Review Article

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Precision medicine for focal segmental glomerulosclerosis

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Focal segmental glomerulosclerosis (FSGS) is one of the common causes of nephrotic syndrome in adults and children worldwide. FSGS consists of a group of kidney diseases classified based on specific histopathological features. The current classification of FSGS makes it difficult to distinguish individual differences in pathogenesis, disease progression, and response to treatment. In recent years, the spread of next-generation sequencing, updates in biological techniques, and improvements of treatment have changed our understanding of FSGS. In this review, we will discuss the use of genetic testing in patients with FSGS, explore its clinical significance from a genetic identification perspective, and introduce several new biomarkers, that may help in the early diagnosis of FSGS and guide the development of specific or targeted therapies, so as to understand the biological characteristics in FSGS. This will certainly help develop more effective and safer treatments and advance precision medicine.

Keywords: Biomarkers, Focal segmental glomerulosclerosis, Genomics, Podocytes, Therapy

Introduction

Focal segmental glomerulosclerosis (FSGS) is not a specific renal disease, but a histological change in glomerular injury, in which podocytes are the primary target, glomerular injury is due to podocyte loss, the pathologic findings are focal, and there is segmental glomerulosclerosis with diffuse disappearance of the foot process. FSGS is classified as collapsing, tip, cellular, perihilar, and not otherwise specified variants according to the location and character of the sclerotic lesion. The primary clinical feature is varying degrees of albuminuria with or without nephrotic syndrome (NS). In primary nephropathy, FSGS accounts for nearly 20% of the NS in children and adults [1]. The incidence of FSGS is increasing year by year and it is one of the kidney diseases most likely to develop into end-stage renal disease (ESRD), accounting for 10% to 15% of children with end-stage kidney disease and 3% of adults with ESRD in the United States. In addition, FSGS recurs in 20% to 25% of transplanted kidney patients [2].

The etiology of primary FSGS is still unknown. However, knowledge on FSGS is growing quickly, and new advances need to be translated into clinical practice. Big data in respect of the genome has changed our understanding of FSGS and shows that there may be specific genetic mutations in FSGS. Kidney biopsies are invasive with potential complications, histological examination of kidney tissue may not reliably distinguish between idiopathic and secondary FSGS, and there is an urgent need for reliable non-invasive biomarkers for accurate diagnosis. Biomarkers

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such as circulating permeability factors (CPFs), exosomes, and metabolomics comprise one research direction, and the discovery of new biomarkers also provides a new idea for clinical treatment. In terms of treatment, unlike minimal change disease (MCD) patients, most FSGS patients respond poorly to glucocorticoids, and the effectiveness and safety of immunosuppressors are unknown. Therapeutic research is now moving in the direction of precision medicine, and several new drugs that selectively target different disease pathways are currently being evaluated, some with promising results (Fig. 1).

This review will discuss the genetic analysis, biomarkers research, and the update of clinical treatment, so as to understand the biological characteristics of FSGS and make further progress in precision medicine.

Focal segmental glomerulosclerosis genetics and epigenetics

With the popularization of next-generation sequencing, many monogenic causes of FSGS have been found and their manifestations are various; these varied causes explain the lack of diagnosis to a large extent. Identification of the monogenic causes of FSGS has moved the podocyte to the center of its pathogenesis. Over 50 genes are currently known to be involved in FSGS, revealing that the proteins they encode are essential for glomerular function [3]. Starting with nephrin gene (*NPHS1*), many pathogenic genes are involved in the maintenance of the structure and function of podocytes. Most genes have specific variants that are reported to contain protein products that are important regulators of the podocyte skeleton, signal transduction, slit diaphragm components, and the basement membrane. Any damage to the glomerular filtration barrier leads to albuminuria, some of which can develop into FSGS.

Multiple genetic forms of FSGS have been reported; Gast et al. [4] listed 39 genes associated with FSGS, Preston et al. [5] listed 48, and De Vriese et al. [3] listed 55, which may account for a significant proportion of FSGS with steroid-resistant NS (SRNS) [6]. Whole exome sequencing was performed in 187 SRNS patients by Bierzynska et al. [7] and pathogenic variants were detected in 26.2% of patients. Au-

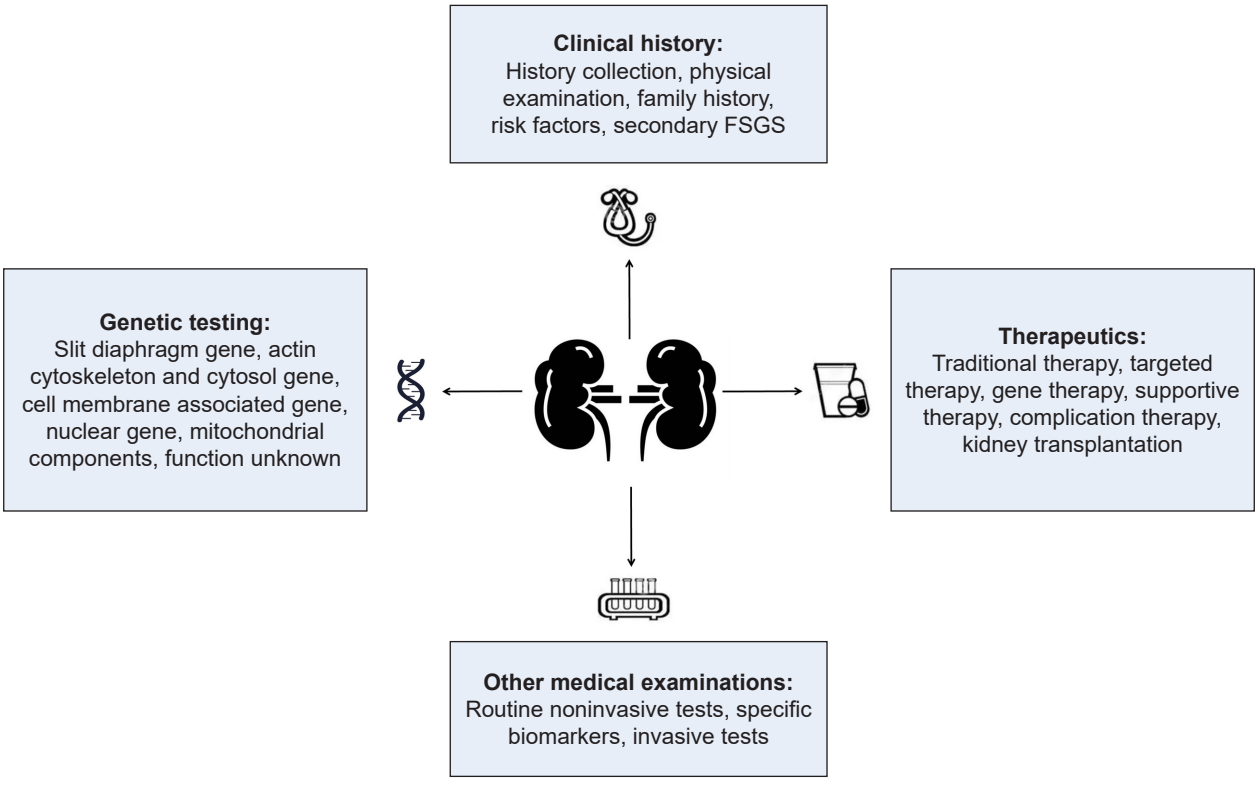

Figure 1. Precision medicine approaches for focal segmental glomerulosclerosis.

tosomal recessive FSGS mostly develops in childhood, and common genes are *NPHS 1*, *NPHS2*, *PLCE1*, and *TTC21B* [7]. Among the 14 gene mutations reported for the PodoNet cohort, abnormalities in *NPHS2*, *WT1*, and *NPHS1* were the most common [6]. Autosomal dominant FSGS is more common in older children, adolescents, and adults, and the most common single gene pathogenic mutation is *COL4A3–5* [8], followed by *INF2*. Other major genes involved in autosomal dominant FSGS are *TRPC6*, *WT1*, *ACTN4*, *LMX1B*, and *PODXL* [9]. A small number of mutations can be inherited through X-linked recessive models, such as *OCRL1* and *NXF5* [9,10]. In addition, there is the possibility of mitochondrial gene mutation, suggesting that mitochondrial gene detection should be perfected when necessary. Although extrinsic phenotypes can provide clues to genetic disease, not all patients exhibit traits of genetic disease, so the phenotypic combination with whole exome sequencing is an effective tool for early identification of the etiology of FSGS.

