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FSGS Recurrence Collaboration: Report of a Symposium

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State of the Problem

General Overview

FSGS describes a histologic lesion secondary to podocyte injury and depletion [1–3]. We currently have very few tools to discriminate between primary or idiopathic, genetic, or forms of FSGS secondary to diverse conditions (hypertension, obesity, drugs, viruses, and others) and the risk of recurrent disease [4]. The incidence of the disease is fairly constant across the lifespan accounting for approximately 10–20% of cases of new-onset nephrotic syndrome. Moreover, FSGS is

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the underlying disease in 5–10% of all patients who have progressive loss of kidney function and require renal replacement therapy [4, 5].

Pediatric Population

The highest risk of FSGS recurrence is found in pediatric patients with FSGS attributed to immune-mediated mechanisms. Acute (<2 weeks) and intermediate (2 weeks–3 months) timing of recurrence is typically attributed to immune dysfunction. The mechanisms related to delayed (e.g., >3 months) recurrence may be distinct and confounded by de novo occurrence of FSGS in the graft. Based on retrospective cohort studies in the USA, Europe, and Australia/New Zealand, clinical risk factors associated with disease recurrence in children include white race, progression to kidney failure within 48 months from disease presentation, and mesangial hypercellularity. Most of these factors have very low positive predictive values and are not robustly reproducible. More recently, initial complete proteinuria remission with corticosteroid therapy (steroid sensitivity in the native kidneys) followed by subsequent steroid resistance has been associated with an approximately 90% risk of post-transplant recurrence [5–7]. Systematic reviews of recurrent FSGS in pediatric patients studies indicate that there is marked variability in treatment regimens for recurrent FSGS that differ from center to center. There is no widely accepted, evidence-based therapeutic intervention that safely and effectively achieves remission of proteinuria and maintains long-term graft function [8].

Adult Population

In order to illustrate the wide variability in treatment protocols, the TANGO study represents an available resource. This observational study is the largest retrospective cohort that included adolescents and adults (>16 years of age), FSGS recurrence was documented in 57 of 176 patients (32%; 95% CI: 25–39%), and the median time to recurrence was 1.5 months [9]. Factors associated with a higher risk of recurrence included older age at diagnosis, native nephrectomy, white race, and lower BMI. Like in the pediatric population, most of these factors cannot reliably predict the risk of recurrence. Genetic testing may help in some cases with risk stratification [6], while measuring other potential circulating factors remains to be validated. Treatment of recurrence in published retrospective studies is highly variable. In the TANGO cohort, 81% of patients received plasmapheresis with or without rituximab [9], with 56% of individuals achieving either complete or partial remission [9]. These results are comparable to other published patient series [10, 11]. When achieving at least a partial remission, relatively stable medium-term eGFR has been demonstrated. Some patients with a response to plasmapheresis may require long treatment to maintain remission [12], while those without a response after a few weeks are unlikely to have any benefit from continuation of treatment. Other experimental drugs or interventions such as abatacept, ACTH gel, and LDL apheresis have only yielded limited benefits in selected case reports. Unfortunately, if the transplant fails due to FSGS recurrence, the risk of recurrence on the second and third transplant is significantly higher, raising questions about the ethics of pursuing re-transplantation in the absence of novel treatments under these circumstances. Overall, FSGS recurrence is associated with a 5-fold higher risk of graft loss in adults and treatment response is only achieved in about half of patients [9]. The RESOLVE study is an ongoing clinical research effort established at the University of Michigan to create a data registry and sample biorepository for pediatric patients with recurrent FSGS using a paradigm comparable to the TANGO study for adult patients with this urgent clinical problem.

