in other reports [10, 28, 29]. According to our experiences with 38 children with FSGS, most of those with a later onset (≥6 yrs. old) and a progression to ESRD in the 24–72 months after onset of NS experienced recurrence, whereas those who had an earlier onset (<6 yrs.) of NS with a faster progression (<18 months) did not have recurrence [11]. There has been controversy over the onset age group that is at risk of recurrence; generally younger patients are considered to be at a higher risk than older patients [13], but some studies have reported no differences between adults and children [30] and even higher risks in adults than in children [25]. The main reason for these differences could be the small sample size of the study populations in most of the reports. In addition, two more aspects should be considered.

First, there are several genetic defects that cause FSGS [31–33], and the frequency and distribution of the genetic types of FSGS differ between populations. For example, the *NPHS2* mutation is the dominant cause of genetic FSGS in European countries, but it is rare in Koreans and the Japanese [31, 34–36]. Although idiopathic steroid-resistant NS (SRNS) with FSGS pathology is believed to be caused by some circulating factors [37] and is therefore prone to recur after kidney transplantation, most genetic FSGS have defective components of the kidneys, particularly podocytes, and therefore their risk of recurrence is low if not zero [16, 32, 36, 38–40]. Some genetic FSGS are characterized by an early onset of SRNS; some syndromic FSGS are accompanied by extrarenal symptoms that may not be evident at the onset of SRNS, thus mimicking idiopathic SRNS. Because a genetic diagnosis of SRNS-FSGS has not yet been incorporated as a routine component of clinical practice in most parts of the world, we do not know how many of the patients previously categorized as SRNS-FSGS have genetic FSGS. In fact, some of the cases that we previously reported as idiopathic SRNS-FSGS were recently found to have mutations in *COQ6* [41] (in patients with progressive hearing loss) or a newly found FSGS-causing gene *NUP107* [42] (unpublished data); these patients had an earlier onset (<6 yrs.) of NS with a faster progression (<18 months) and did not have recurrence [11]. A recent report by Ding et al. showed that children with SRNS who initially responded to steroid treatment were at risk of recurrence after kidney transplantation [43]. This finding may also imply that these cases have nongenetic FSGS. In other words, the wide range of risks of recurrence found in the literature seems to have been dependent on whether genetic testing was broadly performed in the studied cohort.

Second, FSGS is a pathologic diagnosis, and there are multiple causes other than idiopathic SRNS that lead to FSGS [44]. Some of the causes are evident, such as chronic infection (e.g., HIV infection) or reflux nephropathy; however, others are not. Therefore, the distinction between "secondary" and primary (idiopathic) FSGS is not always clear. Similar to genetic FSGS, FSGS secondary to other causes does not recur after kidney transplantation if the causes no longer exist after kidney transplantation; some of the reported FSGS cases without recurrence may in fact have been secondary FSGS. In our own clinical experience, patients who presented with proteinuria but without edema did not experience recurrence; although there was no identifiable cause of FSGS and they were therefore categorized as primary FSGS, we speculate that these cases may have had obscure causes leading to FSGS [11]. Therefore, the pathologic diagnosis of FSGS itself does not mean that the disease could recur after kidney transplantation.

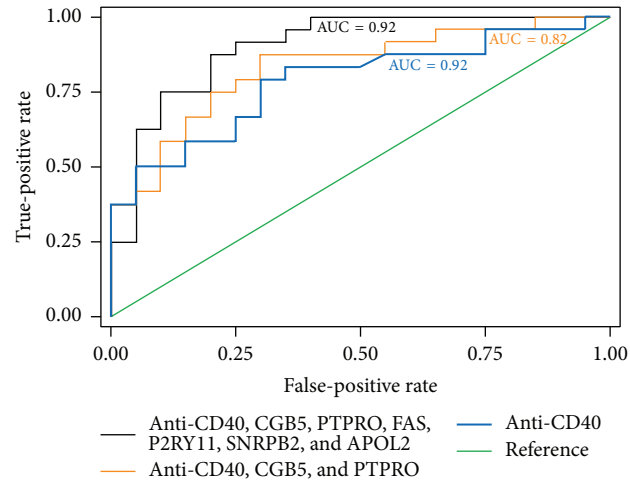


Figure 1: Validation of the FAST Ab panel in rFSGS and the predictive accuracy of the subsets of this panel. ROC analysis for three fitted logistic regression models. The outcome was recurrence versus nonrecurrence of FSGS, and the independent predictors were the log-transformed relative fluorescent signal values of seven Abs: CD40, PTPRO, FAS, CGB5, SNRPB2, APOL2, and P2RY11. The three logistic regression models fitted are shown. Model 1 used the FAST (FSGS antibody serological test) panel with all seven Abs, resulting in an AUC = 0.9. Model 2 used three Abs (CD40, PTPRO, and CGB5), and its ROC curve had an AUC of 0.82. Model 3 used only CD40 Ab data for the ROC analysis, resulting in an AUC of 0.77. Reproduced with permission from The American Association for the Advancement of Science © 2014 (AAAS), Delville et al. [45].

3. Are There Biomarkers Predicting Recurrence?

Despite a decades-long search for circulating "permeability factor(s)" causing FSGS [47], these factors remain elusive [37]. When soluble urokinase receptor (suPAR) was reported to be a candidate permeability factor [48], this news was met with excitement. However, contradicting reports followed [49], and therefore the usefulness of suPAR as a biomarker predicting recurrence is currently doubted. Cardiotrophin-like 1 (CLC-1) is another candidate that has been proposed by Savin's group [50] and is awaiting validation. Similar to other circulating factors, autoantibodies including anti-CD40 antibody have been proposed that have shown a good predictive accuracy of recurrence (Figure 1), but these candidates require further validation in clinical trials [45]. Another approach to identifying biomarkers is the assessment of podocyte changes in response to suspicious factor(s). Vasodilator-stimulated phosphoprotein (VASP) in human podocytes has been shown to be phosphorylated in response to plasma from patients with posttransplant recurrence but not to plasma from non-FSGS, and genetic FSGS cases did not

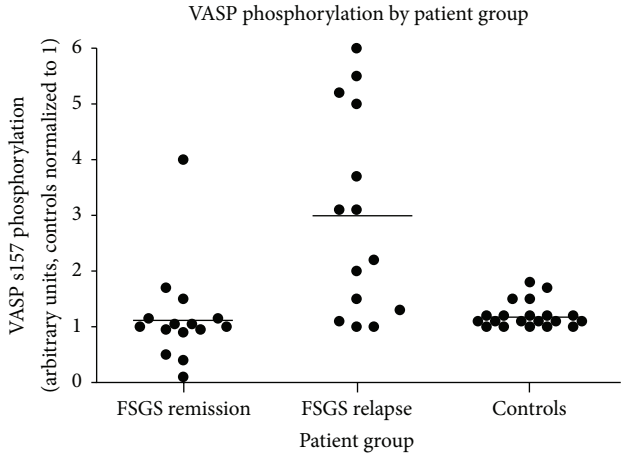


Figure 2: Scatter plot of VASP phosphorylation levels by patient group. Phosphorylation for each individual sample was assigned a densitometry value relative to the control (normal) plasma sample from the same gel, which was normalized to 1. Reproduced with permission from Wiley © 2012 Pathological Society of Great Britain and Ireland, Harris et al. [46].

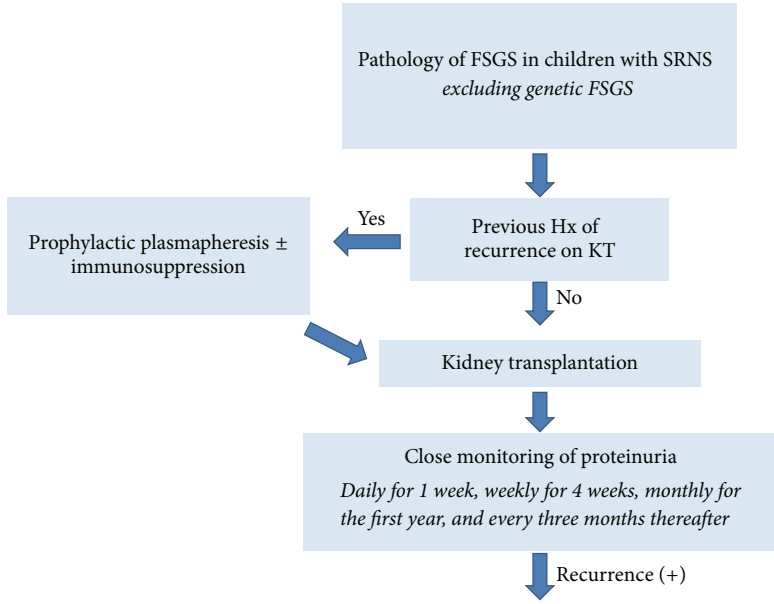
show this effect on podocytes (Figure 2). Once these promising biomarkers are validated and incorporated into clinical practice, we will be able to better predict whether recurrence will occur in a certain patient after kidney transplantation [46]. This would enable us to properly evaluate the efficacy of prophylactic management such as prophylactic plasmapheresis/immunoadsorption or immunosuppression and to possibly conduct preventive measures before transplantation [9].

4. How to Treat Recurrence

Although the recurrence of FSGS is a significant risk factor of graft loss [24, 29, 30, 51, 52], the outcomes of recurrent FSGS have so much improved that primary FSGS is no longer considered a contraindication of transplantation. The remission rate of pediatric recurrent FSGS has been reported to be as high as 70% [11, 12]. The mainstay of treatment for recurrent FSGS is plasmapheresis (removing 1.5 plasma volumes with 5% albumin replacement)/immunoadsorption because recurrence is believed to be caused by circulating factor(s) [53–55]. There have been no prospective randomized clinical trials to compare the efficacies of plasmapheresis and immunoadsorption, and the outcomes of studies using either method seem similar; therefore, the choice between these two methods depends on their availability and the preference of the treating physician [55–57]. It is important to begin treatment as soon as possible because removal or replacement of the FSGS-causing circulating factor(s) should be instituted before irreversible damage can be inflicted to the glomeruli [58]. While there is no evidence that anuric status at the time of transplantation prevents recurrence, recurrence can be detected more promptly if the patient had been anuric, and therefore native kidney nephrectomy can be considered in patients with residual urine output. Once the factors are removed, maintenance immunosuppression against graft rejection would also suppress the resurgence of the source of FSGS. It seems that, for some patients, plasmapheresis/immunoadsorption is sufficient to induce remission of recurrent FSGS [55, 59]. For others, intense immunosuppression with high-dose methylprednisolone, cyclosporine [12], cyclophosphamide [9], or rituximab [60] at various combinations is necessary. Although high-dose cyclosporine has been advocated as necessary by some [9, 12] and tacrolimus as a replacement for cyclosporine has been questioned [9], in our own clinical experience, tacrolimus trough levels of 12 to 15 ng/mL in combination with high-dose methylprednisolone and rituximab in addition to immediate and intense plasmapheresis have worked well, achieving remission in more than 80% of recent recurrent cases (unpublished data). The addition of rituximab to the current strategy against recurrent FSGS seems beneficial, demonstrating a response rate of up to 79% in recurrent FSGS [60, 61]. Although the optimal dosage (375 mg/m^2 up to 6 doses or a single dose of 100 mg [62]) and mechanism of action (eradication of B lymphocytes or binding to sphingomyelin phosphodiesterase acid-like 3b [SMPDL-3b] on podocytes [63]) of this medication remain elusive, a single dose or two of 375 mg/m^2 rituximab have been satisfactory in achieving sustained complete remission in our practice (Figure 3).

There are several promising therapeutic agents that are awaiting validation. Cytotoxic T-lymphocyte-associated antigen 4-immunoglobulin fusion protein (CTLD4-Ig, abatacept) has been tested on the basis that B7-1 (CD80) induction on podocytes plays an important role in the pathogenesis of proteinuria [64] and could eradicate proteinuria in recurrent FSGS [65]; however, the long-term efficacy of this agent has not been confirmed [66]. Of note, another form of CTLA4-Ig, belatacept, is being evaluated as a long-acting maintenance immunosuppressant against kidney allograft rejection [67]. CTLA4-Ig, a costimulatory inhibitor that competes with B7-1, could be indicated in the suppression of both allograft rejection and proteinuria. However, belatacept has been shown to increase the risk of posttransplant lymphoproliferative disease in Epstein-Barr virus- (EBV-) naïve patients [68]. Because the majority of pediatric recipients are naïve to EBV infection, caution is warranted when considering CTLA4-Ig as a therapeutic agent against recurrent FSGS in children. Another agent of interest is galactose. In the search for a "permeability factor" causing FSGS, galactose was found to bind to the factor(s) and eliminate their proteinuric effect [69, 70]. In addition to the clinical trials of this agent in pediatric SRNS [71, 72], anecdotal cases of significant improvement have been reported in recurrent FSGS [73, 74]. Successful treatment of recurrent FSGS has also been reported with anti-TNF-α treatment [75] (based on the upregulation of TNF-α mRNA in patients with FSGS [76]), adrenocorticotrophic hormone gel [77], and allogeneic mesenchymal stem cells [78, 79].

An important point is that treatment failure on native kidneys does not predict treatment failure for posttransplantation recurrence of FSGS. Why? For recurrent FSGS after transplantation, we start treatment almost immediately, before the formation of sclerosis. This implies that delays in treating the native kidney lead to treatment failure, resulting in progressive renal damage. Therefore, if we are equipped



(i) Immediate plasmapheresis/immunoadsorption until normalization of serum albumin
(ii) Methylprednisolone pulse 30 mg/kg up to 1 g per day, 3 days
(iii) Calcineurin inhibitor tacrolimus trough 12~15 ng/mL or cyclosporine trough 120~150 ng/mL
(iv) Rituximab 375 mg/m^2, till CD19 = 0

Figure 3: Approach for patients with SRNS-FSGS, authors' suggestion.

with reliable biomarkers that indicate which medications will be effective in specific patients at the time of NS diagnosis, we will be able to tailor the treatment of pediatric NS, thus applying "precision medicine" to these patients.

5. How to Prevent Recurrence

While any discussion of preventive treatments efficacy is futile because we do not know whether subjects will experience recurrence after transplantation, patients who had lost their previous allograft to recurrence of FSGS have particularly high risk of recurrence [6, 18, 22]. For these patients, to eliminate circulating factors, preemptive plasmapheresis/immunoadsorption is considered, three to five sessions prior to the transplantation followed by immediate posttransplant sessions of three to five [80–85]. Additional single dose of rituximab (375 mg/m^2) along with immunosuppression of corticosteroid, calcineurin inhibitor, and mycophenolate mofetil for two weeks prior to kidney transplantation was shown to prevent recurrence [81, 84].

6. Conclusion

Recurrence after kidney transplantation is devastating for patients and families. However, the outcomes of recurrent FSGS are quite good with the current management strategy; therefore, the pathologic diagnosis of FSGS itself should not be a cause for postponing allograft kidney transplantation. For patients with a high risk of recurrence, close monitoring of proteinuria as suggested by the Kidney Disease: Improving Global Outcomes guidelines for the management of kidney transplant recipients [86] is necessary, followed by immediate, intensive treatment for recurrence, as suggested in Figure 3.

Competing Interests

The authors declare that there are no competing interests regarding the publication of this paper.

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Review Article

Circulating Permeability Factors in Primary Focal Segmental Glomerulosclerosis: A Review of Proposed Candidates

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Primary focal segmental glomerulosclerosis (FSGS) is a major cause of the nephrotic syndrome and often leads to end-stage renal disease. This review focuses on circulating permeability factors in primary FSGS that have been implicated in the pathogenesis for a long time, partly due to the potential recurrence in renal allografts within hours after transplantation. Recently, three molecules have been proposed as a potential permeability factor by different groups: the soluble urokinase plasminogen activator receptor (suPAR), cardiotrophin-like cytokine factor-1 (CLCF-1), and CD40 antibodies. Both CLCF-1 and CD40 antibodies have not been validated by independent research groups yet. Since the identification of suPAR, different 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. Researchers have suggested that cleaved molecules of suPAR have a pathogenic role in FSGS but further studies are needed to determine this role. In future studies, proposed standards for the research of the permeability factor should be carefully followed. The identification of the permeability factor in primary FSGS would be of great clinical relevance as it could influence potential individual treatment regimen.

1. Introduction

Primary and secondary focal segmental glomerulosclerosis (FSGS) are a major cause of nephrotic syndrome in the United States and often lead to end-stage renal disease (ESRD) [1]. FSGS is diagnosed and classified from renal biopsies [2, 3]. Injury of podocytes initiates the disease process of FSGS, leading to the classic focal distribution of sclerosis with a segmental pattern within the glomeruli [4]. Clinically, patients present with an abrupt onset of proteinuria, hypoalbuminemia, and edema. Causes of FSGS are heterogeneous and this paper will only focus on the pathogenesis of primary FSGS, in particular on circulating permeability factors in primary FSGS.

Primary FSGS is diagnosed if gene mutations and other causes of FSGS (glomerular hyperfiltration, virus infection, drugs, etc.) have been ruled out. Primary FSGS accounts for approximately 40% of idiopathic nephrotic syndromes. Even though the idiopathic nephrotic syndrome is a rare disease with an incidence of 7 per 1 million [5], it often leads to severe renal impairment and ESRD and the response to immunosuppressive therapy is poor.

The etiology of primary FSGS is still unknown. However, circulating permeability factors have been implicated in the pathogenesis of FSGS for a long time due to the following observations [6]. First, proteinuria recurs in patients with primary FSGS after renal transplantation in more than 30% of cases [7]. Interestingly, this proteinuria may develop within hours after transplantation and some patients benefit from plasmapheresis [8, 9]. Second, infusion of plasma from FSGS patients causes proteinuria in rats [10–12]. In a model for testing glomerular permeability, sera from some FSGS patients also increased permeability to albumin in isolated rat glomeruli [13]. Third, transmission of a potential permeability factor from a pregnant woman with primary FSGS to her newborn infant has been published. The infant presented with transient proteinuria [14]. Lastly, a patient with primary FSGS who received a kidney transplant from his healthy sister developed proteinuria and a decline of renal function shortly after transplantation. FSGS recurrence

was confirmed by renal biopsy and, despite treatment with plasmapheresis, the transplant did not regain function. Two weeks after transplantation, the allograft was removed and transplanted into another recipient who had ESRD due to diabetic nephropathy. Proteinuria declined rapidly and the histological lesions disappeared on biopsy samples. Kidney function remained stable for at least 8 months after transplantation [15].