Studies in recent years have found that *ApoL1* mutations cause higher rates of FSGS in African Americans than in European Americans. *ApoL1* is the trypanolytic factor of human serum that confers resistance to certain trypanosomes. The *APOL1* gene may have undergone natural selective pressure to counteract these trypanosoma adaptations [11]. The *APOL1* gene is one of six members of the *APOL* gene family on human chromosome 22; only *APOL1* has acquired a secretory signal peptide permitting cellular export of the APOL1 protein into the bloodstream. Intracellular APOL1 protein lacking a signal peptide is retained within the cell; it is this protein isoform that leads to kidney injury within the cell [12]. Approximately 75% of black FSGS patients have high-risk *APOL1* genotypes. Black patients with primary FSGS were more likely to have *APOL1*-related diseases. It was found that APOL1 protein was expressed in endothelial cells, podocytes, and proximal tubule cells outside the glomeruli. *APOL1* causes kidney cell injury via three mechanisms: 1) *APOL1* is overexpressed in podocytes related to cell injury and glomerular injury, leading to cell injury. 2) Increased expression of *APOL1* risk variants has been shown to induce inflammasome activation and cell death pathways. 3) *APOL1* variants G1 and G2 overexpress activated protein kinase R in cultured human podocytes, resulting in reduced protein synthesis, cell stress, or death [13]. Furthermore, differences in mitochondrial gene regulation appear to underlie many of the differences observed between high-risk and low-risk black FSGS patients [14].

The CureGN (Cure Glomerulonephropathy) cohort study included 650 participants with biopsy diagnoses of FSGS. A total of 476 (73%) underwent genetic testing, of whom 87 (18%) were at high risk, and it was found that high-risk *APOL1* genotypes were the primary factor associated with faster loss of kidney function [15]. A cohort study of 90 black patients with FSGS or MCDs showed that while *APOL1*-associated FSGS is associated with having two risk alleles, both one and two risk alleles are associated with cellular/tissue changes in FSGS patients [16]. Expression of *APOL1* risk variants in transgenic mouse models was found to increase susceptibility to lipid-dependent podocyte injury, ultimately leading to mitochondrial dysfunction [17]. Ge et al. [17] found that in an FSGS model, APOL1 protein G1 expression worsened albuminuria and renal function in mice, characterized by podocyte-induced activated nuclear factor expression. APOL1 protein G1 expression in this FSGS model also resulted in increased triglyceride and cholesterol ester contents in kidney cortices, where lipid accumulation correlated with loss of renal function [17]. Recent studies have also found that *APOL1*'s interaction with the environment and infection may be of greater clinical importance in triggering kidney disease in black patients [18].

Genetic FSGS may either be limited to the kidney or be part of a broader syndrome with extrarenal involvement. Genetic FSGS may be less responsive to immunosuppressive therapy, and clinicians should consider discontinuing immunosuppression to protect patients from the potential side effects of such therapy if no clinical benefit is demonstrated after receiving positive gene test results. In addition, patients with monogenic FSGS have a lower rate of disease recurrence after renal transplantation, whereas those caused by CPFs have a higher risk of recurrence [19]. If living-related transplantation is considered, it is safest to have a genetically unaffected family member donate. Likewise, future children of people with hereditary FSGS may inherit the disease, and the identification of mutations is important for genetic counseling of pregnant families [20]. An unequivocal molecular diagnosis can enable individualized treatment approaches to reduce immunosuppressive therapy, avoid kidney biopsy, and provide accurate, informed

genetic counseling [10]. In addition to these clinical benefits, identifying known and pathogenic variants involved in monogenic FSGS will better define genotypic-phenotypic associations, and provide new avenues for *in vitro* and *in vivo* studies of the pathophysiological mechanisms of proteinuria.

Focal segmental glomerulosclerosis and biomarker

In addition to monogenic factors, biomarkers are also a current research direction. FSGS biomarkers cover a broad range of biochemical entities, such as proteins, nucleic acids, small metabolites, and cytogenetics. They can be used for risk assessment, diagnosis, prognosis, and the prediction of treatment efficacy and recurrence. The discovery of reliable FSGS biomarkers plays a crucial role in disease management (Table 1).

Circulating permeability factors

CPFs have long been the focus of research. First, in renal transplantation, proteinuria was observed in some patients within hours after transplantation and was improved by plasma exchange. Second, allograft function gradually recovered after transplantation from FSGS patients to non-FSGS patients, suggesting a pathogenic role of one or more CPFs in recurrent primary FSGS [21,22]. Recent research has developed a novel *in vitro* assay to detect possible CPF activity in plasma in patients with relapsing and primary FSGS, suggesting the presence of a delicate balance between CPFs and a CPF inhibitory factor, which is disturbed in patients with active disease [23]. Identification of the presence of CPFs is very important for the treatment and prognosis of patients, because patients with CPFs have a higher risk of recurrence after transplantation, or they may choose to use plasma exchange or immunosorbent therapy. At present, although some molecules that may be considered CPFs have been found, the specific characteristics and mechanisms are still unclear.

Soluble urokinase-type plasminogen activator receptor

Urokinase-type plasminogen activator receptor (uPAR) is a membrane-bound glycosylated single-chain protein with a molecular weight of 45 to 55 kD and three extracellular domains (D1, D2, and D3) associated with a glycosylated phosphatidylinositol anchor in cell membranes. Under the stimulation of inflammation, infection, and other related factors, uPAR is cleaved by proteases from the cell surface, shedding from the cell surface to form soluble uPAR (suPAR). Full-length suPAR (suPAR D1–3) can be cleaved into two other soluble forms with different biologic properties, suPAR D2D3 and suPAR D1; suPAR D2D3 is a chemotactic agent [24]. suPAR is widely present in a variety of body fluids such as plasma, serum, and urine, and participates in a variety of physiological pathways. Under pathological conditions, suPAR acts as a chemotactic agent that activates the immune system, leading to elevated suPAR levels in serum or urine [25]. In the kidney, the main injury mechanism involves circulating suPAR entering the glomeruli and binding with αvβ3 integrin. The increased level of circulating suPAR activates the αvβ3 integrin of the podocyte, mediating the actin skeleton recombination of the podocyte and the fusion of the foot process. In addition, pathological mitosis of the podocyte is induced, which leads to apoptosis of the podocyte and causes proteinuria [26].

Wei et al. [26] first reported that suPAR was elevated in two-thirds of patients with primary FSGS, but not in other patients with glomerular disease. They also found that higher concentrations of suPAR before transplantation underlie the increased risk of FSGS recurrence after transplantation, suggesting that serum suPAR may be a circulatory factor in FSGS [26]. Subsequently, suPAR levels were found to be significantly positively correlated with FSGS and with posttransplantation recurrent FSGS but negatively correlated with MCD and membranous nephropathy, suggesting that suPAR levels can distinguish primary FSGS from other glomerular diseases [27]. A recent meta-analysis found that serum suPAR levels were elevated in FSGS patients compared to controls; this finding was consistent when compared to MCD, but not for membranous nephropathy or immunoglobulin A nephropathy (IgAN) [28]. Although suPAR is closely related to the development of FSGS, its role is still controversial [29]. In another study involving mouse models, the urokinase-type plasminogen activator (uPA) knockout group was found to have higher plasma suPAR levels, glomerular cell apoptosis, and Th1/Th2 imbalance, characterized by rapid disease progression and increased albuminuria levels. These data suggest the levels of serum uPA may be an indicator of the progres-