Use of Genetics as a Research Accelerator for FSGS Recurrence

Genetic Architecture of FSGS Recurrence

The genetic predisposition to develop FSGS recurrence is obscure. Monogenic causes of FSGS have been reported in up to 30% of individuals presenting with FSGS, with ample variability depending on age of onset of disease and response to treatment [13–17]. Lower frequency is observed in adults and individuals with steroid-responsive NS, while higher frequency is observed when steroid-resistant disease presents in infancy (<1 year of age) and in certain ancestral populations, such as individuals from Finland where congenital NS, mostly caused by two founder mutations in *NPHS1*, is identified as the cause of end-stage kidney disease in pediatric renal transplant recipients [18]. Importantly, Mendelian forms of NS discovered thus far are usually resistant to immunomodulating agents and do not recur following kidney transplantation [19, 20]. These observations indicate that the population of FSGS that is not caused by Mendelian FSGS mutations (non-Mendelian FSGS) is enriched for forms of disease that have potential to recur after transplantation. Moreover, the absence of clear causal variants with large effect size in non-Mendelian FSGS supports a complex genetic determination of recurrent FSGS that would be approachable by genome-wide association studies (GWASs). Accruing large and adequately

powered cohorts of recurrent FSGS for GWAS, although ideal for understanding the genetic architecture of disease, is challenging due to the inherent rarity of the FSGS and, even more, the recurrent sub-phenotype. While progress is being made in establishing such cohorts, one way to gain insights into the etiology of recurrent FSGS is to conduct genetic studies of non-Mendelian FSGS. This approach carries at least two advantages: first, it tackles a subset of FSGS that has a high risk of recurrence; second, rare Mendelian variants are likely to mask the effect of common variants with small-to-moderate effect size; hence, removing genetic forms of FSGS is likely to increase power in such GWAS. To date, no GWAS for non-Mendelian forms of FSGS has been conducted, identifying a unique opportunity for dissecting the genetic basis of this condition. Large collaborative efforts are, therefore, required to assemble adequate cohorts of patients with FSGS for GWAS analyses to identify genetic risk factors for both native kidney non-monogenic FSGS and disease recurrence following kidney transplantation.

HLA Biology and FSGS Recurrence

Numerous GWAS analyses have revealed variants in HLA class II genes that are associated with glomerular diseases such as IgA nephropathy, membranous nephropathy, and childhood-onset steroid-sensitive nephrotic syndrome. The role of these genetic variations in adaptive immune genes in FSGS is unknown [21, 22]. The involvement of HLA in NS is likely proximal to the initial immune dysregulation event, involving the presentation of glomerular autoantigens within the context of inflammation. The dissection of the exact role of HLA alleles linked to each specific glomerular disease is anticipated to be complex and will require combinatorial approaches of precise imputation in different populations, deep resequencing at the HLA locus on chromosome 6p21, as well as integration of sequencing data with blood and immune cell transcriptomic and epigenomic data during different state of disease activity and response to therapy. Interrogation into potential autoantigen targets, HLA binding potential of these autoantigens, and tissue expression patterns for these autoantigen targets is needed for better understanding of NS in native kidney and recurrence following kidney transplant [23–25]. Modeling should explore whether HLA risk alleles alone predispose some patients to develop NS/recurrence or if HLA is working in concert with other genetic risk variants and environmental factors [26]. We anticipate that HLA genotyping or, better, genome-wide approaches by either array or genome sequencing, performed early in disease presentation, may provide clearer disease characterization and allow individualized interventional therapies.

APOL1 Nephropathy

In the light of well-established association of high-risk APOL1 genotype and high incidence of idiopathic FSGS in native kidneys of black/African American patients [27], two relevant questions emerge: (1) Do Black patients have a higher incidence of FSGS recurrence post-transplant? (2) Does high-risk APOL1 genotype of the recipient or donor increase the incidence of FSGS recurrence or allograft survival? Available evidence suggests that in general, black /African American patients do not have higher incidence of FSGS recurrence [9, 10, 28]. While an early study shows that high-risk APOL1 genotype in deceased donor kidney is associated with allograft failure [29], it is unknown whether this observation extends to post-transplant recurrence of disease. An intriguing question is whether a transplanted kidney from a donor with a high-risk APOL1 genotype will carry a higher risk for the development of de novo FSGS. At present, there are insufficient data on whether high-risk APOL1 genotype of the donor impacts FSGS de novo occurrence after transplant. An initial study suggested that APOL1 genotype of recipients does not affect 5-year allograft survival [30]. However, a recent study challenged this finding [31]. The ongoing APOLLO study aims to answer questions regarding the contribution of donor and recipient APOL1 genotype on kidney allograft survival [32] (ClinicalTrials.gov Identifier: NCT04910867).