Taken together, these observations strongly suggest a causative role of one or more circulating permeability factor(s) in recurrent primary FSGS.

2. Circulating Permeability Factors in Primary FSGS

A recent review on nephrotic syndromes described among other things the historical perspectives of the permeability factors identification in idiopathic nephrotic syndrome [6]. Many investigators have used different models to test permeability factors and comparisons amongst these studies are therefore difficult due to the lack of strict criteria of how putative disease-causing permeability factors are defined. Maas et al. have now proposed criteria to define pathogenic circulating factors in MCD and FSGS [6]. We agree with the authors in the attempt to standardize these criteria, even though as a result research in this field will become much more complicated.

The molecular characteristics of permeability factors have been derived from observations that the active fraction of sera from patients with FSGS precipitates in 70–80% ammonium sulfate solution independent of the immunoglobulin fraction. The putative permeability factor(s) are bound to protein A and had a molecular size between 30 and 50 kDa [13]. Immunoabsorption with a protein A column reduced proteinuria in a patient with recurrent FSGS [16]. When the 30–50 kDa fraction was infused into rats, proteinuria developed [17]. In addition, it was proposed that the circulating factor in FSGS interacts with the glycocalyx of the podocytes. To prevent this interaction, galactose was tested and had a high affinity to the active fraction of FSGS sera that was greater than 30 kDa [18]. Furthermore, oral galactose caused a decrease in the active fraction of FSGS serum in a patient with recurrent, plasmapheresis resistent FSGS. Harris et al. reported that FSGS sera increased protease activated receptor-1 mediated phosphorylation of the vasodilator stimulated protein (VASP) in human podocytes, indicating a pathological role for circulating proteases in FSGS [19]. Recently, a novel *in vitro* assay to test the probability of FSGS recurrence was published [20]. Sera from patients with FSGS recurrence disrupted podocyte focal complexes imaged by immunofluorescence.

Recently, three candidate proteins have been proposed to be the circulating factor in FSGS (Table 1). These will be reviewed in more detail.

2.1. Soluble Urokinase Plasminogen Activator Receptor (suPAR). Urokinase plasminogen activator receptor (uPAR) is a cell membrane glycosylphosphatidylinositol- (GPI-) anchored protein expressed in many cell types, for example, immune cells [21–23], endothelial cells [24], tumor cells [25], tubular epithelial cells [26], and podocytes [27]. uPAR is composed of three domains (D_{I}, D_{II}, and D_{III}) that bind to their ligand urokinase plasminogen activator (uPA). Through interaction with transcellular receptors, such as integrins, uPAR promotes cell migration, proliferation, and survival [28].

Through cleavage of uPAR from its GPI-anchor, the soluble urokinase plasminogen activator receptor (suPAR) is released. Further cleavage between the D_{I} and $D_{\mathrm{II}}/D_{\mathrm{III}}$ domains of suPAR generates other cleaved suPAR fragments. suPAR and uPAR are heavily glycosylated proteins. Depending on the amount of glycosylation and the size of the cleaved proteins, suPAR's size ranges from 25 to 50 kDa. suPAR can be detected in plasma, serum, urine, and other body fluids. In healthy individuals, suPAR is present at low levels regulating neutrophil trafficking and stem cell mobilization [29]. Infections and inflammatory diseases lead to an increase in suPAR levels indicating a role as an acute phase reactant [30–34].

2.1.1. suPAR as Biomarker. Recently, suPAR has emerged as a biomarker in different disease conditions. For example, suPAR concentrations were associated with increased risk of cardiovascular events in the general population [35, 36]. In patients with myocardial infarction (MI), suPAR levels predicted recurrent MI and mortality [37, 38]. In addition, suPAR concentration correlated with mortality in critically ill patients beyond validated score systems [34, 39]. Patients with chronic kidney disease (CKD) are known to be at increased risk of cardiovascular events. In line with the observations in the general population, suPAR was associated with mortality and new-onset cardiovascular disease in a mild-moderate CKD cohort [40].

Several studies have described an inverse correlation of suPAR levels with the estimated glomerular filtration rate (eGFR) [41–44]. Recently, Hayek et al. investigated the role of plasma suPAR levels and the incidence of CKD in a prospective cohort study of patients with cardiovascular disease [45]. In this cardiovascular patient cohort, suPAR levels were independently associated with the decline in eGFR and the development of CKD (defined as eGFR <60 mL/min/1.73 m^2). In addition, suPAR levels were positively correlated with the incidence of proteinuria. However, proteinuria data from this study needs to be interpreted with caution, as the absolute patient numbers with proteinuria were low and proteinuria was diagnosed only semiquantitatively via urine dipstick.

2.1.2. suPAR in FSGS. The first evidence that uPAR plays a role in podocyte biology was published by Wei et al. in 2008 [27]. Quite recently, the same group published an article in which they identified suPAR as a possible causal factor in FSGS [46]. The authors found increased concentrations of suPAR in patients with FSGS. However, patients with minimal change disease (MCD), membranous nephropathy (MN), and preeclampsia did not display a significant elevation of suPAR levels. The highest suPAR concentrations

Table 1: Circulating permeability factors in primary FSGS: summary of proposed candidates.

Circulating factor	Molecular weight (kDa)	Experimental findings	Clinical data for FSGS and CKD
suPAR	25–50	Administration of suPAR caused albuminuria in uPAR −/− mice [46], however not in WT mice [41, 59] Activation of podocytic $\alpha_v\beta_3$-integrin leading to cytoskeletal rearrangement [46] Decrease of nephrin expression via suppression of WT-1 [57]	suPAR levels are inversely correlated with eGFR, no discrimination of primary FSGS to other proteinuric diseases [41, 42, 44, 49, 50] suPAR seems to be a microinflammatory marker in FSGS [40] suPAR predicts CKD in a cardiovascular cohort [45] Significance of suPAR levels as a biomarker for FSGS in patients with preserved renal function unclear [53]
CLCF-1	22	Binds to galactose columns [18, 61, 65] and galactose blocked increase in glomerular permeability by FSGS sera [65] Administration of CLCF-1 increases glomerular permeability and proteinuria in mice [60] Decreases nephrin expression and disrupts the podocytic cytoskeleton [60] Inhibitors of the Jak/Stat3 pathway abolish CLCF-1 and FSGS sera effects [61]	Concentration of CLCF-1 in FSGS patients up to 100-fold higher than in controls, however available assay too insensitive at the moment [60] Current data do not support therapy of FSGS patients with galactose [64] Due to measurement difficulties not tested in FSGS cohorts yet
CD40 autoantibodies	150	Expressed in glomeruli from FSGS patients [58] Disrupt podocyte actin cytoskeleton [58] Injection of CD40 autoantibodies leads to albuminuria only if recombinant suPAR is coadministered [58] Administration of CD40 autoantibodies does not increase glomerular permeability in CD40 −/− mice [58]	Identified in autoantibody panel from sera of patients with recurrent FSGS [58]

suPAR, soluble urokinase plasminogen activator receptor; CLCF-1, cardiotrophin-like cytokine factor-1; WT, wild-type; WT-1, Wilms tumor-1; FSGS, focal segmental glomerular sclerosis; eGFR, estimated glomerular filtration rate; Jak, Janus-kinase; Stat3, signal transducer and activator of transcription 3.

were found in patients with recurrent FSGS. In addition, suPAR levels correlated with the presence but not with the level of proteinuria. The authors also proposed a pathological cut-off for suPAR. Levels of 3000 pg/mL and above were present in two-thirds of FSGS patients, however much less in other proteinuric kidney diseases. To prove the causal impact of suPAR, Wei et al. performed cell culture and mouse experiments that are described in more detail below.

2.1.3. Does suPAR Discriminate Primary FSGS from Other Proteinuric Kidney Diseases? In the FSGS CT (70 adults) and Podonet (94 children) cohort, Wei et al. tested suPAR levels in patients with primary FSGS using the proposed cut-off level of 3000 pg/mL [47]. The three major findings of this study were that suPAR levels were elevated in 84.3% (CT cohort) or 55.3% (Podonet cohort) of patients with primary FSGS. Second, suPAR levels did not correlate with inflammation measured by C-reactive protein (CRP) values and, third, mycophenolate mofetil (MMF) therapy was associated with a decline in suPAR levels. There was an inverse correlation of suPAR levels with eGFR. Interestingly, female patients had higher suPAR levels in both cohorts. Li et al. confirmed in their cohort (109 primary FSGS, 20 MCD, 22 MN, and 96 healthy controls) that suPAR levels were elevated in about half of their patients with FSGS and could therefore discriminate between FSGS and other proteinuric kidney diseases [48]. In addition, suPAR levels predicted steroid-responsiveness of FSGS. There was no association of suPAR levels with eGFR, but only patients with eGRF >40 mL/min were included in the study and therefore these results need to be interpreted with caution.

Since the original description of suPAR as a potential causal factor in primary FSGS, many researchers have tested suPAR levels in human adult and pediatric cohorts with conflicting results [41, 42, 44, 49, 50]. For example, Meijers et al. measured suPAR levels in control patients with CKD (476) and biopsy-proven FSGS patients (44) [42]. Multivariate analysis revealed a strong inverse association of suPAR with eGFR and serum albumin, while there was a positive association with age and CRP. No differences in suPAR levels were identified amongst FSGS and control patients. In the Nephrotic Syndrome Study Network (NEPTUNE including adults and children) cohort, suPAR levels were analyzed in 241 patients with FSGS (60), MCD (104), IgA nephropathy (57), and MN (82) [41]. In this cohort of proteinuric kidney disease, suPAR levels inversely correlated with eGFR and proteinuria in all disease groups. Multivariate linear regression depicted that plasma suPAR concentration was not associated with FSGS after adjustment of eGFR. With regard to the clinical endpoints in the NEPTUNE cohort, plasma suPAR levels did not predict the occurrence of end-stage renal disease (ESRD), 50% loss of eGFR, and complete or partial remission after adjustment of eGFR or proteinuria. Wada et al. confirmed the relationship of suPAR levels and eGFR in a Japanese cohort with primary glomerular diseases including FSGS [44]. In patients with eGFR >60 mL/min/1.73 m^2, suPAR levels did not discriminate primary FSGS from other glomerular pathologies. Even though Huang et al. reported elevated suPAR concentrations in FSGS in a Chinese cohort, suPAR levels did not differentiate between primary and secondary FSGS [49]. In addition, several pediatric cohort studies did not confirm the initial reports that serum or plasma suPAR levels could serve as a biomarker for primary FSGS [43, 51, 52].

Taken together from the evidence presented above, plasma and serum suPAR levels do not discriminate primary FSGS from other proteinuric kidney diseases. However, suPAR seems to be a biomarker for reduced renal function. Due to its molecular size (25–50 kDa), suPAR is probably filtered by the glomerulus. Reduced eGFR will lead to a reduction of filtered suPAR resulting in potentially increased serum and plasma levels of suPAR. Nothing so far is known about the tubular processing of suPAR.

Evidence points towards a role of suPAR as a microinflammatory marker in FSGS [42]. However, in a CKD population, suPAR seems to have additional prognostic value beyond conventional microinflammatory markers [40]. Elevated suPAR levels predict development of CKD in patients with cardiovascular disease [45]. However, in patients with preserved renal function, elevated suPAR levels cannot be explained by the theory of reduced suPAR filtration [53]. Further studies will need to clarify the role of suPAR as a biomarker in patients with preserved renal function.

2.1.4. Does suPAR Cause Podocyte Injury and Proteinuria in FSGS? In the studies of Wei et al., the hypothesis that suPAR causes FSGS derived from *in vitro* and *in vivo* experiments [46]. As described above, FSGS sera led to robust staining with an AP5 antibody indicating activated $\alpha_v\beta_3$-integrin in human podocytes and glomeruli as the pathomechanism of primary FSGS. In contrast, Yu et al. reported that $\alpha_v\beta_1$ was the essential integrin in five patients with FSGS (one with primary FSGS and four with recurrent FSGS) [54]. Mechanistically, B7-1 (CD80) deactivated $\alpha_v\beta_1$ but not $\alpha_v\beta_3$-integrin in podocytes of these patients who were also glucocorticoid and rituximab resistent. Abatacept, a costimulatory inhibitor of B7-1, induced remission in all of these patients. Interestingly, some biopsy specimen from patients with other proteinuric kidney diseases had positive B7-1 staining indicating that B7-1 might be a biomarker of podocyte injury and could identify patients that may benefit from therapy with abatacept [54]. However, conflicting results have also been published by Benigni et al. and Delville et al. [55, 56].

Following the original article of Wei et al., three different mouse models confirmed further the hypothesis that suPAR caused proteinuria in mice. In *Plaur* −/− mice, infusion of recombinant suPAR (recombinant mouse suPAR-Fc) caused proteinuria and these mice became protected from LPS induced proteinuria. Furthermore, wild-type mice with transplanted kidneys from *Plaur* −/− mice were challenged with LPS and developed proteinuria. Lastly, wild-type mice that were treated with gene transfer ($sPlaur_{WT}$ or $sPlaur_{E134A}$ mutant potentially defective of β_3-integrin binding) were analyzed for protection from LPS induced proteinuria. Mice that received gene transfer of the defective integrin binding suPAR mutant were protected. In line with

these results, Alfano et al. showed that high dosages of suPAR (recombinant mouse suPAR-Fc) induced proteinuria in *Plaur* −/− mice [57]. Providing further insights into the potential pathomechanism of suPAR action, Alfano et al. described that suPAR decreased nephrin expression in podocytes via suppression of Wilms tumor-1 (WT-1) transcription factor. Interestingly, only the full-length suPAR molecule interacted with β_3-integrin and caused podocyte damage. Cleaved suPAR molecules were not able to activate β_3-integrin. Delville et al. revealed that suPAR (recombinant human suPAR) exacerbated proteinuria in an anti-CD40 antibody mediated proteinuria model [58].

In contrast, Spinale et al. did not find proteinuria after 24 h in wild-type mice after administration of recombinant suPAR (recombinant mouse suPAR-Fc) even though high suPAR levels were detected [41]. In addition, ectopic expression of the full-length suPAR (D_{I}–D_{III}) molecule from the liver did not induce proteinuria for 44 days despite elevated suPAR levels. Similarly, Cathelin et al. were not able to demonstrate that short-term and prolonged administration of suPAR (recombinant mouse suPAR-Fc and monomeric mouse uPAR produced in S2-cells) caused proteinuria in wild-type mice [59].

This conflicting data can partly be explained by the different genetic backgrounds of the mice (Plaur −/− versus WT) investigated in the different studies. In addition, Wei et al. used a splice variant of mouse suPAR containing a retained intron 4 in their gene transfer experiments. If not spliced out, intron 4 would have led to a premature stop within uPAR domain 2 [41]. The expression of this suPAR variant seems to be rare and the homologous splice variant has not yet been identified in humans [41].

Many questions about the potential causal role of suPAR for podocyte damage remain. Due to the conflicting data at the moment, more evidence is needed that circulating suPAR causally leads to podocyte damage in primary FSGS patients.

2.2. Cardiotrophin-Like Cytokine Factor-1 (CLCF-1). CLCF-1 is a member of the IL-6 family of cytokines with a predicted molecular weight of 22 kDa [60]. CLCF-1 is secreted and forms heterodimers with either cytokine receptor like factor 1 (CRLF1) or soluble ciliaric neurotrophic receptor alpha (sCNTFRα) resulting in composite cytokine [61].

2.2.1. CLCF-1 in FSGS. The identification of CLCF-1 as a potential permeability factor in primary FSGS was the result of studying FSGS plasma for more than 20 years by Savin's group [11, 13, 17, 18, 60–65]. Through systematic investigation of the biochemical characteristics of the active fraction of FSGS plasma, Savin's group was able to isolate the permeability factor by galactose affinity chromatography and mass spectrometry [18]. After dialyzation of the eluate from the galactose column, the permeability factor was identified in the fraction <30 kDa [18]. Finally, CLCF-1 was found in the active fraction of plasma from patients with recurrent FSGS [18, 65]. The concentration of CLCF-1 in plasma from patients with recurrent FSGS was up to 100 times higher than in controls [65]. In preliminary studies, the concentration of CLCF-1 in healthy subjects was only 100 pg/mL [60]. Therefore, available assays to measure CLCF-1 are not sensitive enough to detect CLCF-1 levels in patient samples at the moment [60]. In addition, CLCF-1 levels have not been investigated in other disease states or in the urine of FSGS patients so far [60]. In the future, assays for CLCF-1 detection need to be developed. Evaluation of CLCF-1 levels in clinically well defined cohorts (e.g., NEPTUNE, FSGS CT) is necessary to prove CLCF-1's pathophysiological role only in primary FSGS. If confirmed, CLCF-1 is an excellent candidate for therapy as no essential role for CLCF-1 is described after fetal development [60]. Antibodies targeting CLCF-1 or its receptors could be potential future and individualized treatment strategies.

2.2.2. Does CLCF-1 Cause Podocyte Injury and Proteinuria in FSGS? Several years ago, Savin's group developed an *in vitro* assay to study glomerular permeability [62]. In isolated rat glomeruli, an isotonic albumin oncotic solution was replaced by a solution with a lower albumin concentration. This led to an increase in glomerular size through swelling if the permeability barrier was intact. Incubation of the glomeruli with FSGS sera led to a decrease of glomerular size compared to control glomeruli. This indicated a disruption of the oncotic gradient through an increase in glomerular permeability. Permeability to albumin (*Palb*) was expressed as 1 minus the difference in glomerular size. CLCF-1 mimicked the effects of FSGS plasma on *Palb,* while a CLCF-1 antibody abolished this effect. In addition, CLCF-1 decreased nephrin expression in glomeruli and podocytes. Incubation of murine podocytes with CLCF-1 disrupted the actin cytoskeleton in a time and concentration dependent manner and led to a motile phenotype of the podocytes [60]. More recently, recombinant monomeric human CLCF-1 increased *Palb* in isolated rat glomeruli [61] as well as albuminuria in mice after acute and chronic infusion [60]. However, heterodimers of CLCF-1 with CRLF-1 blocked the increase in *Palb* from FSGS sera [61]. In addition, inhibitors of the Jak-Stat3 signaling pathway abolished the increase in *Palb* from CLCF-1 or FSGS sera [60, 61].