Table 1. Summary of biomarkers in primary FSGS

Biomarkers	Mechanism of action	Findings for FSGS	Limitations
CPFs			
suPAR	Increased level of circulating suPAR activates the αvβ3 integrin of the podocyte, mediating the actin skeleton recombination of the podocyte and the fusion of the foot process.	suPAR increased in patients with primary FSGS. suPAR levels were found to be significantly positively associated with FSGS and post-transplantation recurrent FSGS.	Did not cause podocytosis or proteinuria in animal models. In a further study serum suPAR levels did not differ significantly in children.
CLCF1	CLCF-1 increased the phosphorylation of STAT3, activated podocytes leading to the formation of lamellipodia and a decrease in basal stress fibers, and increased glomerular albumin permeability.	The plasma concentration of CLCF1 in patients with recurrent FSGS was significantly higher than that in the control group. CLCF1 or FSGS plasma increased permeability to albumin and activated JAK2/STAT3 pathways in the glomeruli.	Its pathophysiological effects need to be clearly identified and validated in different patient cohorts.
Anti-CD40 autoantibody	In FSGS, suPAR-mediated podocyte injury may lead to the uncovering of hidden podocyte epitopes that are easily recognized by anti-CD40, leading to the production of autoantibodies.	Increased antiCD40 antibodies before transplantation had the best correlation with the risk of rFSGS after transplantation. In wild-type mice, the injection of anti-CD40/rFSGS antibodies enhanced suPAR-mediated proteinuria.	suPAR and anti-CD40 autoantibodies may play a cooperative role in the development of rFSGS, but the main cause of the disease is not fully understood.
ApoA-Ib	ApoA-Ib is a misprocessed form of proApoA-I. and impair the correct ApoA-I reabsorption in the proximal tubular cells.	ApoA-Ib was detected in the urine of patients with rFSGS and was only found in patients with rFSGS.	Studies are needed in larger cohorts of patients with rFSGS and ApoA-Ib cannot be tested in anuria patients.
B7-1	B7-1 promotes disease-associated podocyte migration through β1 integrin inactivation. B7-1 mediates podocyte injury and glomerulosclerosis through communication with the Hsp90ab1-LRP5-β-catenin pathway.	B7-1 staining was observed in biopsy specimens from patients with rFSGS. Some patients with posttransplant FSGS who received abatacept therapy, followed by improvement of proteinuria, and stable renal function.	The exact molecular mechanisms of B7-1 and the clinical response to abatacept remain the subject of debate.
Exosomes	Exosomes comprise a class of extracellular membrane-bound vesicles released from host cells via the fusion of the multivesicular body with the cell membrane. Specific microRNA profiles are associated with cellular processes.	Mice expressing transgenic miR-193a rapidly progressed to FSGS. Urine exosome miR-193a levels were significantly higher in patients with primary FSGS. Plasma miR-186 and miR-150 levels were correlated with the degree of proteinuria in patients with FSGS. Urinary miR-196a was significantly increased in active FSGS. The miRNA group is also a potential independent diagnostic and prognostic factor for FSGS.	The sample size was small and further studies are needed to verify the results. Serum or plasma exosomes may not be suitable as they may contain non-nephrogenic exosomes.
Metabolomics	Enzymes catalyze chemical reactions that are essential for cell function. The upstream biological disruption leads to a series of metabolomic changes, and as a result, metabolomics holds a wealth of information.	A significant increase in *myo*-inositol in 70 urinary metabolites was found in FSGS patients.	The biological interpretation of metabolomic data depends on the ability to accurately identify metabolites.
Single-cell transcriptome	Gene expression is examined by partial sequencing of complementary DNA clones, revealing the sequence and abundance of the corresponding RNA.	Type 1 IFN-mediated STAT1 activation could be a key mechanism involved in glomerular disease progression for patients with endothelial cell activation.	Providing only a partial sample of the cellular transcriptome and preferring highly expressed genes, being sensitive to sample quality, and high costs.
TNF-pathway urine markers	TNF pathway activation leads to adverse outcomes.	Two TNF-pathway urine markers were identified, which could be used to predict an individual's TNF pathway activation score.	Need to expand clinical subgroups.

ApoA-Ib, apolipoprotein A-Ib; CLCF1, cardiotrophin-like cytokine factor 1; CPFs, circulating permeability factors; FSGS, focal segmental glomerulosclerosis; IFN, interferon; JAK2: Janus kinase 2; rFSGS, recurrence FSGS; STAT3: signal transducer and activator of transcription 3; suPAR, soluble urokinase-type plasminogen activator receptor; TNF, tumor necrosis factor.

sion of FSGS in clinical subjects and animal models [30]. In wild-type (WT) and uPAR–/– mouse trials, suPAR was deposited within the glomeruli but only in the endothelium not in podocytes, and only in WT mice. Neither trial produced a loss of podocytes or albuminuria [31,32]. In a further study, serum suPAR levels did not differ significantly in children and it is recommended that future studies should be better age-stratified [33].

There are several possible reasons for the difference in results: 1) Different isoforms and glycosylation statuses of suPAR may have different impacts. 2) Modifying factors may have mediated suPAR-induced activation of αvβ3 integrin. 3) Major confounders of suPAR need to be controlled. 4) No appropriate statistical methods were chosen [28]. In addition, a low glomerular filtration rate (GFR) may increase suPAR levels due to impaired clearance. A recent study that analyzed suPAR values in patients with chronic kidney disease (GFR below 60 mL/min/1.73 m^2) and patients without chronic kidney disease confirmed that suPAR was negatively correlated with estimate GFR (eGFR), highlighting the significance of suPAR in the assessment of FSGS independent of renal function [34]. Although the discovery of suPAR as a CPF and biomarker for recurrence FSGS (rFSGS) has attracted significant attention, its use in the clinical diagnosis of rFSGS still needs to be better understood.

Cardiotrophin-kike cytokine factor 1

Cardiotrophin-like cytokine factor 1 (CLCF1) is a member of the IL-6 family of cytokines, with a predicted molecular weight of 22 kDa. CLCF1 is considered to be a heterodimer composite cytokine that is secreted and circulates with either of two proteins, namely cytokine receptor-like factor-1 (CRLF1) or soluble receptor alpha for ciliary neurotrophic factor. CRLF1 is essential for normal kidney development, and CRLF1 and CLCF1 appear to function as heterodimers in neuronal differentiation and kidney development.

An earlier study identified CLCF1 in the active fraction from galactose affinity chromatography. CLCF1 is present in the plasma of active FSGS patients and decreases nephrin expression by glomeruli and cultured podocytes. In the aforementioned study, the plasma concentration of CLCF1 in patients with recurrent FSGS was significantly higher than that in the control group. The authors suggested that CLCF1 might be a permeability factor for recurrent FSGS [35]. The team then found that CLCF-1 increased the phosphorylation of signal transducer and activator of transcription (STAT) 3 in multiple cell types, activated podocytes leading to the formation of lamellipodia and a decrease in basal stress fibers, increased glomerular albumin permeability, and increased STAT3 phosphorylation of peripheral blood cells and the renal cortex. CLCF-1 has also been shown to increase the urine albumin/creatinine ratio in mice and increase the B-cell expression of immunoglobulin G in mouse spleens [36]. Research by Savin et al. [36] also showed that CLCF1 or FSGS plasma increased permeability to albumin and activated Janus kinase 2 (JAK2)/STAT3 pathways in the glomeruli. Sharma et al. [37] compared the effect of CLCF1 sera from FSGS patients on glomerular albumin permeability *in vitro* using anti-CLCF1 antibody or inhibitors of JAK2 and STAT3. The results showed that the monomer CLCF1 or FSGS serum increased, while the heterodimer CLCF1-CRLF1 attenuated the effect [37]. These studies provide a direction for the role of CLCF1 and its related molecules in the etiology of relapsing FSGS.

Anti-CD40 autoantibody

The CD40 receptor and CD40L ligand are transmembrane proteins that belong to the tumor necrosis factor (TNF) receptor superfamily and are critical to the initiation and sustainment of the inflammatory response. The CD40–CD40L pathway is the backbone of cell-cell communication in the immune system. It is involved in the pathogenesis of immune system diseases, transplant rejection, nervous system regulation, cardiovascular diseases, and tumors. Renal epithelial cells and podocytes have been shown to express CD40 and identified as antigen-presenting cells, and circulating anti-CD40 autoantibody is considered a permeability factor for FSGS [38].

