NEPHROTIC SYNDROME IN PEDIATRIC PATIENTS

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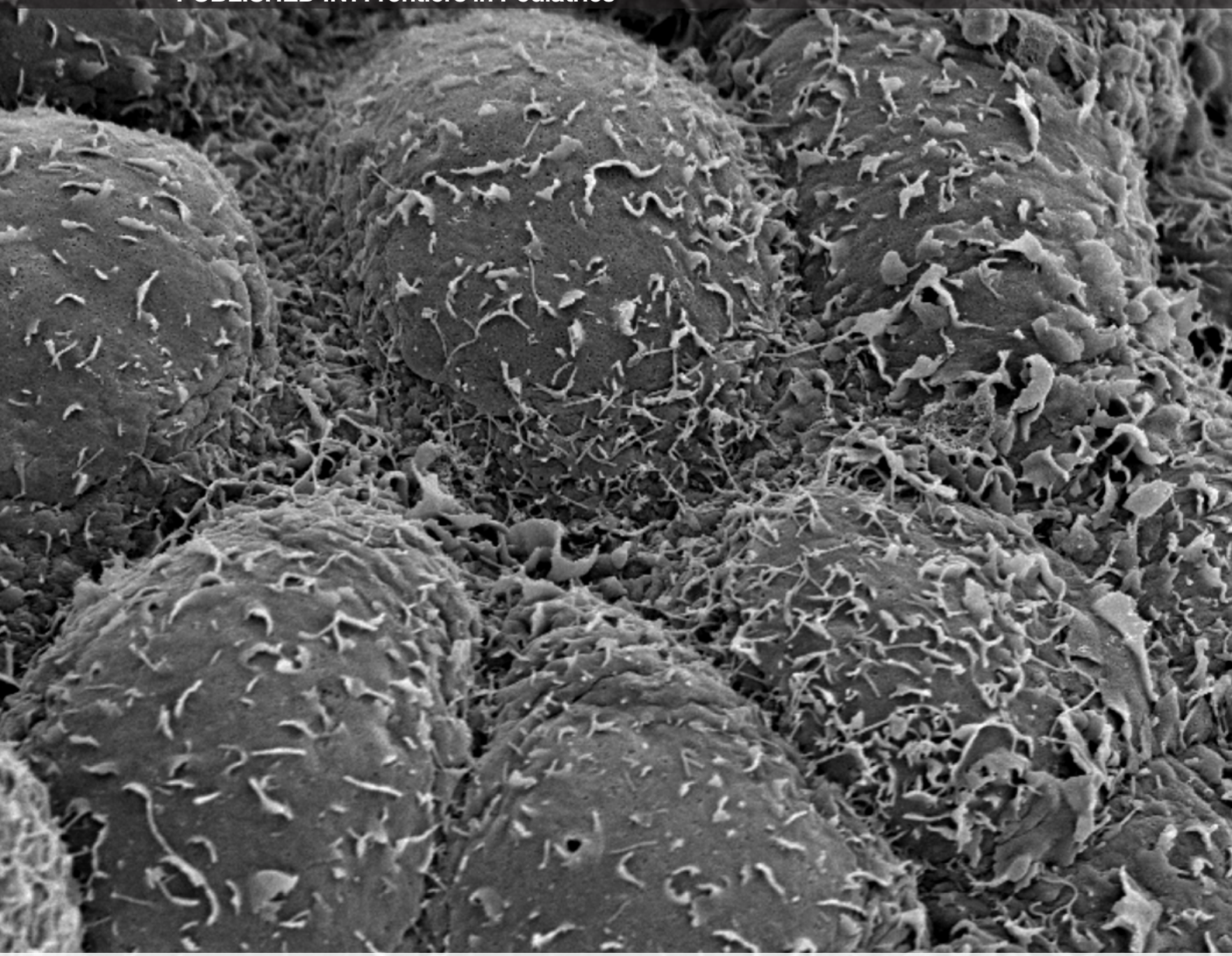

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NEPHROTIC SYNDROME IN PEDIATRIC PATIENTS

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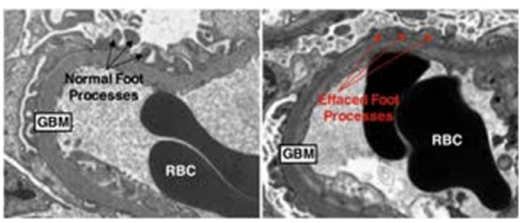

Electron micrograph images showing glomerular capillary loops of normal (left) and diffusely fused or effaced (right) podocyte foot processes resting on the glomerular basement membrane (GBM). Red blood cells (RBC) are seen inside the open capillary loops. Foot process effacement is an invariable morphological feature of glomerular disease presenting with nephrotic syndrome. 40,000x. Image: Dr. Sarangarajan Ranganathan, Children's Hospital of Pittsburgh of UPMC, University of Pittsburgh School of Medicine.

Cover image: Human podocytes were cultured in Dr. Swiatecka-Urban's laboratory at Children's Hospital of Pittsburgh of UPMC, University of Pittsburgh School of Medicine. Cover image was obtained by Dr. Donna Beer Stolz from the Imaging Center at the University of Pittsburgh.

Nephrotic syndrome (NS) - characterized by heavy glomerular protein loss (proteinuria), edema, hypoalbuminemia, and hyperlipidemia - has diverse causes and frequently leads to chronic kidney disease. This E-book encompasses articles on a variety of topics in NS, including a historical perspective on understanding and treatment of NS, followed by state-of-the-art reviews of the molecular pathomechanisms, clinical outcomes, as well as current and emerging treatment strategies for NS. We hope that this comprehensive review will help to reduce the gaps between the research and the day-to-day care of patients with NS and inspire new research efforts towards updating and expanding the treatment armamentarium for the future.

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EDITORIAL
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Editorial: Nephrotic Syndrome in Pediatric Patients

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Keywords: podocytopathy, APOL1, FSGS, calcineurin inhibitor, outcomes, edema, endocytic trafficking, obesity

Editorial on the Research Topic

Nephrotic Syndrome in Pediatric Patients

Nephrotic syndrome (NS) remains a clinical diagnosis, encompassing proteinuria, dyslipidemia, hypoalbuminemia, and gravity-dependent edema. We came a long way since the initial gross descriptions of NS as dropsy, lipoid nephrosis, and nil disease to the most recent classification of podocytopathies as causes on NS. Recent advances demonstrate that mutations of a number of genes regulating structure and/or function of glomerular filtration barrier are causally associated with histologic diagnosis of focal segmental glomerulosclerosis (FSGS), diffuse mesangial sclerosis, and clinical unresponsiveness to glucocorticosteroids in patients with NS. These findings explain why a subset of children who do not respond to treatment have genetic causes of NS. While the genetic causes represent only a minority of childhood NS, the exact molecular mechanisms underpinning the steroid-responsive NS remain largely unknown. Newer data explain that remission of NS after treatment with glucocorticosteroids, and an overall favorable prognosis are not consistent across all pediatric age groups and ethnic backgrounds.

In this special pediatric nephrology series, we have combined contributions from experts in childhood NS working in a variety of fields, including basic science, clinical medicine, and epidemiology to provide the reader with a comprehensive and the most up-to-date information on different aspects of NS. We were fortunate to enlist a prominent group of 31 authors to contribute a wide range of articles. In total, 10 papers have been included, with 3 original research articles, 6 reviews, and 1 historical perspective (Pal and Kaskel; Ranganathan; Swiatecka-Urban; Ellis; Hjorten et al.; Beins and Dell; Spino et al.; Chanchlani and Parekh; Woroniecki et al.; Canetta and Radhakrishnan).

We introduced the topic by historical perspective authored by Pal and Kaskel. The article walks readers through inspiring stories of tremendous efforts and international collaborations pawing the milestones in understanding of the pathophysiology and treatment of NS. Progress in the field is measured by a huge decrease in mortality related to NS from above 50% to less than 3% in the span of half of a century. As authors note, continued multicenter collaborations are critical to achieve new milestones and progress to personalized approach in the treatment of this rare childhood disease. Ranganathan reviewed the histopathology of congenital NS, minimal change disease, and its variants, and FSGS (Ranganathan). Swiatecka-Urban focused on the role of endocytic trafficking at the mature podocyte slit diaphragm and reviewed the critical role of signaling in maintaining the integrity of slit diaphragm that protects from to glomerular protein loss (proteinuria), manifesting as NS. Ellis described current pathophysiology of edema formation in NS (Ellis). The author reviewed "the under and overfill hypothesis" and current evidence for loss of plasmin and other serine proteases in the urine resulting in upregulation of the epithelial sodium channel (ENaC) causing Na+ and fluid retention (Ellis). Hjorten et al. reviewed the long-term outcomes of NS in

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childhood noting the importance of steroid responsiveness as a prognostic indicator. While steroid-resistant NS (SRNS) is associated with a high-risk (HR) of developing end-stage renal disease (ESRD), long-term complications of steroid-sensitive NS are under-recognized (Hjorten et al.). Beins and Dell in their retrospective chart review surmise that although majority of SRNS patients initially respond to calcineurin inhibitor therapy, a significant percentage still progresses to ESRD, despite achieving short-term remission. Spino et al. reviewed current therapeutic options for FSGS and discussed the problem of varied efficacy related to specific therapy, patient characteristics, cost, and common side effects, and noted that this variability of response to treatment is likely caused, at least in part, by the heterogeneity in the etiology of FSGS.

Chanchlani and Parekh further reviewed the emerging role of genetic factors determining the response to treatment within an ethnic group, noting that among African-Americans (AA), the risk variants in *APOL1* are associated with a more than 10-fold increase in risk of FSGS and HR carriers have a twofold greater risk of progression to ESRD. Woroniecki et al. presented original data indicating that AA children with HR *APOL1* genotype and FSGS have increased prevalence of obesity and left ventricular hypertrophy (LVH) despite a later age of FSGS onset, while adjusting for socioeconomic status. They noted that treatment of obesity may be an important component of chronic kidney disease and LVH management in this population (Woroniecki et al.). Finally to contrast with children, Canetta and Radhakrishnan reviewed the evidence-based approach to adult-onset NS.

Our overarching goal was to stimulate interest of academic and practicing physicians and scientists in this relatively rare childhood disease with diverse pathophysiology and outcomes. We hope that we will be seeing more studies and research in this field in the near future.

AUTHOR CONTRIBUTIONS

RW wrote and reviewed the editorial, AS-U and FK contributed to review process and editing.

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REVIEW
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History of Nephrotic Syndrome and Evolution of its Treatment

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The recognition, evaluation, and early treatment of nephrotic syndrome in infants and children originate from physicians dating back to Hippocrates. It took nearly another 1000 years before the condition was described for its massive edema requiring treatment with herbs and other remedies. A rich history of observations and interpretations followed over the course of centuries until the recognition of the combination of clinical findings of foamy urine and swelling of the body, and measurements of urinary protein and blood analyses showed the phenotypic characteristics of the syndrome that were eventually linked to the early anatomic descriptions from first kidney autopsies and then renal biopsy analyses. Coincident with these findings were a series of treatment modalities involving the use of natural compounds to a host of immunosuppressive agents that are applied today. With the advent of molecular and precision medicine, the field is poised to make major advances in our understanding and effective treatment of nephrotic syndrome and prevent its long-term sequelae.

Keywords: nephrotic syndrome, early recognition, evaluation, natural history, treatments

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HISTORY OF NEPHROTIC SYNDROME

Nephrotic syndrome, as we know it today, is a combination of proteinuria, hypoalbuminemia, hyperlipidemia, and edema, a concept that took some time to be developed. Interestingly, the effective treatments became available only recently in the mid 1900s, with the advent of steroids, antibiotics, diuretics, and other immunomodulators. Even today, there is a gap in our understanding of the etiology(s) of nephrotic syndrome of childhood, and better treatments are still required in the more resistant forms. Nephrotic syndrome as such is a combination of clinical and laboratories findings that is seen with a variety of pathologic lesions affecting the glomerulus.

Generalized edema, referred to as dropsy in the earlier literature, has been documented from the times of Hippocrates (1, 2), although the differentiation between various causes (heart vs. liver vs. nutritional disturbances vs. renal abnormalities) was not made. One observation by Hippocrates was: "when bubbles settle on the surface of the urine, it indicates a disease of the kidney and that the disease will be protracted" (1). Cornelus Roelans of Belgium described in 1484 a child with nephrotic syndrome and "whole body swelling." He went on to recommend the treatment as follows: "take the tops of elder plant and daneswort, cook in white wine and wrap the child in hot clothes by applying the poultice in whole or in part, and so cure him" (3).

During the sixteenth to eighteenth centuries, dropsy was considered a disease *per se*, the patient had dropsy, without differentiating between the causes (4). One of the first accurate descriptions of nephrotic syndrome in children was made by Theodore Zwinger of Basel in 1722 (5). He also noted decreased urine output and attributed this to "obstruction and compression of the tubules of the kidney," thus placing the seat of the disease in the kidney, since at that time, it was recognized that in pediatric practice heart and liver disease rarely manifest. His important observation had little influence on the scientific community, and his findings were not quoted by any author in

the following 200 years. Of note, these observations were made even before Morgagni was able to establish the idea that disease might arise from specific organs (6). Morgagni's disciple William Heberden went on to say: "Dropsy is very rarely an original distemper but generally a symptom of some other which is too often incurable" (7).

Later in the eighteenth century, dropsy was divided into those dependent on morbid viscera (liver and heart) and a general form, supposedly inflammatory. With humoralism, a prevalent concept in Europe at that time, bloodletting was a common practice for these so called "inflammatory diseases" and was a commonly prescribed. Around the same time, several observers [namely, Cotugno (8), Cruikshank (9), Wells (10), and Brande (9)] noted coagulability of urine in the patients. It was finally in 1827 that Richard Bright (1789–1858) was able to put together the triad of generalized edema, proteinuria, and kidney disease, as presenting features of this disease (11). John Bostock, a colleague of Bright, also noted that when protein in the urine was highest, it was lowest in the serum (11). Christison confirmed these findings in 1829 (12). Hence by 1830, nephrotic syndrome of profound albuminuria, hypoalbuminemia, and edema, resulting from diseased kidneys was established. Subsequently, on the postmortem analysis, these kidneys revealed a diseased state. Bright described three varieties of postmortem appearance of the kidneys, Chritison seven, Pierre Rayer six in 1840, and Carl Rokitansky no less than eight in 1846, including the "specknierre" or bacon kidney, recognized later as amyloidosis (9).

Over the next few years, the various lipid abnormalities in nephrotic syndrome became the forefront of discussion. Latescent or milky appearance of the serum was known to be present in nephrotic syndrome (noted with bloodletting sessions). Christison showed this to be from ether soluble fat (13). In 1846, Johnson not only described the fatty nature of the kidneys on gross appearance in many such patients but also fat globules and fat casts in the tubules (14). More attention was given to the cellular and tubular components of the kidneys as they were more obvious by the histological techniques available at that time.

Virchow introduced the term "parenchymatous nephritis" for a pathological picture with primary tubular involvement (15). With advances in microscopy, glomerular involvement became clearer along with that of the parenchyma. In acute nephritis, the presence of pale exsanguinated glomeruli was long known. In 1872, Klebs coined the term "glomerulonephritis" to describe the exudative glomerular changes seen under the microscope (16). In 1905, the term "nephrosis" was coined by Müller to describe all "non-inflammatory" diseases of the kidney as a substitute for parenchymatous nephritis, contrasting it with exudative and inflammatory disease, which would retain the name nephritis (17). This concept of "nephritis in contrast to nephrosis" was further popularized by F. Volhard, T. Fahr, and C. Munk.

TREATMENTS FOR NEPHROTIC SYNDROME

Introduction of the renal biopsy in the mainstream clinical practice of nephrology during the 1950s–1960s added a new dimension to the understanding of the histological findings in nephrotic syndrome. The histological classifications were based on the light microscopic appearance and included membranous glomerulonephritis, proliferative glomerulonephritis, a mixed membranous and proliferative glomerulonephritis, diabetic nephropathy with hyaline nodules, and focal segmental glomerular sclerosis; these are summarized in a case series from 1958 (18). The availability of electron microscopy and immunofluorescent localization of proteins was a welcomed coincidence. These new techniques transformed the ideas on morphology and pathogenesis, and made definite correlations between clinical presentation and pathological picture. The 1961, a Ciba Foundation symposium on the use of the renal biopsy was a landmark in these regards (19).

Antibiotics played an important role in treating the infections that arose from nephrotic syndrome, and the mortality rate reduced drastically from two-third to 35% (20). It is important to point out that prior to availability of steroids multiple desperate treatments were tried. A study from Boston noted the various treatments that were attempted from 1926 to 1948 for nephrotic syndrome. Dietary modification and low salt diet were probably the most effective treatments at that time. There were some weak mercurial diuretics with little if any action. Other drastic measures, such as the induction of measles and vaccinia, were instituted. Many of the children inoculated had some form of post-infection diuresis and decrease in proteinuria (11 out of 14), only to recur. Other supportive measure included blood transfusions, antibiotic therapy, treatment with thyroid extract, decapsulation of the kidney, testosterone, multiple vitamins, horse antiserum, and parathyroid hormone.

Steroid hormones were first isolated and identified in 1936 (21, 22). By 1946, cortisone was first prepared by partial synthesis from bile acids (23). On the other hand, adrenocorticotropic hormone (ACTH) was extracted from pig and sheep pituitary gland by isoelectric precipitation (22, 24) in the 1940s. One preparation of ACTH was commercially available as sterile powder reconstituted with isotonic saline for intramuscular injection. In the early 1950s, intramuscular injections of Cortisone (25) and ACTH (26) were first being used for treatment for nephrotic syndrome in children by Arneil and Wilson from Glasgow. These were relatively short courses, 5 days of daily 100–300 mg of intramuscular cortisone in the first study and 40–80 mg of an ACTH intramuscular injection for 12 days in the second study (25, 26). Later on, prednisone was first synthesized by oxidation of cortisone (27, 28). ACTH and cortisone were quickly replaced by prednisolone and prednisone as they could be administered orally without the need for daily injections (29, 30). With the advent of steroid therapy, mortality from nephrotic syndrome dramatically decreased to 3% (31).

An important series of studies, in the era after renal biopsy and steroids, to better understand the management of childhood nephrotic syndrome were heralded by the International Study of Kidney Disease in Children (ISKDC) established in 1965 with participants from North America, Europe, and Asia. A series of prospective, multicenter cooperative studies by the ISKDC established definitions, clinicopathological correlations, and recommendations for therapy that provided a basis for diagnosis

and management of pediatric nephrotic syndrome that persists today. Between January 1967 and June 1974, children with the nephrotic syndrome who were older than 12 weeks and younger than 16 years were enrolled into the clinical survey from the 24 participating clinics (32–34). Of the 521 entered, 76.6% had minimal-change nephrotic syndrome, 7.5% had membranoproliferative glomerulonephritis, and 6.9% had focal segmental glomerulosclerosis (35). All participants had biopsies prior to starting steroid therapy with prednisone. Initial treatment was 60 mg/24 h/m^2 (maximum dosage 80 mg/24 h) in divided doses for 4 weeks, followed by 40 mg/24 h/m^2 in divided doses, three consecutive days out of seven for 4 weeks. A seminal observation was made that patients with non-minimal lesions had a varied and limited response to steroids (36).

Azathioprine, a purine synthesis inhibitor, was also tested by this group in a randomized placebo-controlled trial (37). Patients that were considered to be early non-responders to steroids (not responding to the initial 8-week therapy) or frequently relapsing were randomized to receive every other day prednisone with azathioprine (test group) or placebo (control group). No significant decrease in proteinuria or number of relapses was noted in the test group vs. the control group (37).

The use of an alkylating agent in steroid-resistant nephrotic syndrome was first initiated with nitrogen mustard as early as 1958 (38). This was extended to other alkylating agents, cyclophosphamide and chlorambucil, in the 1960s and prompted the ISKDC to conduct a randomized control trial to define the role of cyclophosphamide in children with early non-responders and frequently relapsing nephrotic syndrome. Cyclophosphamide was shown to remit proteinuria in early non-responders and decrease the number of relapses of nephrotic syndrome (39). Thus, it proved to be an important agent to decrease steroid use and prevent steroid toxicity, although it caused gonadal failure in post-pubertal males (39). Chorambucil, although noted to be as effective as cyclophosphamide (40), had concerning side effects of acute leukemia and renal carcinoma (41).

The initial 8-week steroid regimens (4 weeks daily and 4 weeks every other day) were then compared with a longer steroid treatment duration in the late 1980s (42). Mounting evidence supported a longer duration of steroids, which helped decrease the number of future relapses and steroid dependence, and the initial 8-week steroid regimen has been recommended to be increased to at least 12 weeks (43, 44).

Levamisole, known for its anthelmintic, was first reported to be used in children with nephrotic syndrome in 1980 (45) and continues to be used as a steroid sparing agent in many countries. Cyclosporine A (CsA), a calcineurin inhibitor, was first isolated from the fungus *Tolypocladium inflatum* found in a soil sample obtained in 1969 from Norway, by Hans Peter Frey (46). It was initially used in humans for renal transplantation in 1978 and has since changed the face of transplantation (47). The first use for CsA was reported in 1986 among adults with difficult to treat nephrotic syndrome (48). By the late 1980s, there were reports of its successful use in children with steroid-resistant or steroid-dependent nephrotic syndrome that had not responded well to alkylating agents with the target levels of CsA between 50 and 200 ng/ml (49, 50).

Tacrolimus, another calcineurin inhibitor, was first extracted from *Streptomyces tsukubaenis* in 1987 by a Japanese group (51) and was initially used as a drug in human organ transplantation. Its use for nephrotic syndrome was first started in adults in the early 1990s (52–54) and then reported to be used children by the early 2000s (55–57). It was shown to be similar to CsA with regard to efficacy and renal toxicity but without the cosmetic side effects of hirsutism and gingival hypertrophy (58).

Mycophenolate mofetil (cellcept®) and, more recently, mycophenolate sodium (myfortic®) are prodrug forms of mycophenolic acid, another purine synthesis inhibitor, identified from *Penicillium* species. It was first reported to be used in pediatric nephrotic syndrome in 2000 (59) after being successfully applied in other glomerular diseases (60) and renal transplantation. It has been shown to be useful in steroid-dependent and frequently relapsing steroid-sensitive nephrotic syndrome, as a first-line agent, although there is evidence to indicate that it is probably less effective that calcineurin inhibitors (43, 61–63).

Rituximab, a monoclonal antibody against a B-lymphocyte antigen CD20, came into the armamentarium after case reports describing the incidental finding of a beneficial effect of rituximab on childhood nephrotic syndrome (64). Benz et al. first used rituximab to treat ITP, resistant to steroids and immunoglobulin therapy, in a 16-year-old boy who also suffered from steroid-dependent nephrotic syndrome. As well as effectively resolving the ITP, rituximab also improved the nephrotic syndrome, inducing a relapse-free period for over 12 months on low-dose CsA (65). In the other two cases, rituximab was used to treat post-transplant lymphoproliferative disease (PTLD) in boys who also had recurrence of FSGS in their transplants (66, 67), with beneficial effects on proteinuria. Its efficacy was recently demonstrated as a steroid-sparing agent in childhood onset frequently relapsing and steroid-dependent nephrotic syndrome by a multicenter, double-blind, randomized, and placebo-controlled trial (68) conducted by Iijima et al. Patients in the rituximab-treated group were relapse-free for prolonged period of time despite being weaned off other immunosuppressants such as mycophenolate or cyclosporine. There have been few case reports of its efficacy in refractory primary FSGS; however, controlled clinical trails are needed to define its exact role (69, 70). It has been proven to be effective in combination with plasmapheresis for post-transplant recurrence of FSGS (70, 71). Part of this effect may be mediated by its direct action on the podocyte (72).

FUTURE TREATMENTS AND CONCLUSION

Interestingly, ACTH is currently remerging as a potential treatment for nephrotic syndrome. In Europe, it is available as a synthetic depot formulation (Synacthen®) and in the US as a highly purified formulation (Acthar® gel) from porcine or bovine sources (73). There are reports on its efficacy in multiple causes of nephrotic syndrome, including idiopathic membranous nephropathy, FSGS, minimal-change disease, and mesangial glomerulonephritis, with a response rate varying from 29 to 100%

(73–79). Recently, it has been shown to be particularly beneficial in idiopathic membranous nephropathy. Although the exact mechanism of action for ACTH is not known, it is thought to act directly on the podocytes *via* melanocortin receptors on the podocytes. There is need for a multicenter randomized control trial to assess its use in idiopathic nephrotic syndrome.

With discovery of various gene defects associated with nephrotic syndrome, there is an expanding knowledge of various podocyte signaling pathways that play a role in the pathogenesis of nephrotic syndrome (80). A better understanding of these pathways is needed to develop future targeted therapy. The high risk variant genotype of APOL1 gene (codes of apolipoprotein 1, known for its trypanolytic properties), was found to confer increased risk to kidney disease by gene-wide association studies (GWAS) (81–83). However, its association and role in nephrotic syndrome and podocyte biology are yet to be defined (80). New drugs on the horizon, such as losmapimod (p38 MAPK inhibitor), sparsentan (endothelin receptor type 1A antagonist), and biologics, such as adalimumab (anti-TNF-α) and abatacept (anti-CD80), hold promise in the treatment of steroid-resistant nephrotic syndrome and prevention of renal progression (79).