As described above, CLCF-1 was found in the active fraction of FSGS sera and was isolated by galactose affinity chromatography. Furthermore, application of galactose blocked the increase of Palb by FSGS sera [65]. However, several case reports have shown conflicting results on the treatment of FSGS patients with galactose [66–68]. Recently, the FONT II trial was published as a phase I/II open-label randomized controlled trial. The trial compared standard conservative therapy (SCT) versus SCT plus adalimumab (antibody against tumor necrosis factor-α/TNF-α) versus SCT plus galactose [64]. Patients with biopsy-proven primary FSGS or genetic FSGS, with proteinuria of >1 g/g and eGFR >40 mL/min/1.73 m^2, were included. The patients received therapy over 26 weeks and the primary end point was a 50% reduction in proteinuria with stable GFR. Of the 21 patients included in the study, 7 received SCT plus galactose. Three out of seven patients met the primary end point. No improvement was noted with treatment of SCT plus adalimumab. Even

though primary FSGS is a rare disease, further and larger studies are needed to confirm the potential benefit from galactose treatment in FSGS.

Taken together, the identification of CLCF-1 as a potential circulating permeability factor is very promising. However, its pathophysiological role needs to be validated in well characterized patient cohorts and by different research groups in the future.

2.3. Anti-CD40 Antibodies. The costimulatory molecule CD40 is a member of the TNF receptor superfamily [69]. CD40 is an important molecule in immunity and inflammation. It is expressed in various tissues especially on the surface of antigen presenting cells (APCs), macrophages/monocytes, and dendritic cells [69]. CD40 is also expressed in endothelial and epithelial cells. CD40 ligand binds to CD40 and is expressed also in many different cell types such as immunological, endothelial, and epithelial cells [69]. CD40 ligand activates endothelium and leads to increased expression of chemokines, metalloproteases, uPA, and suPAR [58].

2.3.1. Anti-CD40 Autoantibodies in FSGS. Delville et al. described the identification of a panel of autoantibodies in recurrent FSGS before transplantation [58]. Using array data and an enzyme linked immunosorbent assay (ELISA), pretransplant sera from 20 patients with FSGS and biopsy-proven FSGS were analyzed [58]. 10 patients had disease recurrence in the first year of transplantation (recurrent FSGS) and 10 had no recurrence of proteinuria or histological disease after transplantation (nonrecurrent FSGS). IgG profiles from the sera of recurrent and nonrecurrent FSGS varied significantly and, after validation with different tools, autoantibodies against CD40 were the most promising antibodies to pursue further.

2.3.2. Do Anti-CD40 Autoantibodies Cause Podocyte Injury and Proteinuria in FSGS? CD40 is expressed in human cultured podocytes and its expression cannot be induced by challenging *in vitro* [58]. However, in patients with FSGS, CD40 was detected in glomeruli from recurrent FSGS patients. Interestingly, the autoantibodies against CD40 did not recognize human CD40 and anti-CD40 antibody reactive regions differed between recurrent and nonrecurrent FSGS sera. Even though autoantibodies against CD40 from recurrent FSGS sera did not detect recombinant human CD40, purified CD40 autoantibodies from recurrent FSGS sera disrupted the podocyte (human) actin cytoskeleton *in vitro*. This finding points to a posttranslational modification of the CD40 molecule *in vivo* that is necessary for detection with CD40 autoantibodies. The data of Delville et al. further suggested that suPAR-β_3-integrin pathway could be involved. In addition, injection of anti-CD40 antibodies from recurrent FSGS patients into wild-type mice was not sufficient to cause robust albuminuria. However, if full recombinant suPAR was coadministered, albuminuria developed. An antibody against suPAR or a small molecule targeting the activation of $\alpha_v\beta_3$-integrin blocked the effect of CD40/suPAR. There was no increase in glomerular permeability in *CD40* −/− or wild-type animals injected with recombinant CD40. The authors concluded that CD40 autoantibodies have a pathogenetic role in the development of recurrent FSGS potentially through interaction with suPAR.

The size of IgG antibodies is approximately 150 kDa [70]. The size of intact CD-40 autoantibodies therefore contradicts previous findings that the active fraction of FSGS sera was smaller than 30–50 kDa [17].

Besides these exciting findings, the role of CD40 antibodies in human disease needs to be validated. Anti-CD40 blocking antibodies (ASKP1240 or lucatumumab) are already commercially available and could become potential treatment options tested in clinical trials [71].

3. Conclusion

The clinical evidence presses for the existence of circulating permeability factors in primary FSGS. Some molecules have been proposed but have not finally proven their pathogenic role. So far, none of the proposed molecules have been validated by different research groups in different FSGS disease models. We expect additional promising data for known and novel candidates in near future. Hopefully, this will enable us to treat patients with primary FSGS individually based on their pathogenic circulating factor.

Competing Interests

The authors declare that they have no competing interests.

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Review Article

Recent Advances in Treatments of Primary Focal Segmental Glomerulosclerosis in Children

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Focal segmental glomerulosclerosis (FSGS) is a nephrotic syndrome. Up to around 80% of cases of primary FSGS are resistant to steroid treatment. A large proportion of patients with steroid-resistant FSGS progress to end-stage renal disease. The purpose of treatment is to obtain a complete remission of proteinuria, a necessary step that precedes improved renal survival and reduces the risk of progression to chronic kidney disease. When this is not possible, the secondary goal is a partial remission of proteinuria. Reduction or remission of proteinuria is the most important factor predictive of renal survival. We will review the current updated strategies for treatment of primary FSGS in children, including traditional therapies consisting of corticosteroids and calcineurin inhibitors and novel therapies such as rituximab, abatacept, adalimumab, and fresolimumab.

1. Introduction

Idiopathic nephrotic syndrome (INS) is a primary renal disorder defined by the three signs of proteinuria, hypoalbuminemia, and edema. Histopathological categories of INS include minimal change disease, focal segmental glomerulosclerosis (FSGS), and diffuse mesangial proliferation [1]. Based on the response to steroid treatment, two types of INS can be characterized: steroid-sensitive nephrotic syndrome (SSNS), in which the proteinuria resolves, and steroid-resistant nephrotic syndrome (SRNS), in which the proteinuria does not resolve [1]. Most patients with minimal change disease respond well to steroid treatment and, currently, renal biopsy is not routinely performed in patients with SSNS. Therefore, the terminology of minimal change disease has become synonymous with SSNS [1]. However, genetic forms of nephrotic syndrome have been emphasized since the discovery of causative mutations in cases with infantile and juvenile SRNS and familial nephrotic syndrome [1]. Histologic findings in the kidney reveal FSGS in most patients with both syndromic and nonsyndromic forms of SRNS [1]. Therefore, it is important to rule out genetic forms of FSGS beforehand to avoid trying ineffective therapies.

A report of the International Study of Kidney Disease in Children (ISKDC) demonstrated that, among patients who were initial steroid responders and those who were not, 3% and 47.5% had FSGS, respectively [2]. Up to around 80% of cases of primary FSGS are resistant to steroids [3]. A large proportion of patients with steroid-resistant FSGS progress to end-stage renal disease [4]. Therefore, nephrologists are challenged and are struggling to treat patients with FSGS. We will review current strategies for medical treatment of primary FSGS in children, omitting any discussion of genetic forms or relapses of FSGS after kidney transplantation.

2. Corticosteroids

Steroid therapy is the most basic and standard treatment [1]. Initial steroid therapy is with oral prednisone at a dose of 60 mg/m^2 or 2 mg/kg per day with a maximum dose of 60 mg per day for 4 to 6 weeks followed by 40 mg/m^2 or 1.5 mg/kg every other day for 4 to 6 weeks [2, 5, 6]. Ehrich and Brodehl reported the advantages of a 12-week compared to an 8-week steroid treatment protocol for an initial attack of INS [7]. The cumulative rate of patients with sustained remissions after 2 years was significantly higher after the 12-week course than after the 8-week treatment [7]. Several comparative studies have reported that a longer duration of steroid treatment (3–7 months) after an initial 4–8-week daily steroid treatment followed by an alternative protocol significantly reduced the relapse rate compared with the 8-week protocol [7–10]. An alternative treatment for patients who do not achieve remission of nephrotic syndrome after the initial 4 weeks is to administer an intravenous pulse of methylprednisolone, 30 mg/kg per dose, with a maximum of 1 g every other day for 2 weeks [1, 11]. Two-thirds of initial steroid nonresponders eventually became responders with a 2-week course of methylprednisolone [12].

The "Mendoza" protocol is an aggressive treatment strategy based on high-dose methylprednisolone pulse therapy and oral prednisolone, with or without an alkylating agent, administered for a total of 82 weeks (Table 1) [4, 12]. When this protocol was used to treat cases of SRNS, approximately 65% went into complete remission and only 25% progressed to chronic kidney disease [4]. However, prolonged steroid treatment for nephrotic syndrome causes significant side effects, including growth impairment, obesity, hypertension, cataracts, osteoporosis, immune suppression, diabetes mellitus, psychosis, hirsutism, and striae [11].

Table 1: The "Mendoza" protocol for steroid-resistant nephrotic syndrome in children.

Week	Methylprednisolone, 30 mg/kg	Oral prednisone
1-2	Every other day, 6 doses	None
3–10	Every week, 8 doses	2 mg/kg every other day
11–18	Every 2 weeks, 4 doses	With/without taper
19–50	Every 4 weeks, 8 doses	Slow taper
51–82	Every 8 weeks, 4 doses	Slow taper

3. Cyclophosphamide

Treatment with a 12-week course of cyclophosphamide at a single dose of 2 mg/kg per day with a maximum cumulative dose of 168 mg/kg reduced the rate of relapse in steroid dependent nephrotic syndrome (SDNS) [6, 13]. A longer remission was observed when cyclophosphamide treatment was used in combination with steroids compared to cyclophosphamide given alone for SDNS [14]. There is little data indicating that cyclophosphamide is effective in SRNS with FSGS and updated version of the Kidney Disease: Improving Global Outcomes (KDIGO) guidelines (http://kdigo.org/home/glomerulonephritis-gn/) suggests that cyclophosphamide should not be given to children with SRNS [15–17]. However, renal failure progresses less frequently in SRNS patients who are partial responders to cyclophosphamide [18, 19].

Cyclophosphamide can be used as monthly intravenous boluses at a dose of 500 mg/m^2 for 6 months for infrequently relapsing nephrotic syndrome [20]. There are divergent opinions about intravenous pulse cyclophosphamide for steroid-resistant FSGS [21]. However, intravenous pulse cyclophosphamide has been considered as an adjunctive therapy for steroid-resistant FSGS [22].

4. Calcineurin Inhibitors

4.1. Cyclosporine. Cyclosporine reduces the relapse rate by 80% in patients with SDNS [23]. However, the patients tend to become cyclosporine dependent just as they did with steroids [1]. The remission rate is significantly higher when the therapeutic dosage of cyclosporine is 4–6 mg/kg/day in two divided doses to maintain a trough level between 60 and 80 ng/mL in patients with SDNS [1, 6, 24]. Some research has demonstrated that cyclosporine in combination with steroids is significantly effective in reducing the relapse rate in patients with SRNS [25, 26]. Another study showed that prolonged combination therapy with cyclosporine and steroids, including intravenous pulses of methylprednisolone, maintained the remission rate at 84% for patients with SRNS [27]. Although relapses were frequent, long-term cyclosporine treatment in children with SRNS reduced the progression of chronic kidney disease by reducing proteinuria [28, 29]. Therefore, the Canadian Society of Nephrology (CSN) recently recommended using a calcineurin inhibitor, either cyclosporine or tacrolimus, following standard therapy with steroids for children with SRNS [6]. In recent years, however, cyclosporine has been found not to work in all FSGS patients because patients with a hereditary background of the disease rarely benefit from cyclosporine therapy [30, 31]. Side effects of cyclosporine include hypertrichosis, gum hypertrophy, hypertension, and nephrotoxicity [11].

4.2. Tacrolimus. Tacrolimus has recently been highlighted as an alternative to cyclosporine for SRNS [11]. Tacrolimus at a dose of 0.1–0.2 mg/kg per day divided into two doses was effective and well tolerated in children with SRNS, with a complete remission rate of 81% [32, 33]. A target trough level of 5–7 ng/mL should be recommended for SRNS [6, 34]. Cosmetic side effects such as hypertrichosis and gum hypertrophy are not seen with tacrolimus, but any difference between cyclosporine and tacrolimus in the incidence of nephrotoxicity has not been proven [11]. Other side effects, including tremor, arterial hypertension, and diabetes mellitus, can occur. Recently, the efficacy of multidrug therapy consisting of tacrolimus, mycophenolate mofetil (MMF), mizoribine, or leflunomide in combination with steroids for children with SRNS has been reported [35, 36]. While the relapse rate was significantly decreased after multidrug

therapy in children with SRNS, long-term safety has not yet been proven.

5. Mycophenolate Mofetil

The CSN does not recommend the widespread use of MMF due to limited cost-effectiveness [6]. However, a retrospective study in children with nephrotic syndrome reported that the combined rate of complete and partial remission for MMF was 67% in SRNS, and for refractory cases, combination therapy with MMF, tacrolimus, and steroids yielded a 75% response rate [37]. Compared to tacrolimus, the response rate for MMF was not superior, but MMF has milder side effects than calcineurin inhibitors [11]. MMF was not inferior to calcineurin inhibitors in preventing relapses but only if administered in high doses [38]. MMF can be an alternative agent in patients with adverse effects due to calcineurin inhibitors [1, 6]. MMF can be given at a dose of 1200 mg/m^2 per day divided into two doses. Area under the concentration-time curve (AUC) measurements are essential in the use of this drug. Commonly reported adverse effects of MMF include metabolic acidosis, infection, diarrhea, abdominal pain, and hyperlipidemia.

6. Mizoribine

Mizoribine inhibits inosine monophosphate synthetase and guanosine monophosphate synthetase, resulting in the inhibition of DNA synthesis and cell division. Mizoribine has fewer side effects than azathioprine, which also inhibits an enzyme required for DNA synthesis [39, 40]. Although the mechanism of action for mizoribine is similar to the more widely used azathioprine and MMF, there is little data as to whether it is effective in maintaining remission in nephrotic syndrome [41]. However, there are some case series reporting successful treatment with combination therapy using mizoribine, tacrolimus, or plasmapheresis for children with refractory nephrotic syndrome in Japan [35, 42]. Mizoribine can be administered at a dose of 3 mg/kg once daily before breakfast [35].

7. Renin-Angiotensin System Blockade

The KDIGO Work Group recommended renin-angiotensin system (RAS) inhibition with angiotensin converting enzyme inhibitor (ACE-I) or angiotensin II receptor blockers (ARBs) for children with SRNS [6]. RAS blockade combined with MMF was markedly effective in children and young adults with steroid-resistant FSGS [43]. A comparative study of ACE-I and calcium channel blockers in SRNS demonstrated that both ramipril and verapamil reduced proteinuria in patients with SRNS [44]. Another randomized trial of 45 children (10 with FSGS) with normotensive SRNS suggested that fosinopril significantly reduced proteinuria [45]. RAS blockade is especially effective for those patients with chronic renal insufficiency. Monitoring adverse effects of RAS inhibition, notably hyperkalemia and reduction of glomerular filtration rate, is necessary during treatment [11].

8. Galactose

Oral galactose, a monosaccharide sugar, inhibits the circulating permeability activity that is the cause of FSGS [46]. However, currently, there is little evidence that it improves proteinuria in children with FSGS [47]. There are only a few case reports about partial remission after oral galactose therapy at a dose of 0.2 g/kg twice a day as a nontoxic and adjunctive agent for SRNS [48, 49].

9. Rituximab

Rituximab is a chimeric monoclonal antibody that inhibits CD20-mediated B lymphocytes [11]. It has been used in hematologic malignancies such as B cell non-Hodgkin lymphoma [50]. Rituximab is administered by two to four intravenous infusions at a dose of 375 mg/m^2 weekly or biweekly [41]. There are several reports of rituximab therapy being an effective steroid-sparing agent for children with SDNS [51, 52] or SRNS [50, 53, 54]. Rituximab and other immunosuppressants such as cyclosporine or MMF combination therapy for SRNS have been reported to be successful in decreasing the relapse rate [55, 56]. However, some patients with SDNS become rituximab dependent as they do with steroids or calcineurin inhibitors [51]. Anti-rituximab autoantibodies may be a concern after repetitive infusion of rituximab [57]. Therefore, the presence of anti-rituximab autoantibodies should be monitored if a severe infusion reaction occurs and B cell depletion is not observed despite rituximab therapy [57, 58].

10. Synthetic Adrenocorticotropin Analog

Injection with adrenocorticotropin (ACTH) was used many decades ago as a therapeutic agent for children with nephrotic syndrome, but it has been replaced by cheaper oral steroids [59]. Response to ACTH among patients with FSGS is about 30%, but ACTH gel may be an alternative treatment option for some patients with SRNS [60, 61].

11. Abatacept

Abatacept (cytotoxic T-lymphocyte-associated antigen 4–immunoglobulin fusion protein [CTLA-4–Ig]) is an inhibitor of the T-cell costimulatory molecule B7-1 (CD80) [62]. It is currently approved for the treatment of rheumatoid arthritis. Podocyte B7-1 expression was found in patients with proteinuric kidney [62]. Yu and colleagues reported five patients with FSGS, four patients with rituximab-resistant recurrent FSGS, and one patient with steroid-resistant FSGS, whose high proteinuria resolved after abatacept treatment [62]. The authors explained that abatacept may attenuate β1-integrin activation in podocytes and decrease proteinuria in patients with B7-1-positive glomerular disease [62].

12. Adalimumab

Adalimumab is a human monoclonal antibody directed against tumor necrosis factor-α (TNF-α), which triggers an autoimmune response [61]. The Novel Therapies for Resistant FSGS (FONT) Study Group conducted a phase I trial of adalimumab in FSGS [63]. Adalimumab was injected subcutaneously every 2 weeks at a dose of 24 mg/m^2 with a maximum of 40 mg for 16 weeks (total, nine doses) in 10 patients with resistant FSGS [63]. Adalimumab was well tolerated with no serious side effects and 4 out of 10 patients with proteinuria had it decreased by more than 50% [63]. The FONT Study Group formed the basis for a phase 2 study of adalimumab in resistant FSGS [63].

13. Fresolimumab

Fibrosis represents the final common pathway of glomerular damage in FSGS, leading to chronic kidney disease. The experience with a wide range of antifibrotic agents was summarized in a recent review article [61]. Fresolimumab is a recombinant, fully human monoclonal antibody and inhibits the activity of all isoforms of transforming growth factor (TGF-β) [64]. Trachtman and colleagues conducted a phase 1, single-dose study of fresolimumab in adults with treatment-resistant FSGS [64]. They reported one case of complete remission and two cases of partial remission of proteinuria among a total of 16 patients with resistant FSGS. Further studies are needed to confirm the efficacy of fresolimumab for the treatment of resistant FSGS.