Epigenetics in Kidney Disease

Apart from the subgroup of cases that are due to single-gene defects, most FSGS and glomerular diseases are likely attributable to complex interaction between multiple genes and environmental factors. More recently, the role of epigenetic changes (changes in gene accessibility rather than the genetic code) has been shown to play a significant role in phenotypic expression of different glomerular diseases. For example, epigenomic wide association studies in diabetic kidney disease revealed specific epigenetic signatures that are associated with risk of kidney fibrosis in kidney biopsy samples and diabetic kidney disease progression [33, 34]. Although histone modification was found to be more pronounced in kidney tubules in diabetic kidney disease compared to peripheral blood, there was significant concordance between changes in blood and kidney tissue [35, 36]. In addition, cytosine methylation changes occur prior to pathology, suggesting that it may provide insight into early intervention strategies to slow disease progression as well as identifying mechanistic pathways leading to disease. These findings justify further research into the role of epigenetic changes in native kidney FSGS and disease recurrence.

Identifying Disease Pathways: Podocytes and Beyond

Molecular Mechanisms of FSGS

Pre- and post-reperfusion biopsies in transplant recipients with FSGS have been analyzed by microarray and gene enrichment pathway analysis and compared with data obtained from FSGS patients enrolled in other cohorts (NEPTUNE, ERCB) [37]. Using an ex vivo cell-based assay to identify genes specifically activated in podocytes exposed to sera of FSGS patients, inflammatory signaling pathways, including the TNF pathway, were activated in glomeruli immediately after exposure to sera of FSGS patients. TNF pathway activation correlated with clinical and histological parameters of disease. Similarly, a set of genes was identified that specifically activated in podocytes when cultured in the presence of serum of patients with recurrent FSGS. These clinical translational study results strengthen the hypothesis that an integrated system biology approach may be leveraged to risk stratify FSGS patients.

High Sensitivity Flow Cytometry

Efficacy of immune-suppressive agents and the evidence that FSGS may recur rapidly in a transplanted kidney graft support the concept that the immune system plays a critical role in the disease pathogenesis. Flow cytometric approaches have been used to interrogate the phenotype of peripheral blood mononuclear cells of children with idiopathic NS, which includes two main histological entities, minimal change disease (MCD) and FSGS. These analyses suggested a role for reduced regulatory T cells and increased effector T cells in disease pathogenesis [38]. Efficacy of B-cell-depleting treatments enabled identification of an association between memory B cells and disease relapses [39], suggesting a pivotal role for this B-cell subpopulation, which is also increased at the onset of steroid-sensitive forms of NS in children [40, 41]. In the past decade, the field of cytometry has witnessed advances in hardware that enabled a dramatic increase in measurable parameters per cell [42]. For example, spectral flow cytometry uses multiple detectors to capture full spectrum emission of every fluorochrome across each laser, allowing for increased resolution and sensitivity. This technology allows to use up to 40 markers with regular fluorophores. Time-of-flight mass cytometry, which utilizes heavy metal-labeled antibodies, instead of fluorophores, allowed performance of an in-depth phenotyping of peripheral lymphocytes in idiopathic nephrotic syndrome patients treated with B-cell-depleting antibodies [43]. Despite the small sample size, this approach was able to detect granular differences in B-cell subsets between patients who did vs. those who did not relapse. Innovative and simplified technologies are being developed and they will enable investigators to study the immune system of idiopathic nephrotic syndrome patients using peripheral blood, biopsy, and urine samples in a standardized fashion not requiring specialized research facilities [44].

Mapping Immune Cell Activity: An Example from Lupus Nephritis

The Accelerating Medicines Partnership is a joint effort of the NIH, pharmaceutical companies, and nonprofit organizations to deconstruct lupus kidney disease to identify promising biological targets for diagnostics and drug development. A study of 156 kidney biopsies from patients with active lupus nephritis used single-cell technologies and collected ~74,000 immune cells [45]. Focusing on the intrarenal myeloid compartment, analyses revealed that (1) a higher frequency of myeloid vs. lymphoid cells was associated with treatment response and nonresponse, respectively; (2) macrophage differentiation into a phagocytic state was associated with ongoing histopathologic kidney damage; (3) phagocytic macrophage was derived from infiltrating monocytes entering tissue from the peripheral blood. This work reveals features of the intrarenal immune compartment, including new myeloid cell types, which are associated with important clinical features of lupus kidney disease for future studies. This example provides a potential strategy for the exploration of the intrarenal immune compartment in recurrent FSGS. The possibility that recurrent FSGS could be linked by an autoimmune process is also suggested by the identification of autoantibodies such as anti-CD40 in translational studies [46] and is further supported by the evidence of anti-nephrin autoantibodies in a subset of patients with MCD [47].