In summary, the seminal contributions of the ISKDC identified the importance of global collaborations in order to conduct meaningful multicenter trials aimed at effectively treating children with nephrotic syndrome, preventing renal progression and facilitating the opportunity for each child to reach their full potential and quality of life. The availability of advanced genetic and molecular applications to personalized medicine offer unique opportunities along this promising journey.

AUTHOR CONTRIBUTIONS

The authors contributed to the manuscript. Dr. AP is the first author as he did most of the work in reviewing the literature and formatting the style and content of the submission. Dr. AP is the corresponding author.

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REVIEW
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Pathology of Podocytopathies Causing Nephrotic Syndrome in Children

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Nephrotic syndrome (NS) in children includes a diverse group of diseases that range from genetic diseases without any immunological defects to causes that are primarily due to immunological effects. Recent advances in molecular and genomic studies have resulted in a plethora of genetic defects that have been localized to the podocyte, the basic structure that is instrumental in normal filtration process. Although the disease can manifest from birth and into adulthood, the primary focus of this review would be to describe the novel genes and pathology of primary podocyte defects that cause NS in children. This review will restrict itself to the pathology of congenital NS, minimal change disease (MCD), and its variants and focal segmental glomerulosclerosis (FSGS). The two major types of congenital NS are Finnish type characterized by dilated sausage shaped tubules morphologically and diffuse mesangial sclerosis characterized by glomerulosclerosis. MCD has usually normal appearing biopsy features on light microscopy and needs electron microscopy for diagnosis, whereas FSGS in contrast has classic segmental sclerosing lesions identified in different portions of the glomeruli and tubular atrophy. This review summarizes the pathological characteristics of these conditions and also delves into the various genetic defects that have been described as the cause of these primary podocytopathies. Other secondary causes of NS in children, such as membranoproliferative and membranous glomerulonephritis, will not be covered in this review.

Keywords: congenital nephrotic syndrome, Finnish type, diffuse mesangial sclerosis, minimal change disease, focal segmental glomerulosclerosis, C1q nephropathy

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INTRODUCTION

The kidney is the main organ of filtration in the body, and the daily protein loss is only a small portion of the total protein ingested. Nephrotic syndrome (NS) is characterized by heavy proteinuria that exceeds 1.66 g/1.73 m^2/day in children, edema, hypoalbuminemia, and hyperlipidemia (1). Although the causes of NS are many and diverse, it is a frequent cause of renal disease in children with an annual incidence of about 2 to 7 children per 100,000 (1). The International Study of Kidney Disease in Children lists minimal change disease (MCD) as the commonest cause of primary NS in children affecting 77% of the children followed by focal segmental glomerulosclerosis (FSGS) at about 8% followed by membranoproliferative glomerulonephritis (MPGN) and membranous glomerulonephritis (2). The causes of NS also vary to some extent with the age of presentation with the congenital forms more directly associated with specific genetic defects and those appearing in later childhood related more often to secondary causes.

PATHOPHYSIOLOGY OF NS

The primary defect in NS is loss of proteins in the kidney. Although lack of tubular reabsorption could lead to proteinuria, NS range proteinuria usually implies permeability defects in the glomerular membrane that results in this excessive protein loss. This leads to albuminuria and hence the associated hypoalbuminemia and edema, the two main manifestations of NS. The hyperlipidemia is usually due to the increased lipoprotein synthesis induced by the hypoalbuminemia, and this may lead to increased platelet aggregation and thrombosis, one of the complications of NS. The loss of other proteins, minerals, and vitamins with the proteinuria may also predispose to malnutrition and infections. The most dramatic advances for understanding the pathophysiology of NS has occurred in the area of podocyte biology and the structure of the slit diaphragm (3–5). The glomerular filtration barrier consists of the fenestrated capillary endothelium, the extracellular basement membrane, and the intercalated podocyte foot processes. NS is associated with the biopsy finding of effacement of podocyte foot processes. Effacement is characterized by flattening of the podocyte, retraction of foot processes, and sometimes microvillous transformation. It has also been understood that MCNS and FSGS can be classified as podocytopathies, in which disruption of slit diaphragm and normal podocyte function can lead to proteinuria and glomerular disease. Details of the structure and mechanisms of podocyte injury and defects are beyond the scope of this article, and readers are directed to several reviews on podocytes (3–5).

CAUSES OF NEPHROTIC SYNDROME IN CHILDREN

The causes of NS show some variations depending on the age of the child with early onset NS usually representing primary genetic or idiopathic causes, such as congenital NS, due to any of the known genetic defects or MCD and less often FSGS (**Table 1**) (2, 6, 7). As the age increases, the ratio of MCD and FSGS may vary with secondary causes becoming more common in the second decade. Although NS may be the only presentation in children, often the condition is precipitated or can be seen as part of a nephritic syndrome. There is still some controversy on whether MCD and FSGS are related entities with MCD representing the early form of a disease and FSGS the late stage. This is especially so since there is often the finding of an early biopsy with MCD changes with refractory disease and a later biopsy showing features of FSGS. Also, while several genes have now been identified in MCD and FSGS, no distinct genetic defects have been found for these two diseases (7).

TABLE 1 | Causes of nephrotic syndrome in children.

Genetic
Congenital nephrotic syndrome of Finnish type
Diffuse mesangial sclerosis (DMS)
Isolated DMS
Part of Denys–Drash syndrome
Epidermolysis bullosa associated
Steroid-resistant nephrotic syndrome
Familial focal segmental glomerulosclerosis (FSGS)
Infectious causes
Congenital infections including syphilis, toxoplasmosis, and HIV
Cytomegalovirus
HIV-associated nephropathy
Idiopathic
Minimal change nephropathy
Focal segmental glomerulosclerosis
Diffuse mesangial hypercellularity
Membranous glomerulonephritis
Membranoproliferative GN (MPGN) (NS may predominate or with nephritic syndrome)
Others
Lupus nephropathy
IgA nephropathy
Drugs
Malignancies
Hemolytic uremic syndrome (HUS)

GENETICS OF NS

The podocyte, as mentioned before, is the critical structure and has a critical role in pathogenesis of NS (**Table 2**; **Figure 1**). Podocytes form foot processes and slit diaphragms as part of normal development. This leads to the expression of specific proteins, such as ZO-1, in the slit diaphragm and nephrin, podocin, and CD2AP (adhesion molecule CD2-associated protein) are expressed (5). Nephrin gene mutations cause Finnish-type nephropathy (7, 8). Other developmental genes involved in podocyte structure and function include Pax-2 and WT1. The WT1 gene mutation results in the Denys–Drash syndrome and Frasier syndrome. Podocin gene defects cause familial autosomal-recessive steroid-resistant NS in early childhood. The individual genetic defects are described further in the respective disease sections. It is critical to understand the structure of the podocyte and the basement membrane with all its collagen to understand the genetic defects and their potential effect. Discovery of novel mutations has led to further understanding of the biology and prognosis of these diseases.

ROLE OF RENAL BIOPSY IN DIAGNOSIS OF NS

In many instances, there may be no role for pathologist for diagnosis due to the ability to do genetic testing for individual mutations and due to the response to steroid therapy, but increasing incidence of resistance to steroid therapy has increased the role of the pathologist in determining if FSGS is the cause of steroid resistance. Other causes for biopsy are presence of coexisting nephritic syndrome and for determination of other causes of NS. All renal biopsies need triaging for adequacy to perform immunofluorescence and electron microscopy (EM). In view of the broad etiology and associated conditions, the pathologist very often has a critical role in guiding therapy in these patients. Routine histology warrants a PAS and silver stain for assessment of basement membranes and a Masson trichrome stain for assessment of fibrosis and glomerulosclerosis as well as vascular changes. The standard panel for IF includes IgG, IgA, IgM, C3, C4, C1q, and albumin with kappa and lambda where needed. EM is necessary for diagnosis in many instances.

TABLE 2 | Genetic causes of nephrotic syndrome.

Genes	Location (if known)	Protein	Significant clinical association
NPHS1	19q13.12	Nephrin	CNS Finnish type
NPHS2	1q25.2	Podocin	Steroid-resistant NS; rapidly progressive renal disease; FSGS
WT1 (NPHS4)	11p13	Wilms' tumor 1	Denys–Drash syndrome; nephrotic syndrome–FSGS; Frasier syndrome
SMARCAL1	2q35	SW1/SNF related	Schimke immunoosseous dysplasia
PLCE1 (NPHS3)	10q23.33	Phospholipase C_E1	DMS, FSGS
PTPRO	12p12.3	Protein tyrosine phosphatase, receptor-type O	Steroid-resistant NS
LAMB2	3p21.31	Laminin, beta-2	CNS with ocular abnormalities; Pierson syndrome
INF2 (FSGS 5)	14q32.33	Inverted formin 2	FSGS
COQ6	14q24.3	Coenzyme Q10 def, primary 6	Progressive NS in infancy with sensorineural deafness; FSGS, DMS
MYO1E (FSGS6)	15q21	Myosin 1E	FSGS (AR)
TRPC6 (FSGS2)	11q22.1	Transient receptor potential cation channel, subfamily C, member 6	FSGS (AD)
COQ2	4q21.23	Coenzyme Q10 deficiency-1	Steroid-resistant NS
LMX1B	9q33.3	LIM homeobox transcription factor 1, beta	Nail–patella syndrome
ADCK4 (NPHS9)	19q13.2	AARF domain-containing kinase 4	NS (AR)
PDSS2	6q21	Prenyl diphosphate synthese, subunit 2	NS
ACTN4 (FSGS1)	19q13.2	Alpha-actinin-4	FSGS
CD2AP (FSGS3)	6p12.3	CD2-associated protein	FSGS
MYH9	22q13.1	Non-muscle myosin IIA heavy chain	FSGS, collapsing glomerulopathy

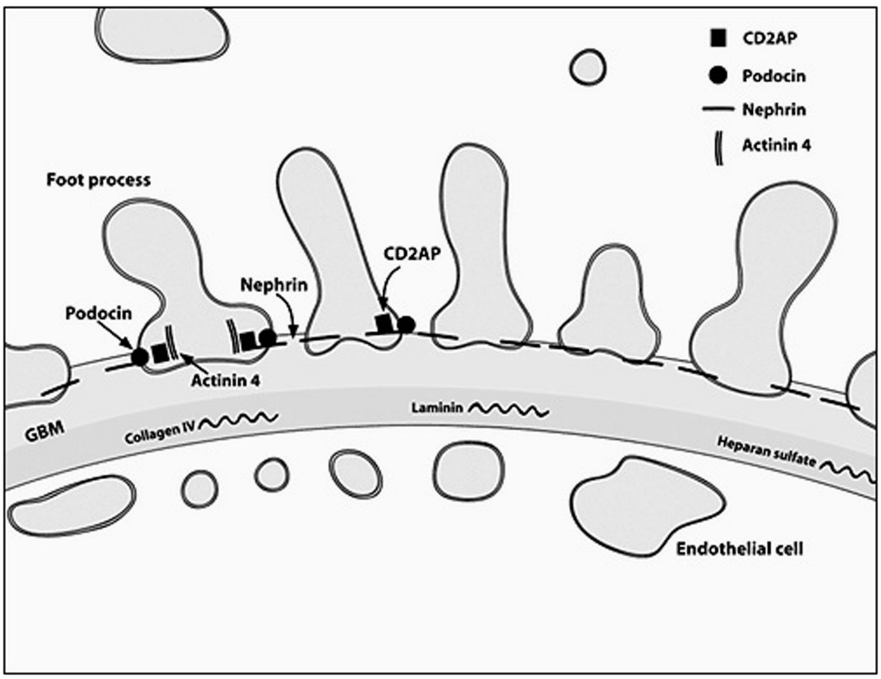

FIGURE 1 | A diagrammatic representation of the basement membrane foot processes with the location of the most common genes implicated in nephrotic syndrome.

PATHOLOGY OF COMMON CAUSES OF NS IN CHILDHOOD

Congenital Nephrotic Syndrome of Finnish Type

It is an autosomal-recessive disorder with an incidence of 1:8200 births in Finland (9). It is also seen in North American and European populations and is a cause of congenital NS with prenatal presentation in many cases. Diagnosis has been made *in utero* by amniocentesis that shows elevated alpha-fetoprotein as early as 16–18 weeks gestation (10). It results in small for gestational age neonate with prematurity, deformities due to contractures and an abnormally enlarged placenta. It results in heavy proteinuria in the neonatal period with selective proteinuria in

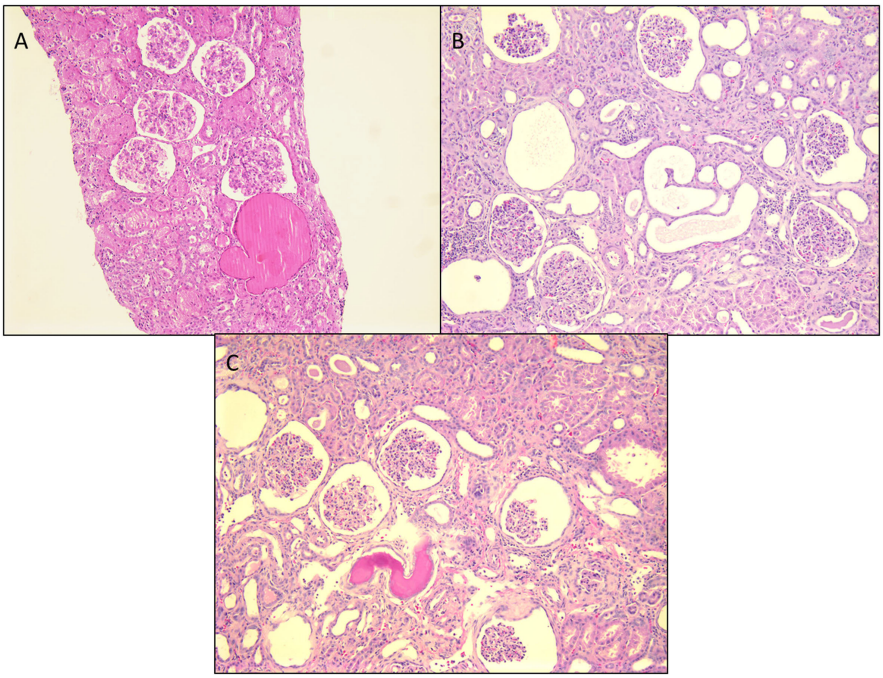

FIGURE 2 | **Histological panel for congenital nephrotic syndrome of Finnish type**. **(A)** A photomicrograph showing a cluster of normal appearing glomeruli with dilated proximal tubules with proteinaceous contents (H&E 100×). **(B)** Another area from this resected renal specimen showing the varying shapes of the dilated proximal tubules, a characteristic feature of congenital NS (H&E 100×). **(C)** Another image from the opposite kidney showing the same morphological features with no segmental sclerosis evident at this time (H&E 100×).

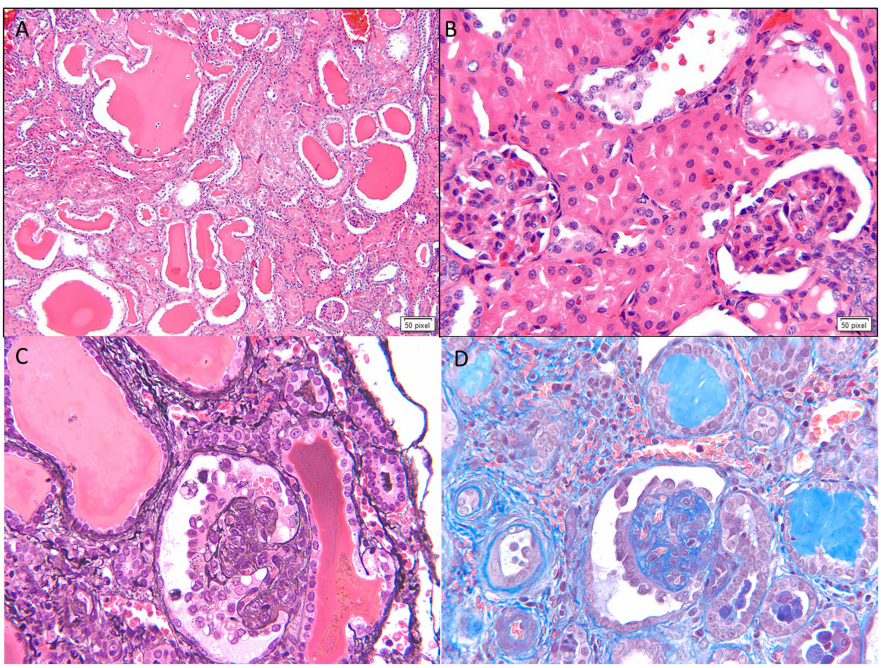

FIGURE 3 | **Histological images for diffuse mesangial sclerosis**. **(A)** An image from another nephrectomy specimen showing the numerous dilated tubules with small occasional glomeruli (H&E 40×). **(B)** Higher magnification of the glomeruli showing a solidified appearance on light microscopy (H&E 200×). **(C)** A Jones silver stain highlighting the solidified loops with accentuation of the epithelial cells on the surface. No capillary loops are identified (Jones methanamine silver 200×). **(D)** A trichrome stain showing the mesangial sclerosis characteristic of this disease. Note again the prominent epithelial cells. Progressive disease leads to glomerular obsolescence and interstitial fibrosis seen in this image (Trichrome 200×).

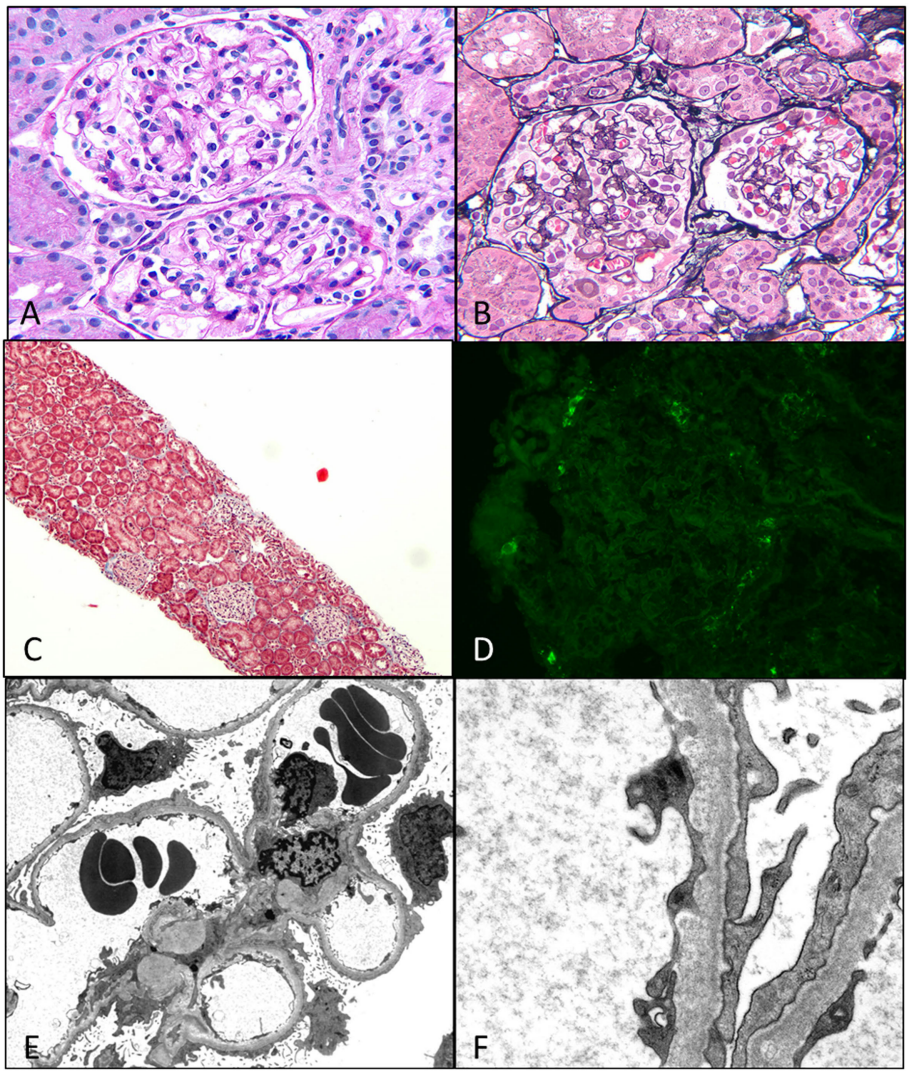

FIGURE 4 | **Minimal change disease**. **(A)** A photomicrograph showing normal appearing uniform sized glomeruli in the cortex (H&E 400×). **(B)** A silver stain showing the normal loops with accentuation of the epithelial cells (Jones 400×). **(C)** A low magnification image of a trichrome stain showing no glomerular or interstitial fibrosis (Trichrome 40×). **(D)** A negative immunofluorescence panel for immunoglobulins or C3 (Direct immunofluorescence 400×). **(E,F)** An electron micrograph image showing open capillary loops with effacement of foot processes better visualized in **(F)**, which shows the diffuse fusion of foot processes [6200× **(E)** and 46,000× **(F)**].

the early stages and more non-selective proteinuria in the later stages as the disease progresses. Most infants do not survive beyond 1 year of life, usually due to infections and sepsis related to loss of immunoglobulins. The gene for Finnish nephropathy (FN) is *NPHS1* mapped to the long arm of chromosome 19 (19q13.1) that codes for nephrin (7, 11, 12). Several nephrin mutations have now been identified. The typical mutations are a 2-bp deletion in exon 2 (Fin-major) or a nonsense mutation in exon 26 (Fin-minor). Renal biopsy shows normal glomeruli or some with mesangial hypercellularity, hyperlobulated capillary tufts, and some scarring. Microcystic dilatation of proximal and distal tubules is also seen, and there may be associated interstitial fibrosis and inflammation (**Figure 2**). Immunofluorescence is negative, whereas EM shows diffuse foot process effacement with or without villous transformation. Immunohistochemical stain for nephrin, now commercially available will show negative staining of podocyte. This stain helps in differentiating FN from other causes of congenital NS. Development of anti-nephrin antibodies and recurrence in allograft kidney has been reported (13).

Diffuse Mesangial Sclerosis

This is the second most common cause of congenital NS diagnosed on renal biopsy. Although the presentation is similar to that of FN, diffuse mesangial sclerosis (DMS) can present later up to 4 years of age. It presents with unremitting NS within the first 9 months of life usually. Infants develop hypertension with rapid onset of renal failure. The combination of DMS, Wilms tumor, and male pseudohermaphroditism constitutes Denys–Drash syndrome (14). Other associations include cataract, strabismus, nystagmus, myopia and aniridia, mental retardation, microcephaly,

TABLE 3 | Common forms of familial FSGS.

Gene (protein effected)	Inheritance	Typical age of onset	Distinguishing clinical features
NPHS1 (nephrin)	AR	Infancy	Congenital nephrotic syndrome (Finnish type); severe nephrosis leading to ESRD
NPHS2 (podocin)	AR	3 months to 5 years	10–20% of SRNS in children
WT1 (Wilms tumor 1)	AD	Child	Diffuse mesangial sclerosis/ FSGS ± Wilms tumor or urogenital lesions
PLCε1 (phospholipase Cε1)	AR	4 months to 12 years	Diffuse mesangial sclerosis/ FSGS
CD2AP (CD2-associated protein)	AR	<6 years	Rre, progresses to ESRD
INF2 (inverted formin 2)	AD	Teen/young adult	Mild nephrotic syndrome, but progressive CKD
ACTN4 (α-actinin 4)	AD	Any age	Mild nephrotic syndrome may develop progressive CKD
TRPC6	AD	Adult (age 20–35 years)	Nephrotic, progressive CKD
$tRNA^{Leu(UUR)}$ gene	Mitochondrial DNA	Adult	May be associated deafness, diabetes, muscle problems, retinopathy (maternal inheritance)

deafness, musculoskeletal abnormalities, and cleft palate. DMS is also part of the Galloway–Mowat syndrome, Pierson syndrome (LAMB2 mutations), and Frasier syndrome (7). It is caused by WT1 gene mutations in exon 8 or 9 (14). Frasier syndrome is usually caused by a splice variant mutation in exon 9 (15). The earliest light microscopic feature is increased mesangial matrix that is global and diffuse. There is a gradient of changes seen from the outer to the inner cortex with the most severe sclerosis being seen in the outer cortex, DMS in the mid zone and milder sclerosis in the inner zone (**Figure 3**). In some cases, prominent focal or global glomerulosclerosis may be seen. Podocytes may be prominent over the tufts. Severe tubulointerstitial damage with cysts and tubular ectasia may be seen. Immunofluorescence shows no immune deposits or non-specific mesangial IgM, C3, and C1q, whereas EM shows variable effacement of foot processes. Glomerular basement membrane lamellations and splitting similar to Alport's syndrome may be seen. In late stages, there is widening of the BM with thickened capillary loops and finally a sclerotic glomerulus.

Minimal Change Disease

Minimal change disease is more common in boys than girls with a ratio of 2:1. Almost 80% of cases occur in children <6 years of age (median age 2.5 years) (1, 16). It can be idiopathic, secondary, or familial. The most common form is sporadic MCD that is usually steroid sensitive. The genes mutated in MCD include NPHS1 and NPHS 2 (7). Mutations in the dysferlin gene have also been described. More recently, mutations in epithelial membrane protein 2 (EMP2) gene have been shown to cause childhood-onset NS. Crumbs homolog 2 (CRB2) defects have been associated with steroid-resistant NS. NPHS2 mutations have been associated with steroid resistance (17, 18).