14. Conclusions

FSGS is a heterogeneous disorder and response to treatment is individually diverse. SRNS is an indication for a renal biopsy and genetic study. If the renal biopsy shows FSGS and if no pathogenic mutations in podocyte genes can be identified, efforts to minimize drug toxicity are important in pediatric FSGS. However, nephrologists still depend on traditional treatment approaches based on glucocorticoids plus calcineurin inhibitors. A steroid-based combination therapy with one or two drugs such as calcineurin inhibitors, MMF, or RAS blockade for refractory cases can induce partial remission of proteinuria. Severe FSGS cases experiencing treatment toxicities may try novel therapies such as rituximab, abatacept, adalimumab, or fresolimumab. In the future, further investigations of the disease mechanism will assist in the introduction of novel targeted individual approaches for treating FSGS.

Disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the paper.

Competing Interests

The authors have no potential competing interests to disclose.

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Review Article

FSGS Recurrence in Adults after Renal Transplantation

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Recurrence of focal segmental glomerulosclerosis (FSGS) in the allograft occurs in 30–50% of patients, and it is associated with poor renal allograft survival. Major risk factors for recurrence are younger age at diagnosis, rapid progression to end-stage renal disease, white race, and the loss of previous allografts due to recurrence. Recent data support the hypothesis that circulating permeability factors play a crucial role in podocyte injury and progression of FSGS. Due to lack of controlled trials, the management of recurrent FSGS is inconsistent and highly empirical. Prophylactic and perioperative treatment with plasmapheresis and high-dose (intravenous) cyclosporine represent the main cornerstones of immunosuppressive therapy. In recent years, therapy with rituximab has shown promising results. Despite evidence of activation of the renin-angiotensin system (RAS) in recurrent FSGS and its association with progression, only limited data exist on the renoprotective role of RAS blockade in this setting. Further well designed studies are needed on pathogenesis risk factors and therapeutical options in FSGS and its recurrence after transplantation.

1. Introduction

Focal segmental glomerulosclerosis (FSGS) is the leading cause of nephrotic syndrome in the adult population. FSGS is either termed primary (i.e., idiopathic), when a specific cause cannot be identified, or secondary to a variety of etiologies, such as genetic (specific mutations of podocyte genes), viral-associated (e.g., HIV, parvovirus B19, simian virus 40, cytomegalovirus, and Epstein-Barr virus), drug-induced (e.g., pamidronate, heroin, lithium, interferon, calcineurin inhibitors, and sirolimus), and adaptive (e.g., structural-functional responses to glomerular hypertension, such as conditions with reduction of renal mass and hyperfiltration of the remaining nephrons) [1]. In general, only primary FSGS recurs following kidney transplantation.

Within 10–20 years from diagnosing a substantial proportion (approximately 40–70%) of patients with FSGS progress to end-stage renal disease (ESRD), making FSGS the most common primary glomerular disorder in the dialysis population with a prevalence of 4% [1–3]. The first case report of FSGS recurrence was published by Hoyer et al. in 1972 [4]. Currently, the reported FSGS recurrence rate averages approximately 30% [5, 6]. However, it is likely that the recurrence rates of idiopathic FSGS are even higher (up to 50%) due to the fact that the cause of ESRD is difficult to ascertain and it is often not clear if the patient had primary FSGS or FSGS related to other causes [7]. The clinical hallmark of FSGS recurrence is proteinuria, which is often diagnosed within days after transplantation, and sometimes the full picture of the nephrotic syndrome may be present [8]. Diffuse foot process effacement as detected by electron microscopy is the only initial finding of FSGS in early allograft biopsies. As shown by Chang et al. this characteristic histological feature may already appear within 1–2 hours after reperfusion, predicting the recurrence of nephrotic range proteinuria 3–7 days posttransplant with a sensitivity of 71% and a specificity of 92%. Furthermore, in this study there was an association of the degree of foot process effacement with proteinuria, suggesting a key role of podocyte injury in the pathogenesis of recurrent FSGS [9].

Among patients with biopsy-proven FSGS as cause of ESRD the recurrence of the disease is associated with an increased risk of allograft loss [10]. In a large study from the Australia and New Zealand Dialysis and Transplant Registry (ANZDATA) the incidence of allograft loss at 10 years due to recurrent FSGS was 12.7% (95% CI 7.3–21.6). Furthermore, those patients with recurrent FSGS had a twofold higher risk of allograft loss as compared to patients with other

glomerulonephritides (adjusted HR 2.03, 95% CI 1.19–3.44) [11].

2. Pathogenesis of FSGS Recurrence

Gallon et al. reported an interesting case of FSGS recurrence after kidney transplantation [12]. A 27-year-old man with ESRD due to primary FSGS received a kidney transplant from his healthy 24-year-old sister. Despite pre- and perioperative plasmapheresis and standard immunosuppressive therapy, nephrotic range proteinuria developed on postoperative day 2. Allograft biopsy on day 6 revealed marked podocyte foot process effacement and loss of the interdigitating arrangements, consistent with recurrence of FSGS. On day 14 the renal allograft was removed due to severe hypoalbuminemia, progressive acute kidney injury, and an abdominal hematoma. After consultation of the institutional review board and obtaining informed consent, the kidney was transplanted into a 66-year-old man with ESRD caused by diabetes mellitus type 2. Within days after retransplantation kidney function improved and proteinuria decreased significantly. Furthermore, allograft biopsies performed on day 8 and 25 after retransplantation showed reversal of the glomerular lesions. This report supports the theory of a circulating factor as cause of primary FSGS, and it provides evidence that podocyte injury might be reversible at least before renal scarring occurs.

An extensive review of the pathogenesis of recurrent FSGS is beyond the scope of this chapter. In brief, the hypothesis that both primary FSGS in the native kidneys and also recurrent disease in the allograft are likely due to circulating factors or the absence of a normally present factor in the plasma is supported by several lines of evidence: first, it has been shown that pretransplant serum of patients with FSGS may increase the permeability of glomeruli to albumin *in vitro*. The serum of patients with recurrent FSGS after transplantation had significantly higher permeability values as compared to controls. Furthermore, the *in vitro* tested permeability was reduced to control values after plasmapheresis, which was associated *in vivo* with a decrease in proteinuria [13]. Second, insights from the Buffalo/Mna rat model of spontaneous FSGS further support the hypothesis of a circulating factor. The Buffalo/Mna rats develop proteinuria and present with renal histological features of human FSGS. Treatment with steroids, cyclosporine, or cyclophosphamide leads to proteinuria reduction. When a kidney from a healthy control rat is transplanted into a Buffalo/Mna rat, FSGS recurs. On the other hand, when Buffalo/Mna rat kidneys are transplanted into control rats, proteinuria and renal lesions regress [14]. Third, the frequent occurrence of a relapse of proteinuria early after transplantation, the rapid recovery of allograft function following retransplantation into a patient without FSGS, and improvement of proteinuria after plasmapheresis or rituximab suggest that injury of the podocytes is caused by a circulating factor, supposedly an autoantibody or a factor released by T cells upon interference with B cells. Finally, in genetic forms of FSGS the recurrence rate is low (but not zero). In patients with homozygous FinMajor-NPHS1 (nephrin) mutations, the recurrence of proteinuria posttransplant is probably due to preexisting antinephrin antibodies in the recipient [15]. In the case of FSGS due to homozygous NPHS2 (podocin) mutations, the existence of antipodocin antibodies has not been proven to date [16]. Jungraithmayr et al. identified genotype-phenotype correlations of NPHS2 mutations and recurrence of FSGS in a cohort of 53 children with primary FSGS. Interestingly, none of the 11 children who were homozygous or compound heterozygous for NPHS2 mutations developed a recurrence of FSGS, compared with 45% of the patients without mutations [17]. In a similar manner Weber et al. described FSGS recurrence only in one of 32 patients with two NPHS2 mutations [18]. In contrast to these findings Bertelli et al. found an equal recurrence rate in genetic and nongenetic forms of FSGS; however, they included heterozygous NPHS2 mutations in their analysis [19]. Circulating permeability factors such as the soluble urokinase receptor (suPAR) [20, 21] and autoantibodies directed against actin, adenosine triphosphate synthase, angiotensin II type 1 receptor, nephrin (NPHS1), protein tyrosine phosphatase receptor type O (PTPRO), and Thy1 have been implicated in the pathogenesis of FSGS recurrence [15, 22–24].

Although several potential permeability factors have been identified, current evidence on the nature of this factor and on the pathogenesis of recurrent FSGS remains frustratingly inconclusive and is an ongoing subject of extensive speculation.

3. Risk Factors for Recurrence

Several clinical risk factors have been associated with an increased risk of FSGS recurrence after transplantation (reviewed in [25]), including younger age at onset of initial disease (particularly between 6 and 15 years of age) [26, 27], rapid progression of primary FSGS to ESRD (<3 years) [28–30], white race [10], and the loss of previous allografts due to recurrence [28]. Retrospective data from the United States Renal Data System (USRDS) suggests that ethnicity and genetic background may have an impact on the risk of recurrence. In this analysis the risk of recurrent FSGS was higher in white than in nonwhite patients, and particularly white recipients of African-American kidneys had an increased risk of recurrence [10]. Patients who received pretransplant bilateral nephrectomy may experience a higher risk of recurrence [27]. It is speculated that native kidneys act as absorbers of permeability factors, although data on nephrectomy are contradictory [31].

Steroid-resistant nephrotic syndrome due to FSGS in the native kidneys may indicate lower risk of recurrence. In a recent study of 125 children with steroid-resistant nephrotic syndrome (>95% biopsy-proven FSGS), it was shown that 92.9% of those patients who initially were steroid-responsive (defined as complete remission of proteinuria on at least one episode after steroid therapy) experienced a recurrence of FSGS compared to only 30.2% of steroid-resistant patients (OR 30; 95% CI 6.62–135.86) [32].

Data are inconclusive on the role of induction therapy and initial immunosuppression and the risk of FSGS recurrence.

In a retrospective single center study the use of antilymphocyte sera, particularly antithymocyte globulin (ATG), was associated with a higher risk of recurrence, as compared to no induction therapy [33]. On the contrary, Pascual et al. showed that induction therapy with ATG was associated with a reduced risk of recurrence of any primary glomerulonephritis, including FSGS, as compared to alemtuzumab or interleukin-2 receptor antagonists [34]. No differences in the rate of graft loss due to recurrent FSGS were seen in a large retrospective analysis ($n = 4502$ patients with FSGS as initial disease) from the Organ Procurement and Transplant Network/United Network of Organ Sharing (OPTN/UNOS) between those patients treated with mycophenolate mofetil versus those treated with azathioprine in cyclosporine based regimens [35].

Five morphological variants of FSGS exist, namely, NOS (not otherwise specified), perihilar, cellular, tip, and collapsing variant [36]. Although it has been recognized that the histological subtype predicts the course of disease in the native kidneys, it is not clear if the same subtype is observed when FSGS recurs in the allograft [37, 38]. One can speculate that recurrent FSGS in the allograft may initially represent the same histological variant as in the native kidney (maybe with the same risk of progression to ESRD). However, ischemia-reperfusion injury and the effect of calcineurin inhibitors on the single graft may cause a mismatch between nephron number and metabolic demand leading to a mixture of primary and adaptive (i.e., secondary) form of FSGS thus changing histology and prognosis [39].

As mentioned above the risk of recurrence is low in genetic or familial forms of FSGS, depending on the disease causing mutation [15, 17, 29].

Several molecules have been proposed as biomarkers to quantify the risk of recurrence after transplantation. One of the most extensively studied candidates is suPAR, which not only serves as a mere biomarker, but also has been proposed as being involved in the pathogenesis of FSGS by activating podocyte $\beta 3$ integrin causing foot process effacement [21]. Higher levels of suPAR before transplantation were associated with an increased risk of recurrence of FSGS in the allograft [21]. Reduction of very high suPAR levels during FSGS recurrence by a combination of plasmapheresis and immunoadsorption was associated with remission of proteinuria [40], while immunoadsorption alone did not alter suPAR levels [41]. However, elevated suPAR levels have also been identified in a wide range of inflammatory diseases, including pneumonia, malaria, tuberculosis, HIV, sepsis, and also in various cancers [42]. Furthermore, the role of suPAR as a marker of FSGS and its recurrence was questioned by data showing no difference in suPAR levels between primary FSGS, secondary FSGS, and also minimal change disease (MCD). Also response to steroid therapy was not predicted by suPAR levels [43]. However, in this report an inverse correlation of suPAR levels with eGFR was found, independently of histological diagnosis. Recently, suPAR was identified also as a predictor of incident chronic kidney disease in the Emory Cardiovascular Biobank Cohort [44]. These inconclusive results can be explained by the fact that not all molecular forms of suPAR are equally pathogenic to podocytes or can be measured easily in human subjects [45], questioning the straightforward clinical usefulness of suPAR as a marker of FSGS recurrence.

In a recent study Delville et al. identified a circulating antibody panel as predictor of FSGS recurrence [46]. By high-throughput screening of pretransplant sera from patients with recurrent FSGS and patients without recurrence, the authors identified antibodies targeting glomerular antigens. Of these a panel of seven antibodies (CD40, PTPRO, CGB5, FAS, P2RY11, SNRPB2, and APOL2) was validated in an independent cohort of patients with and without FSGS recurrence. The panel was able to predict FSGS recurrence with 92% accuracy. Of these antibodies anti-CD40 alone had the best correlation with risk of FSGS recurrence (78% accuracy). Purified anti-CD40 antibodies from humans with recurrent FSGS disrupted the cytoskeleton of podocytes—possibly in a suPAR dependent manner—and also induced proteinuria in murine experiments, suggesting a role of CD40 perturbations also in the pathogenesis of FSGS and its recurrence.

4. Treatment of Recurrent FSGS

Despite advances in the understanding of FSGS recurrence and progression, management remains difficult and treatment decisions are often based on evidence from small case series. Data from controlled trials comparing the efficacy of various approaches are lacking.

One of the most commonly used therapies for recurrent FSGS is plasmapheresis. Since the original description of a successful treatment in 1985, numerous case reports and case series have been reported with varying degrees of success [47]. In established FSGS recurrence plasmapheresis can induce remission in 70% of children and in 63% of adults, as summarized by Ponticelli in a systematic review [48]. However, one can assume that the published treatment effect is overestimated due to publication bias, retrospective and uncontrolled design, and short-term follow-up. Best results seem to be achieved when plasmapheresis is started early after transplantation, immediately when recurrence becomes clinically evident. A typical plasmapheresis prescription is 1–2 times plasma volume exchanges, 3–4 treatments per week, and a total of 8–12 treatments until remission is achieved. However, in some cases weaning protocols or intensive plasmapheresis for up to several months has been reported [26]. Prophylactic plasmapheresis therapy during the perioperative period has also been proposed. Gohh et al. prospectively treated 10 patients with at least 8 plasmapheresis sessions during the perioperative period. Recipients of living donor kidneys received plasmapheresis from 1 week before to 1 week after the transplantation. Recipients of deceased donor kidneys received the first plasmapheresis 24 hours before implantation. Interestingly, FSGS recurrence was diagnosed in none (0 of 4) of the high-risk patients with rapid progression to ESRD and in only 50% (3 of 6) of the patients who lost their first graft due to recurrent FSGS. The authors concluded that these rates might be less than expected from historical reports [49]. However, other authors did not find any benefit from prophylactic treatment [26, 50]. Some case reports have suggested the combined use of plasmapheresis

and immunoadsorption, but data on this treatment are very limited and difficult to interpret [51, 52].

The use of standard oral doses of cyclosporine has not been shown to prevent FSGS recurrence; however, higher intravenous doses have been associated with proteinuria reduction (reviewed in [39]). The rationale behind maintaining high cyclosporine blood levels is explained by the lipophilic characteristics of cyclosporine. Cyclosporine is incorporated into peripheral lymphocytes via binding to LDL receptors on the cell surface. High blood levels of LDL cholesterol as often seen in patients with nephrotic syndrome reduce the amount of the free drug. Thus, hypercholesterolemia inhibits the effect of cyclosporine on lymphocytes, and high blood levels may overcome this effect. In one prospective yet uncontrolled cohort study, intravenous cyclosporine at a dose of 3 mg/kg/day for 3–4 weeks, followed by an oral dose maintaining blood levels between 250 and 350 ng/mL, induced remission in 14 or 17 patients [53]. Raafat et al. have reported encouraging results with high-dose oral cyclosporine [54]. However, well-known complications of high doses of cyclosporine limit long-term safety of such a treatment.

Rituximab is a chimeric mouse/human monoclonal antibody targeting the CD20 surface antigen on B-lymphocytes, selectively depleting these cells. Furthermore, rituximab seems to have a direct protective effect on podocytes. Rituximab partially prevented the downregulation of sphingomyelin phosphodiesterase acid-like 3b (SMPDL-3b) protein and acid sphingomyelinase (ASMase) that was observed in podocytes treated with the sera of patients with recurrent FSGS [55]. Its beneficial effect on recurrent posttransplant FSGS was reported initially in 2006 [56]. Since then several reports on the efficacy of rituximab treating recurrent FSGS have been published (summarized in [39]). A systematic review of 39 reported cases indicates a complete or partial remission in 64% of the patients [57]. Normal serum albumin at recurrence and younger age were associated with treatment response. Interestingly, in the univariate analysis fewer rituximab infusions were also associated with a better treatment effect, which is in line with data from idiopathic membranous nephropathy and its recurrence after transplantation [58]. It remains to be elucidated if titrating the dose of rituximab to obtain B-cell depletion is the optimal strategy in this setting. In the published reports a typical rituximab regimen is 2–6 doses of 375 mg/m^2/dose given once every one to two weeks. Some case reports suggested better efficacy when rituximab is combined with plasmapheresis [59, 60]. In the reports of 4 children by Tsagalis et al., rituximab was administered at a dose of 1 g, two doses two weeks apart. After rituximab infusion, plasmapheresis was not performed during the next 72 hours to prevent removal of the antibody. Complete remission was achieved in 2 and partial remission in the other 2 patients, while renal function remained stable and no severe infectious complications occurred during a follow-up time of 18–60 months [59].

Despite evidence that activation of the renin-angiotensin system (RAS) is also crucially involved in progression of recurrent FSGS [61], only few case reports have been published addressing the beneficial effect of RAS blockade on proteinuria reduction in recurrent FSGS [50, 62, 63]. Reduction of proteinuria in this setting nicely illustrates that recurrent FSGS is not entirely immunologically mediated but rather includes components of both primary and adaptive FSGS.