Delville et al. [39] processed 141 serum samples from 64 rFSGS patients and 34 non-FSGS control patients from four hospitals and selected 10 antibodies against glomerular antigens for enzyme-linked immunosorbent assay validation. A panel of seven antibodies (CD40, PTPRO, CGB5, FAS, P2RY11, SNRPB2, and APOL2) could predict rFSGS after transplantation with 92% accuracy. Increased antiCD40 antibodies before transplantation had the best correlation with the risk of rFSGS after transplantation (78% accuracy). The authors found that anti-CD40 antibodies purified from

rFSGS patients were particularly pathogenic in human podocyte cultures. In WT mice, the injection of anti-CD40/rFSGS antibodies enhanced suPAR-mediated proteinuria, but no sensitization was observed in mice deficient in CD40 or in WT mice receiving CD40-blocking antibodies [39]. It was found that suPAR-mediated podocyte injury in FSGS may lead to the uncovering of hidden podocyte epitopes, leading to the production of autoantibodies. In rFSGS serum, two β-hairpin peptides (NSQCC and ESEF) were found between the two antiparallel beta-strands of the CD40 structure. The flexible folding of these peptides makes them particularly exposed and easily recognized by anti-CD40 from rFSGS patients [39].

Apolipoprotein A-Ib

In addition to the possible presence of CPFs in the blood, there also appear to be meaningful biomarkers in the urine. Apolipoprotein A-Ib (ApoA-Ib) is the heavier form of ApoA-I, and the increase in molecular weight may correspond to a posttranslational change in the conventional form of ApoA-I. Lopez-Hellin et al. [40] detected ApoA-Ib in the urine of patients with relapsed FSGS, and since it was only found in relapsed patients, it was speculated that ApoA-Ib might be a biomarker associated with the disease activity of relapsed FSGS. In a retrospective cohort study of 61 renal transplant patients (37 FSGS and 24 non-FSGs), the sensitivity, specificity, and negative predictive value of ApoA-Ib diagnosis of FSGS relapses were 93.3%, 90.9%, and 95.2%. In the prospective cohort, ApoA-Ib predated the recurrence in four out of five episodes observed. In the non-relapsing group (n = 9), ApoA-Ib was negative in 37 of 38 samples. ApoA-Ib has the potential to be a good diagnostic biomarker for FSGS relapses [41]. The mass spectrometry analysis revealed three extra amino acids at the N-terminal end of ApoA-Ib that were not present in the standard plasmatic form of ApoA-I. These amino acids corresponded to half of the propeptide sequence of the immature form of ApoA-I (proApoA-I), indicating that ApoA-Ib is a misprocessed form of proApoA-I [42]. The team's latest study showed that ApoA-I was localized at the brush border of tubule cells in patients with recurrent FSGS, while ApoA-I was distributed along the cytoplasm of tubule cells in non-FSGS patients. This suggests that ApoA-I staining in a kidney biopsy, coupled with the determination of ApoA-Ib in urine, can be used as a diagnostic tool for the relapse of primary FSGS after kidney transplantation due to its highly specific tubular distribution [43].

B7-1 (CD80)

Recent studies have found that the expression of B7-1 on podocytes is considered to be both a biomarker and a therapeutic target. B7-1 (CD80) is a new biomarker of podocyte injury discovered in 2004. B7-1 is a transmembrane protein normally expressed by antigen-presenting cells, playing a key role in initiating and modulating immune responses by activating (when combined with CD28) or inactivating (when combined with CTLA-4) T cells. B7-1 may play a role in proteinuria, actin recombination, and podocyte migration [44].

Yu et al. [45] observed B7-1 staining in biopsy specimens from patients with relapsed FSGS and treated five FSGS patients with abatacept, an inhibitor of the T-cell costimulatory molecule B7-1. Abatacept partially or completely alleviated proteinuria in these patients, suggesting that B7-1 may be a useful biomarker for the treatment of FSGS [45]. Recently, the team described nine patients with posttransplant FSGS who received abatacept therapy, followed by improvement or resolution of proteinuria, and stable renal function. The presence of B7-1 in podocytes in the kidney transplant biopsies of FSGS recipients after transplantation suggested a subset of patients who may benefit from abatacept therapy [46]. The mechanism may be that B7-1 promotes disease-associated podocyte migration through β1 integrin inactivation, which is reversed by abatacept. In addition, B7-1 mediates podocyte injury and glomerulosclerosis through communication with the Hsp90ab1-LRP5-β-catenin pathway [47]. The exact molecular mechanisms of B7-1 and the clinical response to abatacept, however, remain the subject of debate. Novelli et al. [48] analyzed the expression of B7-1 in the kidneys of Balb/c mice injected with adriamycin, and the model was very similar to the histological lesions of human FSGS; however, the expression of B7-1 in the glomerulus was not detected in either polyclonal or monoclonal antibodies. Eroglu et al. [49] found that CD80 expression was positive in only six of 19 FSGS biopsies. Cd80-positive biopsies showed a decreased number of FOXP3-positive CD4 T cells, suggesting that a decreased anti-inflammatory milieu may be

a cause of increased CD80 expression [49]. A systematic review including 11 studies (n = 32) showed that the efficacy of abatacept therapy for FSGS or MCD was only 43.8%. Abatacept should only be used in patients with positive B7-1 staining on kidney biopsies, as these patients tend to respond to treatment [50]. Although B7-1 offers new hope as a biomarker of podocytes, many questions remain about the current data.

Exosomes

Exosomes comprise a class of extracellular membrane-bound vesicles released from host cells via the fusion of the multivesicular body with the cell membrane. Specific microRNA (miRNA) profiles are associated with cellular processes such as fibrosis, inflammation, and epithelial-mesenchymal transformation. Exosomal miRNA has also been found to be associated with the development of FSGS. Gebeshuber et al. [51] revealed that mice expressing transgenic miR-193a rapidly progressed to FSGS, presenting extensively as the effacement of podocyte foot processes. Meanwhile, upregulation of miR-193a levels has been observed in the isolated glomeruli of adults with FSGS [51]. It was found that urine exosome miR-193a levels were significantly higher in patients with primary FSGS than in patients with minimal change nephropathy (MCN) and IgAN. The area under the receiver operating characteristic values for distinguishing primary FSGS from MCN or IgAN were 0.85 and 0.821 [52,53]. Possible mechanisms include the following: 1) miR-193a inhibits the expression of Wilms' blastoma protein (WT1), and decreased WT1 expression leads to downregulation of its target genes *PODXL* (podocalyxin) and *NPHS1* (nephrin), as well as several other genes critical to podocyte structure, affecting podocyte stability [51]. 2) Overexpression of miR-193a *in vivo* results in the upregulation of human parietal epithelial cell (PEC) markers and the loss of podocyte markers in isolated glomeruli [54]. 3) MiR-193a drives inflammatory response activation, upregulation of complement and fibrinogen pathways, and several protease inhibitors, leading to changes in the extracellular matrix [55]. Other studies showed that plasma miR-186 and miR-150 levels were correlated with the degree of proteinuria in patients with FSGS [56,57]. Urinary miR-196a was significantly increased in active FSGS, and patients with higher levels of urinary miR-196a had lower renal survival than patients with lower levels of urinary miR-196a [58].

The miRNA group is also a potential independent diagnostic and prognostic factor for FSGS. It was found that four miRNAs (miR-17, miR-451, miR-106a, and miR-19b) were significantly downregulated in the plasma of FSGS patients, which may lead to enhanced apoptosis of podocytes during FSGS development [59]. Urinary miR-196a, miR-30a-5p, and miR-490 discriminated active FSGS from FSGS in remission. After steroid treatment, their levels were lower in steroid-responsive patients with FSGS but were unchanged in steroid-resistant patients [60]. Yildirim et al. [61] found that miR-34c, miR-132, and miR-214 play a role in autophagy regulation and prorenin receptor expression in podocytes by regulating the expression of key autophagy proteins. Urine miR-155-5p was also significantly increased in experimental models of FSGS [62].