Podocyte Dropout and FSGS

Glomerular hypertrophy after kidney transplantation can destabilize allograft glomeruli and cause an accelerated detachment of podocytes even in healthy allografts [48]. Furthermore, the rate of podocyte detachment predicts long-term allograft survival. To further understand the mechanisms of podocyte detachment, the transcriptomic profile of glomerular cells, at a single-cell resolution, in first-year stable and healthy kidney allografts was compared to glomerular cells of biopsies from a healthy 2-kidney state. Downregulation of the integrin α3 (ITGA3) transcript that codes for a protein forming the ITGα3β1 heterodimer was identified. ITGα3β1 is the principal integrin by which podocytes attach themselves

to the glomerular basement membrane [49]. These findings were confirmed at a protein level with immunohistochemistry. Investigating the interaction of podocyte-specific proteins with the glomerular basement membrane can allow for obtaining mechanistic insight into the various processes that affect this interaction including in patients with recurrent FSGS.

Application of Urine and Tissue Proteomics to Study FSGS

Proteomic analysis can be applied to study both FSGS pathophysiology and biomarkers of diagnosis and recurrence. FSGS urine studies were performed in specimens from the CureGN cohort [50]. Urine samples were processed and tagged with an isobaric ion tag and analyzed by 2D-LC-MS/MS. Over 1,100 proteins were identified by two-peptide analysis. We identified a 9-protein signature that discriminated between FSGS and other glomerulopathies. For proteomic analysis of kidney tissue, we used laser capture microscopy to sample discrete glomeruli that are subjected to analysis by LC-MS/MS [51, 52]. In recent work, we compared extracellular matrix protein composition in the collapsing form of FSGS (cFSGS) and FSGS not otherwise specified (FSGS-NOS). The proteomic analysis identified several proteins with markedly increased expression in cFSGS compared to FSGS-NOS. Differentially expressed proteins included annexin A3 and cathepsin C and B and increased expression was confirmed by immunohistochemistry [53]. Cathepsin c and annexin A3 expression was increased in HIV-associated cFSGS (unpublished data). In summary, proteomic analysis of urine and tissue can identify proteins that discriminate between FSGS and other glomerulopathies and revealed marked differences in tissue protein expression between cFSGS and FSGS-NOS.

Biomarkers of FSGS Recurrence

Reliable biomarkers of FSGS recurrence will be valuable to predict, identify, and ultimately treat this difficult clinical condition. Using an experimental model of stimulation of human podocytes in vitro with recurrent disease plasma, activation of a signaling marker, phosphorylated VASP, has been described from both 2D and 3D glomerular models [54]. This signaling pattern, along with additional molecules that correlate with increased podocyte motility, is replicated by stimulating human podocytes with supernatant from Th17 cells. A variety of circulating factors or absence of inhibitory factors including CLC-1, VEGF, hemopexin, suPAR, and TNF have been associated with FSGS recurrence. A panel of seven antibodies including CD40, PTPRO, CGB5, FAS, P2RY11, SNRPB2, and APOL2 and specific HLA haplotypes may represent a novel tool to identify children at high risk of recurrent FSGS [46]. Emerging data suggest that anti-nephrin autoantibodies are associated with some cases of podocytopathy and have been documented in a few cases of FSGS recurrence [47]. There was a substantial decline in titer in remission versus active disease. This is consistent with a pathogenic role for the autoantibodies; however, more research is required to clarify this point and the role of anti-nephrin antibodies in recurrent FSGS. At present, there is insufficient evidence to support the identification of any of these molecular candidates as the circulating "factor" that triggers FSGS or their use as a validated prognostic biomarker for recurrent FSGS. Preliminary epigenetic research of patient lymphocytes appears to distinguish circulating factor disease from other forms of nephrotic syndrome. (Saleem, personal communication) The distinction between the cases that recur within the first 24–48 h from those with later may be valuable in assessing the heterogeneity of the mechanisms of disease enhanced by the development and validation of timing- and mechanism-specific biomarkers.