Light microscopy usually shows normal glomeruli and tubules with the only change being prominence and swelling of visceral epithelial cells (2, 16). Patchy mild expansion of the mesangium may be seen in some cases (**Figure 4**). Histological finding of even a single glomerulus with segmental sclerosis, hyalinosis, or synechiae is enough to warrant a diagnosis of FSGS. Immunofluorescence is usually negative for any immune deposits, although there may be weak staining for IgM or C3 in a mesangial location in some cases. Albumin is positive within tubules reflecting the albuminuria. EM shows diffuse foot process effacement with prominent villous transformation, directly correlating with the severity of proteinuria and less prominent and patchy following treatment. Although this is the usual pattern, variants with increased mesangial hypercellularity are described. Immature glomeruli may be seen with this mesangial hypercellularity variant. In those patients presenting with acute renal failure, acute tubular injury and interstitial inflammation may be seen. Tubular atrophy is, however, not a feature of MCD. In those cases where IF shows deposits, the most likely ones are IgM (IgM nephropathy) or C1q (C1q nephropathy). In those where IgA is the dominant immunoglobulin, coexistent IgA nephropathy must be considered (19). The differential diagnosis for MCD is FSGS and as mentioned earlier, presence of any of the glomerular changes or foci of tubular atrophy in the absence of typical FSGS lesion, should raise the possibility of FSGS. Foot process effacement is not specific to MCD and can be seen in any cause of severe proteinuria. The debate over C1q being a specific subtype is still open as more recent data seems to suggest that though the initial response to therapy is poorer in this group, the overall long-term outcome is not different from cases of MCD.

The diffuse mesangial hypercellularity variant of MCD defined by presence of more than four mesangial cells per mesangial region affecting at least 80% of the glomeruli is a rarer cause of NS in children (16, 20). These patients can present with hematuria and hypertension unlike classic MCD children. IF shows mainly IgM and C3 in the mesangium, whereas EM shows paramesangial deposits besides the diffuse foot process effacement. This variant is usually associated with increased initial resistance to steroid therapy, but the overall remission rates are similar to MCD.

IgM nephropathy is another unusual variant of MCD characterized by at least 2+ staining intensity for IgM on IF (20). The distribution is mainly mesangial but could be membranous, but EM shows only a few small paramesangial deposits, suggesting that this deposition may be more of protein trapping rather than an immune complex disease. Initial steroid resistance is followed by remission (21).

Focal Segmental Glomerulosclerosis

Focal segmental glomerulosclerosis is defined by the presence of segmental sclerotic lesions within glomeruli causing NS. It is the cause of NS in about 10–20% of cases in children. There is an association with low birth weight infants, especially for secondary FSGS (22). The causes are manifold and include

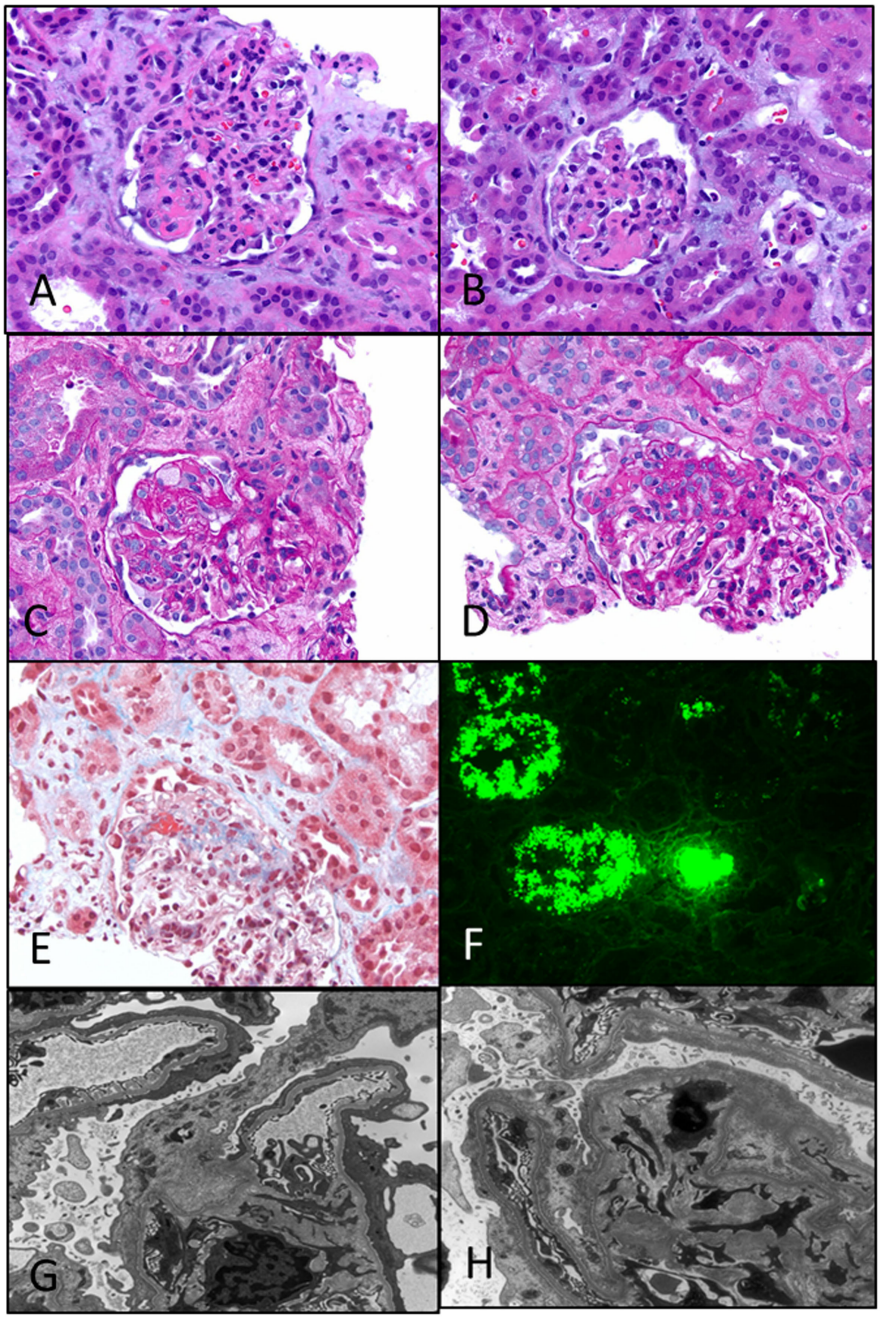

FIGURE 5 | **Primary focal segmental glomerulosclerosis**. **(A,B)** Photomicrographs showing two glomeruli with a segmental lesion characterized by obliteration of capillary loops with solidification and eosinophilia of the segment due to sclerosis (H&E 200×). **(C,D)** PAS stained sections showing a focal sclerosing lesion in a segment in **(C)** and in the perihilar region in **(D)** close to the vascular pole. Note again the preservation of capillary loops in other segments of the glomeruli (PAS 200×). **(E)** A trichrome stain showing the perihilar zone of sclerosis as evidenced by the blue staining of that segment. Note also some fibrin deposition in that area (red) (Trichrome 200×). **(F)** Immunofluorescence showing strong staining for albumin in the tubules, a characteristic feature of nephrotic syndrome in general. Not shown is the associated IgG deposition (DIF 100×). **(G,H)** Two electron photomicrographs showing collapsed loops in the **(H)** with effacement of foot processes in both images. Typically, the foot process effacement may be segmental and over the sclerosed segments (EM 3400×).

primary idiopathic FSGS, genetic, and familial cases included under secondary FSGS, and secondary FSGS due to systemic diseases, which are more common in adults but can be seen in older children too (**Table 3**).

Primary (Idiopathic) FSGS

This is the most common pattern of FSGS in children with a slight male preponderance and increased incidence in the African-American population (23). It presents with NS in 90%

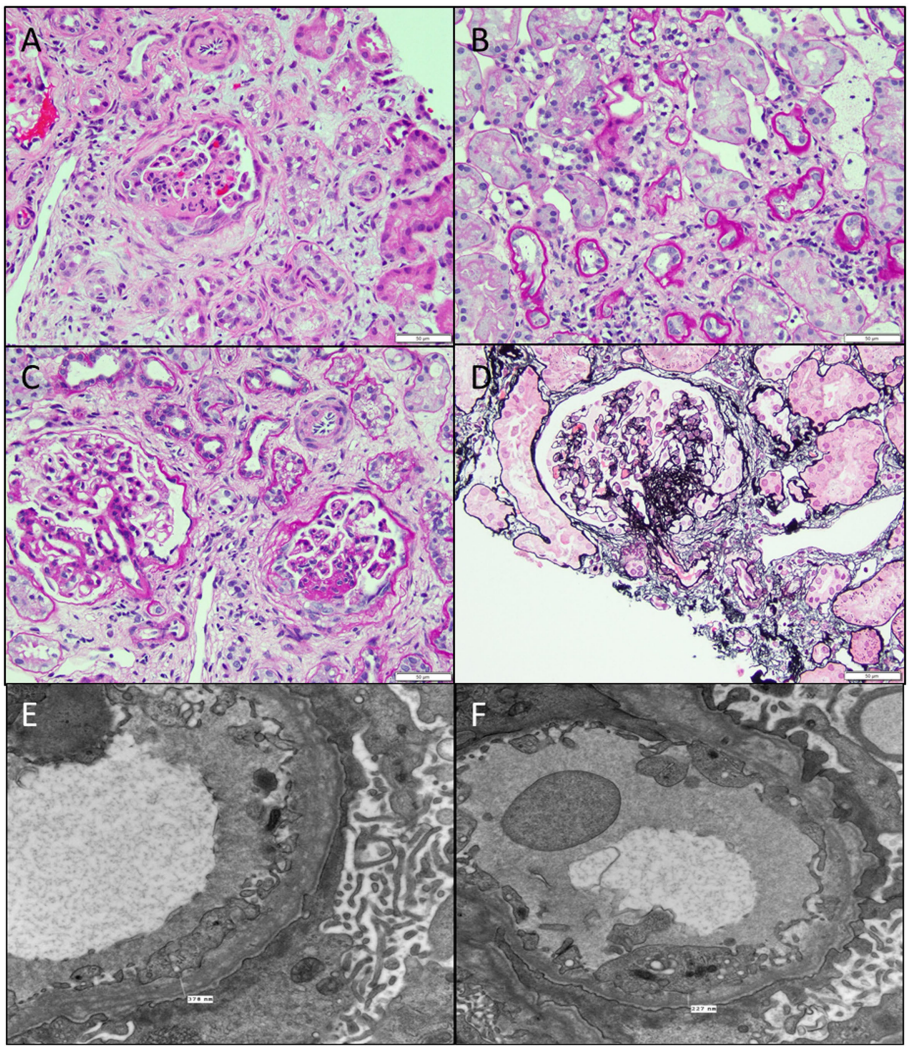

FIGURE 6 | **Secondary FSGS**. **(A)** An image showing a biopsy with interstitial fibrosis, tubular atrophy, and a segmentally sclerosed glomerulus (H&E 100×). **(B)** PAS stain showing the extensive tubular atrophy (PAS 100×). **(C)** PAS stain showing two glomeruli, one larger than the other with the smaller one showing an area of sclerosis associated with epithelial cell proliferation in that area (PAS 200×). **(D)** A silver stain showing the segmental sclerosis in the perihilar region (Jones 200×). **(E,F)** Electron micrographs showing two images of the basement membranes with variable diameters showing prominent splitting of lamina densa with a basket weave appearance characteristic of hereditary nephritis, proven by collagen studies. Note also the microvillous transformation of foot processes and effacement in this patient with nephrotic range proteinuria (11,500×).

of cases but may present with renal insufficiency, hypertension, and hematuria in a proportion of cases. The histological variants described for FSGS include the classic or FSGS NOS variant, cellular variant, tip lesion, FSGS with mesangial hypercellularity, perihilar FSGS, and collapsing FSGS. Light microscopy for FSGS NOS is characterized by discrete segmental solidification of the glomerular tuft with a predilection for the juxtaglomerular region in the early stages of the disease. They commonly affect the vascular pole or the periphery of the tuft. The capillaries are occluded by acellular matrix material that produces intramembranous hyalinosis lesions highlighted as bright pink with a PAS stain and red with a trichrome stain, with progressive wrinkling of the membranes, development of adhesions and synechiae with the Bowman's capsule with a prominence of visceral epithelial cells over this tuft (**Figure 5**). While focal lesions are the rule in early cases, with progression, glomerular obsolescence results. Tubular atrophy and interstitial fibrosis are evident in the vicinity of the sclerosed glomerulus involving the same nephron unit. Tubules contain protein resorption droplets and lipid droplets (24–26). IF usually shows focal and segmental granular IgM and C3, both within the membranes of the sclerosed segment and mesangium. Albumin will be noted in tubules. EM shows wrinkling and retraction of the glomerular basement membrane with accumulation of hyaline. There is complete effacement of foot processes overlying the sclerotic region with podocyte hypertrophy and focal microvillus transformation. Effacement may be mild to severe in the adjacent non-sclerotic glomeruli but affects more than half the surface membrane.

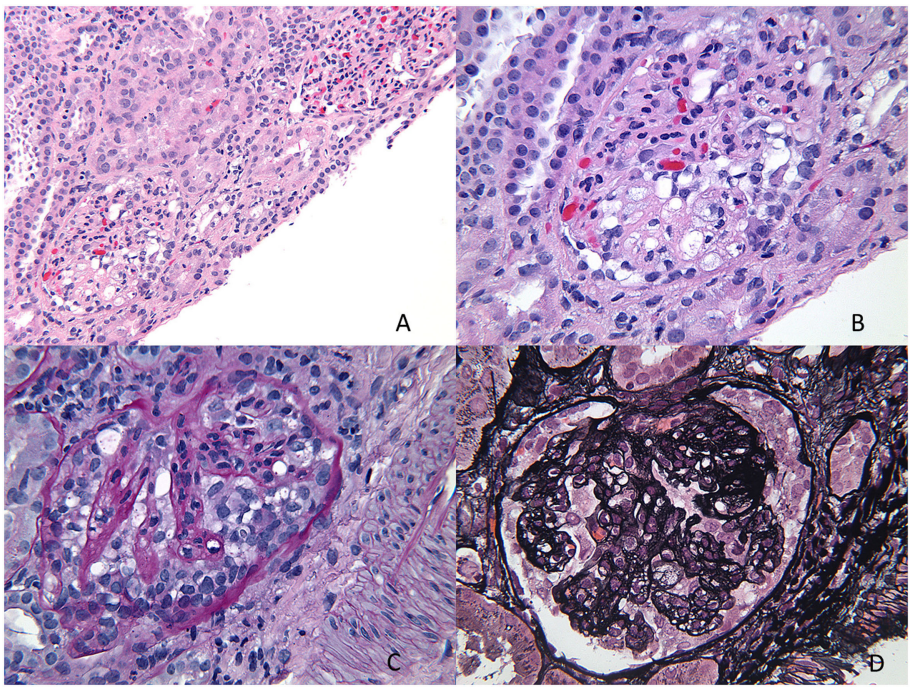

FIGURE 7 | **Collapsing glomerulopathy**. **(A,B)** H&E images showing a low power and higher magnification appearance of a glomerulus with prominent epithelial cells and closed capillary loops better visualized on special stains (H&E 100× and 200×). **(C)** A PAS stain showing the collapsed cords of basement membranes with exuberant epithelial cell proliferation that seems to "choke" the capillary loops (PAS 400×). **(D)** A silver stain showing another glomerulus with wrinkling of the basement membranes and prominent epithelial cell proliferation (Jones 400×).

Cellular FSGS

This variant is characterized on light microscopy by segmental hypercellularity resembling focal proliferative GN with areas of the tuft showing endocapillary proliferation with luminal obliteration of capillaries, increased mesangial cells, and prominent increase in inflammatory cells, including neutrophils, foam cells, and monocytes, with marked podocyte hyperplasia giving the appearance of crescents (pseudocrescents). IF shows some IgM and C3, whereas EM shows no significant deposits but prominent foot process effacement with intact basement membranes.

Tip Lesion

This is characterized by proliferation of swollen podocytes and endocapillary proliferation with progressive obsolescence located at the tip of the tuft at the origin of the proximal tubule. As the lesion progresses, it results in a segmental scar in that area. There is associated tubular atrophy and interstitial fibrosis develops over time and may not be a feature in the early classic tip lesions. IF again shows IgM and C3, whereas EM resembles the cellular variant.

Primary FSGS with Mesangial Hypercellularity

In this variant, the histology reveals classic segmental sclerosing lesions associated with podocyte hyperplasia but with the associated finding of mesangial hypercellularity in the non-sclerotic glomeruli. The IF shows a diffuse presence of IgM and C3 in the mesangium of non-sclerotic glomeruli and in the zone of sclerosis. The EM shows extensive foot process effacement with no electron dense deposits.

Familial FSGS

Familial FSGS probably accounts for about 20% of cases of FSGS and can manifest at any age (27). The incidence increases to two-thirds of cases discovered in the first year of life. Several newer mutations have now been described to be associated with FSGS pattern of injury including mutations in the *NPHS2* gene (podocin defect, chromosome 1q25-31), with an autosomal-recessive mode of inheritance, actinin 4 defect (*ACTN4*, autosomal dominant), TRPC6 (transient receptor potential cation channel, subfamily C, member 6), and CD2AP (7, 28, 29). Of these the podocin defect is the most common and can be detected by immunohistochemistry that shows loss of podocin staining. Other mutations and their known associations are shown in **Table 3**.

Secondary FSGS

Segmental sclerosis can occur commonly in other diseases, not only in adults but also in children. The most common pediatric diseases that can have a component of associated NS in addition to a nephritic picture with histological evidence of FSGS include IgA nephropathy, Hereditary nephritis (Alport's syndrome), and lupus nephritis The histological picture and immunofluorescence usually reveal the underlying disease, for example, mesangial hypercellularity and mesangial IgA in IgA nephropathy, full-house pattern in lupus nephritis, and thin basement membrane

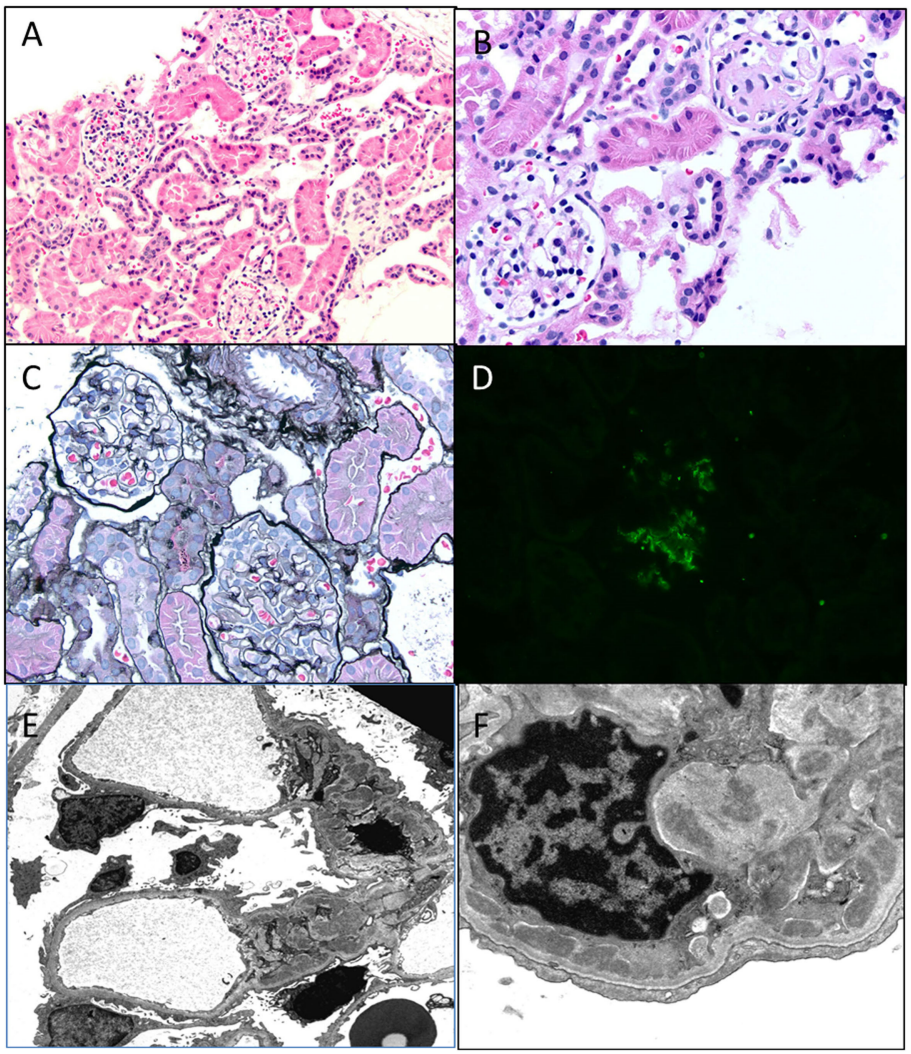

FIGURE 8 | **C1q nephropathy**. **(A,B)** Light microscopy showing a low and high magnification images of normal appearing glomeruli in this child with nephrotic syndrome. Note one glomerulus in **(B)** is undergoing normal obsolescence (H&E 100× and 200×). **(C)** A silver stain showing normal glomeruli with no tubular atrophy (Jones 100×). **(D)** Immunofluorescence for C1q shows a 2+ staining in a mesangial location within a glomerulus. All other stains were negative, including C3, IgG, and IgM (DIF 100×). **(E,F)** Electron micrographs showing open capillary loops with mesangial, paramesangial, and even some subendothelial deposits. Note diffuse foot process effacement (3400× and 7100×).

disease with basement membrane abnormalities on EM in Alport's syndrome (**Figure 6**).

OTHER HISTOLOGICAL VARIANTS OF FSGS

Collapsing Glomerulopathy

Collapsing variant of FSGS is characterized by progressive obliteration of capillary lumina due to wrinkling and shrinking of the basement membranes (23, 25, 30). It is relatively uncommon in children and was originally described in patients with HIV, but subsequently also seen in the absence of HIV. Other causes of collapsing glomerulopathy (CG) include infections, such as malaria and visceral leishmaniasis, drugs, such as interferon-alpha, bisphosphonates, and valproic acid, and autoimmune diseases, such as thrombotic microangiopathy and hematologic malignancies (31). They are as a group associated with progressive renal failure and have more severe disease at presentation. In children, rare forms associated with mitochondrial disorders have been reported. The COQ2 mutation is the most common and inherited as an autosomal-recessive condition, encoding for para-hydroxybenzoate-polyprenyl-transferase enzyme of the CoQ10 synthesis pathway (32). The MYH9 gene, an encoding non-muscle myosin IIA heavy chain gene, has been implicated in HIV associated CG and genetic FSGS in patients of African descent (33). A renal biopsy shows characteristic CG with glomerular epithelial cells showing abnormal mitochondria. It is important to recognize

TABLE 4 | Pathological features of podocytopathies in children.

Diagnosis	Light microscopy glomeruli	LM tubulointerstitial changes	Immunofluorescence	Electron microscopy
CNS Finnish type	Normal immature glomeruli early; later mesangial increase; sclerosis over time	Early cystic changes in tubules, prominent over time. No casts, epithelium attenuated	Initial none; later IgM and C3	Foot process effacement. No deposits. Non-specific
DMS	Increased mesangial matrix diffuse; segmental sclerosis possible; podocyte hyperplasia	Tubular ectasia, small cysts, casts, progress to atrophy and interstitial fibrosis	IgM and C3 in mesangium	Extensive foot process effacement; increased mesangial matrix, no deposits. BM thickened and lamellated
Minimal change Disease (MCD)	Normal appearance of glomeruli, some podocyte prominence	Normal	Negative	Diffuse effacement of foot processes; partial if treated
FSGS cellular	Segmental hypercellularity – endothelial cells, foam cells, and inflammatory cells, podocyte hyperplasia – pseudocrescents	Atrophy variable with blood in tubules; interstitial inflammation	C3, IgM	Foot process effacement restricted. Capillary lumina occluded
FSGS NOS	Segmental sclerosis, podocyte hyperplasia, random distribution of sclerotic segment; hyalinosis	Atrophy with interstitial inflammation and fibrosis variable	C3 and IgM	Foot process effacement in region of sclerosis. No deposits usually but small paramesangial
FSGS with mesangial hypercellularity	Segmental sclerosis, mesangial hypercellularity of non-sclerotic glomeruli	Tubular atrophy and interstitial fibrosis	IgM and C3 in sclerosis and diffusely in mesangium	Extensive podocyte effacement segmental. No deposits
Collapsing glomerulopathy	Diffuse basement membrane wrinkling with collapse of capillary BM with obliteration of lumina, diffuse podocyte hyperplasia	Extensive, atrophy, interstitial inflammation, edema, fibrosis, tubular regeneration	IgM, C3	Wrinkling of BM, podocyte prominence, foot process effacement. Rare paramesangial deposits, tubuloreticular inclusions only in HIV
C1q nephropathy	Normal or FSGS	Normal or tubular atrophy. Variable interstitial changes	C1q at least 2+; IgG, IgM, C3	Mesangial and paramesangial deposits; rare subendothelial, foot process effacement

this variant, as it responds to ubiquinone replacement therapy. Histologically, CG is characterized by an implosive collapse of the capillary loops with wrinkling and contraction of the basement membrane with compensatory hypertrophy and hyperplasia of the podocytes, which tend to fill the Bowman's space resembling crescents (**Figure 7**). There is no endothelial proliferation in this condition and no hyaline droplets or lipid is noted. Prominent tubulointerstitial changes are a feature. Typically, no significant deposits are noted in the membrane besides some IgM and C3. EM shows wrinkling with little to no thickening of the basement membranes with marked hypertrophy of the overlying podocytes. There is marked foot process effacement involving even the glomeruli without the collapsing lesions. In general, patients with CG progress rapidly to renal failure and show resistance to steroid therapy.