Therefore, exosomes may be a potential source of new biomarkers for FSGS. The noninvasive acquisition of urinary exosomes is another advantage, whereas serum or plasma may not be suitable as they may contain non-nephrogenic exosomes.

Metabolomics

Metabolomics studies also provide potential early biomarkers for FSGS. A significant increase in *myo*-inositol in 70 urinary metabolites was found in FSGS patients, which was inversely proportional to the initial eGFR and correlated with plasma suPAR levels. In an *in vitro* FSGS model, *myo*-inositol treatment improved the decreased expression of ZO-1 and synaptopodin, and as *myo*-inositol increased, *myo*-inositol oxygenase tissue expression decreased proportionally to eGFR. It has been suggested that *myo*-inositol in urine may be an important indicator for the diagnosis and treatment of FSGS patients [63].

Single-cell transcriptome

In recent years, RNA sequencing approaches at the single-cell transcriptome level have also been applied in the diagnosis of FSGS. Menon et al. [64] found that type 1 interferon (IFN)-mediated STAT1 activation could be a key mechanism involved in glomerular disease progression for patients with endothelial cell activation. They identified

two distinct FSGS groups with significant differences in intrarenal *A2M* gene expression levels. The group with higher *A2M* gene expression was associated with poor prognosis [64]. Although single-cell studies have certain limitations, such as providing only a partial sample of the cellular transcriptome and preferring highly expressed genes, being sensitive to sample quality, and high costs limiting the ability to perform large-scale analysis, they also provide a direction for precision therapy.

In addition, renal tubule interstitial injury and fibrosis have been shown to be closely related to a decreased GFR and treatment response. Although FSGS is a glomerular disease, it is possible to detect pathways associated with disease progression in the renal tubulointerstitial profile. Mariani et al. [65] used an unbiased transcriptomic-driven approach to identify a subgroup of patients with MCDs or FSGS that shared renal TNF pathway activation and adverse outcomes. Two TNF-pathway urine markers were identified, i.e., tissue inhibitor of metalloproteinases-1 and monocyte chemoattractant protein-1, which could be used to predict an individual's TNF pathway activation score [65]. The study of the renal tubulointerstitial TNF pathway may represent the pathogenesis and chronic progression of glomerular disease.

Focal segmental glomerulosclerosis and treatment

The most commonly used drugs for FSGS include glucocorticoids and calcineurin inhibitors (CNIs). Other drugs such as mycophenolate mofetil, adrenocorticotropic hormone, or rituximab (RTX), have been used for some time, but their efficacy is still debatable [66,67]. The strategy of protecting podocytes based on precision medicine is the development direction in respect of progressive kidney disease. The molecular pathways associated with FSGS are targets for several emerging therapies and, while they are still being explored, they can also serve as a framework for precision medicine to identify appropriate drugs for specific patients with FSGS (Table 2).

Immunosuppressors are usually used in combination with renin-angiotensin system inhibitors (RASIs) in FSGS, but immunosuppressors have many adverse effects. Studies have shown that endothelin (ET) can damage podocytes through a variety of molecular mechanisms, including promoting inflammatory response and fibrosis. ET type A (ETA) receptor antagonists enhance the effects of RASI, and small-molecule ET-receptor antagonists or RASIs improve parenchymal injury and reduce proteinuria in FSGS rodent models [68]. DUET was a phase 2 randomized, double-blind, active-control study of the efficacy and safety of the dual ET-receptor and angiotensin-receptor blocker sparsentan (RE-021) in patients with FSGS. Compared with irbesartan, patients treated with sparsentan had a greater decrease in proteinuria and experienced no adverse effects [69]. Therefore, dual inhibition of the renin-angiotensin-aldosterone system and ETA receptors seems to be a promising approach, and further clinical trials are needed to clarify the efficacy.

Table 2. Summary of clinical trials for drugs targeting the FSGS

Drug	Target	Status	References and NCT number
Sparsentan	ARB and ETA	Phase III	NCT03762850
ION532	*APOL1*	Phase I	[76]
VX-147	*APOL1*	Phase II	NCT04340362
AZD2373	*APOL1*	Phase I	NCT04269031
Adalimumab	TNF-α	Phase II	NCT00814255
Ofatumumab	CD20	Phase II	NCT02394106
Ofatumumab	CD20	Phase II	NCT02394119
GFB-887	TRPC5	Phase IIa	NCT04387448
PF-06730512	ROBO2	Phase II	NCT03448692
Bardoxolone methyl	NRF2	Phase II	NCT03366337
LNA-antimiR-150	miR-150	Preclinical	[57]

APOL1, apolipoprotein L1; ARB, angiotensin-receptor blocker; ETA, endothelin type A; FSGS, focal segmental glomerulosclerosis; NCT, National Clinical Trial; NRF2, nuclear factor erythroid 2-related factor 2; ROBO2, roundabout guidance receptor 2; TNF-α, tumor necrosis factor alpha; TRPC, transient receptor potential channel.

Genetic testing is recommended for highly suspected genetic problems. Although some cases of hereditary SRNS partially respond to CNIs, genetic FSGS is usually resistant to immunosuppression and does not recur after kidney transplantation. The identification of a monogenic (such as *TRPC6*, ARHGDIA, *PLCE1*, and *ADCK4*) cause could result in new treatment possibilities for specific patients. Coenzyme Q10 is an important part of the mitochondrial respiratory chain. The recessive mutation of the gene encoding the protein in the biosynthetic pathway of coenzyme Q10 can lead to the deficiency of coenzyme Q10 and cause FSGS/SRNS [70]. In one study, genes involved in the coenzyme Q10 biosynthesis pathway (*PDSS2*, *COQ2*, *COQ6*, and *COQ8B*/*ADCK4*) were found to be mutated in about 1% to 2.7% of SRNS cases [71]. Once the genetic diagnosis is clear, early treatment with coenzyme Q10 can benefit patients [72,73]. Researchers suggest that all patients diagnosed with primary CoQ 10 deficiency should receive early and lifelong coenzyme Q10 supplementation to slow the progression of kidney disease and prevent further damage to other organs [74]. Some reports suggest that patients with FSGS caused by genetic mutations, such as *PLCE1*, *TRPC6*, and *APOL1*, may occasionally have a partial response to immunosuppressive agents, although data are limited [75].

APOL1-mutation–induced FSGS has been found in African patients. The current treatment for *APOL1* nephropathy is nonspecific and the efficacy is not satisfactory. Aghajan et al. [76] developed a transgenic mouse model of *APOL1* and discovered the first *APOL1* inhibitor, IONIS-APOL1Rx, which is a potent antisense treatment of *APOL1* and can systematically inhibit the expression of *APOL1* in the liver and kidney. IONIS-APOL1Rx effectively improved IFN-γ–induced albuminuria in *APOL1* G1 mice in a dose-dependent manner [76]. Yang et al. [77] administered antisense oligonucleotides (ASO) targeting *APOL1* to podocyte-specific G2*APOL1* mice and observed an efficient reduction in *APOL1* levels. They concluded that APOL1 ASO1 may be an effective therapeutic approach for *APOL1*-associated glomerular disease. Two therapies that inhibit *APOL1* function have undergone clinical trials. One was a pharmacokinetic study of escalating single doses of a subcutaneously administered ASO (AZD2373) against *APOL1* conducted by AstraZeneca; this was terminated, seemingly because the sponsor felt that there were sufficient data available for further study and that higher doses were associated with injection site reactions (NCT04269031). The other was a phase II study sponsored by Vertex Pharmaceuticals testing VX-147, an oral small-molecule *APOL1*-active inhibitor, in patients with *APOL1*-associated FSGS (NCT04340362). In addition, JAK inhibitors may have therapeutic benefits for managing cytokine-induced, *APOL1*-mediated podocytopathy [78]. Although the therapeutic effect is still unknown, targeted therapy for patients with *APOL1* high-risk alleles will be an important advance in the accurate treatment of FSGS.