Organoids

Human kidney organoids may have the potential to model recurrent FSGS, which would provide an assay to dissect mechanisms of disease or test therapeutic approaches. Organoids are complex heterocellular cultures that contain podocytes, proximal tubules, and distal tubules in arrangements resembling kidney tissue. These models can express general features of FSGS. Even "healthy" organoid podocytes express certain transcriptomic and morphological features that are characteristic of FSGS [55]. Gene-edited PODXL −/− organoids that lack podocalyxin exhibit defects in junction and microvillus formation, which parallel phenotypes in Podxl −/− mice [56, 57]. Second, circulating factors relevant to FSGS can be analyzed in organoids. For example, interferon-γ treatment of organoids induces apolipoprotein L1 (APOL1), including FSGS high-risk variants [58]. Furthermore, implantation of organoids into immunodeficient mice produces vascularized glomerulus-like structures, which are exposed to the circulation, although these structures remain immature [58–60]. Thus, exposing organoids to conditions simulating recurrent FSGS, such as patient sera and select mediators, could provide disease models for mechanistic analysis and therapeutic screening. Dedicated efforts are needed to innovate such models.

Glomerular Spheroids

A novel 3D model of the glomerulus has recently been described that demonstrates more complete podocyte architecture compared to 2D cultures and upregulated expression of podocyte-specific proteins [56]. This has been demonstrated to be a rapid screening tool for effects of FSGS plasma on podocyte loss and GBM production and is a promising tool for further development.

Systems Biology

Systems biology is the analysis of complex interactions and pathways within biologic systems. We identified a 272-gene transcriptional signature of TNF activity in kidney tissue from individuals in NEPTUNE (Nephrotic Syndrome Study Network) with poor outcomes in FSGS and MCD [61]. These individuals demonstrated increased loss of kidney function and decreased likelihood of complete remission. Kidney organoids generated from human pluripotent stem cells treated with TNF recapitulated this transcriptional signature of disease. Additionally, TNF-treated organoid cells expressed and secreted MCP-1 and TIMP-1, both identified as urinary biomarkers of TNF activity in NEPTUNE. Further, a proteomics analysis of TNF-treated organoid cultures revealed C3 and VCAM-1 to be additional putative biomarkers of TNF activity. Indeed, expression of both genes was significantly higher in tubular cells from individuals in NEPTUNE with high versus low TNF activity. Together, these findings demonstrate that TNF gene activity associated with poor outcomes in FSGS/MCD is present to a significant extent in kidney cells, identifying kidney tissue as potentially amenable to anti-TNF-targeted therapies in a subset of affected individuals. Additional work is needed to determine whether the TNF signaling pathway and activation signature identified in patients with FSGS is relevant to other glomerular disorders and whether it can be used to apply precision medicine approaches to treatment in these conditions. Human pluripotent stem cell-kidney organoids provide a powerful platform to study mechanisms involved in FSGS and development of precision medicine therapies.

Therapy Development and Rare Disease Trial Design

Lessons Learned from Trials

Rituximab lowers proteinuria in patients at high risk of recurrent disease. In the absence of CD20, rituximab interacts with SMPDL-3b on the podocyte cell surface to exert its intra-glomerular actions [62]. Serum from patients with recurrent FSGS reduces the expression of SMPDL-3b in podocytes and disrupts the actin cytoskeleton. Rituximab reverses both of these findings. SMPDL-3b also plays a role in the regulation of innate immunity in podocytes. Mediators of the immune system that are expressed on podocytes include cGAS-STING (innate) and CD80 (adaptive). Agonists of STING promote glomerular inflammation and disease [63]. In contrast, abatacept, in select case series, reduces proteinuria in patients with recurrent FSGS. While the contribution of impaired ABCA1-dependent cholesterol efflux has been largely described [64] and a small molecule oral agent is being studied in phase 2 trial in primary FSGS (NCT05267262), the contribution of this pathway to recurrent FSGS remains to be established. These translational studies have defined target pathways and potential therapies for patients with recurrent FSGS post-transplantation.