C1q Nephropathy

As mentioned before, the existence of C1q nephropathy as a distinct subgroup of NS has been debated. The original description of this entity was by Jennette and Hipp (34). Although originally described as a form of FSGS, the histological spectrum for C1q nephropathy (**Figure 8**) could range from MCD to mild mesangial proliferation to FSGS (35). The clinical manifestations include nephrotic range proteinuria with or without hematuria, with hypertension and renal insufficiency in a subset of patients (36, 37). Serology is usually negative, and serum C3 and C4 levels are normal. Most patients seem to present with steroid-resistant NS. The hallmark is presence of strong (2+ and above) staining for C1q on IF in a mesangial pattern (35, 38). IgG and/or IgM may also be present to some extent as can IgA. EM would show features of either MCD or FSGS with some mesangial electron dense deposits. The deposits may be predominantly seen in a "paramesangial" location. Foot process fusion will also be noted (34, 35). The important differential diagnosis to exclude for this condition is lupus nephritis and IgA nephropathy. These two conditions are distinguished by their distinct immunofluorescence pattern and in the case of lupus nephritis with finding of tubuloreticular inclusions on EM. A membranoproliferative pattern of histology would also not favor a diagnosis of C1q nephropathy.

The exact significance of this entity is still debated with its relationship to outcome being variable. They do not necessarily portend a worse outcome as previously believed with a median renal survival in C1q nephropathy/FSGS being about 81 months (35). They may be, however, associated with relapses, steroid-resistant, and steroid-dependent NS (21).

In conclusion, the pathology of podocytopathies have some unique and some overlapping features and are frequently associated with specific genetic mutations. A summary of the pathological findings have been summarized in **Table 4** for the reader.

AUTHOR CONTRIBUTIONS

The author has reviewed the literature and provided a succinct account of the genetics and pathology of nephrotic syndrome in children.

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Endocytic Trafficking at the Mature Podocyte Slit Diaphragm

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Endocytic trafficking couples cell signaling with the cytoskeletal dynamics by organizing a crosstalk between protein networks in different subcellular compartments. Proteins residing in the plasma membrane are internalized and transported as cargo in endocytic vesicles (i.e., endocytosis). Subsequently, cargo proteins can be delivered to lysosomes for degradation or recycled back to the plasma membrane. The slit diaphragm is a modified tight junction connecting foot processes of the glomerular epithelial cells, podocytes. Signaling at the slit diaphragm plays a critical role in the kidney while its dysfunction leads to glomerular protein loss (proteinuria), manifesting as nephrotic syndrome, a rare condition with an estimated incidence of 2–4 new cases per 100,000 each year. Relatively little is known about the role of endocytic trafficking in podocyte signaling and maintenance of the slit diaphragm integrity. This review will focus on the role of endocytic trafficking at the mature podocyte slit diaphragm.

Keywords: nephrotic syndrome, proteinuria, endocytic trafficking, endocytosis, podocytes, nephrin, podocin, glomerular slit diaphragm

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MECHANISMS OF ENDOCYTIC TRAFFICKING

All living cells process information by trafficking cargo from the plasma membrane in endocytic vesicles (i.e., endocytosis) and by returning much of the internalized membrane to the cell surface by a reciprocal process called recycling. The balance between endocytosis and recycling controls the plasma membrane composition and provides cells with information that has been resolved in time and space. Endocytic trafficking controls the supply of adaptor proteins and cargo, sorts the internalized cargo to specific intracellular compartments, and orchestrates the crosstalk in intracellular vesicles (1–3). As a result, cells turn over the equivalent of the entire plasma membrane one to five times per hour. Although endocytosis and recycling are ubiquitous, specific endocytic motifs and an assortment of protein adaptors guide cargo to diverse trafficking pathways. Defective endocytic trafficking has been associated with human disease, including congenital malformations, cancer, inflammation, and immunodeficiency (1). The mechanisms of endocytic entry of protein cargo, endocytic compartments, and mechanisms of cargo recycling were recently reviewed and will not be discussed here (4).

ENDOCYTIC TRAFFICKING AT THE MATURE PODOCYTE SLIT DIAPHRAGM

The kidney glomerulus is a filtering apparatus that allows passage of water and solute into the urinary space while retaining the vast majority of plasma proteins within the circulation. The functional

unit of glomerular filtration is formed by the epithelial cells podocytes, glomerular basement membrane (GBM), and fenestrated capillary endothelial cells (5). The mature podocyte consists of the cell body, primary and secondary processes with microtubule and intermediate filament-based cytoskeleton, and the actin-based foot processes. Resting on the GBM, foot processes form interdigitating extensions linked by the slit diaphragm, which provides the only cell–cell contact between mature podocytes (6). Slit diaphragm is critical for (i) providing selective permeability between the blood and urinary space, (ii) separating the apical and basolateral plasma membrane, and (iii) serving as a signaling platform (4). Structurally, a network of proteins residing at the slit diaphragm membranes connects with the actin cytoskeleton through a juxtaposed cytoplasmic protein scaffold (7). Strong evidence demonstrates that the slit diaphragm is a dynamic unit (8, 9), suggesting that endocytic trafficking of membrane proteins plays an integral role in regulating the dynamics (**Figure 1**). Recent data provided genetic, functional, and high-resolution ultra-structural evidence highlighting a model of dynamic and multilayered architecture of the slit diaphragm (10). According to the model, the mammalian intercellular slit has a specific arrangement of the integral membrane proteins nephrin and Neph1 at a ratio of 1:2.5. The homophilic interactions between extracellular domains of nephrin are formed more apically, while the homophilic interactions of Neph1 localize closer to the basement membrane at the intercellular slit (10). Difference in the length of extracellular domain of nephrin and Neph1 results in a different length of the intercellular strands formed by the homophilic interactions that subsequently determine the width of the slit (10). Nephrin is an immunoglobulin-type cell adhesion molecule critical to the slit diaphragm function (11, 12). Mutations in the *Nphs1* gene encoding nephrin protein lead to congenital nephrotic syndrome characterized by absence or profound impairment of the slit diaphragm and manifesting as severe proteinuria or nephrotic syndrome (13, 14). Although nephrin was shown to undergo endocytosis (15–19), relatively little is known how this process dynamically controls the slit diaphragm integrity. Data reviewed below demonstrate current understanding of the protein–protein interactions that regulate nephrin endocytic trafficking. The role of Neph1 is discussed in a separate section.

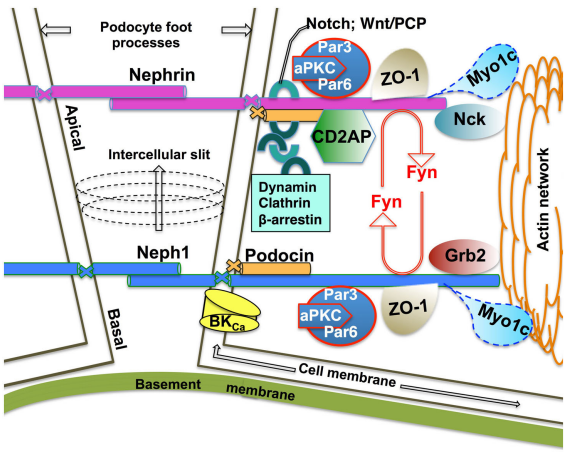

FIGURE 1 | Schematic of the functional Neph1–nephrin protein complex at the mature mammalian slit diaphragm. Homophilic interactions of the integral membrane proteins nephrin and Neph1 form the mature mammalian slit diaphragm with a ratio of 1:2.5, respectively (10). Nephrin homophilic interactions are located more apically while the Neph1 strands localize more basally at the intercellular slit (10). The number of Ig repeats in the extracellular domain (9 and 5 for nephrin and Neph1, respectively) determines, at least in part, the width of the intercellular slit (10). The PHB domain-containing protein podocin anchors nephrin and Neph1 at the cell membrane. The intracellular domain of nephrin and Neph1 interacts with the cortical actin cytoskeleton *via* the juxtaposed cytoplasmic protein networks of junctional scaffolding proteins, channels, adaptor proteins, including endocytic adaptors, protein kinases, and motor proteins. Fyn-mediated phosphorylation of nephrin and Neph1 is essential for the podocyte architecture and signaling. Growing evidence supports a model where endocytic trafficking of the integral membrane proteins dynamically controls podocyte architecture and signaling. While the endocytic itineraries of nephrin are better understood, very little is known about the Neph1 endocytic mechanisms. Published data demonstrate that the motor protein Myo1c recruits Neph1 to the podocyte membrane and controls the cell membrane turnover of Neph1 in an actin-dependent manner (20, 21).

Nephrin localization at the slit diaphragm is determined by podocin, a lipid raft-resident protein encoded by the *Nphs2* gene (22, 23). The prohibitin homology (PHB) domain—a lipid recognition motif present in podocin may target the podocin–nephrin complex to the lipid raft domains of slit diaphragm membranes (24, 25). Certainly, the *Nphs2* gene mutations that mislocalize podocin also mislocalize nephrin and lead to steroid resistant, hereditary, and sporadic nephrotic syndrome (22, 26). The podocin C-terminal $T^{339}VV$ motif regulates the cell surface localization as well as the lipid raft-independent podocin stability (27). While tagged podocin resides specifically in the late endosomal/lysosomal compartment (27), mislocalized endogenous podocin co-localizes with an early endosomal marker Rab5 in the puromycin nephrosis rat model (28). Despite the above data, a large gap in understanding of the podocin endocytic itineraries still exists. Flotillins are members of the PHB domain-containing protein family. PHB domains contain hairpin-forming, hydrophobic regions that help to insert a protein into the inner leaflet of plasma membrane. The PHB domain and a region upstream of the N-terminus, modified posttranslationally by palmitoylation and myristoylation, mediates binding of flotillins to the inner leaflet of plasma membranes (24). The predicted topology of mouse podocin has the intracellular N- and C-terminal regions, a transmembrane domain, and the PHB domain, which unlike the PHB of flotillins, is not involved in hairpin formation (22). Flotillins form homo- and heterophillic interactions mediated *via* the C-terminal region conserved only within the flotillin family while in podocin both the N- and C-terminal domains are predicted to mediate homooligomerization (22, 24). As of now, nothing is known about the molecular organization of the podocin hairpin domain or whether podocin undergoes palmitoylation and/or myristoylation. The PHB domain of flotillins plays an essential role in endocytic trafficking of several receptors, including those activated by the Src family kinase Fyn (29). Nephrin is phosphorylated by Fyn, and the phosphorylation augments nephrin interaction with podocin and facilitates nephrin endocytosis *via* a clathrin-independent pathway, activating nephrin signaling

(15, 30). By contrast, dephosphorylation of the nephrin-Y^{1193} increases nephrin endocytosis mediated by β-arrestin, decreases nephrin signaling, and impairs the slit diaphragm integrity (31). These data suggest that different endocytic pathways may play opposite roles in modulating nephrin function and that the endocytic itineraries of nephrin are regulated by the phosphorylation/dephosporylation cycle (see below).

PKC-α, stimulated by the high glucose concentration, induces nephrin endocytosis and leads to proteinuria while depletion of PKC-α stabilizes nephrin in diabetic nephropathy (32, 33). PKC-α phosphorylates two residues in the intracellular domain of nephrin, T^{1120} and T^{1125}, and facilitates nephrin interaction with β-arrestin in murine podocytes (16). These data suggest that PKC-α promotes β-arrestin-mediated nephrin endocytosis, most likely *via* the clathrin-dependent pathway. Following clathrin-dependent endocytosis, receptors are transported either to the fast or slow recycling route, depending on the stability of interaction between the receptor and β-arrestin. In addition to regulating endocytosis, β-arrestin controls post-endocytic itineraries of several receptors. Nothing is currently known about post-endocytic sorting of nephrin. Understanding the structural basis and kinetics of binding between nephrin and β-arrestin may help to elucidate the fate of internalized nephrin, whether it undergoes lysosomal degradation or recycling to the plasma membrane or both.

CD2AP may control nephrin endocytic trafficking by regulating actin assembly and vesicle sorting. The importance of CD2AP in podocytes is demonstrated by glomerulosclerosis and foot process effacement, leading to renal failure in mice lacking CD2AP (34). CD2AP localizes in the slit diaphragm cytoplasmic region juxtaposed to the lipid raft membranes and co-localizes with the actin-related protein 2 (ARP2) and ARP3 (Arp2/3) and cortactin. CD2AP regulates endosomal sorting and vesicle trafficking by regulating actin assembly (35). Moreover, CD2AP associates with the dynamic actin pool and co-localizes with the endocytic adaptors Rab5, Rab4, the clathrin-dependent endocytic adaptor assembly polypeptide-2 complex (AP-2), and participates in formation of multivesicular bodies (35, 36). CD2AP interacts with podocin and nephrin, anchoring the proteins to the actin cytoskeleton (37, 38). CD2AP and nephrin interact with the p85 regulatory subunit of the phosphoinositide 3-OH kinase (PI3K), leading to its recruitment to the plasma membrane (39). PI3K plays an essential role in endocytic trafficking, including regulation of membrane lipid composition, release of clathrin-coated vesicles, and the Rab5 recruitment (40). The interactions of PI3K with nephrin, CD2AP, and other slit diaphragm proteins suggest that PI3K plays an important role in endocytic trafficking at the slit diaphragm (41).

$CIN85/Ruk_L$ is a closely related homolog of CD2AP (42). In podocytes, CD2AP blocks $CIN85/Ruk_L$ expression by sumoylation while CD2AP depletion increases $CIN85/Ruk_L$ abundance and leads to $CIN85/Ruk_L$-mediated nephrin ubiquitination and endocytosis (43, 44). $CIN85/Ruk_L$ depletion has the opposite effect and preserves nephrin expression at the slit diaphragm membranes and reduces proteinuria in diabetic mice (45). Studies showed that unlike CD2AP, CIN85 does not contain an actin-binding domain (43, 46). Absence of CD2AP may facilitate nephrin interaction with CIN85, impair nephrin partitioning to lipid rafts, and induce nephrin endocytosis. However, another study has shown that CIN85 interacts directly with actin and together with CD2AP bundles actin filaments and modulates podocyte migration (47). Moreover, CIN85 clusters Src family kinases, providing a scaffold for Arp2/3-mediated actin assembly, and regulates cell polarization and motility. Expression of multiple CIN85 splice variants in different cell types may explain the reported difference of CIN85 function.

Notch activation alters foot process architecture in mature podocytes and induces proteinuria by stimulating the dynamin-dependent and lipid raft-independent nephrin endocytosis (17). The Wnt/planar cell polarity (PCP) pathway increases clathrin/β-arrestin-dependent nephrin endocytosis, and depletion of the PCP protein Vngl2 increases nephrin abundance at the cell surface and disturbs glomerular maturation (19).

The following protein networks have been shown to play a role in endocytic trafficking in podocytes, either directly or by regulating actin dynamics but their role in nephrin trafficking has not been demonstrated. α-Actinin-4 provides structural stability at the slit diaphragm by cross-linking and connecting actin filaments with nephrin and membrane associated guanylate kinase inverted (MAGI-1) (7, 48). The α-*actinin-4* gene mutations are associated with nephrotic syndrome called focal segmental glomerulosclerosis (49). Direct interactions between members of the multi-protein scaffold organized by α-actinin-4, MAGI-1, and the endocytic adaptor megalin indicate a role of the scaffold in endocytic trafficking at the slit diaphragm (50, 51). As a member of the cytoskeleton-associated recycling or transport (CART): an Hrs/actinin-4/BERP/myosin V, α-actinin-4 mediates constitutive recycling of transmembrane receptors *via* the rapid recycling route on actin filaments (52). The role of α-actinin-4 or other members of the CART complex in cargo recycling at the slit diaphragm remains unknown. Endocytic adaptors, synaptojamin 1, and endophilin 1–3 are critical for the foot process formation, while dynamin I and II play a role in the foot process maintenance (18).

STRUCTURAL BASIS FOR NEPHRIN ENDOCYTIC TRAFFICKING

A protein frequently utilizes different endocytic itineraries to diversify its function (1). Clathrin-dependent endocytosis is one of the most important internalization routes in eukaryotic cells (1). Endocytic adaptors recognize linear internalization signals located in the intracellular C-terminal tail domains of transmemembrane proteins and recruit these proteins as cargo to clathrin-coated pits, which are plasma membrane deformities coated with clathrin and clathrin-dependent endocytic adaptors. Subsequently, additional adaptors cleave off clathrin-coated pits and release them into the cell interior as clathrin-coated vesicles [reviewed in Ref (4)]. Clathrin-independent endocytosis includes a diverse group of internalization mechanisms sharing a requirement for free cholesterol, proteins, and lipids that reside in sphingolipid-rich lipid raft membranes (53).

Little is known about linear endocytic motifs in nephrin or other integral membrane proteins at the slit diaphragm. For example, the nephrin-Y^{1193} and subsequent amino acid residues D–E–V conform to a canonical, tyrosine-based endocytic signal of the YxxØ type, which is essential for clathrin-mediated endocytosis. Phosphorylation of the tyrosine residue inhibits the interaction of the YxxØ motif with the μ2 subunit of AP-2 and prevents endocytosis. By contrast, dephosphorylation of the tyrosine residue allows the YxxØ interaction with AP-2 and facilitates endocytosis. Thus, the phosphorylation state of the tyrosine residue serves as a regulatory switch controlling protein retention at the plasma membrane or its endocytosis *via* the clathrin-dependent pathway (54). Consistent with this model, Quack et al. demonstrated that β-arrestin mediates clathrin-dependent endocytosis of nephrin dephosphorylated at the conserved Y^{1193} residue (31). By contrast, phosphorylation of nephrin-Y^{1193} by Fyn augments nephrin interaction with podocin, prevents nephrin interaction with β-arrestin, attenuates β-arrestin-mediated nephrin endocytosis, and augments nephrin signaling (31). Moreover, the Fyn-mediated phosphorylation facilitates nephrin endocytosis *via* the clathrin-independent pathway (15, 30). These data demonstrate that the phosphorylation state of nephrin-Y^{1193} regulates nephrin signaling by directing nephrin to different endocytic pathways, either the clathrin-independent endocytosis to augment signaling or the clathrin-dependent endocytic to attenuate nephrin signaling. Although β-arrestin is known to interact predominantly with the clathrin-dependent adaptors, it was also found to modulate the clathrin-independent pathway by interacting with Arf6, and it may also utilize ubiquitination to inactivate receptor signaling (55, 56). At this time, the role of β-arrestin in nephrin ubuquitination or clathrin-independent internalization is unknown. Presence of several sequences conforming to the tyrosine-based endocytic motifs in nephrin cytoplasmic C-terminal domain suggests that regulation of nephrin endocytic itineraries is even more complex. The complexity is further increased by additional protein–protein interactions between nephrin, Fyn, and the actin cytoskeleton. Nephrin tyrosine phosphorylation is critical for recruitment of actin adaptors, such as p85/PI3K, Cas/Crk, and Nck, facilitating cytoskeletal dynamics in the podocyte foot processes (39, 41, 57–60). Nck facilitates Fyn-mediated nephrin phosphorylation, while Nck depletion leads to decreased nephrin tyrosine phosphorylation and foot process effacement (61, 62). Three conserved, Nck-binding Y–D–x–V motifs in the C-terminal nephrin tail, Y^{1176}DEV, Y^{1193}DEV, and Y^{1217}DQV mediate these effects (63). Replacing the Y^{1176}, Y^{1193}, and Y^{1217} residues with the non-phosphorylated tyrosine mimic phenylalanine leads to proteinuria associated with foot process effacement, irregular thickening of the GBM, and dilated capillary loops in a mouse model (63).

THE ESSENTIAL ROLE OF NEPH1 AT THE MAMMALIAN SLIT DIAPHRAGM

Neph1, a transmembrane protein partially homologous to nephrin is another key component of the mammalian slit diaphragm (10, 64). Nephrotic syndrome resulting from mutations in the *neph1* gene in humans as well as severe proteinuria, foot process effacement, and early postnatal death in *neph1* KO-mice demonstrate its importance for the glomerular function (64). Although the extracellular domain of Neph1 can interact with nephrin, recent data show that such heterophilic interactions are rare while the hemophilic Neph1 and nephrin interactions are critical for the mammalian slit diaphragm architecture (10, 65). Similar to nephrin, the Neph1 cytoplasmic domain engages directly with podocin, anchoring the integral membrane proteins at the cell membrane and the scaffolding protein ZO-1 that connects with the cortical actin cytoskeleton (66, 67) (**Figure 1**). Upon signaling engagement, Neph1 and nephrin form a phosphorylation-dependent unit transmitting outside-in signaling through a multi-protein scaffold of junctional proteins to the podocyte actin network (68, 69). While the Fyn-mediated tyrosine phosphorylation of the Neph1 intracellular domain leads to recruitment of protein adaptor Grb2 necessary for the Neph1-mediated actin polymerization, tyrosine phosphorylation of nephrin intracellular domain leads to recruitment of Nck, necessary for nephrin-mediated actin polymerization (58, 69–71). Activation of the Neph1–nephrin functional complex occurs during junction formation and during podocyte injury, both requiring intense signaling and actin cytoskeleton reorganization (69). One study demonstrated that inhibiting Neph1 signaling in podocyte culture preserves podocyte architecture and function in the puromycin aminoglycoside (PAN) injury model, while maintaining high Neph1 levels protects podocytes from injury in *in vivo* zebrafish PAN and adriamycin injury models (72). Another group demonstrated that renal ischemia induces a rapid loss of Neph1 interaction with ZO-1 in an *in vivo* rat ischemic model (73). Induction of injury by ATP depletion in podocyte culture resulted in rapid loss of Neph1 and ZO-1 interaction and redistribution of both proteins from the cell membrane to the cytoplasm while recovery from the injury resulted in increased Neph1 tyrosine phosphorylation, restoring Neph1 and ZO-1 membrane localization and interaction (73). The structural basis for the Fyn-mediated Neph1–ZO-1 interaction essential for the podocyte function has been recently elucidated. The ZO-1 PDZ1 domain interacts directly with the PDZ-binding region in the C-terminus of Neph1 where the T^{787}HV and L^{761}T residues are critical for protein–protein binding and its stability (67, 74). The functional Neph1–nephrin unit also engages with the Par3–Par6–atypical protein kinase C (aPKC) complex to maintain a three-dimensional foot process architecture and integrity of the slit diaphragm (75). Neph1 may influence localization of other proteins in the foot process. For example, Neph1 interaction with the large-conductance Ca^{2+}-activated (BK_{Ca}) channel affects membrane localization of the channel (76). Data reviewed above attest to a dynamic role that Neph1 plays in the slit diaphragm remodeling. Yet, little is known about Neph1 trafficking itineraries. Emerging data demonstrate that Neph1 is recruited to the podocyte membrane by a motor protein Myo1c that interacts with Neph1 intracellular region directly *via* cargo-binding, C-terminal domain in an actin-dependent manner (20, 21). Moreover, Myo1c may control the cell membrane turnover of Neph1 (21). Myo1c also binds nephrin directly. However, by contrast to the effect on Neph1, Myo1c reduces nephrin localization at the podocyte

membrane (20). It remain unknown how Myo1c exerts opposite effects on the membrane localization of Neph1 and nephrin.

SUMMARY

Recent data confirm that endocytic trafficking allows rapid regulation of the signaling strength and duration at the slit diaphragm. Identifying endocytic motifs of the integral membrane proteins and examining the structural basis of the protein–protein interactions that control endocytic trafficking may unravel novel mechanisms and enrich our understanding of the role of this essential biological function at the podocyte slit diaphragm, as well as teach about the mechanisms of nephrotic syndrome and measures to correct it.

AUTHOR CONTRIBUTIONS

AS-U reviewed the literature and wrote the manuscript.

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REVIEW
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Pathophysiology, Evaluation, and Management of Edema in Childhood Nephrotic Syndrome

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Generalized edema is a major presenting clinical feature of children with nephrotic syndrome (NS) exemplified by such primary conditions as minimal change disease (MCD). In these children with classical NS and marked proteinuria and hypoalbuminemia, the ensuing tendency to hypovolemia triggers compensatory physiological mechanisms, which enhance renal sodium (Na^+) and water retention; this is known as the "underfill hypothesis." Edema can also occur in secondary forms of NS and several other glomerulonephritides, in which the degree of proteinuria and hypoalbuminemia, are variable. In contrast to MCD, in these latter conditions, the predominant mechanism of edema formation is "primary" or "pathophysiological," Na^+ and water retention; this is known as the "overfill hypothesis." A major clinical challenge in children with these disorders is to distinguish the predominant mechanism of edema formation, identify other potential contributing factors, and prevent the deleterious effects of diuretic regimens in those with unsuspected reduced effective circulatory volume (i.e., underfill). This article reviews the Starling forces that become altered in NS so as to tip the balance of fluid movement in favor of edema formation. An understanding of these pathomechanisms then serves to formulate a more rational approach to prevention, evaluation, and management of such edema.