Because TNF-α and peroxisome proliferator activator gamma pathways are involved in FSGS, and galactose has been found to neutralize circulating FSGS permeability factor activity, the FONT phase II clinical trial (NCT00814255) was designed to assess the efficacy of adalimumab and galactose compared to standard medical therapy, which usually comprises lisinopril, losartan, and atorvastatin. Oral administration of galactose resulted in at least a 50% reduction in albuminuria in three of the seven subjects, with stable eGFR in two subjects. The findings suggest that adalimumab is not a viable drug for further study in resistant FSGS patients and galactose seems to have potential value [79].

B-cell–targeting drugs reduce the B-cell production of antibodies and have been shown to be effective and relatively safe in a variety of immune-mediated kidney diseases. RTX is a chimeric monoclonal antibody that specifically binds to CD20-positive lymphocytes and can kill B cells by binding to CD20. A meta-analysis of 221 adult patients in 16 studies found that overall remission and relapse rates were 53.6% and 47.3%, respectively, and there were fewer serious adverse effects [80]. B-cell-targeting drugs reduce the B-cell production of antibodies and have been shown to be effective and relatively safe in a variety of immune-mediated kidney diseases [81,82]. The toxicity of anti-CD20 monoclonal antibodies has been limited to post-infusion side effects in children with more complex diseases. Adverse reactions were more common in patients treated with ofatumumab [83].

The transient receptor potential canonical-5-Ras-related C3 botulinum toxin substrate 1 (TRPC5-Rac1) pathway is central to the pathogenesis of podocyte injury. Rac1 activation leads to elevated reactive oxygen species, which promote cytoskeletal remodeling and podocyte death. Therefore, reducing the Rac1 level by inhibiting TRPC5 has

potential as a targeted therapy for podocytosis. GFB-887 is a podocyte-targeting, small-molecule inhibitor of TRPC5 designed specifically to treat patients with glomerular kidney diseases characterized by overactivation of the TRPC5-Rac1 pathway. TRACTION-2 was a phase 2a, double-blind, placebo-controlled, multiple escalation dose study (NCT04387448) of GFB-887, in which GFB-887 was found to be safe and well tolerated, having a pharmacokinetic profile allowing once-daily dosing, and dose-dependently decreased urinary Rac1 in healthy adults [84].

Roundabout guidance receptor 2 (ROBO2) is a cell adhesion molecule that belongs to the Ig superfamily of single-pass transmembrane receptors. In humans, mutations in ROBO2 or its ligand slit guidance ligand 2 (SLIT2) can cause urinary birth defects. Studies have shown that the inhibition of glomerular ROBO2/SLIT2 signaling can stabilize podocyte adhesion. ROBO2 may be a therapeutic target for podocyte injury and podocyte disease [85,86]. PODO (NCT03448692) is an open-label, phase IIa, multicenter trial in adults with FSGS. The ongoing trial will evaluate the initial efficacy and safety of ROBO2/ SLIT2 inhibition with the ROBO2 fusion protein PF-06730512 in patients with FSGS [87].

MiRNA also plays an important role in the treatment of FSGS. It was found that upregulation of miR-150 in human podocytes increases profibrotic proteins and decreases the antifibrotic suppressor of cytokine signaling 1. MiR-150 inhibitors improve doxorubicin-induced FSGS in mice, and the renal protective mechanism may be mediated by antifibrosis, anti-inflammation, and reduction of T-cell infiltration in the kidney [57]. A significant increase in miR-155-5p was also found in an FSGS mouse model and Nrf2 is the direct target of miR-155-5p; in that model, anti-miR-155-5p mitigated kidney damage and delayed the progression of renal fibrosis. MiR-155-5p was significantly increased in urine, suggesting that urine miR-155-5p may be useful in the diagnosis of FSGS [62]. Glomerular PECs are speculated to be pathogenic and comprise extracapillary proliferation in FSGS. MiR193a is a novel mediator that connects podocyte loss and PEC activation. In a mouse model of primary FSGS, induction of miR193a led to the downregulation of WT1, leading to podocyte dedifferentiation [88]. Thus, downregulation of miR193a with a similar drug appears to be promising for improving podocyte morphology in pathologic conditions.

Conclusion

At present, FSGS still represents a difficult problem in the field of glomerular diseases, with limited therapies and poor prognosis. In this paper, we reviewed the rapid developments in precision medicine in FSGS. Genomics is the foundation of precision medicine research, and proteomics and transcriptomics can provide diagnostic biomarkers and therapeutic targets. The search for genetic tests and biomarkers in FSGS is continuing, and therapies targeting specific molecular markers or other biomarkers are being further developed. This reveals the complexity of FSGS and requires a major shift in treatment modalities, from histologically type-centric to gene-level or molecular-marker-oriented diagnosis and treatment that is intended to provide individual choices for each patient. At present, precision medicine research still faces many challenges, such as the lack of random, large sample, and high-matching clinical trials; the identification of meaningful biological markers; the need for advanced technical support; and the difficulty in obtaining targeted therapeutic drugs. We believe that with the continuous expansion of precision medicine approaches in FSGS, there will be increasing patient benefits by which to achieve diagnosis and treatment at the individual level.

Conflicts of interest

All authors have no conflicts of interest to declare.

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Data sharing statement

The data presented in this study are available upon reasonable request from the corresponding author.

Authors' contributions

Conceptualization, Validation: YX, FL
Investigation, Supervision: FL

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References

1. Filler G, Young E, Geier P, Carpenter B, Drukker A, Feber J. Is there really an increase in non-minimal change nephrotic syndrome in children? *Am J Kidney Dis* 2003;42:1107–1113.
2. Kiffel J, Rahimzada Y, Trachtman H. Focal segmental glomerulosclerosis and chronic kidney disease in pediatric patients. *Adv Chronic Kidney Dis* 2011;18:332–338.
3. De Vriese AS, Sethi S, Nath KA, Glassock RJ, Fervenza FC. Differentiating primary, genetic, and secondary FSGS in adults: a clinicopathologic approach. *J Am Soc Nephrol* 2018;29:759–774.
4. Gast C, Pengelly RJ, Lyon M, et al. Collagen (COL4A) mutations are the most frequent mutations underlying adult focal segmental glomerulosclerosis. *Nephrol Dial Transplant* 2016;31:961–970.
5. Preston R, Stuart HM, Lennon R. Genetic testing in steroid-resistant nephrotic syndrome: why, who, when and how? *Pediatr Nephrol* 2019;34:195–210.
6. Trautmann A, Bodria M, Ozaltin F, et al. Spectrum of steroid-resistant and congenital nephrotic syndrome in children: the PodoNet registry cohort. *Clin J Am Soc Nephrol* 2015;10:592–600.
7. Bierzynska A, McCarthy HJ, Soderquest K, et al. Genomic and clinical profiling of a national nephrotic syndrome cohort advocates a precision medicine approach to disease management. *Kidney Int* 2017;91:937–947.
8. Yao T, Udwan K, John R, et al. Integration of genetic testing and pathology for the diagnosis of adults with FSGS. *Clin J Am Soc Nephrol* 2019;14:213–223.
9. Liu J, Wang W. Genetic basis of adult-onset nephrotic syndrome and focal segmental glomerulosclerosis. *Front Med* 2017;11:333–339.
10. Giglio S, Provenzano A, Mazzinghi B, et al. Heterogeneous genetic alterations in sporadic nephrotic syndrome associate with resistance to immunosuppression. *J Am Soc Nephrol* 2015;26:230–236.
11. Genovese G, Friedman DJ, Ross MD, et al. Association of trypanolytic ApoL1 variants with kidney disease in African Americans. *Science* 2010;329:841–845.
12. Yusuf AA, Govender MA, Brandenburg JT, Winkler CA. Kidney disease and APOL1. *Hum Mol Genet* 2021;30:R129–R137.
13. Ekulu PM, Nkoy AB, Adebayo OC, et al. A focus on the association of Apol1 with kidney disease in children. *Pediatr Nephrol* 2021;36:777–788.
14. McNulty MT, Fermin D, Eichinger F, et al. A glomerular transcriptomic landscape of apolipoprotein L1 in Black patients with focal segmental glomerulosclerosis. *Kidney Int* 2022;102:136–148.
15. Kallash M, Wang Y, Smith A, et al. Rapid progression of focal segmental glomerulosclerosis in patients with high-risk APOL1 genotypes. *Clin J Am Soc Nephrol* 2023;18:344–355.
16. Zee J, McNulty MT, Hodgin JB, et al. APOL1 genotype-associated morphologic changes among patients with focal segmental glomerulosclerosis. *Pediatr Nephrol* 2021;36:2747–2757.
17. Ge M, Molina J, Ducasa GM, et al. APOL1 risk variants affect podocyte lipid homeostasis and energy production in focal segmental glomerulosclerosis. *Hum Mol Genet* 2021;30:182–197.
18. Friedman DJ, Pollak MR. APOL1 nephropathy: from genetics to clinical applications. *Clin J Am Soc Nephrol* 2021;16:294–303.
19. Maas RJ, Deegens JK, van den Brand JA, Cornelissen EA, Wetzels JF. A retrospective study of focal segmental glomerulosclerosis: clinical criteria can identify patients at high risk for recurrent disease after first renal transplantation. *BMC Nephrol* 2013;14:47.
20. Snoek R, Nguyen TQ, van der Zwaag B, et al. Importance of genetic diagnostics in adult-onset focal segmental glomerulosclerosis. *Nephron* 2019;142:351–358.
21. Gallon L, Leventhal J, Skaro A, Kanwar Y, Alvarado A. Resolution of recurrent focal segmental glomerulosclerosis after retransplantation. *N Engl J Med* 2012;366:1648–1649.
22. Shoji J, Mii A, Terasaki M, Shimizu A. Update on recurrent focal segmental glomerulosclerosis in kidney transplantation. *Nephron* 2020;144 Suppl 1:65–70.
23. den Braanker DJ, Maas RJ, Deegens JK, et al. Novel in vitro assays to detect circulating permeability factor(s) in idiopathic focal segmental glomerulosclerosis. *Nephrol Dial Transplant* 2021;36:247–256.
24. Thunø M, Macho B, Eugen-Olsen J. suPAR: the molecular crystal ball. *Dis Markers* 2009;27:157–172.
25. Saleem MA. What is the role of soluble urokinase-type plasminogen activator in renal disease? *Nephron* 2018;139:334–341.
26. Wei C, El Hindi S, Li J, et al. Circulating urokinase receptor