In one case report an intravenous infusion of galactose in a patient with recurrent FSGS reduced circulating permeability factor activity [64]. The current use of cyclophosphamide in recurrent FSGS is not frequent due to contradictory results and concerns about long-term toxicity [25]. In a case series of 4 patients with recurrent FSGS resistant to plasmapheresis and rituximab treatment with abatacept, a soluble fusion protein that blocks the T-cell costimulatory protein B7-1 (CD80) was associated with almost complete remission [65]. However, others were not able to reproduce these beneficial results neither with abatacept nor with belatacept [66–68].

5. Living Kidney Donation in Patients with FSGS

Data from large registries such as the Recurrent Allograft Disease Registry (RADR), the USRDS, the North American Pediatric Renal Transplant Cooperative Study (NAPRTCS), and the UNOS demonstrated a similar rate of FSGS recurrence and graft loss among recipients of kidneys from living versus deceased donors [7, 10, 69, 70]. However, recipients of living donor transplants may lose their survival advantage which is usually observed over recipients of deceased donor transplants [69]. Analysis of the USRDS data showed that death-censored graft survival was significantly better in patients with FSGS who received a zero-mismatch kidney from living donors as compared to patients who received a zero-mismatch organ from cadaveric donors [71]. In the case of genetic or familial FSGS, donor selection has to be performed with great caution. In the cohort examined by Jungraithmayr et al., not only homozygous NPHS2 mutations but also heterozygous NPHS2 mutations or variants (e.g., R229Q) were identified. The authors proposed to perform genetic screening of the related donor when the recipient has a mutation of NPHS2. In the case of recessive (i.e., homozygous) mutations, one could accept a heterozygous donor. In the case of heterozygous mutations or variants such as R229Q, it is not clear if this is a dominant-negative variant, which would pose the donor at risk. In such case the donor should not be accepted. In particular the long-term clinical significance of heterozygous NPHS2 mutations is currently unknown, and some centers do not accept heterozygote donors.

6. Conclusions

The frequent recurrence of FSGS in the allograft is associated with poor graft survival. Despite novel insights into the pathogenesis of FSGS and its recurrence, outcomes did not substantially change in the last decade. Plasmapheresis, high-dose cyclosporine, and more recently rituximab represent the most promising therapeutical options. Recent advances on the pathology and pathophysiology of podocyte injury in FSGS, some of them hypothesis-driven and some

hypothesis-generating, may transform not only into better risk-stratification, but also into more specific therapies for patients with FSGS.

Competing Interests

The author declares that there are no competing interests regarding the publication of this paper.

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Review Article

Treatment Strategies of Adult Primary Focal Segmental Glomerulosclerosis: A Systematic Review Focusing on the Last Two Decades

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Adult primary focal segmental glomerulosclerosis (FSGS) remains a therapeutic challenge for the treating physician. With the advent of novel immunosuppressive measures, our arsenal of therapeutic options increased considerably. The aim of this review was to summarize reports published over the last two decades which reported on treatment outcome. Most reports included patients with a steroid-resistant (SR) disease course, yet the cohort with the highest unmet need, since persistent nephrotic range proteinuria is associated with a poor renal prognosis and portends a high risk of developing end-stage renal disease. While in first-line treatment, steroid treatment remains the recommended standard with an overall remission rate of 50% and higher, optimal treatment strategies for steroid-dependent/multirelapsing (SD/MR) and SR patients have to be defined. In both entities, calcineurin inhibitors showed good efficacy, while mycophenolate mofetil was less effective in SR cases compared to those with SD/MR. The same was true for rituximab, a monoclonal antibody targeting B-cells. In resistant cases, addition of extracorporeal treatment options or treatment with alkylating agents may be considered. To shape the future for treatment of FSGS, international collaborations to conduct larger clinical trials are needed to identify potential novel efficacious immunosuppressive or immunomodulatory therapies.

1. Introduction

The incidence of focal segmental glomerulosclerosis (FSGS) has increased over the past decades and it is assumed to be one of the leading causes of idiopathic nephrotic syndrome in adult patients. Racial disparities have been reported with African American being two to three times more often affected than Caucasian [1]. Despite an increased arsenal of therapeutic options, treatment of this glomerular lesion is remaining a challenge for nephrologists. In contrast to other primary forms of nephrotic syndrome spontaneous remission is rare (<5%) and initiation of immunosuppressive measures should be commenced once diagnosis is confirmed by renal biopsy. Presence of nephrotic syndrome (>3–3.5 g/d) portends a poor prognosis with 50% of patients progressing to end-stage renal disease (ESRD) 6–8 years after initial diagnosis, whereas patients with nonnephrotic proteinuria in particular have a favorable outcome. Those with massive nephrotic syndrome (proteinuria > 10 g/d) tend to have an even more aggressive disease course with half of the patients reaching ESRD after 3 years. Serum creatinine above 1.3 mg/dL (approximately 114 μmol/L) was associated with a poorer prognosis than a preserved renal function [2]. Analysis of the United States Renal Data System revealed that FSGS is the leading cause of glomerulonephritis-associated ESRD in the United States [3], highlighting the importance of improved surveillance (diagnosis early in the disease course) and improved strategy options to overcome treatment unresponsiveness.

Thus, the aim of this systematic review was to summarize the literature published over the last two decades focusing on the treatment of adult primary FSGS.

2. Materials and Methods

To evaluate literature-based publications over the last two decades, the MEDLINE database search was restricted to

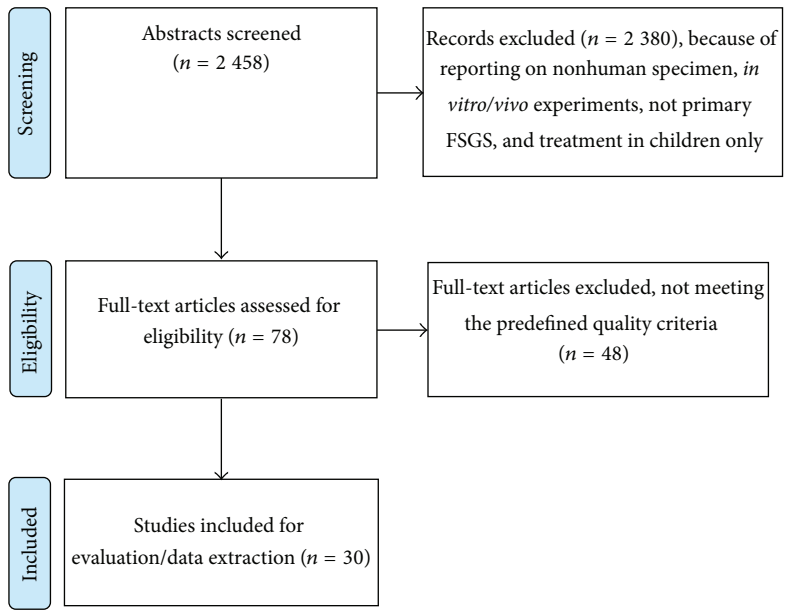

Figure 1: The search strategy "focal segmental glomerulosclerosis" AND "treatment" yielded a total of 2 458 abstracts which were evaluated regarding the predefined criteria. After initial evaluation, 78 articles were accessed in full text. Of these, 48 could be excluded due to not meeting the predefined criteria. Thus, data of 30 articles were extracted (modified from [4]: Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement).

a time period ranging from January 1995 to 31 October 2015. The search was conducted using the keywords "focal segmental glomerulosclerosis" AND "treatment". Restricting the time frame to the last two decades would allow access to a majority of identified manuscripts.

We predefined the following exclusion criteria for further data analysis: (i) presenting data on recurrence of FSGS after kidney transplantation, (ii) combining treatment data of children and adults with a predominance of the former, (iii) reporting no concrete outcome data (i.e., not reporting on the number of patients achieving either partial/complete remission or treatment failures and/or not the mean decrease of proteinuria following initiation of treatment), (iv) no differentiation whether patients had an underlying primary FSGS or other entities leading to nephrotic syndrome, (v) no differentiation of treatment modalities (i.e., combining results of patients receiving prednisolone and other immunosuppressive measures in final analysis), (vi) no differentiation between steroid-dependent (SD)/multirelapsing (MR) and steroid-resistant (SR) patients in the analysis of the results, or (vii) reports including a low number of patients (arbitrary cut-off ≥ 5 patients).

3. Results

3.1. Search Strategy. The systematic search resulted in an overall number of 2 458 records. A large number of articles ($n = 2\,380$) could be excluded since these records reported on *in vitro* experiments, non-FSGS related studies, other entities leading to nephrotic syndrome, or findings in children. A total of 78 articles were left over after initial screening. After access of full text we could exclude another 48 articles, which did not meet our predefined inclusion criteria. Data were extracted from 30 articles reporting on treatment outcome of patients with primary FSGS (see Figure 1).

We divided the results obtained from the included studies into three categories, namely, first-line treatment, SD/MR, and SR.

3.2. First-Line Treatment Options in Focal Segmental Glomerulosclerosis. Most studies reported on first-line treatment consisting of daily oral prednisolone and in some cases in combination with other immunosuppressive measures. The total number of patients treated with prednisolone ranged from 8 to 79 patients in the respective studies. The overall response rate reported in these studies ranged from 50% [5] up to 68.8% in a prospective study conducted in India [10]. Follow-up of patients was highly diverse, ranging from 16.2 to 62 months. As expected, in the study with the shortest follow-up the relapse rate was the lowest (27.3%) [10], while Rydel and colleagues reported a relapse rate of 67% [5]. In the study reporting a single center experience, a majority received high dose prednisone for at least one month (87% $\geq$ 60 mg/d) and those remaining on high dose prednisolone treatment showed a significant trend towards better response. A multicenter retrospective analysis from Italy revealed remission of 31 out of 52 patients treated with steroids (either 1 mg/kg body weight for 8 weeks with subsequent tapering or three intravenous pulses of 1 g each followed by 0.5 mg/kg body weight oral prednisolone with subsequent tapering). All included patients had nephrotic range proteinuria measured at least twice ahead of treatment initiation. Of the 38 patients who did not achieve either complete or partial remission, 26 were retreated with either prolonged corticosteroid or other

Table 1

First author	Year	Design	Study	Country	PRED	TAC	CSA	MMF	AZA	No	CR	PR	NR	PR (BL)	PR (FU)	RL (%)	FU (m)
Rydel [5]	1995	Retro	Cohort	USA	1	0	0	0	0	30	12	3	15	13.6 ± 10		67	62
Ponticelli [6]	1999	Retro	Cohort	Italy	1	0	0	0	0	53	21	10	22			25.8	
Choi [7]	2002	Retro	Cohort	USA	0	0	0	1	0	10	3	2	5	4.5 ± 3.1	2.6 ± 2.9		7.9
Duncan [8]	2004	Pro	Cohort	UK	0	1	0	0	0	6	0	6	0	11 ± 4.5	2.8 ± 2.5	0	12.8
Goumenos [9]	2006	Retro	Cohort	UK/Greece	1	0	0	0	0	8		5	3				
Goumenos [9]	2006	Retro	Cohort	UK/Greece	1	0	1	0	0	7		6	1				
Goumenos [9]	2006	Retro	Cohort	UK/Greece	1	0	0	0	1	5		4	1				
Senthil Nayagam [10]	2008	Pro	RCT	India	1	0	0	1	0	17	10	2	5			33.3	15.3
Senthil Nayagam [10]	2008	Pro	RCT	India	1	0	0	0	0	16	9	2	5			27.3	16.2
Jafry [11]	2012	Retro	Cohort	Egypt	1	0	0	0	0	79	36	4	39	6 ± 4.4	4.6 ± 5.1	35	26

AZA: azathioprine, CR: complete remission, CSA: cyclosporine A, FU: follow-up, MMF: mycophenolate mofetil, No: number, NR: no remission, PRED: prednisone/prednisolone, PR: partial remission, PR (BL): proteinuria baseline, PR (FU): proteinuria follow-up, Pro: prospective, Retro: retrospective, RL: relapse, and TAC: tacrolimus.

immunosuppressive measures (azathioprine, cyclosporine A, or cyclophosphamide). Among those receiving steroids two of the patients achieved complete and partial remission (out of six), while cytotoxic drugs and cyclosporine A (CSA) treatment led to one and zero complete as well as five and seven partial remissions (out of 11 and 9 patients) [6]. A study from Egypt included a total of 79 patients. Of these, a majority had nephrotic syndrome at the time of treatment. In total, 40 patients achieved remission followed induction treatment with prednisolone therapy (1 mg/kg body weight for 6 weeks, followed by 0.75 mg/kg body weight for another 6 weeks, and subsequent tapering). Mycophenolate mofetil (MMF) efficacy was evaluated in two studies. One retrospective cohort reported by Choi and colleagues treated patients either with or without concomitant steroid due to impaired renal function ($n = 7$) or nephrotic syndrome ($n = 7$). MMF dosage varied from 1.0 to 2.0 g per day and five out of ten patients achieved remission (3 complete and 2 partial). Follow-up was rather short with 7.9 months [7]. In a prospective study from India MMF (target dose 2 g/day for 6 months) was given along with a reduced steroid dose (0.5 mg/kg body weight as initial dosage, total treatment duration 2-3 months). Out of 17 patients, 70.8% achieved remission (10 complete and 2 partial). Remission rates were comparable to those receiving prednisolone monotherapy (initial dosage 1 mg/kg body weight, cumulative prednisolone dosage 7.3 ± 0.9 g versus 1.9 ± 0.3 g in the MMF group). Mild gastrointestinal discomfort was noticed in one patient and two patients in the MMF group required hospitalization due to severe infection. However, side effects were not reported independent of disease entity (patients with membranous nephropathy and FSGS). Subsequent relapse rate was similar in both groups as well [10]. Other studies reporting on first-line treatment in FSGS included a small number of patients only (<10). Among these, one reported on single center experience with tacrolimus monotherapy in six patients. All subjects achieved partial remission with a median reduction of proteinuria from 11 ± 4.5 to 2.8 ± 2.5 g/d, while serum albumin improved from 26.8 ± 4.6 to 37.7 ± 1.9 g/L. During a follow-up period of 12.8 months, no relapse was observed [8]. One study retrospectively analyzed patients treated with either prednisolone (1 mg/kg body weight) alone, prednisolone (0.5 mg/kg body weight) with azathioprine (AZA, 2 mg/kg body weight), or CSA (3 mg/kg body weight). Remission rates were higher in the latter two groups, whereas side effects were observed in the prednisolone group (3 patients became cushingoid) and leukopenia was observed in two patients being treated with AZA [9]. More details related to the single studies are highlighted in Table 1. Taken together, remission rates after steroid treatment are reported to be at least 50%. Alternative treatment strategies, such as MMF or tacrolimus, either in combination or as monotherapy, may yield similar remission rates. In patients with absolute or relative contraindication towards steroid treatment, these agents may have a role in the first-line treatment.

3.3. Steroid-Dependent/Multirelapsing Focal Segmental Glomerulosclerosis. We used "steroid-dependent" for patients achieving remission after steroid induction but having relapses upon steroid tapering or within two weeks after discontinuation [12], while we used "multirelapsing" for patients with a relapsing disease. Various studies/case reports investigated the effect of additional immunosuppressive agents on top of ongoing steroid treatment. In a prospective study from Korea, all patients (total $n = 5$) achieved remission (complete remission in 4 patients). At baseline all patients received daily oral prednisolone (total dosage 10 mg) with subsequent tapering and CSA in an initial dosage of 5 mg/kg body weight (with the aim of achieving a trough level between 100 and 200 ng/mL). If the trough level was not maintained above

100 ng/mL or patients had an incomplete response, CSA dose was increased up to 7 mg/kg body weight a day [13].

Experience with MMF is limited to one report including several entities leading to nephrotic syndrome. Among patients with FSGS, the median proteinuria decreased from 5.1 g/day at baseline to 1.9 g/day during follow-up. Remission was recorded in eight patients (3 complete and 5 partial), while 5 patients were nonresponsive towards MMF (target dose 1.5–2 g/day). Side effects were mainly restricted to gastrointestinal symptoms [14]. Sirolimus, targeting mammalian target of rapamycin (mTOR), was tested in a phase 2 open-label clinical trial. All patients ($n = 7$) had nephrotic range proteinuria at trial initiation. The target trough level during the first four months was 5–15 ng/mL and was further increased to 10–20 ng/mL during the following eight months. Therapy was stopped in five patients due to inefficacy and overall no patient experienced response to treatment [15]. Adrenocorticotropic hormone (ACTH) gel (80 units twice weekly) was tested in either SD or SR patients. Among those with SD FSGS, 2 patients showed a partial response (one with a not otherwise specified and one with a tip lesion histology), while four patients had no response (two not otherwise specified, one cellular, and one tip lesion). During follow-up, serum creatinine stabilized from a baseline median value of 1.5 mg/dL to 1.45 mg/dL (at follow-up), whereas serum album increased from 2.44 g/dL to 3.04 g/dL.

In their prospective trial, Ruggenenti et al. recruited patients with complete remission. The median relapse rate before and after rituximab (RTX) therapy was significantly reduced in the overall cohort. Among the eight patients with FSGS, three patients relapsed within a period of 12 months. In general, patients received a B-cell driven protocol (one infusion of 375 mg/m^2, which was repeated when B-cells were present in peripheral blood after one week). Overall, the concomitant steroid use could be reduced and the authors did not report serious adverse events following B-cell depletion [16]. All reports included in our systematic review are summarized in Table 2. CSA and MMF may be useful measures in the treatment of patients with SD/MRFSGS and among the novel immunosuppressive measures, remission maintenance was achieved following RTX treatment in most patients. However, cohorts including larger numbers of patients treated in this indication are clearly needed to draw definite conclusions.