Keywords: edema, nephrotic syndrome, children/pediatrics, pathophysiology, management

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INTRODUCTION

Edema is an essential clinical feature of the diagnosis of nephrotic syndrome (NS) of various etiologies. It is defined as a palpable swelling resulting from an accumulation of fluid in the interstitial fluid compartment. Massive generalized edema (anasarca) is common, especially in children with primary minimal change disease (MCD) and serves as the main clinical justification for hospital admission for "diuretic management." In such children, the selective loss of large amounts of albumin in the urine leads to hypoalbuminemia and decreased plasma oncotic pressure favoring fluid sequestration in the interstitial fluid compartment, and secondarily triggers renal Na^+ and fluid retention so as to preserve intravascular volume and blood pressure, hence preventing an "underfill" state. In contrast to MCD, in NS associated with glomerulonephritis the magnitude of the proteinuria is variable and reduction in GFR is common. Hence, in disorders, such as focal and segmental glomerulosclerosis, acute post-streptococcal glomerulonephritis, Henoch Schoenlein nephritis, lupus nephritis, and several other glomerulonephritides, intravascular fluid volume is typically normal or expanded because of

inappropriately stimulated Na^+ and fluid retention which together with decreased GFR results in an "overfill" state. The clinical distinction of these two predominant physiological states is critical to the proper management of edema in children with NS. However, the role of hypoalbuminemia has been questioned, and it is apparent that other mechanisms such as water retention and vascular permeability factors, and activation of channels that promote Na^+ reabsorption, may also play an important role in edema formation in diverse causes of NS. Thus, multiple mechanisms acting simultaneously may explain the clinical variability in response to measures aimed at removing excessive fluid among individual children with NS. This has led to inconsistent or improper clinical evaluation and management of this relatively common disorder.

While the precise mechanism(s) of edema formation may be controversial and turn-around time of several laboratory tests used to confirm the underlying mechanism(s) may not be sufficiently rapid so as to influence acute management decisions, in most cases, there is adequate information to aid the clinical differentiation of the underlying basic mechanism of edema. This review discusses the pathophysiological alterations that favor edema formation in NS. This background then serves as the basis for developing selection criteria for hospital admission of children with NS who may benefit from edema fluid removal, establishing more standard guidelines for the clinical evaluation of nephrotic edema, and delineation of more uniform management guidelines aimed at providing effective management of the edema. Ultimately, the goal of therapy is to minimize the risk for hypovolemia, acute kidney injury (AKI), and other potentially serious complications ensuing from inappropriate diuretic regimens.

PATHOPHYSIOLOGY OF EDEMA FORMATION IN NS

Compared to adolescents and adults, neonates and younger children have a greater proportion of total body and interstitial (IS) fluid volume, which can double or triple because of edema related to NS (1). Nephrotic edema is a transudate with low protein concentration (<3 g/dL) and a few or no cells. In contrast to other forms of edema, it is pitting, tends to be generalized and is more prominent in dependent body areas in which capillary hydraulic pressure (P_{CAP}) is high (ankles and feet), or in tissues with low resistance or low interstitial hydraulic pressure (P_{IF}) (eyelids, gastrointestinal tract/abdomen, and scrotum).

It is apparent that while total body water remains constant during health, the fluid within each compartment is not static; there is continuous movement between compartments. The process of *diffusion* across cell membranes by far accounts for most fluid turnover (about 80,000 L/day in a 70 kg adult). By contrast, nephrotic edema represents net movement of water from the intravascular (IV) into the IS fluid compartment through the process of *filtration* across the capillary wall. Such filtration is dependent on the balance or net gradient, between the hydrostatic pressure and the oncotic pressure gradients across the capillary, as initially described by Starling in 1896 (2–4). Starling's equation or "Law" was later modified as it became apparent that the net filtration is also determined by **Lp**, **S**, and **s**, as shown below:

$$\text{Net filtration} = \text{LpS} \times (\Delta \text{ hydraulic pressure} - \Delta \text{ oncotic pressure})$$
$$= \mathbf{LpS} \times [(\text{Pcap} - \text{Pif}) - \mathbf{s}(\pi\text{cap} - \pi\text{if})]$$

Lp is the unit permeability (known as porosity or "hydraulic conductivity") of the capillary wall, which in the kidney refers to the glomerular capillary; **S** is the surface area available for fluid movement; **Pcap** and **Pif** are the capillary and interstitial fluid hydraulic pressures; **πcap** and **πif** are the capillary and interstitial fluid oncotic pressures; and **s** represents the reflection coefficient of proteins across capillary walls which ranges from 0 for vessels completely permeable to proteins and approaches a value of 1.0 in protein-free ultrafiltrate. The interstitial fluid oncotic pressure is derived primarily from filtered plasma proteins and to a lesser degree from proteoglycans in the interstitium.

Because of unique anatomy, physiological properties, and Starling forces of glomerular capillaries, the kidney plays a central role in total body fluid and electrolyte homeostasis as depicted elsewhere (5). Of note, the mean net ultrafiltration filtration pressure in glomerular capillaries is quite small and amounts to only 6–8 mmHg. Because normal glomerular capillaries are essentially impermeable to protein (so oncotic pressure in the filtrate is zero), this is a true ultrafiltrate with a high reflection coefficient (**s** is about 1.0). Several forces are altered in NS so as to favor net fluid filtration both in glomerular and in peripheral capillaries. These include reduction in **πcap**, increased **Lp**, elevation in **Pcap** depending on the underlying etiology of NS, increased **πif** and lymphatic vessel obstruction which may interfere with removal of filtered fluid thereby enhancing edema formation. Children with MCD tend to have more marked proteinuria and lower πcap than adults, while those with acute post-streptococcal glomerulonephritis tend to have Na^+ retention and rise in Pcap, favoring edema formation.

While mathematically and conceptually sound in providing a theoretical framework, direct measurement of Starling forces is complex and particularly challenging to assess in NS in which there are multiple alterations acting in concert to alter all components of Starling's equation to different degrees in any given individual. For example, **Lp** can differ based on histopathological cause of NS, type and extent of elaboration of permeability factors that affect pore size and protein permeability, secretion of neuroendocrine hormones involved in preserving **Pcap**, and the concentration of plasma and interstitial albumin and other proteins that influence **πcap** and **πif**. Thus, in clinical practice, a glimpse of the basic operative mechanisms is often inferred by clinical assessment of hypovolemic symptoms or signs and by measurement of a limited number of blood and urinary conformational biochemical studies. Consequently, from a practical standpoint, two major mechanisms of edema formation are prevalent. First, in relation to edema associated with MCD or other non-inflammatory conditions resulting in massive proteinuria, an increase in transcapillary oncotic pressure gradient is the single most important driver of edema formation. This is because albumin which contributes 6 mmHg of oncotic pressure per g/dL, or, 24 out of 26 mmHg of normal plasma oncotic pressure, is markedly reduced in such disorders. According to the *underfill hypothesis* reduction in plasma oncotic pressure promotes net

movement of fluid out of the intravascular compartment, leading to volume depletion, or underfilling (6–12). This then causes appropriate or compensatory physiological activation of several mechanisms ultimately resulting in secondary Na^+ and fluid retention aimed at restoring intravascular volume and blood pressure. This is the most prevalent mechanism in edematous children presenting with NS. This is in contrast to the second mechanism of nephrotic edema, or *overfill hypothesis*, exemplified by acute post-streptococcal glomerulonephritis and other inflammatory proteinuric disorders in which the pathophysiological process activates several mediators sub-serving primary Na^+ retention, resulting in intravascular volume expansion, increased capillary hydraulic pressure and edema. A schema depicting the two major mechanisms of nephrotic edema is presented in **Figure 1**.

CONTROVERSY OF MECHANISMS OF NEPHROTIC EDEMA

Primary vs. Secondary Na^+ Retention

While previous schemes of nephrotic edema depicted Na^+ retention through distinct secondary (in underfill) and primary (in overfill) mechanisms, there is now compelling evidence that primary Na^+ retention may occur by a mechanism common to all individuals with "nephrotic urine," independent of underlying histology or blood volume status [see below, Ref. (13)]. Several studies implicate loss of plasmin and other serine proteases in the urine in the up-regulation of the epithelial sodium channel (ENaC) causing Na^+ and fluid retention (see below). This is also highlighted in **Figure 1**.

Blood Volume Status

Based on clinical observations and theoretical grounds, the long held prevailing opinion has been that children and adults presenting with NS edema, are in a state of intravascular volume depletion. Although a few studies support the presence of hypovolemia in adults with MCD compared to other histological forms of NS (14), and in some but not all children with MCD (15, 16), other investigations indicate that most adults and some children with NS of any cause have an adequate or expanded effective blood volume and do not exhibit orthostatic hypotension or other hypovolemic symptoms and signs (16–18).

To gain greater insight into the pathophysiology of edema in children with NS, Vande Walle et al. (19) studied children in early relapse of NS and found that they grouped into three presentations: (a) incipient proteinuria without edema, Na^+ retention, and slightly elevated circulating aldosterone, increased renal plasma

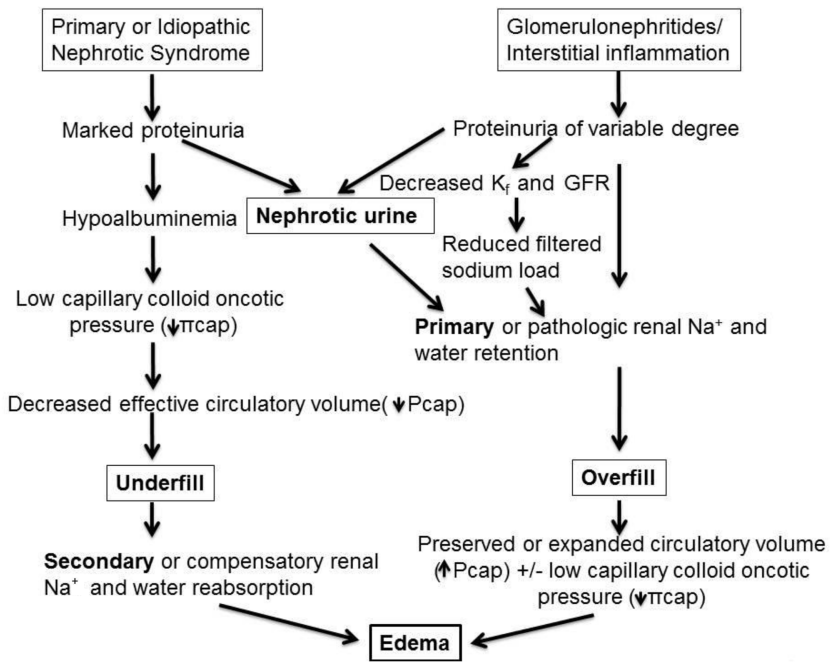

FIGURE 1 | Pathophysiology of edema formation in NS. In disorders with massive proteinuria and marked hypoalbuminemia but minimal or absent renal inflammatory infiltrate, as in most children with minimal-change disease (MCD), the reduction in capillary colloid oncotic pressure (πcap) favors net fluid exit from the vascular to the interstitial fluid compartment thereby reducing effective circulatory blood volume, denoted as "underfill." This then triggers secondary, or compensatory, Na^+ retention and hemodynamic alterations aimed at achieving blood pressure homeostasis. By contrast, various glomerulonephritides and inflammatory renal disorders, such as acute post-streptococcal glomerulonephritis, may be associated with variable degrees of proteinuria and with pathologic release of mediators, which promote primary renal Na^+ and water retention, as well as vasoconstrictive hormones that are released despite an intact or even expanded intravascular volume. These factors together with reduction in glomerular ultrafiltration coefficient (K_f, or LpS in the Starling equation), lead to reduced glomerular filtration rate (GFR) and combine to further limit Na^+ excretion, resulting in an "overfill" state and rise in capillary hydraulic pressure (Pcap). In turn, this causes net fluid accumulation in the interstitial fluid compartment. Note that nephrotic urine may be a common pathway for primary Na^+ retention in both undefill and overfill disorders (refer to text and **Tables 1**–**3** for the mediators sub-serving each of these main mechanisms of edema formation).

flow, and normal norepinephrine (NE) levels; (b) symptoms of hypovolemia, edema, Na^+ retention, elevated plasma renin, aldosterone and NE levels, low atrial natriuretic peptide, and reduced GFR; and (c) edema, no hypovolemia, no active Na^+ retention, normal plasma hormones, and normal GFR.

These data are very important because they indicate that there is both clinical and biochemical heterogeneity in children with NS. Distinguishing these fundamental mechanisms aids, the clinical prediction of safety and benefit of diuretic therapy: diuretics are well tolerated in children with primary renal Na^+ retention but, if underfilling is the predominant mechanism, diuretic use can exacerbate hypovolemia and tissue hypoperfusion.

Role of Hypoalbuminemia in Nephrotic Edema

Figure 1 highlights the role of hypoalbuminemia in children with nephrotic edema. However, with the exception of marked hypoalbuminemia (<2.0 g/dL) and plasma oncotic pressures below 8–10 mmHg, several clinical and experimental observations call into question the central role of hypoalbuminemia in the pathogenesis of nephrotic edema (8). For example, studies in analbuminemic rats show no significant change in transcapillary oncotic pressure gradient or tendency to edema compared to controls (20). Also, 81% of individuals with congenital analbuminemia (dysfunction of the albumin gene resulting in marked hypoalbuminemia) have little or no edema (21). It appears that in this disorder, $\boldsymbol{\pi}$**cap** is partially compensated over time by other plasma proteins and may explain the parallel decrease in $\boldsymbol{\pi}$**if**.

Other observations in experimental animals as well as in humans undergoing repeated plasmapheresis to intentionally produce modest reductions in serum albumin concentrations show that the transcapillary oncotic gradient is preserved; only with greater degree or more rapid achievement of hypoproteinemia does blood volume fall and edema develops (22, 23). This is because as plasma oncotic pressure falls, a parallel reduction in tissue oncotic pressure also occurs. Moreover, administration of albumin concentrates does not appreciably mobilize edema fluid in many nephrotic subjects, although Na^+ excretion can increase transiently in some (24–26). Finally, patients with MCD who are treated with corticosteroids often undergo a diuresis and natriuresis well before the serum albumin concentration starts to rise; this finding suggests that correction of the hypoalbuminemia might not be essential in steroid-induced natriuresis (27). However, several of the experimental conditions investigating the role of albumin *per se* in the pathomechanism of nephrotic edema may not be appropriate models for this condition in humans in whom vascular permeability factors and cytokines may, in fact, play an important role in preventing the counterbalance of oncotic pressure gradient evident in congenital analbuminemia or in experimental plasmapheresis.

Despite the controversy surrounding the "central role" of hypoalbuminemia in the development of edema in NS, nearly all children with NS and edema have hypoalbuminemia and in the author's opinion this controversy has little baring on the clinical evaluation and management of edema.

Factors That Protect Against Edema Formation in NS

Because normally there is a small net pressure gradient favoring net filtration across capillaries, it might be expected that only a minor change in these hemodynamic forces would lead to edema. However, experimental and clinical observations indicate that there must be at least a 15 mmHg increase in the net pressure gradient favoring filtration before edema can be detected. With lesser reduction of this gradient, edema is unlikely to occur because of three compensatory factors (27, 28). First, experimental evidence indicates that there is increased lymphatic flow which, by bulk flow, will remove albumin as well, and help remove some of the excess filtrate (29, 30). Second, fluid entry into the interstitium will eventually raise the interstitial hydraulic pressure, thereby oppose filtration and interstitial fluid accumulation (28). Third, fluid accumulation in the interstitium simultaneously reduces interstitial oncotic pressure in subcutaneous tissue which in humans it is normally 12–15 mmHg (7). Thus, a gradual fall in plasma oncotic pressure in NS is associated with a parallel decline in interstitial oncotic pressure and rise in interstitial hydraulic pressure (7, 27), which minimizes the change in the transcapillary pressure gradients favoring net fluid movement out of the vascular space and results in relative preservation of plasma volume.

As a result of these compensatory physiological responses, there is usually little change in the transcapillary oncotic pressure gradient in children with NS, and therefore little tendency to plasma volume depletion, unless the hypoalbuminemia is severe. Similarly, as long as children with NS are not overdiuresed, plasma volume is typically preserved during diuretic therapy for edema removal.

VOLUME REGULATORY HORMONES SUB-SERVING UNDERFILL AND OVERFILL MECHANISMS OF EDEMA FORMATION IN NS

In children who are hypovolemic or "underfilled," there is interplay of several volume regulatory hormones and nephron channels listed in **Table 1**, which tend to attenuate the effect of vascular underfilling mainly through modulation of renal Na^+ and fluid retention. Activation of the RAAS is an important mechanism in the majority of children presenting with nephrotic edema. It is notable that several of the hormones shown in **Table 1**, including AT II and anti-diuretic hormone (ADH), have dual Na^+ or water retaining, and vasoconstriction properties, and are therefore very suitable in preserving systemic blood pressure in children with NS and reduced circulatory volume. Similarly, NE release in response to stimulation of low pressure cardiopulmonary receptors mediates neuronal control of renal tubular Na^+ reabsorption (31). Edema may thus be viewed as a byproduct of these adaptive processes.

In addition, there may be several relatively unique pathological perturbations that promote edema formation in NS. Thus, children with acute post-streptococcal glomerulonephritis manifesting edema and hypertension often have increased

TABLE 1 | Contribution of volume and blood pressure regulatory hormones and channels which mediate or participate in renal Na^+ and edema formation in NS.

Hormones and channels	Function
RAAS activation	Direct stimulation of active Na^+ reabsorption in the PCT by AT II; aldosterone-mediated Na^+ retention
Non-osmotic ADH/vasopressin release	Water retention in CD, vasoconstriction
Norepinephrine (NE) release	α-Adrenergic stimulation of renal tubular Na^+ reabsorption; vasoconstriction
Atrial natriuretic peptide (ANP) release	Promotes natriuresis and diuresis in DCT and CD, but tubular epithelium is resistant to these effects in NS
Urodilatin activation	Promotes natriuresis and diuresis in DCT and CD, but tubular epithelium is resistant to these effects in NS
Phosphodiesterase activation	Promotes degradation of ANP and urodilatin
Sodium-hydrogen exchanger 3 (NHE3) activation	Mediates Na^+ reabsorption in PCT
Epithelial sodium channel (ENaC) activation by plasmin loss in nephrotic urine	Stimulates Na^+ reabsorption in the DCT and CD
Sodium potassium ATPase (Na^+/K^+ ATPase) activation	Provides energy for pumps involved in active Na^+ transport and facilitates peritubular uptake of Na^+ by exporting Na^+ out of cells in the anti-lumenal side of CCT

RAAS, renin angiotensin aldosterone system; AT II, angiotensin II; Na^+/K^+ ATPase, sodium potassium adenosine tri-phosphatase; DCT, distal convoluted tubule; CD, collecting duct; PCT, proximal convoluted tubule; CCT, cortical collecting tubule.

circulating ANP and reduced ADH, plasma renin activity, aldosterone, and NE concentrations. Experimental models of unilateral NS or glomerulonephritis show increased Na^+ reabsorption in the collecting tubules (26, 32), which is also the site of action of ANP and the related renal hormone urodilatin. Urodilatin is an ANP analog or isoform secreted by distal convoluted tubule (DCT) and collecting duct (CD) epithelium and exerts a paracrine function similar to ANP in promoting natriuresis and diuresis. In both experimental and human NS, a state of relative resistance to ANP and urodilatin has been observed (6, 33–35). This defect is due at least in part to urinary plasmin loss in individuals with NS which then stimulates phosphodiesterase activity, leading to more rapid degradation of the second messenger of ANP, cyclic guanosine monophosphate (cGMP), in the collecting tubules. Infusion of a phosphodiesterase inhibitor largely reverses this defect and restores the natriuretic response to volume expansion (33, 35).

Also, in many individuals with inflammatory forms of glomerulonephritis and NS there is activation of several tubular channels and transporters that promote Na^+ reabsorption and edema formation. These include:

1. Increased activity of the Na^+-hydrogen exchanger (NHE3) that mediates a large portion of proximal Na^+ reabsorption (36, 37). However, as demonstrated in experimental unilateral NS, overall Na^+ retention is influenced to a larger degree by mechanisms that promote Na^+ reabsorption in the distal tubule (26).
2. Inactive plasminogen present in nephrotic urine is converted to active plasmin by the action of urokinase-type plasminogen activator. Cleavage and activation of γ subunit of the ENaC is then stimulated by the serine proteinase plasmin found in nephrotic urine, which may contribute to Na^+ retention in the cortical collecting tubule (13, 38–41). This effect may be reversed by amiloride. This provides a common and potentially important mechanism by which filtered proteins cause primary Na^+ retention regardless of the underlying histological form of NS or the child's blood volume status. It also explains the clinical observation that spontaneous diuresis and edema improvement can occur in NS prior to a decrease in urinary protein excretion, despite ongoing hypoalbuminemia.
3. Increased activity of the Na-K-ATPase pump in the cortical collecting tubule but not in other nephron segments (42). This transporter provides the energy for active Na^+ transport by pumping reabsorbed Na^+ out of the cell and aiding it is uptake into the peritubular capillary. However, it is not clear if this represents a primary defect or if it is simply a secondary marker for increased Na^+ transport at this site.

Clinical Evaluation of the Predominant Mechanism of Edema formation in NS

Timely clinical assessment of hemodynamic aspects, including circulatory volume, is the key to determining management approaches to reduce edema in children with NS. This will help avoid exacerbation of the most common complication encountered in hospitalized children with NS and "underfill," AKI (43), or hypertensive and pulmonary complications in those with "overfill." **Tables 2** and **3** summarize the typical clinical and laboratory features, which serve to distinguish children with NS with underfill or overfill physiology.

It should be noted that because Na^+ retention occurs both in underfill and overfill states (**Figure 1**), it is not possible to use the FE_{Na+} to clinically differentiate primary from secondary Na^+ retention in NS. Also, measurement of several hormonal markers shown in **Tables 2** and **3** are not readily measured in hospital laboratories and may not be clinically useful in confirming the underlying operative mechanism or influence point-of-care decisions. However, an increase in RAAS and circulating aldosterone effect can be inferred on the basis of more readily measured values such as an increased transtubular potassium gradient (TTKG) index or, $UK^+/UK^+ + UNa^+$, which is observed in hypovolemic children and not when blood volume is preserved. Similarly, a TTKG index below 60% along with FE_{Na+} above 0.5%, and normal or suppressed plasma aldosterone concentrations, highly implicate a primary Na^+ retention mechanism leading to increased intravascular volume (i.e., overfill) (10, 19). This also suggests a primary role of aldosterone in the intrinsic activation of Na^+/K^+ ATPase in the cortical CD. Such children may benefit from diuretic use. By contrast, diuretic use in children with NS and secondary Na^+ retention triggered by hypovolemia or circulatory

TABLE 2 | Mechanism of edema formation in nephrotic syndrome: "underfilling."

Clinical characteristics
Neuromuscular weakness, pallor, cool extremities, tachycardia, and other signs and symptoms of orthostatic hypotension, abdominal pain secondary to gut edema, abdominal compartment syndrome, or thrombosis of vena cave or renal veins
Laboratory findings
Reduced urine volume
FE_{Na+} < 0.2%
$UK^+/UK^+ + Na^+$ > 60% (increased TTKG index)
Reduced urinary Na^+ and high potassium concentration
Very low serum albumin (≤2 g/dL)
Low serum creatinine level
GFR > 75 mL/min/1.73 m^2
Hemoconcentration
High circulating PRA, aldosterone, vasopressin, and norepinephrine
Low ANP concentration

FE_{Na+}, fractional excretion of sodium; UK^+ and UNa^+, urinary potassium and sodium concentrations; TTKG, transtubular potassium gradient; GFR, glomerular filtration rate; PRA, plasma renin activity; ANP, atrial natriuretic peptide.

TABLE 3 | Mechanism of edema formation in nephrotic syndrome: "overfilling."

Clinical findings
Normal or elevated BP without tachycardia or orthostatic symptoms, and no signs to indicate distal extremity hypoperfusion
Laboratory findings
FE_{Na+} > 0.5% while on no salt restricted diet
$UK^+/UK^+ + UNa^+$ < 60% (decreased TTKG index)
Hematuria and cellular casts
Serum albumin >2 g/dL
Elevated serum creatinine and BUN
GFR < 50 mL/min/1.73 m^2
Decreased vasopressin
Low circulating PRA and norepinephrine
Low or normal plasma aldosterone
High ANP

FE_{Na+}, fractional excretion of sodium; UK^+ and UNa^+, urinary potassium and sodium concentrations; TTKG, transtubular potassium gradient; GFR, glomerular filtration rate; PRA, plasma renin activity; ANP, atrial natriuretic peptide.

insufficiency may have serious deleterious consequences (see below).

MANAGEMENT OF NEPHROTIC EDEMA

Before reviewing the management of edema in children with NS it is worth noting the change in the incidence of known clinical complications of NS that may relate to edema or its improper medical management. In a recent study involving hospital discharges of 4,701 children admitted with NS, Rheault et al. found that the frequency of infectious and thromboembolic complications has not changed much over the past 10 years; however, the incidence of AKI had increased from 3.3 to 8.5% (158%) over the period of 2001 and 2009 (43). The increasing use of nephrotoxic medications such as calcineurin inhibitors and angiotensin converting enzyme/angiotensin receptor blockers to co-manage steroid dependent and steroid resistant NS may be partly responsible for this trend. However, aggressive diuresis in children not recognized as having intravascular volume depletion (underfilling) may enable progression from incipient AKI to established AKI. Furthermore, prevention of AKI is of great importance because it may be a precursor to future development of chronic renal injury and hypertension. Inappropriate diuresis may also promote a thrombotic tendency in this disorder (44–47). Consequently, children with "underfill" physiology may benefit first by circulatory volume expansion using salt-poor albumin infusions, and delayed start of diuretics until after restoration of tissue perfusion is achieved.