Clinical Considerations when Studying Rare Clinical Conditions

Recurrent FSGS is a rare complication of a rare disease. A critical first step in conducting meaningful clinical trials in this area will be to establish a uniform case definition that can be applied in real time. This will facilitate early identification and enrollment into registries and yield a relatively homogeneous population to study natural history, promote biomarker discovery, and enable interventional clinical trials. Recurrent FSGS and Alport syndrome share several features, e.g., the importance of genetics, the lack of approved therapies, and the prominent involvement of pediatric patients. Recurrent FSGS represents its own challenges because it is a sudden and unexpected complication with profound short- and long-term effects on kidney function and overall health. Delineation of the optimal population to enroll in clinical trials is essential. Ensuring that the intervention and objectives are in line with the lived experiences of patients increases acceptance of proposed studies and hopefully promotes participation in the trial. Another challenge in designing clinical trials for patients at risk of recurrent FSGS is that each transplant center may have set protocols in place to guide the prevention or management of the problem. Ensuring that patients and investigators agree with the design of a trial is essential for successful recruitment.

Biostatistical Considerations in Clinical Trials for FSGS Recurrence

Innovative trials in recurrent FSGS require bolstering the operational infrastructure that supports the design, conduct, and analysis of such trials. Optimal design

requires access to relevant literature or unpublished data for estimates. Statistical methods need to be developed to support the size and operating characteristics of new trial designs. A clinical trial simulation framework is required to guide work in this space. Institutional Review Boards and data monitoring committees need to be configured, so they can manage more complex and novel protocols and informed consent forms efficiently. The conduct of these trials may require the capacity for multiple randomizations. Data systems capable of accessing real-world data and allowing real-time capture of "classical" clinical trial data will facilitate interim analyses and trial decisions. Institutional Review Boards must handle adaptations in trial design with greater dispatch and facilitate ongoing interaction with regulatory authorities and data monitoring committees. Analysis requires the development of statistical methods that may appear simple conceptually but not methodologically. Regulatory and clinical trials principles need to be developed for new situations, such as multiplicity in platform trials. Finally, best practices have to be adopted that can reduce time from last subject last visit to analysis, e.g., blinded data reviews.

Novel Approaches to Study Design

There are a number of issues to consider in studying a rare condition like recurrent FSGS [1]: relevance of external data [2]; degree of borrowing of external data [3]; complexity of estimand of interest [4]; interpretation of study results [5]; communication to stakeholders; and [6] shift from usual statistical conditions for success, namely, $\alpha = 0.05$, power = 80%. Because of the rarity and unpredictable occurrence of post-transplant FSGS recurrence, novel study designs may be needed to address this problem in practical terms. Emerging options include (1) linked phase 2/phase 3 trials; (2) adaptive designs such as (a) group sequential, which includes interim analysis for early decision-making; (b) ranking and selection; (c) response-adaptive randomization; and (d) enrichment to identify the target population; (3) small n, sequential, multiple assignment randomized trial (snSMART) design [65]; and (4) platform trials. The primary interest in snSMART designs is stage 1 outcomes operating under the assumption that there is no carryover effect from prior interventions given a washout period and that the condition is a chronic, stable disease. Satisfying these conditions in trials for recurrent FSGS may depend on the intervention and control under study. Platform trials may use a basket – multiple diseases with shared disease-causing pathways – or umbrella – single disease entity with multiple distinct pathogenic pathways – design. The assignment to a test arm in a platform trial can be based on clinical data, biomarker profiles, or other instruments to subcategorize patients more precisely. A master protocol is a key feature of platform trials. These designs coupled with Bayesian analysis can formally incorporate historical or external data and provide the probability of clinical benefit for efficient treatment effect estimates. The choice of one of the novel trial design options should be guided by the specific nature of trial in question and made in consultation with a biostatistician with experience in rare diseases.

Conclusion

The methodologic progress over recent decades, including the development of effective systems for broad spectrum international collaborations and systems biology, provide a sturdy foundation for the scientific advances needed to resolve recurrent FSGS. The heterogeneity of phenotype and outcomes serves as reminders that recurrent FSGS is a spectrum of diseases. Like many complex diseases, recurrent FSGS will require cohorts inclusive of children and adults with deep phenotyping, longitudinal health outcomes, as well as robust biosampling to anchor the research necessary to understand each subgroup and develop precision medicine strategies for prevention and management. In support of the need for expanded prospective and retrospective cohorts with available biosamples for the scientific community, the RESOLVE study has been launched by members of this recurrent FSGS collaboration (NCT05650619). The innovation of functional tools such as human organoids, which may be developed to match or utilize resources from clinical trials, will be essential to dissect mechanism and conduct "clinical trials in vitro" for candidate therapeutics in the pre-clinical setting before they are applied to patient populations.