3.4. Steroid-Resistant Focal Segmental Glomerulosclerosis. In general, steroid-resistance is defined as persistence of proteinuria despite prednisone treatment (1 mg/kg body weight or 2 mg/kg body weight every other day) for at least 4 months [12]. Most reports over the last two decades have focused on treatment options in this cohort with the highest unmet needs. Calcineurin inhibitors have frequently been used in this indication. Overall response of patients receiving CSA ranged from 57.1% to 77.8% in the different studies [6, 17–20]. One randomized trial including 26 patients evaluated relapse rate after 24 months of follow-up. CSA was initiated with a dose of 3.5 mg/kg body weight with subsequent adaption to a trough level of 125–225 μg/L, accompanied by a maximum prednisone dose of 15 mg with subsequent tapering over 26 weeks. Among those 18 patients with response (3 complete and 15 partial), 61% relapsed during the observational period. A decline in renal function defined as rise of 30% occurred in four patients. Increase in dosage or new prescription of an antihypertensive agent was necessary in eight patients [17]. A prospective study from Germany recruited patients failing a 6-week course of prednisolone (1.5 mg/kg body weight) and acetylsalicylic acid (500 mg/d). CSA was initiated with a trough level of 130–180 μg/L. Serum creatinine was preserved (1.5 ± 0.2 mg/dL), while proteinuria was within nephrotic range (5.5 ± 2.6 g/d). Of the 34 patients, 8 (23%) and 13 (38%) of the patients achieved complete and partial remission, respectively [20]. Another controlled trial from Germany included nephrotic patients and showed a decline of proteinuria from 5.4 ± 5.2 g/d to 2.5 ± 1.8 g/d during a follow-up of three years. No concrete details related to duration of treatment or dosage were given [19]. There were two articles reporting a large number of patients with steroid-resistance receiving tacrolimus treatment. Interestingly, Segarra and colleagues recruited patients with either CSA-resistance or CSA-dependence and showed a high response rate following tacrolimus initiation (initial dose 0.15 mg/kg, with a targeted trough level of 5–10 ng/mL; overall remission rate 72%, 10 complete and 8 partial remissions) combined with prednisone (1 mg/kg body weight for 4 weeks, with subsequent tapering) [21], while a prospective study from India revealed an overall response of 52.3% (17 complete and 6 partial remissions) following treatment initiation of tacrolimus (0.1 mg/kg body weight; trough level 5–10 ng/mL) and oral prednisolone (0.15 mg/kg body weight). The predominant histologic pattern was a not otherwise specified pattern in 75%. At the time of treatment initiation most patients exhibited nephrotic range proteinuria (4.5 ± 3.6 g/d) [22]. The relapse rate in those patients achieving remission was 52% [22] and 76% [21] during a follow-up time of 12–14 months.

Larger cohorts reporting on MMF efficacy in steroid-resistance showed lower overall response rates. In a prospective study by Cattran and coworkers 33.3% of patients achieved remission (0 complete and 6 partial). Patients received a maximum dose of MMF 1 g b.i.d. and prednisone in reducing steps (initial 0.25 mg/kg body weight). In general, treatment was well tolerated with one mild gastrointestinal symptom as predominant side effect and herpes zoster in one patient [24]. Medrano et al. reported an even lower response rate with 14.8% of their patients having remission during follow-up (0 complete and 4 partial). Notably, patients in the latter study were resistant towards CSA (trough level: 150–200 ng/mL). All patients had nephrotic range proteinuria when MMF (target dose 2 g/d) was initiated. Among the side effects, dose-dependent gastrointestinal symptoms were most frequent (33.3%), whereas other adverse events may be related to the continuation of CSA treatment (gingival hyperplasia, acute renal toxicity, and worsening of hypertension) [25]. Two prospective studies from Germany highlighted that chlorambucil is an effective immunosuppressive measure in SR FSGS. While Risler et al. showed a reduction in proteinuria from 3.4 ± 4.9 to 2.3 ± 1.1 g/d in 24 subjects during a follow-up time of 36 months [19], 15 out of 23 achieved remission in another study (4 complete and 11 partial, 65.2%)

Table 2

First author	Year	Design	Study	Country	CSA	MMF	RTX	SIR	ACTH	No	CR	PR	NR	PR (BL)	PR (FU)	RL (%)	FU (m)
Lee [13]	1995	Pro	Observational	Korea	1	0	0	0	0	5	4	1	0				18
Cho [15]	2007	Pro	Clinical trial	USA	0	0	0	1	0	6	0	0	6	8.4 ± 6	12.3 ± 5.8		8
Dimkovic [14]	2009	Pro	Cohort	Serbia	0	1	0	0	0	10	3	5	2	5.1	1.9		
Hogan [23]	2013	Pro	Observational	USA	0	0	0	0	1	6	0	2	4	7.7 ± 6.2	8 ± 9.7		
Ruggenenti [16]	2014	Pro	Observational	Italy	0	0	1	0	0	8				0.3	0.2	37.5	12

ACTH: adrenocorticotropic hormone, CR: complete remission, CSA: cyclosporine A, FU: follow-up, MMF: mycophenolate mofetil, No: number, NR: no remission, PR: partial remission, PR (BL): proteinuria baseline, PR (FU): proteinuria follow-up, Pro: prospective, Retro: retrospective, RL: relapse, RTX: rituximab, and SIR: sirolimus.

following treatment with prednisolone (1.5 mg/kg per day) and chlorambucil (0.1 to 0.4 mg/kg per day) [20].

Several articles reported on the use of extracorporeal measures, either plasma exchange or immunoadsorption. In the larger cohorts, high remission rates as well as significant reduction in proteinuria were reported in two studies from Japan including patients with persistent nephrotic syndrome (12/17, 8 complete and 4 partial) as well as reduction of proteinuria from 7.24 ± 3.58 g/d to 2.56 ± 2 g/d using LDL-apheresis (twice a week for 3 weeks in total; total volume 3-4 liters; concomitant treatment not stated) [26, 27] and from Saudi Arabia (8/11, 6 complete and 2 partial) using plasma exchange (5 daily consecutive sessions, followed by twice weekly for 2 weeks, then once a week for two weeks, every two weeks for four weeks, and finally four monthly sessions; total of 17 sessions), alongside oral prednisolone (1 mg/kg body weight for two months) and six pulses of monthly cyclophosphamide (5–10 mg/kg body weight) [28]. In contrast, one study from Austria (using either protein A or immunoglobulin G immunoadsorption, five sessions within two weeks, which was repeated when ineffective) [29] and one from the USA using plasma exchange (six sessions with 1.5 plasma volume exchanged over two weeks) [30] revealed a low remission rate after addition of extracorporeal treatment (20 and 25%, resp., with a complete remission in the former and two partial remissions in the latter study). Sirolimus was tested in a prospective open-label trial including a majority of patients having nephrotic syndrome (76%) despite 3 months of prednisone therapy. In contrast to the experience in SD patients, remission was achieved in a majority of patients (57.1%) with four and eight subjects having complete and partial remission. However, no initial dosage of sirolimus and no respective trough level were given by the authors. Abdominal pain was the most frequent side effect, followed by flu-like symptoms in two and oral ulcers in one patient [31]. In an observational trial, Hogan et al. reported a response rate of 43.8% following ACTH (80 units twice weekly as subcutaneous injections) treatment in SR patients. Of the responders, 2 achieved complete (both with tip lesion) and 2 partial remission (one with tip and one with a not otherwise specified lesion) [23]. Fernandez-Fresnedo and colleagues retrospectively collected data on RTX-treated patients. They found partial remission in two (both having a not otherwise specified lesion on renal histology) out of eight patients treated with RTX, while one patient had a transient decline of proteinuria twice immediately after initiation of treatment. One patient with partial response received eight consecutive weekly infusions (a dose of 375 mg/m^2), while the other showing response had four weekly infusions followed by two more infusions after six months. All others with no or transient response were treated with four consecutive weekly RTX infusions [32]. More efficacy data are needed for other measures, such as galactose (0.2 mg/kg twice a day, maximum dose 15 g) [33], which was tested in a recent trial. Three out of seven patients (2 with subnephrotic proteinuria) showed a partial response, while the others did not respond to galactose. In contrast, the preliminary trial performed by Trachtman and colleagues did not support the use of adalimumab (24 mg/m^2, maximum dose 40 mg fortnightly as a subcutaneous injection), a monoclonal antibody targeting tumor necrosis factor-α, in the treatment of SR FSGS, since all patients recruited failed to show a response [33]. The respective results are summarized in Table 3.

4. Discussion

The aim of this systematic review was to summarize the progress related to treatment strategies in adult FSGS over the past two decades. Clearly, we found most reports including patients with difficult-to-treat disease forms, namely, SR FSGS, indicating the high unmet need in effective immunosuppressive measures in this subgroup of patients.

Several studies have elucidated nonimmunosuppressive effects of treatment options, including calcineurin inhibitors and rituximab. It was shown that the antiproteinuric effect of CSA may be related to stabilization of the actin cytoskeleton in podocytes rather than inhibition of the nuclear factor of activated T-cells (NFAT) pathway. CSA was capable of blocking the calcineurin-dependent degradation of synaptopodin, which colocalizes with 14-3-3ß in the adult mouse kidney. Preservation of this interaction led to protection from cathepsin L-mediated degradation. Furthermore, it was demonstrated that lipopolysaccharide- (LPS-) induced proteinuria was reduced in those severe combined immunodeficiency (SCID) mice receiving CSA treatment [36]. Stabilization of the actin cytoskeleton has also been demonstrated for rituximab. Despite its effects on CD20 bearing cells, rituximab was capable of preventing sphingomyelin-phosphodiesterase-acid-like-3b (SMPDL-3b) and acid sphingomyelinase (ASMase) downregulation. Overexpression of

Table 3

First author	Year	Design	Study	Country	TAC	CSA	MMF/MPA	RTX	CA	ET	SIR	ACTH	GAL	ADA	No	CR	PR	NR	PR (BL)	PR (FU)	RL (%)	FU (m)
Ittel [18]	1995	Retro	Cohort	Germany	0	1	0	0	0	0	0	0	0	0	7	1	3	3	13.7 ± 3.8	4.7 ± 0.4		6
Risler [19]	1996	Pro	RCT	Germany	0	1	0	0	0	0	0	0	0	0	23				5.4 ± 5.2	2.5 ± 1.8		36
Risler [19]	1996	Pro	RCT	Germany	0	0	0	0	1	0	0	0	0	0	24				3.4 ± 4.9	2.3 ± 1.1		36
Yokoyama [27]	1998	Retro	Cohort	Japan	0	0	0	0	0	1	0	0	0	0	14				7.2 ± 3.6	2.6 ± 2.0		
Mitwalli [28]	1998	Retro	Cohort	Saudi Arabia	0	0	0	0	0	1	0	0	0	0	11	6	2	3	5.3 ± 1.2	1.4 ± 0.6		27.5
Haas [29]	1998	Retro	Cohort	Austria	0	0	0	0	0	1	0	0	0	0	5	1	0	4	13.6 ± 8.9	11.9 ± 10.6		
Feld [30]	1998	Retro	Cohort	USA	0	0	0	0	0	1	0	0	0	0	8	0	2	6			0	24
Cattran [17]	1999	Pro	RCT	North America	0	1	0	0	0	0	0	0	0	0	26	3	15	8	6.9 ± 3.3		61	24
Ponticelli [6]	1999	Retro	Cohort	Italy	0	1	0	0	0	0	0	0	0	0	9	0	7	2				
Muso [26]	2001	Retro	Cohort	Japan	0	0	0	0	0	1	0	0	0	0	17	8	4	5	6.2 ± 3.3	2.7 ± 2.7		
Segarra [21]	2002	Retro	Cohort	Spain	1	0	0	0	0	0	0	0	0	0	25	10	8	7	10.3 ± 9.5	2.6 ± 3.2	76	12
Heering [20]	2004	Pro	Observational	Germany	0	1	0	0	0	0	0	0	0	0	34	8	13	13	5.5 ± 2.6			
Heering [20]	2004	Pro	Observational	Germany	0	0	0	0	1	0	0	0	0	0	23	4	11	8	4.2 ± 0.6			
Cattran [24]	2004	Pro	Observational	USA	0	0	1	0	0	0	0	0	0	0	18	0	6	12	9.1 ± 5.2	6.8 ± 6.1		
Tumlin [31]	2006	Pro	Clinical trial	USA	0	0	0	0	0	0	1	0	0	0	21	4	8	9	8 ± 1.2	3.9 ± 0.7		6
Fernandez-Fresnedo [32]	2009	Retro	Cohort	Spain	0	0	0	1	0	0	0	0	0	0	8	0	3	5	14 ± 4.4	11.3 ± 4.2		12
Li [34]	2009	Pro	Observational	China	1	0	0	0	0	0	0	0	0	0	7	3	1	3	7	1.4	25	12
Medrano [25]	2011	Retro	Cohort	Spain	0	0	1	0	0	0	0	0	0	0	27	0	4	23	7.7 ± 3.9	6.0 ± 4.1		12
Hogan [23]	2013	Pro	Observational	USA	0	0	0	0	0	0	0	1	0	0	16	2	2	12	6.3 ± 6	4.1 ± 4.8	14	
Fan [35]	2013	Pro	Observational	China	1	0	0	0	0	0	0	0	0	0	7	3	3	1			17	12
Ramachandran [22]	2014	Pro	Observational	India	1	0	0	0	0	0	0	0	0	0	44	17	6	21	4.5 ± 3.6	0.5 ± 0.5	52	14
Trachtman [33]	2015	Pro	RCT	USA	0	0	0	0	0	0	0	0	1	0	7	0	3	4	5.4 ± 5	6.7 ± 3.5		6
Trachtman [33]	2015	Pro	RCT	USA	0	0	0	0	0	0	0	0	0	1	6	0	0	6	12.2 ± 17	7.6 ± 10.5		6

ACTH: adrenocorticotropic hormone, ADA: adalimumab, CA: chlorambucil, CR: complete remission, CSA: cyclosporine A, ET: extracorporeal treatment, FU: follow-up, GAL: galactose, MMF: mycophenolate mofetil, MPA: mycophenolic acid, No: number, NR: no remission, PR: partial remission, PR (BL): proteinuria baseline, PR (FU): proteinuria follow-up, Pro: prospective, Retro: retrospective, RL: relapse, RTX: rituximab, SIR: sirolimus, and TAC: tacrolimus.

SMPDL-3b or treatment with rituximab of a human podocyte cell culture could prevent podocyte apoptosis or disruption of the actin cytoskeleton induced by sera of patients with recurrent FSGS after kidney transplantation. Moreover, the incidence of recurrent nephrotic range proteinuria and decline in estimated glomerular filtration rate 3 and 6 months following kidney transplantation were lower in the rituximab-treated patients [37]. Given these observations, stabilization of the actin cytoskeleton as a potential nonimmunosuppressive effect has emerged as an explanation of calcineurin inhibitor and rituximab efficacy in the treatment of proteinuric glomerular disease including FSGS.

Patients with primary FSGS should receive RAAS-blockade, either ACE-inhibitor treatment or angiotensin receptor blockade if contraindications are ruled out. An analysis of patients with FSGS highlighted that the use of RAAS-blockade was associated with better renal survival and a slower progression of chronic kidney disease in univariate analysis. Although this association became nonsignificant in multivariate analysis [38], experience from other entities clearly supports its role in the long-term treatment. As a first-line immunosuppressive treatment strategy, daily oral prednisolone has emerged as a suitable therapeutic option with good remission rates. Since spontaneous remission is rare in primary FSGS [2], treatment should be initiated after the result of the renal biopsy is retrieved especially in those with high risk of progression and secondary forms are excluded. The relapse rate following cessation of therapy may be around 30–70%, depending on the length of follow-up. In cases with relative or absolute contraindication towards steroids such as psychosis, severe obesity, or impaired glucose tolerance, other immunosuppressive measures may be used. Remission rates have been shown to be similar for tacrolimus, CPA, or MMF-treated patients.

In those patients with SD/MR disease, experience is limited to single reports. Calcineurin inhibitors (CSA) have shown good remission rates. One limitation is the relative small number of adult patients included in these studies. Most experience in this setting has been gathered for MMF treatment. Again, efficacy is comparable with those treated with CSA. Rituximab has shown encouraging effects in SD/MR adult FSGS as has been shown in the maintenance of remission. We have reported a small case series [39] which again highlighted its potential. Other measures have been tested in single center studies, and there is no recommendation to use ACTH (4 out of 5 patients resistant) [23] as well as sirolimus (overall increase of proteinuria after treatment was commenced) [15] in this setting.

Most experience has been published in patients with steroid-refractoriness. In this form, calcineurin inhibitors have shown good efficacy in diverse ethnicities with a high relapse rate after cessation in those with an initial response. Most patients receiving MMF/MPA on top of their steroid treatment did not achieve remission. Thus, the recommendation in the latest KDIGO glomerulonephritis guidelines in the setting of steroid-refractoriness and contraindication towards calcineurin inhibitors [12] is not supported by our systematic review. Interestingly, no study reported efficacy of CPA in more than five patients over the last two decades. Again, in the KDIGO glomerulonephritis guidelines, CPA should be considered in SR nephrotic syndrome in children (mainly FSGS as entity) [12]. In patients with rapid-progressive deterioration of renal function and idiopathic membranous nephropathy, the addition of an alkylating agent (in this case chlorambucil) was superior to CSA or supportive treatment in abrogating further decline in renal function [40]. This may highlight that, in patients with a refractory disease course, alkylating agents may be of particular interest in FSGS as well. In line with this assumption, two studies from Germany showed either good remission rates (4 and 11 achieved complete and partial remission and eight no remission) or a decline in proteinuria following initiation of chlorambucil treatment [19, 20]. Fernandez-Fresnedo et al. published results from the Spanish GLOSEN registry on the use of rituximab in SR FSGS, highlighting persisting partial remission in two out of eight and a transient partial response in one subject. The others did not show any benefit following rituximab treatment [32]. Conflicting results have been observed and published related to extracorporeal treatment forms (either immunoadsorption or plasma exchange) in the treatment of naïve kidney FSGS. However, addition of immunoadsorption or plasma exchange may be considered in patients unresponsive to several immunosuppressive measures, given its theoretical effects on removing the "circulatory factor(s)." The total number of patients treated with other strategies such as sirolimus, ACTH, adalimumab, or galactose is low [15, 31, 33]. Galactose may be an agent of interest, since it may be effective in some cases and adverse events attributable to its treatment may be absent [33].

In consideration of additional immunosuppressive agents weighing pros and cons taking into account side effects is pivotal. Most reports included herein did not report adverse events. In those recording side effects, most complications have been non-life-threatening. Nevertheless, treating physicians need to be aware of potential side effects, especially when prescribing novel agents such as rituximab. No concrete life-threatening side effects have been reported in a recent meta-analysis from our institute when treatment of idiopathic nephrotic syndrome was analyzed [41]. However, in other autoimmune diseases, such as ANCA-associated vasculitis, B-cell depletion may exhibit a more severe side effect spectrum with fatal infectious complications such as *Pneumocystis jirovecii* pneumonia or septic conditions [42].