TABLE 4 | General aspects of management of edema in children with NS.

Avoid NSAID use
Avoid placement of deep lines to prevent thromboembolic events
Reduce dietary salt
No fluid restriction unless brisk diuresis is achieved
Insure adequate nutrition
Monitor urine output, renal function, electrolytes, serum albumin, body weight, and vital signs
Elevate extremities or use compression stockings when ambulating; water immersion is helpful but impractical
Avoid ACE inhibitors as remission can occur in many children with corticosteroid monotherapy

NON-PHARMACOLOGICAL MEASURES

Apart from managing the underlying condition leading to NS according to established guidelines (48–51), **Table 4** summarizes other basic aspects of care. Because Na^+ and fluid retention is a fundamental feature of all causes of NS and because treatment regimens that include corticosteroids tend to enhance this effect, all children presenting with edema are counseled on dietary Na^+ restriction (35 mg Na^+/kg/day, or approximately1.5 mEq/kg/day) and are monitored for clinical signs of hypovolemia (52). Fluid restriction is usually self-limited in children who adhere well to Na^+ restriction, and it is not recommended in children managed in the outpatient setting.

Attention to nutrition is very important particularly in conditions associated with massive proteinuria, such as Finnish type NS. Given the T1/2 of albumin of 21 days, when provided with adequate calories and amino acids, the liver can produce 200 mg albumin/kg/day to replace albumin catabolism or urinary loss. This normal synthetic function can double when the oncotic pressure in hepatic sinusoids falls as in the setting of NS. However, many children with NS are "picky eaters" and may have intestinal edema and abdominal pain, resulting in poor appetite as well as protein-losing enteropathy that may further compromise nutrition. In addition, the degree of proteinuria may be underestimated because of the large influence of the proximal tubule in albumin catabolism in NS. Thus, recycled amino acids are not used to replace albumin exclusively. Provision of supplemental calories, egg white protein, and nutritional supplements, such as Boost or Pediassure, may be helpful if clinically indicated.

Once a brisk diuresis is achieved, fluid intake may be limited to 2/3 of maintenance, or 1/2 or less of urine output, so as to produce the intended negative Na^+ and fluid balance. Close monitoring of vital signs, fluid input and output, and of electrolytes is required in order to assure safety. Diuretic therapy should be temporarily discontinued if there is an unexplained decrease in urine output, elevation in serum creatinine or clinical manifestations of hypovolemia (e.g., weakness, orthostatic hypotension, and/or cool extremities) (52, 53).

TABLE 5 | Albumin infusion in the management of edema in NS.

Dosing of salt-poor albumin (25% SPA, or, 25 g/100 mL)
0.5 g/kg infused over 1-h, 2–3 times daily. Slower infusion rates may enhance equilibration of albumin between the intravascular and interstitial fluid compartments, thereby undermining fluid mobilization and removal. Larger dosages may be more effective but may cause acute volume expansion and pulmonary congestion
Indications
Tense ascites with abdominal compartment syndrome limiting diaphragmatic excursion, lymphatic flow, and venous return
Severe pleural effusions compromising breathing
Oliguria with incipient acute kidney injury (AKI)
Marked eyelid edema compromising vision
Severe scrotal or labial edema, risking skin breakdown
Precautions
Expensive
Low supply
Obtained from multiple blood donors risking viral transmission, tissue allosensitization, etc.
Pulmonary edema

PHARMACOLOGICAL MANAGEMENT OF NEPHROTIC EDEMA

Albumin Infusion

In hospitalized children with nephrotic edema, excessive fluid can usually be removed, relatively safely, without exacerbating volume depletion (refer to **Table 5**). This is often accomplished through the combined administration of salt-poor, or, 25% albumin (SPA) to facilitate reabsorption of IS fluid, thereby supporting plasma volume and diuretics to enhance fluid removal. The more available 5% albumin solution can increase blood volume but does not raise oncotic pressure, while it delivers fivefold higher Na^+ for each gram of albumin infused. By expanding plasma volume, albumin infusion suppresses vasopressin release induced by hypovolemia, thereby increasing water diuresis and improvement in hyponatremia. Albumin infusion is associated with more profound diuresis, at least in a subpopulation of pediatric patients with NS (54–57), particularly those with reduced effective arterial blood volume. For example, in one series of children with nephrotic edema and fractional excretion of Na^+ (FE_{Na+}) of <0.2% suggesting reduced effective arterial blood volume were treated with diuretics plus albumin infusion (52). Their diuresis rivaled that obtained by diuretic monotherapy in children with $FE_{Na+} > 0.5\%$ suggesting circulatory volume sufficiency or expansion (58).

Table 5 lists specific indications for albumin infusion recommended by the author. Caretakers should also carefully weigh the precautions or potential drawbacks to such therapy, and consider avoiding pharmacotherapy for edema particularly if prompt remission of NS is anticipated. Notably, cosmetic effects related to edema are not an indication for diuresis in children with NS.

TABLE 6 | Diuretics used to manage edema in children.

Diuretic class, name, mechanism, and site of action	Bioavailability % PO/IV ratio		Onset of action (min) PO/IV	Duration of action (h)	Dosing
Loop diuretics					
Furosemide	60	1.5	40/5	6	Neonates: p.o. 1–4 mg/kg/dose, 1–2×/day iv/im 1–2 mg/kg/dose q 12–24 h
Bumetanide	85	1	40/5	4	Children: p.o./iv/im 1–2 mg/kg/dose q 6–12 h
Torsemide, ethacrynic acid					<6 months: p.o./iv/im 0.05–0.05 mg q 24 h
Inhibit the $Na^+/K^+/2Cl^-$ cotransport system in the thick ascending limb of Henle's loop (ALH)					>6 months: p.o./iv/im 0.015 mg/kg q 24 h; max. 0.1 mg/kg/dose
Thiazide diuretics					
Chlorothiazide	11–20		120	24	<6 months: p.o. 20–40 mg/kg/day divided bid iv 2–8 mg/kg/day divided bid
Hydrochlorothiazide	60–75		120	12–24	>6 months: p.o. 20 mg/kg/day divided bid iv 4 mg/kg/day
Inhibit NaCl cotransport in the early distal convoluted tubule (DCT)					<6 months: p.o. 2–3.3 mg/kg/dose divided bid >6 months: p.o. 2 mg/kg/day divided bid
Thiazide-like					
Metolazone	40–60		60	24	Children: 0.2–0.4 mg/kg/day divided q 12–24 h
Similar to thiazides but also proximal tubular inhibition of sodium uptake					

Non-Protein Colloid Alternatives

The clinical utility of non-albumin colloids, such as (hyperoncotic 12% dextran solution given at 1.2–1.8 g/kg body weight daily as a single dose or on 3–5 consecutive days), was previously investigated in children with nephrotic edema (59). The precise mechanism of action has not been clarified. However, despite being effective in achieving a brisk diuresis at a lower cost than albumin, dextran use has not gained clinical favor because of safety concerns including increased blood pressure, headache, gastrointestinal discomfort, pain upon tissue infiltration, and bleeding diathesis with epistaxis. The clinical response to other non-protein colloid alternatives such as gelatin and hydroxyethyl starch to induce diuresis in nephrotic edema has not been investigated.

Angiotensin Inhibition

Nearly, all children with chronic proteinuric disorders receive an ACE inhibitor or an angiotensin II receptor blocker as adjunctive synergistic drugs aimed at averting progressive renal injury caused by the underlying renal disorder. Such agents also exert a significant anti-proteinuric action and rise in serum albumin concentration which enhances the response to diuretics. However, the author recommends not using such agents in children with marked proteinuria because they tend to exaggerate hyponatremia and increase the risk of AKI because of lowering of systemic and intra-glomerular pressure. Although these agents serve as anti-proteinuric agents regardless of the underlying etiology of chronic proteinuria they are of little benefit in the acute management edema or if prompt remission of proteinuria is anticipated after staring steroids, as in the case of childhood MCD.

DIURETIC MANAGEMENT OF NEPHROTIC EDEMA

Recognizing that in the majority of children nephrotic edema is the result of appropriate compensatory physiological mechanisms aimed at restoring diminished circulatory volume and tissue perfusion, use of diuretics to remove edema fluid should be undertaken with great caution so as not to interfere with adaptive responses (refer to **Table 6**).

With the possible exception of children with inflammatory glomerulonephritis, nephrotic edema and coexisting hypertension or clear evidence of "overfilling," the author rarely recommends diuretic use in the outpatient setting. This is because of the potentially catastrophic risks of diuretic use in NS, such as thrombosis and thromboembolism, AKI, and electrolyte imbalance. By contrast, concurrent use of diuretics and salt-poor albumin or diuretic monotherapy is often utilized to manage edema in the inpatient setting.

Many children with NS respond well to loop diuretics, although there is generally lesser natriuresis than when such diuretics are utilized to manage edema associated with other medical disorders (60, 61). Experimental studies in drug-induced NS suggest that the loop of Henle may be relatively resistant to loop diuretics (62). Several factors are thought to play an important role in inducing this relative diuretic resistance:

1. Because most diuretics are highly protein-bound, they tend to become trapped within the vascular compartment, thereby maximizing their rate of delivery to the kidney. In NS however, the degree of protein binding is reduced due to hypoalbuminemia, resulting in a larger extravascular space of distribution and diminished rate of delivery to the kidney (60, 61).
2. In order to function, loop diuretics must exit the vascular capillary, traverse the interstitium, enter the tubular epithelial cell and be secreted into the tubular lumen where they block Na^+, chloride, and other transporters. Typically in NS, there is expansion of the renal parenchymal interstitial fluid compartment which reduces peritubular diuretic uptake.
3. Some of the diuretic that enters the tubular lumen is bound to filtered albumin and rendered inactive (63, 64). In experimental *in vivo* microperfusion of the loop of Henle, the addition of albumin to the perfusate in a concentration similar to that seen in the tubular lumen in NS diminishes the response to intraluminal furosemide by about 50% (63). However, it is uncertain if this mechanism is important in humans. In one study of seven patients with NS, blocking of albumin binding to furosemide by the administration of sulfisoxazole had no effect on the diuretic response (65).

In clinical practice, this diuretic resistant state can be partly overcome by administering a higher diuretic dosage in subjects with nephrotic edema compared with other edematous disorders (19). In younger children, furosemide is most often used to manage nephrotic edema. It is infused at 0.5 mg/kg/dose every 8–12 h, given at the start or at 1 h after administration of SPA. Also, a modest increase in urine Na^+ excretion and volume has been reported in adults with marked hypoalbuminemia by infusing a solution consisting of furosemide added to SPA. In theory, this approach aids trapping of the diuretic within the vascular compartment, thereby increasing the rate of loop diuretic secretion into the tubular lumen (66).

A recent review of loop diuretics in managing nephrotic and other forms of systemic edema indicates several advantages of bumetanide, including greater bioavailability than furosemide as well as the convenience of 1:1 intravenous to oral conversion (67). However, because of high potency at low dosages this agent is somewhat difficult to titrate in smaller sized children. Because of a short half-life, all loop diuretics require multiple dosing. Ethacrynic acid is reserved for children with sulfa allergy. While popular in adults, there is limited experience with torsemide use in children.

In children who do not respond adequately to loop diuretics, it is advisable to add a thiazide type diuretic in order to achieve diuretic synergy by way of sequential nephron blockade. Chlorothiazide can be given orally or intravenously and is particularly useful in small sized children or if gastrointestinal absorption is compromised. For oral use in children over 5 years old, the author prefers short-term use of metolazone (Zaroxolyn),

a long acting thiazide-like diuretic, which is secreted in the proximal tubule and has a plasma half-life of 36-hours; it is given at a dosage of 2.5–5.0 mg once daily. Like most loop diuretics, metolazone is also highly protein bound and, therefore, it is not dependent on normal GFR for it to be effective. When this is combined with bumetanide and SPA, a brisk diuresis is usually achieved.

Although ENac channel activation has been implicated in Na^+ retention in NS of diverse etiologies, the efficacy of blocking this channel by amiloride is believed to be low because of the relatively small amount of Na^+ arriving at the DCT. However, amiloride and other potassium sparing diuretics, such as spironolactone (1.25 mg/kg/dose), may be utilized in conjunction with loop diuretics.

NEW DIRECTIONS IN THE MANAGEMENT OF NEPHROTIC EDEMA

Aquaretics

Aquaretics are a newer group or class of diuretics which unlike conventional diuretics produce solute-free diuresis, or aquaresis. As discussed above, studies in untreated children with NS and underfill physiology have shown increased plasma and urinary concentrations of vasopressin or ADH (19). This together with presence of hyponatremia that is frequently found in these children is highly suggestive of water retention in excess of Na^+ retention. These observations also apply to adults with NS (68–70) and provide the rationale for aquaretic use to manage nephrotic edema. A case report and early clinical trials (71) suggest an efficacy of brief courses of vasopressin 2 receptor anatagonists, such as tolvaptan, or of somatostatin (Octeotide), in inducing aquaresis by inhibiting their common intracellular mediator, cAMP, thereby decreasing aquaporin channel insertion in the renal CD epithelium and abrogating anti-diuresis.

Urea channel inhibitors provide another newer approach to aquaresis (72–75). Use of these agents relies on the fact that humans and other mammals consuming a high protein diet generate nitrogen which is then excreted by the kidneys in the form of urea. The large amount of urea filtered at the glomerulus tends to promote an osmotic diuresis. To attenuate such diuresis the kidney has "UT-A" (coded by the SLC14A2 gene) and "UT-B" (coded by the SLC14A1 gene) channels, which aid the accumulation of urea in the renal medullary interstitium. This action osmotically balances the urea in the CD lumen, thereby preventing urea-dependent osmotic diuresis that would otherwise occur. Li et al. have identified a compound that inhibits the UT-B urea channel (76). Compounds that inhibit the UT-A channels could be potentially more useful as aquaretic agents by blocking both the source of urea in the inner medulla (UT-A1 and UT-A3 channels in the inner medullary CD) and the countercurrent exchanger in the vascular bundles (UT-A2). Also, UT-A inhibitors are predicted to have fewer adverse effects, since UT-A's only known physiological function is in the kidney, whereas UT-B inhibitors could cause hemolysis and other systemic adverse effects.

Like vaptans and somatostatin, urea channel inhibitors are of potential benefit in the treatment of hyponatremic disorders but may also be of benefit in managing edema associated with NS, particularly if excessive water retention is suspected on clinical evaluation. As with diuretics, aquaretics should only be considered in children with sufficient effective circulatory volume.

Other Potential Therapies of Nephrotic Edema

Identification of molecules that initiate proteinuria or directly contribute to edema formation offers the possibility of modulation of a greater number of potential targets so as to neutralize their detrimental effects. Currently, this area of research is in its infancy.

AUTHOR CONTRIBUTIONS

DE was responsible for the concept, literature review, and manuscript preparation.

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Renal and Cardiovascular Morbidities Associated with *APOL1* Status among African-American and Non-African-American Children with Focal Segmental Glomerulosclerosis

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Background and objectives: African-American (AA) children with focal segmental glomerulosclerosis (FSGS) have later onset disease that progresses more rapidly than in non-AA children. It is unclear how *APOL1* genotypes contribute to kidney disease risk, progression, and cardiovascular morbidity in children.

Design, setting, participants, and measurements: We examined the prevalence of *APOL1* genotypes and associated cardiovascular phenotypes among children with FSGS in the Chronic Kidney Disease in Children (CKiD) study; an ongoing multicenter prospective cohort study of children aged 1–16 years with mild to moderate kidney disease.

Results: A total of 140 AA children in the CKiD study were genotyped. High risk (HR) *APOL1* genotypes were present in 24% of AA children (33/140) and were associated with FSGS, $p < 0.001$. FSGS was the most common cause of glomerular disease in children with HR *APOL1* (89%; 25/28). Of 32 AA children with FSGS, 25 (78%) had HR *APOL1*. Compared to children with low risk *APOL1* and FSGS (comprising 36 non-AA and 7 AA), children with HR *APOL1* developed FSGS at a later age, 12.0 (IQR: 9.5, 12.5) vs. 5.5 (2.5, 11.5) years, $p = 0.004$, had a higher prevalence of uncontrolled hypertension (52 vs. 33%, $p = 0.13$), left ventricular hypertrophy (LVH) (53 vs. 12%, $p < 0.01$), C-reactive protein > 3 mg/l (33 vs. 15%, $p = 0.12$), and obesity (48 vs. 19%, $p = 0.01$). There were no differences in glomerular filtration rate, hemoglobin, iPTH, or calcium–phosphate product.

Conclusion: AA children with HR *APOL1* genotype and FSGS have increase prevalence of obesity and LVH despite a later age of FSGS onset, while adjusting for socioeconomic status. Treatment of obesity may be an important component of chronic kidney disease and LVH management in this population.

Keywords: cardiovascular, left ventricular hypertrophy, chronic renal disease, FSGS, children

INTRODUCTION

African-Americans (AA) have higher rates of hypertension (HTN) and kidney disease compared to Americans of European descent (1). In adults of African descent, the presence of high risk (HR) *APOL1* genotype (characterized by the presence of two risk alleles, defined as G1/G1 homozygotes, G2/G2 homozygotes, and G1/G2 compound heterozygotes), preferentially selected by the process of evolution, was found to be associated with non-diabetic or "hypertension-attributed" end-stage renal disease (ESRD), idiopathic focal segmental glomerulosclerosis (FSGS), and HIV-associated nephropathy (2–5). The role of *APOL1* in the adult cardiovascular phenotype is still controversial with some recent findings, suggesting that *APOL1* variants could contribute to atherosclerotic cardiovascular risk, indicating a genetic component to cardiovascular health disparities among adults of African ancestry (6). While studies in adult AA populations demonstrated strong recessive association of *APOL1* G1 and G2 genetic variants with glomerular and vascular disease progression (7), there is limited information on its role in children with chronic kidney disease (CKD), particularly for cardiovascular comorbidities. In NIH FSGS cohort study and in the FSGS-Clinical Trial (FSGS-CT), both of which included children and adults, HR *APOL1* genotype was present in 72% of self-identified AA subjects (8).

Up to 63% of children with early stages of CKD present with arterial HTN (9). In addition to being common, HTN is associated with a greater rate of decline in kidney function and is a known risk of development of ESRD (10). Furthermore, in Chronic Kidney Disease in Children (CKiD), a population with mild to moderate CKD, a high overall prevalence (53%) of systolic HTN was observed (11). Presence of HTN in children with CKD has also been associated with cardiovascular morbidity and development of left ventricular hypertrophy (LVH) (12). LVH is common among hypertensive adults and children with CKD and is more common among AA than in whites (13, 14). While a previous report of two pediatric cohorts representing children with CKD (CKiD) and nephrotic syndrome (Nephrotic Syndrome Study Network; NEPTUNE) showed similar *APOL1* characteristics in terms of FSGS diagnosis and disease progression (15), the association of *APOL1* and cardiovascular comorbidities in the presence of CKD has not been explored. The purpose of this study was to extend previous findings (15), to characterize the distribution of *APOL1* risk alleles in the CKiD cohort and, by comparing children with an underlying FSGS cause of CKD, to describe the prevalence of cardiorenal phenotypes and markers of disease severity associated with the HR *APOL1* genotype.

MATERIALS AND METHODS

Subjects

The study population comprised of children enrolled in the CKiD Study, an ongoing multicenter prospective cohort study of children aged 1–16 years with mild to moderate kidney disease (16).

Clinical and Laboratory Testing

All clinical and laboratory data in the analysis were based on the first available observation, either at study entry or 6 months after, and were categorized by indicators of renal, cardiovascular, and metabolic health. Echocardiography data were collected 1 year after study entry according to study protocol. Race, sociodemographic, and therapy use were self or parental reported.

Genetic Testing

DNA was obtained from National Institute of Diabetes and Digestive and Kidney Disease (NIDDK) genetics repository of immortalized lymphocyte cell lines based on a sample of whole blood taken at the 6-month-old study visit among those who consented to genetic testing. *APOL1* variants G1 (rs73885319, S342G and rs60945101, I384M) and G2 (rs71785313, NY deletion) were genotyped.

The number of kidney risk alleles defined *APOL1* risk groups: low risk (LR) was defined by 0 or 1 risk allele (G0/G0 homozygous, or G1/G0 and G2/G0 heterozygous), and HR was defined by 2 risk alleles (G1/G1 and G2/G2 homozygous, or G1/G2 compound heterozygous), where G0 represents the ancestral alleles at both rs73885319 and rs71785313 sites.

Indicators of renal health included glomerular filtration rate (GFR) measured by plasma disappearance of iohexol or estimated by CKiD-developed equations based on serum creatinine, cystatin C, and BUN, when iohexol GFR was not available. Proteinuria was assessed as first morning urine protein (milligrams)/creatinine (milligrams) ratio (uPCR).

Indicators of cardiovascular health included the average of three in-clinic measurements of blood pressure and left ventricular mass index (LVMI in $g/m^{2.7}$) as measured by echocardiography. Uncontrolled HTN was defined as systolic blood pressure (SBP) or diastolic blood pressure (DBP) ≥95th percentile for age, sex, height according to the fourth report (17). LVH was defined as LVMI ≥ age- and sex-specific 95th percentile based on the normal population (18). All data were measured and defined by the CKiD study protocol, as described previously (11, 16, 19).

Indicators of metabolic health included total, HDL, and LDL cholesterol (milligrams per deciliter), triglycerides (milligrams per deciliter) with age- and sex-defined categorical definitions for abnormally high total cholesterol, low HDL, and high LDL cholesterol, based on the normal population. Hemoglobin (grams per deciliter), calcium (milligrams per deciliter), phosphate (milligrams per deciliter), and calcium × phosphate product were measured. Hemoglobin was categorized by anemia status based on age- and sex-defined levels of the normal population, and phosphate levels were categorized as high based on age-defined levels of the normal population. Intact parathyroid hormone (iPTH) and C-reactive protein (CRP) were also measured (19), with high sensitivity CRP primarily used ($n = 47$) and when missing, informed by wide-range CRP data ($n = 18$; missing both $n = 3$). High CRP was defined as CRP > 3 mg/l.

The CKiD Study protocol was approved by the institutional review boards at the participating institutions, and all subjects gave informed assent or consent. Genetic testing was approved by the NIDDK Institutional Review Board.

Statistical Analyses

Since 89% of children with the HR *APOL1* profile had FSGS, the appropriate comparison group to determine differences related to *APOL1* were children with LR *APOL1* genotype and FSGS. Fisher's exact test for categorical variables and the Wilcoxon rank-sum test for continuous variables were used to compare univariate differences by *APOL1* risk status among those with a diagnosis of FSGS.

Since lower socioeconomic status (SES) was associated with AA race in this North American cohort, and low SES is associated with disease progression (20), SES is a potential confounder as the exposure groups largely differ by race. To adjust for confounding due to SES, we used inverse probability of exposure weights (IPWs) (21, 22). The IPWs were generated from a logistic regression model with HR *APOL1* as the outcome and variables related to SES as the predictors: income less than $36,000, maternal education less than college, the presence of any public insurance and missing at least one dose of antihypertensive therapy in the past 7 days (self-reported). The inverse of the predicted probability of observed exposure was stabilized to the marginal probability of each group (for HR *APOL1*: 25/68 = 0.37; and for LR *APOL1*: 43/68 = 0.63). Selected cardiovascular, metabolic, inflammatory, and other risk factors were compared to exposure group in adjusted analyses using weighting by IPWs in logistic regression models to obtain prevalence odds ratio comparing the HR *APOL1* group to the LR *APOL1* group. The outcomes were obesity, high LDL cholesterol, uncontrolled HTN, LVH, and CRP > 3 mg/l. Multiple imputation was used to account for missing data, and the results are presented unadjusted (univariately, with imputation where applicable) and adjusted to account for the effect of confounding by SES.