Conflict of Interest Statement

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stands to gain royalties from their future commercialization. AF is vice president of L&F Health LLC and is a consultant for ZyVersa Therapeutics, Inc. ZyVersa Therapeutics, Inc., has licensed worldwide rights to develop and commercialize hydroxypropyl-beta-cyclodextrin for the treatment of kidney disease from L&F Research, which was partially funded by L&F Health LLC. She also holds equities in Renal 3 River Corporation. Matthias Kretzler has received research support on behalf of the University of Michigan from Boehringer Ingelheim, Novo Nordisk, Certa, Poxel, Astellas, and Janssen, has received research funding from NIH, JDRF, Chan Zuckerburg Initiative, amfAR, AstraZeneca, Boehringer Ingelheim, Elpidera, Gilead, Goldfinch Bio, Eli Lilly, Angion Biomedica, Certa, Novo Nordisk, Janssen, Chinook, RenalytixAI, Regeneron Pharmaceuticals, Travere Therapeutics, and Ionis Pharmaceuticals, is on the editorial boards for *J Am Soc Nephrology*, *Kidney Int*, and *Kidney Dis*, and is on an advisory board for NephCure Kidney International. Sandra Merscher is an inventor on pending and issued patents aimed to diagnose or treat proteinuric renal diseases. She stands to gain royalties from their future commercialization. She holds equity interest in L&F Research and is a shareholder of ZyVersa Pharmaceuticals, Inc., who has licensed worldwide rights to develop and commercialize hydroxypropyl-beta-cyclodextrin for the treatment of kidney diseases from L&F Research. Paul Hoover, Kelley Kidwell, Moin Saleem, Leonard Riella, Lawrence Holzman, Annette Jackson, and Opeyemi Olabisi have no disclosures. Paolo Cravedi is a consultant for Chinook therapeutics, Repertoire Immune Medicines, and Calliditas Therapeutics. Benjamin Freedman is an inventor on a patent and patent applications related to human kidney organoid differentiation and modeling of FSGS in this system (e.g., "three-dimensional differentiation of epiblast spheroids into kidney tubular organoids modeling human micro-physiology, toxicology, and morphogenesis" [Japan, USA, and Australia], licensed to STEMCELL Technologies). He has ownership interest in Plurexa LLC. Hailey Desmond received research funding through the University of Michigan from Boehringer Ingelheim, Travere, Roche, Novartis, and Reata. Howard Trachtman is a consultant to Travere Therapeutics Inc., Walden, Boehringer Ingelheim (pending), Natera, Otsuka, and Aclipse. He is the board of the Kidney Health Initiative and the editorial board of Pediatric Nephrology and Kidney360.

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Author Contributions

Debbie Gipson conceptualized the symposium, participated in both original writing and revision, supervised activity planning and execution, and acquired funding. Chia-shi Wang, Eloise Salmon, Lawrence Holzman, and Howard Trachtman participated in both original writing and revision and supervised activity planning and execution. Rasheed Gbadegesin, Abhijit Naik, Alessia Fornoni, and Leonard Riella conducted original research presented at the symposium, participated in both original writing and revision, and supervised activity planning and execution. Simone Sanna-Cherchi Gbadegesin, Paul Hoover, Annette Jackson, Opeyemi Olabisi, Benjamin Freedman, Jennifer Harder, and Jon Klein conducted original research presented at the symposium and participated in both original writing and revision. Matthias Kretzler supervised activity planning and execution and participated in revision. Sandra Merscher, Kelley Kidwell, Moin Saleem, Paolo Cravedi, and Hailey Desmond participated in both original writing and revision. Jonathan Himmelfarb and Marina Vivarelli served as chair for a symposium session and participated in both original writing and revision. George Burke led a methodology panel at the symposium and participated in both original writing and revision. Michelle Rheault and Cathie Spino participated in a methodology panel at the symposium and revision.

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