Several limitations need to be taken into account related to our work: (i) a heterogeneity in patients included in the different studies, in terms of pretreatment and proteinuria at the time of treatment initiation; (ii) different definitions used to define steroid-dependence or steroid-resistance (as most studies have not used current KDIGO guidelines); (iii) unclear histopathologic lesions according to the Columbia classification; (iv) lack of clinical variables of interest, such as serum albumin (± increase following treatment response); (v) nonuniform reporting of outcomes (i.e., only proteinuria response and not rates of either complete or partial remission). Thus, we would encourage researchers in the field to add all these variables in future trials in nephrotic

syndrome in general. Clearly, our review highlights the need that more effort in general is necessary to improve patient care (outcome) in FSGS.

5. Conclusion

The identification of the "circulatory factor(s)" is of importance, since we may be able to tailor immunosuppressive agents to its presence and the intensity of removal strategies (i.e., immunoadsorption) may be adapted towards its blood concentration. Despite the improved understanding of podocyte biology with identification of several nonimmunosuppressive targets of immunosuppressive agents used, the ideal treatment strategy has not been discovered in SD/MR or SR patients. In first-line treatment, daily oral prednisolone is a valuable option, whereas calcineurin inhibitors may be considered in those patients with SD/MR or steroid-resistance. Other options in the first case are rituximab or MMF/MPA, whereas both agents may not be effective in steroid-resistance. This patient group may benefit from early switch to alkylating agents, either CPA or chlorambucil, and the addition of extracorporeal options may be considered. Clearly, more studies, favorable in a prospective manner, may pave the way to improve patient care in primary FSGS.

Competing Interests

The authors declare that they have no competing interests.

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Review Article

Secondary Focal Segmental Glomerulosclerosis: From Podocyte Injury to Glomerulosclerosis

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Focal segmental glomerulosclerosis (FSGS) is a common cause of proteinuria and nephrotic syndrome leading to end stage renal disease (ESRD). There are two types of FSGS, primary (idiopathic) and secondary forms. Secondary FSGS shows less severe clinical features compared to those of the primary one. However, secondary FSGS has an important clinical significance because a variety of renal diseases progress to ESRD thorough the form of secondary FSGS. The defining feature of FSGS is proteinuria. The key event of FSGS is podocyte injury which is caused by multiple factors. Unanswered questions about how these factors act on podocytes to cause secondary FSGS are various and ill-defined. In this review, we provide brief overview and new insights into FSGS, podocyte injury, and their potential linkage suggesting clues to answer for treatment of the disease.

1. Introduction

Focal segmental glomerulosclerosis (FSGS) is now considered as a group of clinicopathological syndromes sharing a common histologic lesion characterized by focal and segmental scarring in glomerulus. Although a variety of factors could cause FSGS, the common pathogenic mechanism is podocyte injury. FSGS and a related disorder, minimal change disease, are so called "podocytopathy" [1] whose primary pathologic feature is effacement of the podocyte foot processes. Podocyte (visceral epithelium) is a unique terminally differentiated cell providing the permselectivity for a glomerular filtration barrier. Interdigitating processes of podocyte covering glomerular capillaries develop slits to function as gatekeeper for kidney filtration. Structural changes in podocyte result from podocyte injury, which leads to podocyte loss. Podocytopenia is a major event in the beginning of glomerulosclerosis.

There are two types of FSGS, primary (idiopathic) and secondary forms. The specific cause of primary FSGS has been ill-defined. Recently, clinical evidence suggested that primary FSGS is associated with causative circulating permeable factors including soluble urokinase plasminogen activator receptor (suPAR), although definite cause is not yet documented [2, 3]. Primary FSGS is a representative disorder presenting nephrotic syndrome and is a major type of primary glomerulonephritis [4] and accounts for 4% of end stage renal disease (ESRD) in the United States [5]. In comparison, secondary FSGS often presents with nonnephrotic proteinuria and less clinical severity. Nevertheless, secondary FSGS still has clinical significance; most cases of secondary FSGS are consequences from renal adaptive processes in a variety of renal diseases. Therefore, understanding about secondary FSGS provides clue to how podocyte and glomerulus adapt to renal injury and survive. Here, we review the pathogenic mechanisms underlying secondary FSGS focused on the podocyte injury causing foot process effacement and glomerulosclerosis.

2. Podocyte Injury and Glomerulosclerosis

2.1. Structure of Podocyte and Actin Cytoskeleton. A large body of studies describe the structures and physiologic roles

of podocyte supporting the fact that podocyte is dynamic [6]. Podocyte contains coordinated systems composed of contractile cytoskeletal fibers and associated proteins [7] including actin, myosin II, synaptopodin, talin, vinculin, and α-actinin-4. These systems are critical for maintaining the integrity of podocyte against pathological microenvironmental changes in the glomerulus. Actin cytoskeleton especially plays a major role in maintaining foot process function via integrating all structural components [8, 9], and actin rearrangement is common pathway to develop foot process effacement no matter what causes podocyte injury [10]. The actin cytoskeleton is connected to apical, lateral, and basal areas of podocyte to maintain cooperation between them [11] suggesting that optimal spatial organization of cytoskeleton is crucial for podocyte function. Each area of podocyte is composed of diverse interacting proteins which maintain cell to cell and cell to glomerular basement membrane (GBM) contacts and sense mechanical changes from outer environment to deliver them to the actin cytoskeleton [12, 13].

2.2. Is Podocyte a Major Player to Counterbalance Capillary Distending Pressure? The podocyte foot processes essentially provide the glomerular filtration barrier to filter plasma through slits and also have a tensile strength to oppose capillary distending pressure [14]. But several observations argued that the attribution of podocyte to oppose the hydraulic pressure from capillary was minor because podocyte did not encircle capillary completely [15]. This structural limitation demonstrates that podocyte does not provide enough opposite strength, and integrin connections in basal side of podocyte have a limited role in fixing only individual podocyte to the GBM. Instead, GBM and mesangial cell play a major role in counteracting and balancing the capillary distending pressure [15]. The GBM has basically elastic structure to endure the distending stress and is able to increase resistant force by reinforcing elastic structures according to rise in capillary pressure. Mesangial cell also counterbalances capillary pressures by supporting connections with the GBM and by cell contraction [15].

2.3. Is Foot Process Effacement an Adaptive Process or Just a Result of Disruption of Integrated System? Even though the pathogenic mechanism of foot process effacement has been suggested, it is unclear whether the foot process effacement is an adaptive process to podocyte injury or is merely the result of disintegration of highly organized system. Multiple studies suggested that podocyte responded to mechanical stress originated from capillary pressure [14] and that foot process effacement might be an adaptive mechanism to increase the capability of attachment to GBM against increased capillary pressure. Furthermore, several observations demonstrated that foot process effacement was reversible [16] supporting the adaptive role of foot process effacement. On the other hand, foot process effacement could be induced by nonmechanical injury [9, 17, 18] or via unknown mechanism. Genetic mutations leading to foot process effacement do not seem to have a relationship with an adaptive process [19]. Rather, it is suggested that foot process effacement might be just a result of the disruption of integrated system to maintain the shape of foot process.

2.4. How Does Foot Process Effacement Progress? Multiple studies described ultrastructural findings related to foot process effacement. The universal finding is rearrangement of actin cytoskeleton of podocyte leading to dense network. It is believed that the actin rearrangement is the common cause leading to foot process effacement [10, 20]. Shirato et al. described that, in progress of foot process effacement, the actin cytoskeleton was remodeled to form microfilamentous mat at the sole of podocyte and regular dense bodies within the microfilamentous network served as cross-linker to maintain the dense network. In addition, the surface of effaced process facing GBM had irregular shape, and the dense microfilamentous cytoskeletons connected basal surface of podocyte with lamina densa of GBM. As a result, foot process effacement reinforced the ability of podocyte to counteract the distending forces of capillary [16]. Endlich et al. also reported similar findings. They demonstrated that podocyte processes were thinner and more elongated against a mechanical stress *in vitro*. Stress fibers in podocyte were rearranged from transversal shape into radial shape and actin-rich centers which were described as dense bodies in Shirato's report increased in number and size (Figure 1) [21]. Additionally, it should be noted that molecular compositions of a slit diaphragm can be altered without visible changes in morphology, and foot process structures are reorganized to close filtration slits and to displace the slit diaphragm apically, in early phase of podocyte injury [9, 11]. In an elegant review by Mundel and Shankland, four major causes leading to foot process effacement were suggested: (1) interference with the slit diaphragm complex and its lipid rafts, (2) interference with the GBM or the podocyte-GBM interaction, (3) interference with the actin cytoskeleton and its associated protein α-actinin-4, and (4) interference with the negative apical membrane domain of podocytes (e.g., neutralization of negative cell surface charges) [9, 11]. Overall, the actin cytoskeleton remodeling initiated by either mechanical or nonmechanical stress is probably an important general pathogenic mechanism for foot process effacement of podocyte involving the attachment of podocyte to GBM.

2.5. Podocytopenia Is an Early Event of Glomerulosclerosis. Podocyte has no proliferative potential as a terminally differentiated cell. Therefore, loss of podocyte is not replaced by new podocyte leading to podocytopenia. Podocytopenia is associated with renal outcomes such as increased proteinuria, glomerulosclerosis, and renal disease progression [22]. In addition, clinical nephropathy is closely related with the pathognomonic findings such as glomerulomegaly, mesangial expansion, broadened podocytes, and less number of podocytes than those with normoalbuminuria or microalbuminuria [23]. Consistent with these observations, several studies support the notion that loss of podocyte is positively correlated with the extent of albuminuria, glomerulosclerosis, and disease severity in patients with IgA nephropathy [24] as well as in a puromycin aminonucleoside (PAN) nephropathy [25]. In early stage of FSGS, cellular lesions including

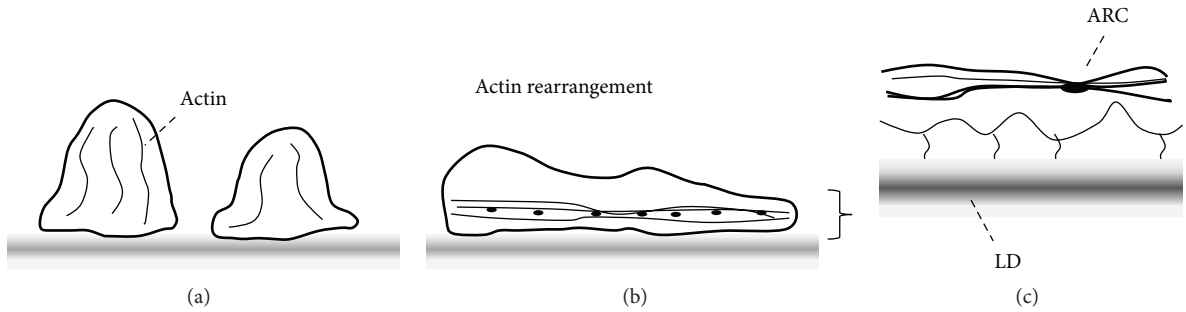

Figure 1: Rearrangement of actin cytoskeletons. (a) Podocyte foot processes and actin cytoskeletons in physiologic condition. (b) Actin cytoskeletons are rearranged into dense network at the basal area of foot process with effacement. (c) Actin-rich center (ARC) is formed within the dense network of actin cytoskeleton to maintain the network. Microfilaments are connected between basal side of foot process and lamina densa (LD) of glomerular basement membrane.

transformed podocytes were accompanied by segmental sclerosis. This observation supports the fact that podocyte damage might be an early event of glomerulosclerosis [26]. Rennke suggested a unique paradigm of glomerulosclerosis development summarized as follows: (1) podocyte injury, (2) foot process effacement and podocyte hypertrophy, (3) endothelial-mesangial hyperplasia and glomerulomegaly, (4) loss of podocytes and denudation of GBM, (5) increased nonselective filtration flow through bare areas of GBM, (6) collapse and occlusion of capillary loops by macromolecules in filtrate on bare areas of GBM, and (7) disruption of glomerular tuft and adhesion to Bowman's capsule [27]. In recent elegant reviews, a novel concept and the essential steps of glomerulosclerosis were suggested as follows: (1) increased glomerular capillary pressure and filtration flow through podocyte slits, (2) foot process effacement as an adaptive response, (3) podocyte hypertrophy and glomerulomegaly, (4) mismatch between glomerular tuft growth and podocyte hypertrophy, (5) stretching and attenuation of podocyte cell body, (6) pseudocysts formation by hindered flow of filtrates beneath the podocyte that is partially detached on bare areas of GBM, (7) complete podocyte detachment by enlarged pseudocysts and adhesion to Bowman's capsule, (8) glomerular tuft's adhesion to Bowman's capsule, (9) spreading of filtrates to interstitium out of nephron through adhesion structure, and (10) interstitial proliferation and nephron degeneration [15, 28–30].

In classic view, podocytes are terminally differentiated cell and have weak motility causing podocytopenia responding to glomerular injury. However, podocytes can be proliferated [2] and replaced by parietal epithelial cells (PECs), which serve as podocyte progenitor [31, 32]. Recently, new paradigms including PECs shed light on glomerular physiology and glomerular diseases [31–36]. PECs exert protective role which responded to podocyte depletion via their progenitor function. Conversely, it has been also suggested that PECs contributed pathological role in the formation of sclerotic lesion in FSGS [33, 34]. The PECs were previously known to be included in the process of glomerular crescents [35]. Similarly, the activated PECs induced adhesion between denuded GBM of tuft and Bowman's capsule in the process of glomerular sclerosis. Then the activated PECs invaded the affected glomerular tuft and increased extracellular matrix leading to glomerular sclerosis [36], suggesting that glomerular sclerosis by activated PECs may represent the active process to prevent further functional deficit beyond the passive result to injury. Cumulated studies argue whether PECs protect podocytopenia via their progenitor function or contribute to glomerular pathology including crescent formation and extracellular matrix accumulation. The selective targeting to the progenitor function of PECs responding to podocyte depletion may provide clues to treatment of the podocytopenia.

2.6. Foot Process Effacement Is the Instinct for Survival. Podocyte detachment is the final destiny of podocyte injury, although the dropped out podocyte is still viable [37]. Podocyte detachment leads to podocytopenia which eventually induces glomerulosclerosis. It therefore should be noted that interaction with GBM is the most important and essential role for podocyte survival. Sometimes podocytes encounter mechanical or nonmechanical stress and face disruption of coordinated structure by loss or dysfunction of endogenous components from genetic mutations. No matter what type of stress is given, podocyte foot process effacement can be induced instinctively not to be apart from the GBM and to survive (Figure 2) suggesting that foot process effacement may be the instinct of podocyte to survive.

3. Secondary FSGS

Various conditions can cause secondary FSGS (see Table 1). Adaptive response to renal injury leads to renal disease progression in the later stage, diverse drugs and infections can cause glomerular injury and sclerosis directly. In addition, loss or dysfunction of coordinated system to maintain glomerular filtration barrier leads to glomerular sclerosis.

3.1. Reduced Renal Mass. Oligomeganephronia, a congenital disease, characterized by larger but fewer glomeruli than normal ones develops FSGS and progresses to chronic renal failure [38]. Vesicoureteral reflux disease is also characterized by reduced renal mass resulting from chronic parenchymal damage and is associated with FSGS [39]. Reduced nephron

Table 1: Causes of secondary FSGS.

Type	Cause
Adaptive (with reduced renal mass)	Oligomeganephronia, vesicoureteral reflux, low birth weight, unilateral renal agenesis, surgical renal ablation, chronic renal allograft nephropathy
Adaptive (with normal renal mass)	Systemic hypertension, obesity, increased lean body mass, renal vasoocclusive disease, cyanotic congenital heart disease, sickle cell anemia
Drug-induced	Heroin, pamidronate, interferon, lithium, sirolimus
Genetic	*NPHS1, NPHS2, INF2, TRPC6, ACTN4, APOL1*
Virus-associated	HIV-1, parvovirus B19, EBV, CMV

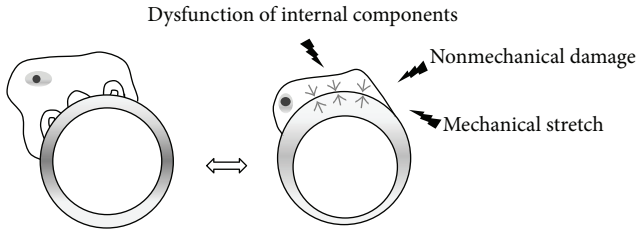

Figure 2: The shape of podocyte is changed with foot process effacement when mechanical or nonmechanical stresses are given or internal components are disrupted.

mass causes glomerular hypertension and hyperfiltration in remaining nephrons. This adaptive mechanism seems to be successful initially but later leads to renal disease progression [40]. As previously discussed, mechanical stretch by glomerular hypertension and hyperfiltration triggers the defense mechanism for podocyte to avoid detachment from GBM and survive. Foot processes of podocyte are effaced to attach the GBM more tightly, and loss of permselectivity of foot process causes proteinuria. However, more important contributing factor to developing glomerular sclerosis is the stimulation of growth which results in endothelial-mesangial hyperplasia and glomerulomegaly [41]. The endothelial-mesangial hyperplasia and glomerulomegaly cause mismatch between tuft growth and podocyte hypertrophy which leads to stretch and attenuation of cell body of podocyte [42]. The loose connection between podocyte and GBM causes podocyte detachment which leads to glomerulosclerosis eventually [15, 43, 44]. Therefore, it should be noted that local soluble factors play an important role in developing FSGS [45]. Several studies demonstrated that mechanical stretch on podocyte increased TGF-β and angiotensin II *in vivo* and *in vitro* which promote glomerular hyperfiltration and glomerular growth [15, 44, 46]. Based on these findings, angiotensin blockers are no longer "new"; they are well-proven substances to retard the progression of renal disease [47]. In summary, the significant reduction in number of nephrons such as low birth weight, unilateral renal agenesis, and unilateral nonfunctioning kidney from trauma or vascular insufficiency has a risk for FSGS and progressive renal disease.