as a cause of focal segmental glomerulosclerosis. *Nat Med* 2011;17:952–960.
27. Jin J, Li YW, He Q. Primary and recurrent focal segmental glomerulosclerosis closely link to serum soluble urokinase-type plasminogen activator receptor levels. *Transplant Proc* 2015;47:1760–1765.
28. Lee JM, Yang JW, Kronbichler A, et al. Increased serum soluble urokinase-type plasminogen activator receptor (suPAR) levels in FSGS: a meta-analysis. *J Immunol Res* 2019;2019:5679518.
29. Harel E, Shoji J, Abraham V, et al. Identifying a potential biomarker for primary focal segmental glomerulosclerosis and its association with recurrence after transplantation. *Clin Transplant* 2019;33:e13487.
30. Chen JS, Chang LC, Wu CZ, et al. Significance of the urokinase-type plasminogen activator and its receptor in the progression of focal segmental glomerulosclerosis in clinical and mouse models. *J Biomed Sci* 2016;23:24.
31. Spinale JM, Mariani LH, Kapoor S, et al. A reassessment of soluble urokinase-type plasminogen activator receptor in glomerular disease. *Kidney Int* 2015;87:564–574.
32. Harel E, Shoji J, Abraham V, et al. Further evidence that the soluble urokinase plasminogen activator receptor does not directly injure mice or human podocytes. *Transplantation* 2020;104:54–60.
33. Bock ME, Price HE, Gallon L, Langman CB. Serum soluble urokinase-type plasminogen activator receptor levels and idiopathic FSGS in children: a single-center report. *Clin J Am Soc Nephrol* 2013;8:1304–1311.
34. Winnicki W, Sunder-Plassmann G, Sengölge G, et al. Diagnostic and prognostic value of soluble urokinase-type plasminogen activator receptor (suPAR) in focal segmental glomerulosclerosis and impact of detection method. *Sci Rep* 2019;9:13783.
35. McCarthy ET, Sharma M, Savin VJ. Circulating permeability factors in idiopathic nephrotic syndrome and focal segmental glomerulosclerosis. *Clin J Am Soc Nephrol* 2010;5:2115–2121.
36. Savin VJ, Sharma M, Zhou J, et al. Renal and hematological effects of CLCF-1, a B-cell-stimulating cytokine of the IL-6 family. *J Immunol Res* 2015;2015:714964.
37. Sharma M, Zhou J, Gauchat JF, et al. Janus kinase 2/signal transducer and activator of transcription 3 inhibitors attenuate the effect of cardiotrophin-like cytokine factor 1 and human focal segmental glomerulosclerosis serum on glomerular filtration barrier. *Transl Res* 2015;166:384–398.
38. Zhang S, Breidenbach JD, Russell BH, George J, Haller ST. CD40/CD40L signaling as a promising therapeutic target for the treatment of renal disease. *J Clin Med* 2020;9:3653.
39. Delville M, Sigdel TK, Wei C, et al. A circulating antibody panel for pretransplant prediction of FSGS recurrence after kidney transplantation. *Sci Transl Med* 2014;6:256ra136.
40. Lopez-Hellin J, Cantarell C, Jimeno L, et al. A form of apolipoprotein a-I is found specifically in relapses of focal segmental glomerulosclerosis following transplantation. *Am J Transplant* 2013;13:493–500.
41. Puig-Gay N, Jacobs-Cacha C, Sellarès J, et al. Apolipoprotein A-Ib as a biomarker of focal segmental glomerulosclerosis recurrence after kidney transplantation: diagnostic performance and assessment of its prognostic value - a multi-centre cohort study. *Transpl Int* 2019;32:313–322.
42. Jacobs-Cachá C, Puig-Gay N, Helm D, et al. A misprocessed form of apolipoprotein A-I is specifically associated with recurrent focal segmental glomerulosclerosis. *Sci Rep* 2020;10:1159.
43. Jacobs-Cachá C, Puig-Gay N, Vergara A, et al. A specific tubular apoA-I distribution is associated to fsgs recurrence after kidney transplantation. *J Clin Med* 2021;10:2174.
44. Novelli R, Benigni A, Remuzzi G. The role of B7-1 in proteinuria of glomerular origin. *Nat Rev Nephrol* 2018;14:589–596.
45. Yu CC, Fornoni A, Weins A, et al. Abatacept in B7-1-positive proteinuric kidney disease. *N Engl J Med* 2013;369:2416–2423.
46. Burke GW, Chandar J, Sageshima J, et al. Benefit of B7-1 staining and abatacept for treatment-resistant post-transplant focal segmental glomerulosclerosis in a predominantly pediatric cohort: time for a reappraisal. *Pediatr Nephrol* 2023;38:145–159.
47. Li J, Niu J, Min W, et al. B7-1 mediates podocyte injury and glomerulosclerosis through communication with Hsp-90ab1-LRP5-β-catenin pathway. *Cell Death Differ* 2022;29:2399–2416.
48. Novelli R, Gagliardini E, Ruggiero B, Benigni A, Remuzzi G. Any value of podocyte B7-1 as a biomarker in human MCD and FSGS? *Am J Physiol Renal Physiol* 2016;310:F335–F341.
49. Eroglu FK, Orhan D, İnözü M, et al. CD80 expression and infiltrating regulatory T cells in idiopathic nephrotic syndrome of childhood. *Pediatr Int* 2019;61:1250–1256.
50. Hansrivijit P, Puthenpura MM, Ghahramani N. Efficacy of abatacept treatment for focal segmental glomerulosclerosis and minimal change disease: a systematic review of case reports, case series, and observational studies. *Clin Nephrol* 2020;94:117–126.
51. Gebeshuber CA, Kornauth C, Dong L, et al. Focal segmental glomerulosclerosis is induced by microRNA-193a and its downregulation of WT1. *Nat Med* 2013;19:481–487.
52. Wang L, Wang J, Wang Z, Zhou J, Zhang Y. Higher urine exoso-