RESULTS

A total of 891 children were enrolled in CKiD, of whom 199 (22%) were AA; **Figure 1** presents the distributions of *APOL1* genotypes and CKD diagnoses by race. Among 140 genotyped AA children, HR *APOL1* status was present in 33 (24%), and among these 33 individuals 28 (85%) had an underlying glomerular cause of CKD, whereas only 5/33 (6%) had a non-glomerular cause of CKD, $p < 0.001$. Of those 28 subjects with HR *APOL1* status and a glomerular disease, 25/28 (89%) had a diagnosis of FSGS. In contrast, among 191 non-AA children with a glomerular CKD cause, only 36 (19%) had an FSGS diagnosis, $p < 0.001$. Of the 28 AA children with LR *APOL1* status (defined as presence of 0 or 1 risk allele), and glomerular CKD, 7 (25%) had a diagnosis of FSGS (difference between HR and LR *APOL1* AA, $p < 0.001$). *APOL1* genotyping was not performed in 59 children: 20 did not consent to genetic testing, 19 dropped out of the study prior to sample collections, 9 were not measured due to missing samples, and 11 samples became available only after genotyping had been completed. Of these 59 children, 47% had a glomerular cause of CKD; 39% of these had a diagnosis of FSGS. The similar proportions of glomerular diagnoses and FSGS among AA LR *APOL1* and non-AA LR *APOL1* children (i.e., 25 and 19%, respectively) suggest potentially similar pathologic mechanisms and were distinct from the distributions observed among AA children with HR *APOL1*. The proportion (47%) of FSGS diagnoses among children with unmeasured *APOL1* status is intermediate between that observed among those with HR (85%) and LR (25%), suggesting that this group with unmeasured genotypes comprises both HR and LR profiles. A total of 37 non-AA children were genotyped, and all were identified as having LR *APOL1* status

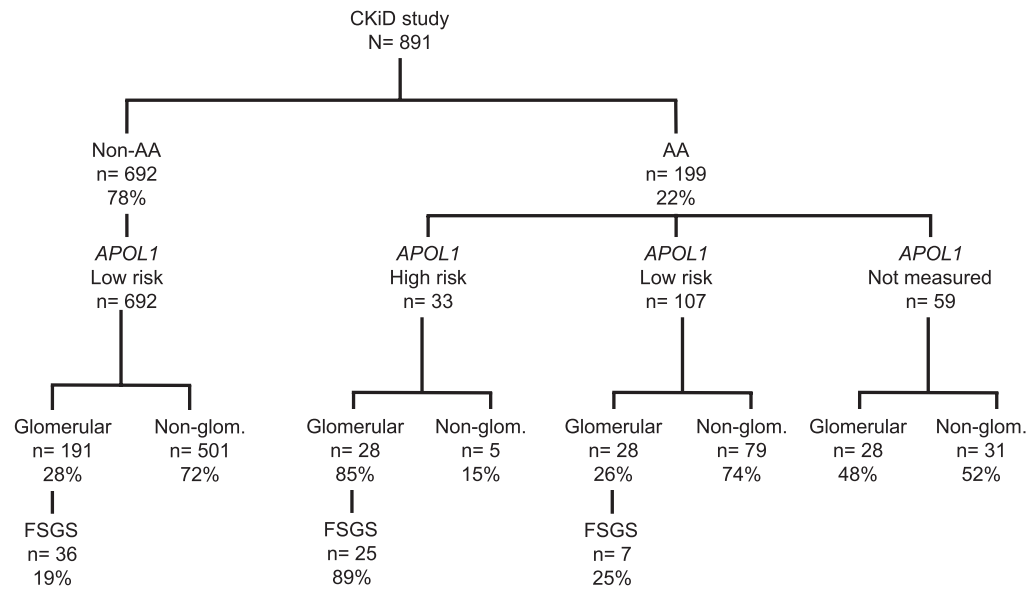

FIGURE 1 | Distribution of CKiD children by race [African-American (AA) vs. non-African-American (non-AA)], *APOL1* genotypes, and CKD diagnoses [glomerular disease (Glom), non-glomerular disease (Non-Glom), focal segmental glomerulosclerosis (FSGS)] All genotyped non-AA (*N* = 37) were found to have LR *APOL1* status, providing a justification for classifying non-AA children as low risk.

(36 had 0 risk alleles and 1 harbored 1 risk allele). This provided justification for classifying non-AA children as LR. A full list of diagnoses by race and *APOL1* status is provided in Table S1 in Supplementary Material.

We compared the prevalence of renal and cardiovascular risk factors by *APOL1* status and AA race within FSGS diagnosis group ($n = 68$). Since there were only seven subjects with LR *APOL1* and AA race, we pooled both AA ($n = 7$) and non-AA ($n = 36$) children as the reference population for statistical comparison with AA children with HR *APOL1* and FSGS ($n = 25$). **Table 1** presents the clinical, sociodemographic, and renal characteristics among those with FSGS, stratified by race and *APOL1* risk status. There were similar proportions of boys and despite similar ages at study entry, those with HR *APOL1* were significantly taller ($p = 0.003$) and heavier ($p < 0.001$), compared to children with LR *APOL1*. This was reflected in the height and weight percentiles standardized to age and gender (i.e., normal, median height, and weight percentiles are equal to 50): those with HR *APOL1* had a median height and weight percentiles significantly higher as compared to those with LR *APOL1*, $p = 0.033$ and $p < 0.001$, respectively. Body mass index (BMI) was significantly higher among the HR *APOL1* children ($p < 0.001$), and this group had a much higher proportion of obesity (BMI > 85th percentile adjusted for age and gender; 48 vs. 19%, $p = 0.01$). Consistent with previously published reports (15), children with HR *APOL1* had a higher prevalence of premature birth than children with LR *APOL1* (29 vs. 5%, $p = 0.011$), but this difference was not observed for low birth weight or small for gestational age.

To account for the potential confounding effects of SES factors related to race, inverse probability weights were used to adjust

TABLE 1 | Descriptive statistics of sociodemographic, renal health, and therapy use among children with FSGS, by race and *APOL1* risk status (LR, low risk vs. HR, high risk; non-AA, non-African-American; AA, African-American), in the CKiD study ($n = 68$), median (IQR), *n* (%); *p*-values based on Fisher's exact test or Wilcoxon rank-sum test.

Variable	Non-AA *APOL1* LR $n = 36$	AA *APOL1* LR $n = 7$	Pooled *APOL1* LR $n = 43$	*APOL1* HR $n = 25$	*p*-Value (pooled LR vs. HR)
Male	21 (58%)	4 (57%)	25 (58%)	11 (44%)	0.318
Black race	0	7 (100%)	7 (16%)	25 (100%)	NA
Age at study entry, years	13.8 [9.6, 15.6]	15.0 [8.2, 16.5]	14.2 [9.5, 15.8]	14.8 [13.0, 15.5]	0.312
Low birth weight (<2500 g)	7 (21%)	1 (17%)	8 (21%)	8 (33%)	0.372
Premature	2 (6%)	0 (0%)	2 (5%)	7 (29%)	0.011
Small for gestational age	7 (23%)	2 (33%)	9 (24%)	7 (30%)	0.765
Low birth weight, premature, or small for gestational age	10 (29%)	2 (29%)	12 (29%)	13 (52%)	0.070
Height, cm	147 [135, 163]	147 [128, 160]	147 [135, 163]	164 [149, 171]	0.003
Height percentile	24 [4, 59]	14 [1, 47]	23 [3, 55]	40 [27, 86]	0.033
Weight, kg	46.4 [34.4, 56.5]	47.1 [27.4, 72.4]	46.4 [34.0, 57.3]	73.5 [60.4, 90.6]	<0.001
Weight percentile	53 [20, 77]	59 [14, 91]	54 [18, 77]	97 [71, 99]	<0.001
Body mass index, kg/m^2	20.2 [17, 23.9]	19.4 [16.6, 27.1]	20 [17.4, 24.1]	25.1 [22.9, 36.3]	<0.001
BMI percentile	74 [28, 93]	77 [38, 91]	76 [33, 93]	93 [82, 99]	<0.001
Obese	7 (19%)	1 (14%)	8 (19%)	12 (48%)	0.014
Socioeconomic variables					
Household income					
<$36,000	15 (43%)	2 (33%)	17 (41%)	12 (48%)	0.409
≥$36,000 and <$75,000	11 (31%)	2 (33%)	13 (32%)	10 (40%)	
≥$75,000	9 (26%)	2 (33%)	11 (27%)	3 (12%)	
Maternal education < college	26 (74%)	6 (86%)	32 (76%)	17 (68%)	0.571
Any public insurance	19 (53%)	2 (29%)	21 (49%)	15 (60%)	0.453
Renal health					
Age at CKD onset, years	6.5 [2.5, 11.5]	4.5 [3.5, 3.5]	5.5 [2.5, 11.5]	12.0 [9.5, 12.5]	0.004
Years with CKD	5.3 [3.4, 7.9]	3.7 [2.2, 2.3]	5.2 [3.3, 7.9]	3.3 [1.1, 4.7]	0.008
ieGFR at entry, ml/min/1.73 m^2	48 [34, 71]	32 [26, 98]	48 [32, 79]	61 [48, 69]	0.132
IeGFR < 45 ml/min/1.73 m^2	14 (39%)	4 (57%)	18 (42%)	6 (24%)	0.190
ieGFR change per year, %	−7.4% [−3.1%, −2.9%]	−2.2% [−14.0%, −14.2%]	−7.4% [−23.0%, −2.9%]	−8.3% [−14.9%, −1.7%]	0.994
ieGFR change per year, ml/min	−4.1 [−12.3, −1.7]	−2.5 [−7.8, 5.9]	−4.8 [−12.8, −1.7]	−4.4 [−9.6, −1.1]	0.903
uPCR at entry, mg/mg creatinine	1.6 [0.2, 5.5]	1.0 [0.1, 1.2]	1.5 [0.2, 5.5]	0.9 [0.3, 1.8]	0.330
Proteinuria, uPCR > 2	15 (43%)	3 (43%)	18 (43%)	3 (13%)	0.025
Therapy use					
Anti-hypertension therapy	31 (86%)	7 (100%)	38 (88%)	25 (100%)	0.150
ACEi/ARB therapy	29 (81%)	7 (100%)	36 (84%)	22 (88%)	0.735
Missed ACEi/ARB in last 30 days	7 (19%)	1 (14%)	8 (19%)	4 (16%)	1.000
Missed ACEi/ARB in last 7 days	6 (17%)	3 (43%)	9 (21%)	10 (40%)	0.103
Steroid therapy	7 (19%)	2 (29%)	9 (21%)	8 (32%)	0.387
Immunosuppression therapy	17 (47%)	5 (71%)	22 (51%)	14 (56%)	0.803

BMI, body mass index; CKD, chronic kidney disease; ieGFR, measured or estimated glomerular filtration rate; uPCR, urine protein to creatinine ratio.

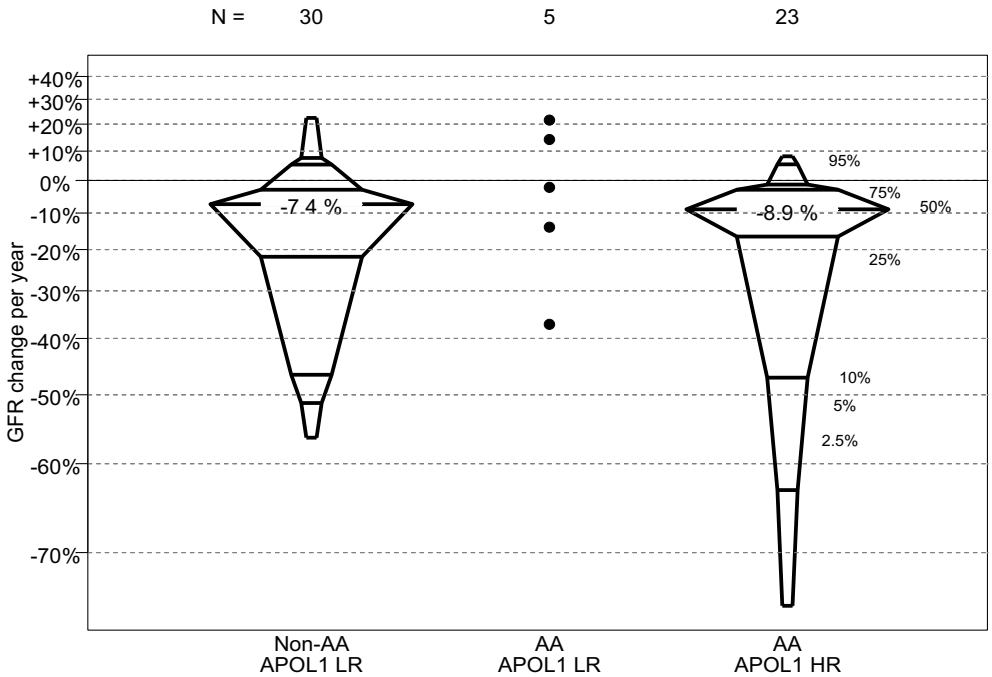

FIGURE 2 | Percentile boxplots of longitudinal GFR changes based on individual regression equations, expressed as percent change per year, by *APOL1* risk and race. A total of six non-AA LR participants, two AA LR participants, and two AA HR participants only contributed one GFR measurement and were not included in this analysis.

for sex, income, maternal education, insurance status, and 7-day adherence to antihypertensive therapy. We found no statistical differences in SES variables between HR and LR *APOL1* genotype groups.

In this comparison, those children with FSGS and HR *APOL1* had a significantly older age at CKD onset, and significantly shorter duration of CKD than those with LR *APOL1*. Children with HR *APOL1* had a higher GFR at entry although this difference was not statistically significant, $p = 0.132$. Changes in GFR over time were similar to *APOL1* status (**Figure 2**). Levels of uPCR at entry and over time were also similar, although the HR *APOL1* group had a lower proportion with nephrotic range proteinuria at study entry, $p = 0.025$.

Table 2 and Table S2A,B in Supplementary Material describe characteristics related to cardiovascular health stratified by race and *APOL1* risk status among those with an FSGS cause of CKD. Children with HR *APOL1* had higher SBP ($p = 0.004$), although SBP percentiles (standardized to the normal population for age, gender, and height) were not statistically significant ($p = 0.132$). No significant differences in DBP were observed. The HR *APOL1* children had a higher prevalence of uncontrolled HTN, although it was not statistically significant. Nearly all children, regardless of *APOL1* status received antihypertensive therapy, the HR *APOL1* group had higher (40 vs. 21%) 7-day non-adherence to ACEi/ARB therapy, $p = 0.103$ (**Table 1**). The proportions receiving glucocorticoid and other immunosuppression therapy were similar by *APOL1* risk status.

Increased left ventricular mass index ($p = 0.004$) and a higher prevalence of LVH ($p = 0.003$) were observed in the HR *APOL1* group. LDL cholesterol was higher in the HR *APOL1* group ($p = 0.047$), but no differences in total cholesterol, HDL cholesterol, and triglyceride levels were observed. Higher phosphate was observed among the LR *APOL1* group ($p = 0.023$), but there was no difference in intact parathyroid hormone. Children with HR *APOL1* had a higher proportion of CRP values greater than 3 mg/l (33 vs. 15%, $p = 0.120$).

Table 3 presents odds ratios to describe the associations of the HR *APOL1* profile and selected risk factors and comorbidities. LVH was highly associated with HR *APOL1* (OR: 6.2, 95%CI: 1.6, 24.9), as was obesity (OR: 4.7, 95%CI: 1.5, 14.4). While not statistically significant, HR *APOL1* had a higher relative odds of uncontrolled HTN, elevated CRP, and high LDL cholesterol.

DISCUSSION

In this cohort of children with FSGS, we demonstrated that AA children with HR *APOL1* genotype were more likely to have FSGS and to have a later age at FSGS onset, compared to AA children with LR genotypes. When restricting analysis to children with FSGS, HR *APOL1* was associated with a greater cardiovascular risk burden, including an increase prevalence of LVH, and obesity, a higher LVMI, BMI, and LDL cholesterol, despite treatment with antihypertensive therapy and adjustment for indicators of SES. Notably, other markers of disease severity were not different between the two groups indicating a potential role for *APOL1* in the etiology of cardiovascular abnormalities.

TABLE 2 | Cardiovascular and metabolic characteristics among children with FSGS, by race and *APOL1* status.

Variable	Non-AA *APOL1* LR *n* = 36	AA *APOL1* LR *n* = 7	Pooled *APOL1* LR *n* = 43	*APOL1* HR *n* = 25	*p*-Value (pooled LR vs. HR)
SBP, mmHg	108 [103, 117]	114 [109, 124]	109 [103, 119]	120 [113, 127]	0.004
SBP percentile	62 [43, 81]	88 [48, 97]	64 [45, 88]	79 [55, 95]	0.132
DBP, mmHg	66 [61, 77]	73 [67, 88]	69 [61, 79]	67 [63, 75]	0.889
DBP percentile	64 [36, 88]	90 [58, 99]	68 [37, 92]	57 [46, 85]	0.377
Uncontrolled hypertension	10 (28%)	4 (57%)	14 (33%)	13 (52%)	0.131
LVMI at V2, g/m$^{2.7}$	30.0 [26.9, 33.0]	28.1 [20.7, 28.6]	29.6 [26.9, 33.0]	40.8 [28.1, 52.9]	0.004
LVH at V2	4 (13.8%)	0 (0%)	4 (12%)	9 (45%)	0.003
Total cholesterol, mg/dl	173 [150, 210]	186 [174, 205]	174 [150, 210]	190 [168, 224]	0.222
High total cholesterol	10 (33%)	2 (40%)	12 (34%)	9 (43%)	0.565
HDL cholesterol, mg/dl	53 [41, 61]	53 [50, 66]	53 [41, 63]	51 [41, 60]	0.883
Low HDL cholesterol	4 (13%)	0 (0%)	4 (11%)	0 (0%)	0.286
LDL cholesterol, mg/dl	90 [71, 120]	98 [97, 123]	92 [71, 122]	112 [101, 145]	0.047
High LDL cholesterol	5 (17%)	1 (20%)	6 (17%)	7 (33%)	0.208
Triglycerides, mg/dl	143 [92, 203]	118 [93, 160]	136 [92, 200]	117 [91, 145]	0.441
High triglycerides	18 (60%)	3 (60%)	21 (60%)	10 (48%)	0.578
Hemoglobin, g/dl	12.5 [11.3, 13.4]	11.7 [10.0, 12.9]	12.3 [11.1, 13.3]	12.4 [11.9, 13.6]	0.165
Anemia	17 (47%)	5 (71%)	22 (51%)	8 (32%)	0.139
Calcium, mg/dl	9.2 [8.5, 9.6]	9.6 [8.0, 9.8]	9.2 [8.4, 9.6]	9.4 [9.1, 9.7]	0.179
Phosphate (mg/dl)	4.5 [4.0, 5.1]	4.1 [3.7, 6.0]	4.4 [4.0, 5.1]	4.3 [3.7, 4.7]	0.154
High phosphate	6 (17%)	2 (29%)	8 (19%)	0 (0%)	0.023
Calcium × phosphate	41.7 [37.4, 44.8]	41.0 [36.3, 48.0]	41.4 [37.0, 45.0]	39.9 [34.0, 45.6]	0.504
iPTH, pg/ml	58.0 [35.8, 89.8]	144.3 [40.0, 267.8]	58.0 [38.0, 95.0]	52.0 [39.5, 67.0]	0.399
CRP > 3 mg/l	4 (12%)	2 (29%)	6 (15%)	8 (33%)	0.120

Median [IQR] or (CI), n (%); p-values based on Fisher's exact test or Wilcoxon rank-sum test.
SBP, systolic blood pressure; DBP, diastolic blood pressure; LVMI, left ventricular mass index; LVH, left ventricular hypertrophy; V2, visit at 12 months post study enrollment; HDL, high density lipoprotein; LDL, low density lipoprotein; iPTH, intact parathyroid hormone; CRP, C-reactive protein (high sensitivity or wide range).
Uncontrolled hypertension was defined as SBP or DBP ≥ 95th percentile for age, sex, and height, regardless of a self-reported history of hypertension or receiving antihypertensive therapy.

TABLE 3 | Relative odds of selected risk factors and comorbidities comparing AA children with FSGS and HR *APOL1* to pooled children (i.e., non-AA and AA) with FSGS and LR *APOL1* based on logistic regression models.

	Unadjusted odds ratios (95%CI)	Adjusted odds ratios (95%CI)
Left ventricular hypertrophy	7.97 (1.90, 33.51)	6.22 (1.55, 24.91)
Obesity	4.04 (1.35, 12.11)	4.65 (1.50, 14.43)
Uncontrolled hypertension	2.24 (0.82, 6.17)	2.54 (0.92, 7.00)
C-reactive protein > 3 mg/l	2.74 (0.82, 9.16)	2.41 (0.67, 8.72)
High LDL cholesterol	1.34 (0.44, 4.08)	1.22 (0.40, 3.72)

Analyses are unadjusted and adjusted for sex, income less than $30,000, maternal education less than college, household having any public insurance, and missing a dose of antihypertensive medication in the past 7 days, as markers of socioeconomic status. Multiple imputation was used to account for missing data.
LDL, low density lipoprotein.

In children with biopsy confirmed FSGS enrolled in the FSGS-CT, 72% of self-identified AA subjects and 6% of children who identified themselves as non-AA had HR *APOL1* risk alleles (8). In the CKiD population, HR *APOL1* risk genotype was strongly associated with both glomerular disease and FSGS. Strikingly, the prevalence of HR *APOL1* among AA children with FSGS was 76% (25/33), and this was congruent with the 72% observed in the FSGS-CT. The distribution of *APOL1* genotypes observed in the adult normal population did not differ from Hardy–Weinberg expectations for genotype distribution; there was no loss of one particular genotype group or gain in another. This provides population-based statistical evidence that there is no preferential loss of HR genotypes between conception and adulthood (2–5). While our study focused on children who have already been diagnosed with CKD, we are unable to make inferences about the general (i.e., non-diseased) pediatric population. Future studies may wish to characterize the distribution of *APOL1* risk alleles in this group to better understand population risk in children.

Previous studies have established that *APOL1* kidney risk variants are strongly associated with kidney disease among adult AA; specifically, HIV-associated nephropathy (odds ratio, 29; 95% confidence interval, 13–68) and FSGS (odds ratio, 17; 95% confidence interval, 11–26) (2–5). In the CKiD cohort, the HR *APOL1* genotype was associated with the glomerular phenotype in children younger than previously reported; previous studies have established that *APOL1*-associated FSGS is characterized by a tendency to present between ages 15 and 39 years old (7) and in FSGS-CT with median age 17 years (13, 23) (8). The median age of FSGS onset among HR children was 12 years in CKiD, which was older than other LR children with FSGS, a finding previously reported alongside the NEPTUNE children with nephrotic syndrome (15). Additionally, this study found a higher prevalence of prematurity among those with HR *APOL1* compared to AA and non-AA children with FSGS, similar to findings with NEPTUNE that employed a different reference group, suggesting that the prematurity effect extends to non-AA children.

It has been well established that AA children with FSGS have later onset disease than non-AA children and progress more rapidly to ESRD (23, 24). In addition, AA children with nephrotic syndrome have a seven times greater risk of having been diagnosed with FSGS than Hispanics (Mexicans and other immigrants from Central America, but not Puerto Ricans and other Caribbean immigrants) (23), and this has been attributed to genetic factors. The present analysis suggest that among children with CKD, AA children with HR *APOL1* genotype present with later onset kidney disease, and have a higher prevalence of cardiovascular and metabolic comorbidities than patients with LR *APOL1* genotype, despite a shorter duration of disease and the same diagnosis of FSGS. This would suggest that in children with HR genotypes, FSGS might have a different pathophysiology with the added influence of distinctive environmental factors (i.e., obesity and potentially birth history, toxin exposure, and/or additional genetic factors). This in turn may lead to a "vascular/endothelial" phenotype clinically presenting as uncontrollable HTN and LVH leading to "enhanced" podocyte injury; in contrast, non-AA primary FSGS may be characterized by a "primary podocyte" phenotype, earlier onset, and slower CKD progression. It is unclear at present how G1 and G2 alleles may cause endothelial or podocyte damage, although the APOL1 protein forms pores in the lysosomal membrane, leading to pathogen lysis, and a variant APOL1 protein was shown in transgenic mice to induce direct tissue injury (25).

The role of *APOL1* in the adult cardiovascular phenotype remains controversial. Ito et al. (6) using data from the Jackson Heart Study (JHS) and Women's Health Initiative (WHI) demonstrated that the *APOL1* kidney risk alleles confer a twofold risk of cardiovascular events, without significant changes in left ventricular mass, whereas the Systolic Blood Pressure Intervention Trial (SPRINT) showed the absence of an *APOL1* association with prevalent cardiovascular disease in a non-diabetic adult sample (26). It is possible that cardiovascular disease–*APOL1* interaction may have an age-dependent relationship pattern, similar to one described in early- and late-onset forms of Alzheimer disease associated with apolipoprotein E, epsilon 4 allele, where subjects at both age spectrum (young-old) seem to be affected by the disease penetrance (27). It is unknown if similar age-dependent relationship pattern could also be seen in cardiovascular disease–*APOL1* interaction.

Furth et al. (19) described cardiovascular disease risk factors in the CKiD cohort and found that only 18% of the cohort exhibited CRP values >3 mg/l (independent of GFR). In our study, elevated CRP was not statistically different between HR and LR *APOL1* children with FSGS, suggesting that inflammation is not a driving factor behind LVH. It is possible that differences in LVH are driven by a combination of *APOL1* genotype, underlying metabolic status, obesity/BMI, HTN, and some other unrecognized socioeconomic or racial variables. Our results showed an excess of obesity among those with HR *APOL*1. Since obesity and metabolic dysregulation are risk factors for both cardiovascular disease and CKD onset and progression, managing weight prior to onset in order to prevent or delay disease may be an important clinical consideration. Future studies should seek to clarify whether the effect of *APOL1* on CKD severity is related to obesity or whether these two outcomes are independently influenced by the HR profile. Our finding of increased cardiovascular and metabolic risks associated with HR genotype in children with CKD underscores the importance of early detection and need for more aggressive treatment of obesity, such as early therapy or adherence support, in this population.