3.2. Obesity. Obesity-related glomerulopathy (ORG) has generally mild presentations of nephropathy and FSGS is the most common type of ORG. Multiple observations demonstrated the clinical characteristics and outcome of ORG [48]. Obesity-related FSGS has significant amounts of proteinuria but they are less than those of idiopathic FSGS without features of nephrotic syndrome [49]. Most patients with ORG also present with mild and visceral obesity, minor proteinuria, and preserved renal function [50]. The pathologic features of ORG include glomerulomegaly, increased foot process width, decreased podocyte density and number, and global and segmental sclerosis. Particularly, decreased podocyte number is correlated with renal function impairment and also with metabolic disturbances such as glycemia, insulin resistance, and hyperinsulinemia [51]. The hemodynamic and metabolic disturbances are associated with dysregulation of hormones acting on podocytes in ORG [52]. The common features in pathogenesis of ORG include glomerular hyperfiltration, activation of renin-angiotensin-aldosterone system, upregulation of local peptide hormones (angiotensin II and TGF-β), insulin resistance and compensatory hyperinsulinemia, and glomerulomegaly [46, 53, 54]. These processes induce oxidative stress, podocyte injury, and apoptosis leading to podocytopenia. Therefore, the drugs blunting those pathways including angiotensin receptor blockers, aldosterone antagonists, and thiazolidinediones may be considered as candidates for the treatment of ORG [49, 53, 55]. A recent study reported a case of obesity-related FSGS, in which 17-year-old girl with obesity-related FSGS unresponsive to medical treatments including angiotensin-converting enzyme inhibitor and steroid and cytotoxic drug showed normalization of proteinuria after bariatric surgery. This observation suggests that body weight reduction is also applicable to improve ORG. Interestingly, normalization of proteinuria was achieved by two weeks after the surgery with 4% reduction of body weight [56].

3.3. Drugs. Heroin has been known as a representative drug causing FSGS. However, several studies argued that heroin-induced FSGS was associated with adulterants added to injection and not heroin itself [57]. In epidemiologic study, the incidence of heroin-associated nephropathy was declined as time passed during study periods because purity of heroin was increasing due to reduction of adulterants use [58]. Nevertheless, several studies demonstrated effects of heroin (or morphine) itself on the kidney. Morphine modulates the proliferation of mesangial cell and fibroblasts and expression of slit diaphragm constituting molecules in podocyte [59–61]. In addition, morphine induces oxidative stress in glomerular epithelial cell [62].

3.4. Genetic Mutations. Podocyte foot process is maintained by elaborately organized system, which is composed of actin cytoskeleton, synaptopodin, podocalyxin, nephrin, podocin, and so forth. Many genetic mutations cause the dysfunction or loss of foot process components leading to secondary FSGS [19, 63]. An elegant review by D'Agati et al. summarized genetic or familial factors of FSGS [1]. Here, we thus briefly introduce recent progress of gene mutations

involving FSGS. Nephrin (*NPHS1*) and podocin (*NPHS2*) are slit diaphragm proteins in podocyte foot process. The patients with genetic mutation of *NPHS1* present with Finnish-type congenital nephrotic syndrome. The mutations of *NPHS1* and *NPHS2* cause nephrotic syndrome resistant to immunotherapy and show less recurrence after renal transplantation [64]. Recently, *APOL1* gene encoding apolipoprotein L1 (ApoL1) issued in the study of an African American has a strong association with FSGS [65]. ApoL1 has the potential to lyse trypanosome causing African trypanosomiasis known as sleeping sickness. The two *APOL1* variants are common in Africa; probably the two gene variants are thought to be evolved to protect Africans against *Trypanosoma brucei*. Recent studies have shown relationships of the two *APOL1* variants with various kidney diseases in Africans or African Americans such as HIV-associated nephropathy (HIV-NP), FSGS, and hypertensive ESRD. Recent studies cumulated the evidences that *APOL1* risk alleles or variants were strongly associated with proteinuric kidney diseases including FSGS [66–68]. However, how the *APOL1* variants act on podocyte to cause FGSG has been ill-defined. Underlying mechanism by which *APOL1* variants regulate podocyte function involving FSGS awaits future investigation.

Additionally, cumulative genetic studies support the fact that genetic mutations play an important role in glomerular diseases including FSGS [69]. The list of genetic mutations causing FSGS probably will continue to grow.

4. Conclusion

Secondary FSGS is not a specific disease but a state representing podocyte injury which is mediated by diverse causes including mechanical and/or nonmechanical stresses and genetic mutations. Podocytes interact with GBM and capillary loops tightly, dysfunction of which is an early event leading to FGSG. FSGS seems like a station to stay in just before arriving to destination. Unanswered questions in the pathogenesis of secondary FSGS are still ill-defined. Uncovering the selective targeting to pathogenesis and underlying mechanism of FSGS may provide clues to answer for treatment of the disease in the future.

Competing Interests

The authors declare that they have no competing interests.

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Review Article

Immunologic Changes Implicated in the Pathogenesis of Focal Segmental Glomerulosclerosis

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Focal segmental glomerulosclerosis is a histological pattern on renal biopsy caused by diverse mechanisms. In its primary form, a circulatory factor is implicated in disease onset and recurrence. The natural history of primary FSGS is unpredictable, since some patients are unresponsive towards immunosuppressive measures. Immunologic changes, leading to a proinflammatory or profibrotic milieu, have been implicated in disease progression, namely, glomerular scarring, eventually leading to end-stage renal disease. Among these, interleukin-1ß, tumor-necrosis factor-α (TNF-α), and transforming growth factor-ß1 (TGF-ß1) have emerged as important factors. Translating these findings into clinical practice dampened the enthusiasm, since both TNF-α and TGF-ß1 blockade failed to achieve significant control of the disease. More recently, a role of the complement system has been demonstrated which in fact may be another attractive target in clinical practice. Rituximab, blocking CD20-bearing cells, demonstrated conflicting data regarding efficacy in FSGS. Finally, the T-cell costimulating molecule B7-1 (CD80) is implicated in development of proteinuria in general. Blockade of this target demonstrated significant benefits in a small cohort of resistant patients. Taken together, this review focuses on immunology of FSGS, attributable to either the disease or progression, and discusses novel therapeutic approaches aiming at targeting immunologic factors.

1. Introduction

Primary or idiopathic focal segmental glomerulosclerosis (FSGS) remains a therapeutic challenge due to its unpredictable disease course. It is assumed to be a lesion rather than a specific glomerular disease and different lesions have been described by the Columbia group which may predict renal outcome of the patients [1]. In primary FSGS one or more putative circulatory factor(s), yet to be identified, are implicated in disease occurrence and recurrence. Immunologic changes attributable to primary FSGS have attracted more attention recently, since targeted therapeutics became available over the last two decades. The aim of this review is to focus on immunologic changes described in primary FSGS and their implication on potential future therapeutic options.

2. T-Cell Involvement in Focal Segmental Glomerulosclerosis

Early reports hypothesized whether T-cell dysfunction is implicated to play a role in FSGS or not. In a small cohort of patients with FSGS, a normal distribution of CD3+, CD4+, and CD8+ T-cells was found [2]. Another investigation highlighted abundant expression of CD8+ T-cells, whereas CD4+ T-cell count was reduced compared to age-matched

controls [3]. The latter was accompanied by an increase in interleukin-2R (IL-2R, CD25) expression on CD4+ cells [3]. CD3-staining of kidney biopsies revealed significantly higher levels in FSGS compared to minimal change disease (MCD) or controls. In contrast, FoxP3+ regulatory T-cells were decreased in FSGS and MCD compared to control biopsies [4] and may increase once remission is achieved [5]. Restoration of FoxP3+ regulatory T-cells was associated with regression of nephropathy in a rat model [6].

Circulating Th17 cells as assessed in peripheral blood mononuclear cells (PBMC) were more abundant in patients with nephrotic syndrome compared to controls and were higher in non-MCD patients. A role for the Th17/interleukin-17 (IL-17) axis was further supported by the finding that IL-17 staining was most abundant in FSGS biopsies compared to MCD and mesangial proliferative glomerulonephritis. In addition, *in vitro* studies revealed a time- and dose-related proapoptotic effect of IL-17 on podocytes [7]. Interleukin-4 positive T-helper cells (Th2) did not differ between FSGS and MCD patients, whereas a significantly higher amount was present in patients with membranous nephropathy. In contrast, the peripheral Th1/Th2 ratio (IFN-γ/IL-4 ratio) was significantly lower in membranous nephropathy when compared to the other entities. Proteinuria correlated with the expression of IL-4 positive cells [8].

3. B7-1 (CD80) and Focal Segmental Glomerulosclerosis

The observation that podocyte expression of the T-cell costimulatory molecule B7-1 (CD80) may be induced during glomerular injury, while being absent in normal kidneys, promoted further explorations in patients with FSGS. In a small cohort of patients with either naïve or recurrent FSGS, positive B7-1 staining was present. In an analysis of diverse glomerular pathologies, patients with lupus nephritis showed the strongest glomerular or mesangial B7-1 staining [9]. However, a subsequent study did not confirm any positive podocyte expression of CD80 in patients with FSGS. Benigni and colleagues failed to show staining of B7-1 in naïve or recurrent FSGS [10]. In order to understand the role of B7-1 in the development of FSGS further studies are required.

4. Complement

The complement system is involved in several glomerular diseases. Recently, Thurman and colleagues analyzed samples from patients with FSGS enrolled in a study comparing efficacy of cyclosporine A (CSA) with mycophenolate mofetil (MMF). Patients with FSGS had higher levels of plasma Ba and C4a compared to healthy controls and patients with antineutrophil cytoplasm antibody- (ANCA-) associated vasculitis or lupus nephritis or healthy individuals. Urinary C4a levels were highest in FSGS patients compared to samples obtained from patients with chronic kidney disease (CKD), ANCA-associated vasculitis, and lupus nephritis. Both plasma and urine sC5b-C9 were significantly higher in patients with FSGS compared to the comparators including CKD patients. Although number of subgroup analyses is low, MMF-treated patients showed a significant decline of plasma sC5b-C9 over time [11].

5. Transforming Growth Factor-ß1

Intrarenal gene expression of transforming growth factor-ß1 (TGF-ß1) revealed a positive predictive value of 90% and a negative predictive value of 80% to identify FSGS compared to other examined histologic lesions. Among the cytotoxic effectors, Fas ligand tended to show coexpression with TGF-ß1, while granzyme B and perforin were expressed in all steroid-resistant cases [12]. In line with this observation, Souto and coworkers showed abundant expression of TGF-ß1 in steroid-resistant cases (majority having FSGS) compared to controls. Moreover, TGF-ß1 expression was highest in patients with a relapsing steroid-resistant disease course [13]. *In vitro* experiments indicated an upregulation of neuropilin-2 (NRP2) following TGF-ß1 stimulation, which was inversely correlated with estimated glomerular filtration rate at the time of biopsy and correlated with subsequent decline in renal function [14]. This highlights a role of TGF-ß1 in FSGS, especially in those with a steroid-resistant disease course who might progress to end-stage renal disease (ESRD).

6. Other Cytokines and Their Role in Focal Segmental Glomerulosclerosis

Other cytokines, namely, interleukin-1ß (IL-1ß) and interleukin-6 (IL-6), were elevated in patients with nephrotic syndrome compared to controls. Renal histopathology revealed higher IL-1ß expression in FSGS kidney biopsy specimen compared to MCD or mesangial proliferative glomerulonephritis [7]. Immunohistochemistry highlighted a differential regulation of glomerular and tubulointerstitial expression of tumor-necrosis factor-α (TNF-α) in MCD and FSGS. Glomerular staining for TNF-α expression was scarce, while tubulointerstitial staining was prominent in FSGS. This was contrary in patients with MCD. Bakr et al. reported on children with MCD and FSGS. TNF-α levels were significantly higher in patients with active nephrotic syndrome and correlated with the degree of proteinuria. Moreover, positive correlation between TNF-α production and the degree of mesangial hypercellularity and glomerulosclerosis was reported. A TNF-α level of greater than or equal to a cut-off of 50 pg/mL was able to predict resistance towards steroids in these patients (predictability 93.2%) [15].

Interleukin-10 (IL-10) levels were almost identical in both entities and increased during nephrotic-range proteinuria. The authors speculated that IL-10 is increasing with the amount of protein loss, whereas TNF-α in the tubulointerstitium may reflect interstitial fibrosis [16]. Serum interleukin-12 (IL-12) was not detectable in the majority of patients with FSGS [17]. Niemir and coworkers observed differential expression in preserved glomeruli compared to sclerotic ones. Whereas IL-1α/ß, IL-1 RII, and IL-1 receptor antagonist (RA) were similarly distributed in nonsclerotic glomeruli of patients with FSGS, glomerulosclerosis was accompanied by a scarce expression of IL-1ß and IL-1 RII

only [18]. Analysis of urinary cytokine excretion revealed significantly higher levels of interleukin-2 (IL-2), interleukin-4 (IL-4), IL-6, IL-10, interferon-γ (IFN-γ), and monocyte chemoattractant protein-1 (MCP-1) in a subgroup of patients with MCD/FSGS, whereas interleukin-17A (IL-17A), TNF-α, and TGF-ß1 were unaltered compared to a control group [19]. Urinary excretion of interleukin-18 (IL-18)/CXCL8, MCP-1/CCL2, and RANTES/CCL5 was not different in an analysis of patients with steroid-resistant or steroid-sensitive nephrotic syndrome. However, there was an association between IL-18/CXCL8 expression and degree of proteinuria [13].

7. Macrophages, HLA, and Myeloid-Derived Suppressor Cells in Focal Segmental Glomerulosclerosis

Interstitial staining for CD68 implicated a significantly higher number of macrophages in childhood FSGS compared to MCD or control biopsy specimens. Patients with a steroid-resistant course had higher numbers compared to steroid-dependent or frequently relapsing patients [4]. Examination of human leukocyte antigen (HLA) by immunohistochemistry indicated that the HLA-DR antigen was present in all patients in glomerular endothelial cells, whereas positivity was present in one quarter in extraglomerular mesangium cells and podocytes [20]. Reduction of diverse HLA class II antigens, namely, –DQ, –DR, –DP, and –DY, was observed in sclerotic glomeruli of patients with FSGS in comparison to healthy kidney tissue [21]. Myeloid-derived suppressor cells (MDSC), characterised by CD11b+HLA-DR-CD14-CD15+ staining, in peripheral blood increased following initiation of steroids in responsive FSGS subjects, whereas no increase was found in steroid-resistant patients. Induction of MDSC was capable of suppressing T-cell proliferation and induced regulatory T-cells *in vitro* [22].

8. B-Cells and Focal Segmental Glomerulosclerosis

Glomerular staining for CD20 positivity was significantly and numerically higher in FSGS compared to controls and MCD, respectively. In contrast, interstitial staining was reduced in FSGS and MCD in comparison to control biopsies [4]. Strassheim and coworkers examined the effect of anti-CD20 treatment and prevention of IgM deposition in a mouse model of FSGS. Approximately 30% of the kidney biopsies examined displayed glomerular IgM deposition, either colocalized with C3 or not. This subgroup may be more susceptible towards a B-cell depleting therapy as shown in their Adriamycin-induced nephropathy [23].

9. Transition into Clinical Practice: Towards Tailored Medicine in Focal Segmental Glomerulosclerosis

9.1. Abatacept. Yu and coworkers demonstrated efficacy of B7-1 blockade with abatacept (CTLA-Ig) in rituximab- and steroid-resistant cases [9]. Mechanistically, they found that abatacept was capable of blocking ß1-integrin activation. Although these data suggest clinical benefit based on mechanistic insights, the initial enthusiasm was seriously dampened by subsequent reports. Proteinuria remained unchanged in one patient with primary and three patients with recurrent FSGS receiving either abatacept or belatacept, the latter having predominant effects on B7-2 (CD86) [24]. Although kidney biopsies from patients with lupus nephritis showed strong B7-1 staining [9, 25], a recent trial comparing abatacept as add-on therapy to cyclophosphamide to a standard-of-care treated control group showed no improvement with the addition of abatacept, again disproving a role of B7-1 blockade in proteinuric kidney diseases [26].

9.2. Adalimumab. As demonstrated above, TNF-α is a promising target in patients with FSGS. Thus, adalimumab, a human monoclonal antibody targeting TNF-α, was tested in a phase I trial including 10 patients with resistant FSGS. Pharmacokinetics revealed an increased clearance by 160% compared to healthy controls and patients with rheumatoid arthritis [27] which was attributable to renal and nonrenal clearance with a direct impact of proteinuria on the former finding [28]. While two patients had a partial remission during follow-up time (proteinuria decreased from a PCR of initially 3.6 and 17 mg/mg creatinine to 0.6 in both subjects), the other eight patients remained nephrotic [27]. Moreover, the authors demonstrated a stabilization of the estimated glomerular filtration rate during follow-up in some patients [29]. A subsequent phase II trial in resistant FSGS failed to recruit significant numbers. Of the seven included patients, no patient had a significant response with worsening of proteinuria in 4/6 patients [30]. Although small numbers limit definite conclusions, a general recommendation related to adalimumab use in resistant FSGS is not warranted.

9.3. Fresolimumab. TGF-ß is implicated in several mechanisms leading to pathologic glomerular changes. Targeting this pivotal cytokine with a human monoclonal antibody, namely, fresolimumab, led to an estimated glomerular filtration rate decline of 5.9 mL/min (annualized 19 mL/min), whereas mean proteinuria measured as PCR decreased by 1.2 mg/mg creatinine in a phase I trial [31]. A further multinational trial investigating the efficacy of this substance has already completed recruitment.

9.4. Rituximab. Although experience of rituximab's use in FSGS is limited to case reports or series, it is widely used to treat resistant cases [32]. Analysis of the Spanish Group for the Study of Glomerular Diseases (GLOSEN) revealed that two patients had a clear and sustained improvement following rituximab treatment, while one patient had a transient response twice and the other five patients did not respond to the treatment [33]. In our analysis including relapsing patients most patients achieved sustained remission with a reduction of relapses following rituximab. However, one patient showed no response following rituximab therapy [34]. This is in line with a recent analysis of childhood onset steroid-resistant and congenital nephrotic syndrome, which

showed equivalent response rates of rituximab compared to calcineurin inhibitors, with 40–45% of the patients achieving complete remission [35]. Treatment with rituximab might lead to life-threatening infections as reported in ANCA-associated vasculitis, for example [36]. No such complications have been reported in adult patients with FSGS treated with rituximab so far. Thus, it might be an option in difficult-to-treat FSGS [37]. One potential para-CD20 effect of rituximab may in fact be stabilization of the actin cytoskeleton. Rituximab partially prevented sphingomyelin phosphodiesterase acid-like 3b (SMPDL-3b) downregulation and prevented disruption of the actin cytoskeleton along with apoptosis of podocytes induced by FSGS sera [38].

10. Conclusions

Several immunologic changes have been identified during the last decades in FSGS. However, lack of replication or failure of translation into useful therapeutic measures limits these findings. With improved laboratory techniques novel potential targets will be elucidated in the future and hopefully therapeutic concepts targeting specific molecules, such as rituximab's effects on SMPDL-3b, will emerge. Overall, the aim has to be identification of the responsible pathogenic factor(s), which may in fact be a useful marker as has been shown in idiopathic membranous nephropathy.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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