mal miR-193a is associated with a higher probability of primary focal segmental glomerulosclerosis and an increased risk of poor prognosis among children with nephrotic syndrome. *Front Cell Dev Biol* 2021;9:727370.
53. Huang Z, Zhang Y, Zhou J, Zhang Y. Urinary exosomal miR-193a can be a potential biomarker for the diagnosis of primary focal segmental glomerulosclerosis in children. *Biomed Res Int* 2017;2017:7298160.
54. Kietzmann L, Guhr SS, Meyer TN, et al. MicroRNA-193a regulates the transdifferentiation of human parietal epithelial cells toward a podocyte phenotype. *J Am Soc Nephrol* 2015;26:1389–1401.
55. Bukosza EN, Kornauth C, Hummel K, et al. ECM characterization reveals a massive activation of acute phase response during FSGS. *Int J Mol Sci* 2020;21:2095.
56. Zhang C, Zhang W, Chen HM, et al. Plasma microRNA-186 and proteinuria in focal segmental glomerulosclerosis. *Am J Kidney Dis* 2015;65:223–232.
57. Qi H, Fu J, Luan J, et al. miR-150 inhibitor ameliorates adriamycin-induced focal segmental glomerulosclerosis. *Biochem Biophys Res Commun* 2020;522:618–625.
58. Zhang C, Liang S, Cheng S, et al. Urinary miR-196a predicts disease progression in patients with chronic kidney disease. *J Transl Med* 2018;16:91.
59. Xiao B, Wang LN, Li W, et al. Plasma microRNA panel is a novel biomarker for focal segmental glomerulosclerosis and associated with podocyte apoptosis. *Cell Death Dis* 2018;9:533.
60. Zhang W, Zhang C, Chen H, et al. Evaluation of microRNAs miR-196a, miR-30a-5P, and miR-490 as biomarkers of disease activity among patients with FSGS. *Clin J Am Soc Nephrol* 2014;9:1545–1552.
61. Yildirim D, Bender O, Karagoz ZF, et al. Role of autophagy and evaluation the effects of microRNAs 214, 132, 34c and prorenin receptor in a rat model of focal segmental glomerulosclerosis. *Life Sci* 2021;280:119671.
62. Liu G, He L, Yang X, et al. MicroRNA-155-5p aggravates adriamycin-induced focal segmental glomerulosclerosis through targeting Nrf2. *Nephron* 2023;147:108–119.
63. An JN, Hyeon JS, Jung Y, et al. Urinary myo-inositol is associated with the clinical outcome in focal segmental glomerulosclerosis. *Sci Rep* 2019;9:14707.
64. Menon R, Otto EA, Hoover P, et al. Single cell transcriptomics identifies focal segmental glomerulosclerosis remission endothelial biomarker. *JCI Insight* 2020;5:e133267.
65. Mariani LH, Eddy S, AlAkwaa FM, et al. Precision nephrology identified tumor necrosis factor activation variability in minimal change disease and focal segmental glomerulosclerosis. *Kidney Int* 2023;103:565–579.
66. Kidney Disease: Improving Global Outcomes (KDIGO) Glomerular Diseases Work Group. KDIGO 2021 clinical practice guideline for the management of glomerular diseases. *Kidney Int* 2021;100:S1–S276.
67. Caster DJ, Magalhaes B, Pennese N, et al. Efficacy and safety of immunosuppressive therapy in primary focal segmental glomerulosclerosis: a systematic review and meta-analysis. *Kidney Med* 2022;4:100501.
68. Komers R, Plotkin H. Dual inhibition of renin-angiotensin-aldosterone system and endothelin-1 in treatment of chronic kidney disease. *Am J Physiol Regul Integr Comp Physiol* 2016;310:R877–R884.
69. Trachtman H, Nelson P, Adler S, et al. DUET: a phase 2 study evaluating the efficacy and safety of sparsentan in patients with FSGS. *J Am Soc Nephrol* 2018;29:2745–2754.
70. Park E, Kang HG, Choi YH, et al. Focal segmental glomerulosclerosis and medullary nephrocalcinosis in children with ADCK4 mutations. *Pediatr Nephrol* 2017;32:1547–1554.
71. Tan W, Airik R. Primary coenzyme Q10 nephropathy, a potentially treatable form of steroid-resistant nephrotic syndrome. *Pediatr Nephrol* 2021;36:3515–3527.
72. Atmaca M, Gulhan B, Korkmaz E, et al. Follow-up results of patients with ADCK4 mutations and the efficacy of CoQ10 treatment. *Pediatr Nephrol* 2017;32:1369–1375.
73. Song X, Fang X, Tang X, et al. COQ8B nephropathy: early detection and optimal treatment. *Mol Genet Genomic Med* 2020;8:e1360.
74. Drovandi S, Lipska-Ziętkiewicz BS, Ozaltin F, et al. Oral Coenzyme Q10 supplementation leads to better preservation of kidney function in steroid-resistant nephrotic syndrome due to primary Coenzyme Q10 deficiency. *Kidney Int* 2022;102:604–612.
75. Rosenberg AZ, Kopp JB. Focal segmental glomerulosclerosis. *Clin J Am Soc Nephrol* 2017;12:502–517.
76. Aghajan M, Booten SL, Althage M, et al. Antisense oligonucleotide treatment ameliorates IFN-γ-induced proteinuria in APOL1-transgenic mice. *JCI Insight* 2019;4:e126124.
77. Yang YW, Poudel B, Frederick J, et al. Antisense oligonucleotides ameliorate kidney dysfunction in podocyte-specific APOL1 risk variant mice. *Mol Ther* 2022;30:2491–2504.
78. Nystrom SE, Li G, Datta S, et al. JAK inhibitor blocks COVID-19 cytokine-induced JAK/STAT/APOL1 signaling in glomerular cells and podocytopathy in human kidney organoids. *JCI Insight*

2022;7:e157432.
79. Trachtman H, Vento S, Herreshoff E, et al. Efficacy of galactose and adalimumab in patients with resistant focal segmental glomerulosclerosis: report of the font clinical trial group. *BMC Nephrol* 2015;16:111.
80. Hansrivijit P, Cheungpasitporn W, Thongprayoon C, Ghahramani N. Rituximab therapy for focal segmental glomerulosclerosis and minimal change disease in adults: a systematic review and meta-analysis. *BMC Nephrol* 2020;21:134.
81. Basu B. Ofatumumab for rituximab-resistant nephrotic syndrome. *N Engl J Med* 2014;370:1268–1270.
82. Colucci M, Labbadia R, Vivarelli M, Camassei FD, Emma F, Dello Strologo L. Ofatumumab rescue treatment in post-transplant recurrence of focal segmental glomerulosclerosis. *Pediatr Nephrol* 2020;35:341–345.
83. Bonanni A, Calatroni M, D'Alessandro M, et al. Adverse events linked with the use of chimeric and humanized anti-CD20 antibodies in children with idiopathic nephrotic syndrome. *Br J Clin Pharmacol* 2018;84:1238–1249.
84. Walsh L, Reilly JF, Cornwall C, et al. Safety and efficacy of GFB-887, a TRPC5 channel inhibitor, in patients with focal segmental glomerulosclerosis, treatment-resistant minimal change disease, or diabetic nephropathy: TRACTION-2 trial design. *Kidney Int Rep* 2021;6:2575–2584.
85. Pisarek-Horowitz A, Fan X, Kumar S, et al. Loss of roundabout guidance receptor 2 (Robo2) in podocytes protects adult mice from glomerular injury by maintaining podocyte foot process structure. *Am J Pathol* 2020;190:799–816.
86. Fan X, Yang H, Kumar S, et al. SLIT2/ROBO2 signaling pathway inhibits nonmuscle myosin IIA activity and destabilizes kidney podocyte adhesion. *JCI Insight* 2016;1:e86934.
87. Beck LH, Berasi SP, Copley JB, et al. PODO: trial design. Phase 2 study of PF-06730512 in focal segmental glomerulosclerosis. *Kidney Int Rep* 2021;6:1629–1633.
88. Bharati J, Chander PN, Singhal PC. Parietal epithelial cell behavior and its modulation by microRNA-193a. *Biomolecules* 2023;13:266.