It is also possible that the increased cardiovascular risk in the HR children is a consequence of the underlying glomerular disease (FSGS) severity and rate of progression. HTN-misattributed kidney disease in AA has been described in the adult literature (28). Given the cross-sectional nature of this study, future research efforts might investigate a large group of AA children with non-glomerular/congenital kidney disease to investigate the effect of HR *APOL1* on cardiovascular risk, in the absence of podocyte injury and glomerular damage. Since the vast majority of HR children in CKiD had a glomerular disease (85%), we were unable to assess the effect of *APOL1* HR on cardiovascular risk among those with a non-glomerular condition.

This study has several limitations. First, the LR *APOL1* subgroup with FSGS was predominantly non-AA. There were only 7 AA with LR *APOL1* compared to 25 AA children with HR *APOL1* and FSGS, thus highlighting the strong association between HR *APOL1* and FSGS among AA children. Since other forms of glomerular disease among the AA LR *APOL1* children, such as hemolytic uremic syndrome, familial nephritis, or SLE, were less common than FSGS in AA HR group, we chose to restrict our analysis to only those with FSGS to determine the effect of HR *APOL1* among those with the same CKD pathology, which necessitated inclusion of non-AA children with FSGS. Although a minority of non-AA subjects were genotyped; all had LR *APOL1* status, providing a justification for classifying non-AA children as LR. It is possible that some children were misclassified, particularly those of Hispanic ethnicity, although this misclassification would lead to more conservative estimates. Ideally, our study would have included a large group of LR and HR *APOL1* AA children with FSGS. As such, we cannot completely discount genetic (non-*APOL1*) or metabolic factors related to race explaining these effects. A related limitation is the potential confounding effect of race and SES, given the disparate distributions of race between the exposed and unexposed groups. While several key SES variables were not associated with our exposure, 7-day antihypertensive adherence was related to HR *APOL1*. Inverse probability or exposure weights were used to account for potential differences in SES variables in adjusted analyses, but there may be other unmeasured SES variables possibly confounding these relationships. The third limitation was the lack of DNA for analysis in 59 subjects (28 subjects with glomerular disease), reducing statistical power. We did not characterize ancestry-informative markers, and therefore, we did not determine the fraction of African ancestry among subjects, although this information would likely not change any inferences, since the prevalence of HR *APOL1* genotype in this study was nearly identical to that previously reported (5, 7, 8). Finally, this study was limited in sample size and may be considered preliminary data for future research to build upon. These findings need to be replicated in larger populations in both children and adults. Despite these limitations, the association of HR *APOL1* genotype

with development of glomerular disease, later onset of FSGS and indications of increased cardiovascular risk among children were compelling.

In conclusion, this study suggests that *APOL1*-associated risks are not restricted to adults only and are present in young children as well. Indeed, in CKiD, cardiovascular abnormalities were more common among AA children with HR *APOL1*. Targeting these established modifiable comorbidities, especially BMI, obesity, and HTN, may be a particularly important component in CKD management to delay ESRD in this HR population.

AUTHOR CONTRIBUTIONS

RW developed the idea for this research, participated in analysis and interpretation of the data, and wrote the manuscript. DN participated in the statistical design, analysis and interpretation of the data, and co-wrote the manuscript. SL, CW, JK participated in sample genotyping, analysis, and interpretation of the data, and edited the manuscript. KR and MS participated in analysis and interpretation of the data, and edited the manuscript. BW, SF, MM, CW, and FK participated in the design of the Chronic Kidney Disease in Children prospective cohort study (source of data in this manuscript) and analysis and interpretation of the data, and edited the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://journal.frontiersin.org/article/10.3389/fped.2016.00122/full#supplementary-material.

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Ethnic Differences in Childhood Nephrotic Syndrome

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Nephrotic syndrome is a common glomerular disease in children with significant variability in both incidence and steroid responsiveness among various ethnic groups. The average incidence of nephrotic syndrome is 2–16.9 per 100,000 children worldwide. Understanding the variability by ethnicity may point to potential factors leading to nephrotic syndrome, which remains elusive, and may highlight factors accounting for differences in medication response. The emerging role of genetic factors associated with steroid responsive and steroid-resistant forms of nephrotic syndrome within an ethnic group can provide insight into potential biological mechanisms leading to disease. For example, among African-Americans, the risk variants in *APOL1* are associated with a more than 10-fold increase in risk of focal segmental glomerulosclerosis and high-risk carriers have a twofold greater risk of progression to end-stage renal disease. Ongoing collaborative studies should consider capturing data on self-reported ethnicity to understand differences in incidence and outcomes. In the future, the availability of whole-genome data will provide an excellent opportunity for new clinical and translational research in childhood nephrotic syndrome and lead to a better understanding of the disease.

Keywords: steroid resistant, steroid dependent, minimal change disease, focal segmental glomerulosclerosis, nephrotic syndrome

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INTRODUCTION

Nephrotic syndrome is a common childhood kidney disease characterized by a constellation of heavy proteinuria (urine protein to creatinine ratio >200 mg/mmol or ≥3 + proteinuria on urine dipstick) resulting in hypoalbuminemia (<25 g/L), hyperlipidemia, and peripheral edema (1). Despite treating nephrotic syndrome with prednisone for over 50 years, the exact mechanism of disease remains unclear. Most studies focus on podocyte injury (2), immunological (3), and environmental factors (4) as potential culprits in the pathogenesis of nephrotic syndrome, but causal factors remain elusive.

Corticosteroids remain the mainstay for treatment of nephrotic syndrome. Based on the response to corticosteroids, children with nephrotic syndrome segregate into a steroid-sensitive group that has a good long-term prognosis, but risk of frequent relapses, and a steroid-resistant group with higher risk of developing chronic kidney disease. Response to medications is quite variable with some children requiring further courses of steroid-sparing agents, while others achieve complete remission after the first course of prednisone. Steroid-sparing agents, such as cyclophosphamide, mycophenolate mofetil, calcineurin inhibitors, and rituximab, are often used to induce or maintain remission with mixed results. It is unclear what leads to this individual variability in drug response and the future risk of relapses.

Minimal change disease (MCD) is the most common histological variant of nephrotic syndrome and accounts for approximately 80% of cases in children based on historical data from 1967 to 1976 (5). Focal segmental glomerulosclerosis (FSGS) is less common but can be progressive with poor long-term outcomes. Data suggest differences in the incidence of FSGS among various ethnic groups, for example, a study involving 86 children from Kansas city, MO, USA, reported the annual incidence of FSGS of 1.6 per 100,000 in African-Americans compared to 0.3 per 100,000 in European Americans (6).

With recent advancements and improved understanding of the human genome, 45 genes are found to be associated with familial and sporadic nephrotic syndrome that primarily affect podocyte structure and function (7). Most recently identified, the gene *APOL1* is a major risk factor for FSGS among African-Americans accounting for progressive kidney disease. The influence of genetic factors among other ethnic groups is less clear.

This review focuses on the current understanding of ethnic differences in childhood nephrotic syndrome, discusses ongoing studies and role of ethnicity, and the influence of genetics within specific ethnic groups.

ETHNIC DIFFERENCES IN NEPHROTIC SYNDROME

Based on our review of the literature, the average incidence of nephrotic syndrome is 4.7 (range 1.15–16.9) per 100,000 persons in studies reported from 1946 to 2014, and the proportion with steroid resistance is 12.4% (range 2.1–27.3%) from 1986 to 2014 (**Figures 1A,B**). Hence, there is a considerable variation in disease burden by country of origin and steroid responsiveness, suggesting the potential role of ethnicity in susceptibility to disease (8–23).

In studies, where there are large diaspora populations, such as the United Kingdom, South Asians are reported to have a higher incidence of nephrotic syndrome ranging from 7.4 to 16.9 per 100,000 persons compared to Europeans (14, 24, 25). Studies from the US report a higher estimated incidence among children of African compared to European descent (**Figure 1A**) (6, 13). The proportion of steroid resistance also varies by ethnicity from 20% among Europeans, 16–27% among Africans, 27–54% among Asians, and 20–39% among South Asians (**Figure 1B**) (26–29). It is possible that these differences are at least partially attributable to variations in clinical management, selection bias, and definitions of outcomes. African-American children are more likely to have biopsy-proven FSGS (42–72%) with worse outcomes as compared to European children and commonly progress to end-stage renal disease (ESRD) (19, 26, 27, 30–32). Comparatively, the proportion of FSGS in India ranges from 15.3 to 39.1% (33, 34). All these reports have differing definitions, inconsistent inclusion, and selection criteria at tertiary centers and variable access to health care, which impact comparison of incidence rates and outcomes such as steroid responsiveness. In addition, most studies combine both steroid resistant and FSGS as a single category, thereby leading to further heterogeneity in study populations and impacting outcomes. On the other hand, registries from New Zealand and Netherlands did not show differences in disease burden by ethnicity primarily due to lack of power or reported ethnic diversity (35, 36). These registries or case series have short follow-up and limited clinical information; hence, differences in treatment response by ethnicity are not well reported. To address these limitations, a number of ongoing registries and cohort studies conducted worldwide deserve mention. These studies include children with various types of nephrotic syndrome with the overall goal to identify clinical, histological and genomic predictors of nephrotic syndrome. The highlights of each study are shown in **Table 1**.

PodoNet is an international registry that includes children with steroid-resistant disease and congenital nephrotic syndrome recruited predominantly from European countries having 90.3% participants of European descent (37). Registry for Rare Kidney Diseases (RaDaR), recently renamed the National Study for Nephrotic Syndrome (NephroS) study, is a web-based UK registry that aims to capture clinical information on all children and adults with nephrotic syndrome and studies to date have focused on genetic testing of steroid-resistant disease primarily involving Europeans (76.6%) (38).

Nephrotic Syndrome Study Network (NEPTUNE) and the Cure Glomerulonephropathy Network (CureGN) are both North American multicenter collaborative longitudinal cohort studies, which enroll both children and adults with biopsy-proven disease (MCD and FSGS). NEPTUNE recruits an incident cohort at the time of first biopsy and follows them closely for up to 5 years (39). It comprises a diverse ethnic cohort based on genetically derived ancestry. CureGN is a new multicenter study enrolling up to 2400 children and adults with incident or prevalent MCD and FSGS. All of these studies to date have limited enrollment of either South Asians or East/Southeast Asians as many may not receive a biopsy or fulfill entry criteria, thus limiting their generalizability to other patient populations.

Canadian Childhood Nephrotic Syndrome (CHILDNEPH) is enrolling children across Canada to study treatment strategies in nephrotic syndrome and system factors driving treatment variation (40). Insight into Nephrotic Syndrome: Investigating Genes, Health, and Therapeutics (INSIGHT) is an ongoing longitudinal study, initially from Toronto, ON, Canada, that aims to detect factors that affect disease susceptibility and treatment response among children with nephrotic syndrome (41). The preliminary results from this study (42) demonstrate that South Asians have approximately 6 times higher incidence of nephrotic syndrome but significantly lower odds of frequently relapsing disease as compared to Europeans.

There is a clear geographical variation in the incidence of nephrotic syndrome. It is possible that the differences in incidence by ethnicity are due to referral patterns at tertiary care centers or publication bias. The reason for variation in response to steroids and other immunosuppressive medications among specific ethnic groups has not been well documented. The results from registries and prospective studies will provide an excellent resource for future clinical and genetic research to understand disease pathogenesis and differences by ancestry.

A major limitation in understanding ethnic differences is the lack of detailed self-reported ethnicity data collected in most

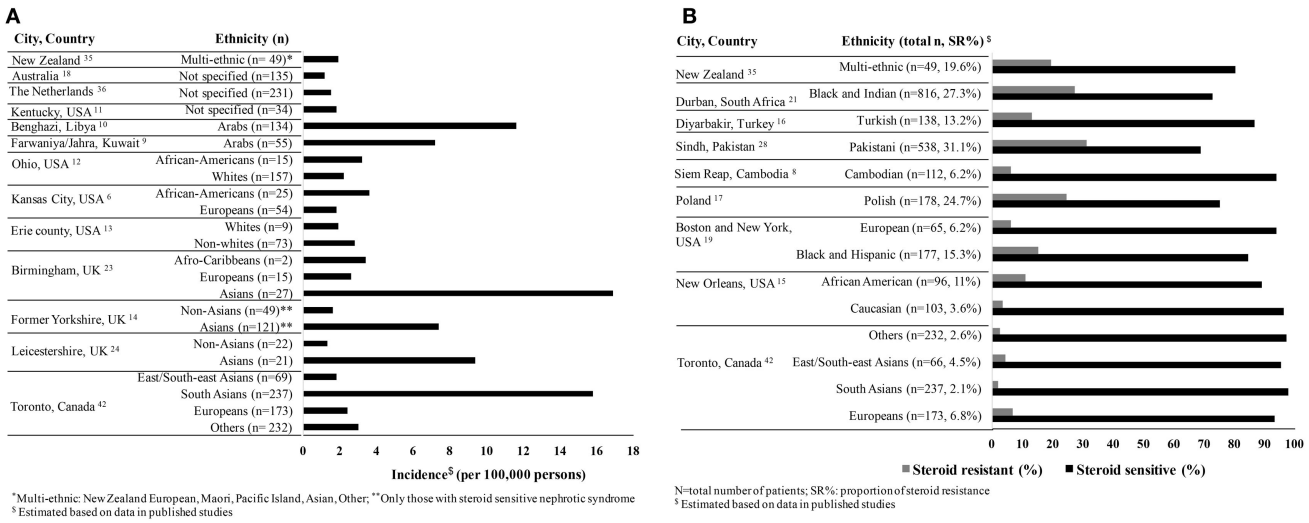

FIGURE 1 | **(A)** Incidence of childhood nephrotic syndrome per 100,000 persons by ethnicity reported from 1946 to 2014. **(B)** Variability of steroid responsiveness by ethnicity among children with nephrotic syndrome in reported studies from 1986 to 2014.

TABLE 1 | Distribution of ethnic groups in ongoing glomerular disease registries and prospective studies.

Study	Participating centers	Enrolled/ projected participants	Initial start date	Inclusion criteria	Ethnic groups			
					Europeans (%)	South Asians (%)	East/South East Asians (%)	Others (%)
Registries								
PodoNet (37)	67	1655	2009	Congenital/steroid-resistant nephrotic syndrome	90.3	0.9	0.4	8.4[a]
RaDaR (38)	Not specified	220	2010	Children/adults with steroid-sensitive and -resistant nephrotic syndrome	76.6	2.6	0.06[d]	20[c]
Cohort studies								
NEPTUNE (39)	18	450	2010	Children/adults with MCD, FSGS, and MN	48	–	9	44[b]
INSIGHT (41, 42)	2	450	2012	All children with nephrotic syndrome	24	33	10	33[e]
CureGN	64	2400	2014	Children/adults with MCD, FSGS, IgA nephropathy, and MN	Not available			

MCD, minimal change disease, FSGS, focal segmental glomerulosclerosis, MN, membranous nephropathy.
[a]*Includes Hispanic, mixed, and Native Americans.*
[b]*Includes admixed Americans and Africans.*
[c]*Includes North African, East African, Afro-Caribbean, mixed, and unknown.*
[d]*Specified as "other Asian".*
[e]*Includes individuals classified as West Central Asian/Middle Eastern, West Indian/Caribbean and African, Latin/Central/South American and Aboriginal, multi-ethnic, and unknown.*

studies. Moreover, due to admixture it can be difficult to establish ethnicity among multi-ethnic children. Additionally, various cultural, environmental, and socioeconomic factors also act as important confounders and may influence individual variability to medication response and frequency of relapses. We provide a few suggestions for future studies in terms of collecting and reporting ethnicity data in children with nephrotic syndrome until genetic ancestry is available in some studies:

1. Ethnicity should be self-reported;
2. Ethnicity of two or more generations should be captured for multigenerational admixture;
3. A uniform definition of ethnic groups should be used to compare across studies;
4. Collaborative studies, both national and international, should include diverse ethnicities.

ROLE OF ETHNICITY AND GENETICS IN CHILDHOOD NEPHROTIC SYNDROME

There is an evolving role of genetic risk and development of nephrotic syndrome in children. The discovery of *NPHS1* and *NPHS2* genes leading to congenital nephrotic syndrome provided the first evidence of a genetic cause of steroid-resistant disease.

Since then, 45 genes have been associated with monogenic forms of nephrotic syndrome and highlight abnormalities in podocyte structure and function leading to disease (7). The current known genes associated with nephrotic syndrome account for only 20–30% of hereditary and 10–20% of sporadic cases. The majority of these genes especially *NPHS2* have been identified in European (43, 44), Japanese (45), and Turkish (46) families but have not been studied across broad ancestral groups where the allele frequency may vary and additional variants may be important. With advancement in genetic analyses, an increasing number of polymorphisms have been detected in children with nephrotic syndrome. It is important to note, however, that some variants are of unknown significance. It is difficult to determine whether these variants are common and/or pathogenic by ethnic or ancestral groups until genetic databases include more comprehensive information across many ethnic groups. Understanding the epidemiological differences in kidney disease by ethnicity may suggest possible genetic risk. For example, African-Americans are known to have a 7.5% lifetime risk of reaching end-stage kidney disease, which is significantly higher than approximately 2% in European Americans (47). In 2008, studies using mapping admixture by linkage disequilibrium identified loci on chromosome 22 that explained the higher incidence of development of FSGS and ESRD among Africans Americans compared to European Americans. Initially, genetic risk was attributed to variants in the *MYH9* gene (48, 49). With new data available in the HapMAP, the major source of genetic risk for African-American non-diabetic ESRD and FSGS was localized to *APOL1*, encoding apolipoprotein L1 (ApoL1), which is only 14 kb from the gene *MYH9* (50, 51). Risk of advanced kidney disease is two to seven times greater for those carrying risk alleles of *APOL1*, as compared to controls. The spectrum of *APOL1*-associated kidney disease is quite diverse and includes nephrotic syndrome (FSGS), non-diabetic chronic kidney disease secondary to hypertension, HIV-associated collapsing glomerulopathy (52), sickle cell nephropathy (53), and lupus nephritis (54). A recent study also demonstrated that the risk of progression of chronic kidney disease is approximately two times higher in African-Americans with *APOL1* variants despite adequate blood pressure control (55). Interestingly, some individuals carrying *APOL1* risk alleles do not develop kidney disease highlighting the role of additional genetic or environmental interactions (56). To further understand the association of *APOL1* variants with different rates of chronic kidney disease across various African countries and potential environmental interaction, the Kidney Disease Network of Human Hereditary and Health in Africa (H3Africa) consortium was established (57).

Recently, a study among 214 children with steroid-sensitive nephrotic syndrome from South Asia, specifically Sri Lanka, reported HLA-DQA1 missense coding variants as possible candidate loci based on exome array in case–control study compared to 149 healthy controls. The study findings were then confirmed among a replication sample of 100 European children with steroid-sensitive nephrotic syndrome and 205 European controls. This study supports the contributory role of immune response in the pathogenesis of nephrotic syndrome (58) and will need to be tested in additional cohorts with diverse ethnic groups. Previous smaller studies in Chinese and Japanese children have also reported association between variants in HLA-DQ3, HLA-DQ8, HLA-DR, HLADQW2, HLA-DQA1, and HLA-DQB1 in nephrotic syndrome (59–62).

ETHNIC DIFFERENCES IN OTHER GLOMERULAR DISEASES

In specific ethnic groups, genome-wide association studies have identified susceptibility loci for membranous and IgA nephropathies. HLA-DQA1 allele on chromosome 6p21 is most closely associated with idiopathic membranous nephropathy in persons of European ancestry (63). IgA nephropathy, the most common cause of glomerulonephritis in adults, has a higher prevalence in Asians as compared to North-Americans or Europeans (64). Recently, in a large diverse cohort of patients with IgA nephropathy, individuals with Pacific Asian origin were shown to have a higher risk of progression to ESRD (65). Studies among East Asians with IgA nephropathy have identified susceptibility loci with the strongest association in the region that include the HLA-DRB1, -DQA1, and -DQB1 genes (66). Similar studies are quite scarce in children with nephrotic syndrome.

CONCLUSION

In children with nephrotic syndrome, incidence and response to treatment varies by ethnicity. It is likely that genetic and environmental risk factors play a substantial role in explaining these ethnic differences and need further study.

AUTHOR CONTRIBUTIONS

RC drafted the manuscript; RP conceptualized the work and critically revised the manuscript.

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Long-Term Outcomes in Children with Steroid-Resistant Nephrotic Syndrome Treated with Calcineurin Inhibitors

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Background: Steroid-resistant nephrotic syndrome (SRNS) is an important cause of chronic kidney disease (CKD) in children that often progresses to end-stage renal disease (ESRD). Calcineurin inhibitors (CNIs) have been shown to be effective in inducing short-term remission in some patients with SRNS. However, there are little data examining their long-term impact on ESRD progression rates.

Methods: We performed a retrospective chart review of all patients treated for SRNS with CNIs at our institution from 1995 to 2013. Data collected including demographics, initial response to medical therapy, number of relapses, progression to ESRD, and treatment complications.

Results: A total of 16 patients met inclusion criteria with a mean follow-up of 6.6 years (range 0.6–17.6 years). Histopathological diagnoses were focal segmental glomerulosclerosis (8), mesangial proliferative glomerulonephritis (4), IgM nephropathy (3), and minimal change disease (1). Three patients (18.8%) were unresponsive to CNIs while the remaining 13 (81.2%) achieved remission with CNI therapy. Six patients (37.5%) progressed to ESRD during the study period, three of whom did so after initially responding to CNI therapy. Renal survival rates were 87, 71, and 57% at 2, 5, and 10 years, respectively. Non-Caucasian ethnicity was associated with progression to ESRD. Finally, a higher number of acute kidney injury (AKI) episodes were associated with a lower final estimated glomerular filtration rate.

Discussion: Despite the majority of SRNS patients initially responding to CNI therapy, a significant percentage still progressed to ESRD despite achieving short-term remission. Recurrent episodes of AKI may be associated with progression of CKD in patients with SRNS.

Keywords: nephrotic syndrome, calcineurin inhibitor, steroid resistant, AKI, FSGS

INTRODUCTION

Nephrotic syndrome is a rare clinical syndrome consisting of high grade proteinuria, hyperlipidemia, hypoalbuminemia, and edema. Nephrotic syndrome affects ~2–7 children per 100,000 and affects all ages and ethnic backgrounds (1–4). Prior studies have demonstrated that ~80% of children diagnosed with nephrotic syndrome will respond to steroid therapy (5). However, there is recent evidence that the incidence of initial steroid resistance is increasing (6). Previous long-term follow-up studies have demonstrated favorable prognosis if remission is achieved with steroids (7, 8).

The majority of patients with steroid-resistant nephrotic syndrome (SRNS) will have focal segmental glomerulosclerosis (FSGS) found on biopsy (2, 5). Historical studies examining SRNS, specifically caused by FSGS, provided evidence that >50% of children who do not respond to initial steroid therapy would progress to end-stage renal disease (ESRD) within 3 years (9, 10). Due to the unfavorable prognosis of SRNS, numerous therapies have been utilized in an attempt to achieve remission, including cyclosporine, tacrolimus, cyclophosphamide, and rituximab. The most predominant therapies utilized are the calcineurin inhibitors (CNIs), cyclosporine, and/or tacrolimus, which are the current recommended first line therapy for SRNS per the 2012 Kidney Diseases Improving Global Outcomes (KDIGO) Guidelines (11). Numerous studies have demonstrated their efficacy in achieving short-term remission (12–14). While several studies have examined long-term outcomes in SRNS (15–18), there are many limitations to these results. The goal of the current study was to examine the long-term (>5 years) follow-up of SRNS patients treated with CNIs at a single institution, with specific attention paid to those who initially showed good response to CNIs.

MATERIALS AND METHODS

A retrospective chart review was performed of all patients with SRNS treated with CNIs from January 1995 through August 2013 at Rainbow Babies and Children's Hospital. Patients were identified via ICD-9 billing code search. Inclusion criteria were (1) diagnosis of SRNS, with a minimum of 6 months follow-up from initial diagnosis; (2) age 1–18 years at the time of diagnosis; and (3) treatment with either cyclosporine A and/or tacrolimus. Exclusion criteria were (1) steroid dependent and/or frequently relapsing NS, (2) late-onset steroid resistance, or (3) incomplete medical records. Definitions of steroid response, remission, and relapse were based upon the 2012 KDIGO guidelines (11).

Data Collection

Data collected from the medical records of children meeting inclusion criteria included basic demographic data (age, gender, and race), clinical features at diagnosis (presence of hematuria, hypertension, serum creatinine, and urine protein/creatinine ratio), histopathology, and growth parameters (weight and height). Longitudinal follow-up data were also collected and included number of relapses/remissions, complications of disease/treatment [infections requiring hospital admission, development of diabetes mellitus, development of hypertension, thrombosis/stroke, and episodes of acute kidney injury (AKI)], and estimated GFR (eGFR). AKI was defined as a 0.3 mg/dL rise in the serum creatinine within a 48-h period per the AKI network criteria (19). eGFR was calculated using the original bedside Schwartz equation and the modified Schwartz equation when serum creatinine was measured using isotope dilution mass spectrometry (IDMS) (20, 21). End follow-up occurred at the completion of the study period or when the patient progressed to ESRD (transplantation or dialysis).

Statistical Analysis

Baseline results were expressed as means with ranges and percentages. Given the small patient sample size normality was not assumed and all statistics were performed non-parametrically. Due to the small sample size, all patients were included in the final statistical analysis regardless of their initial response to calcineurin inhibition. Statistical tests utilized include Pearson chi-squared, Mann–Whitney *U* test, Pearson correlation, Spearman rank correlation, and Kaplan–Meier survival analysis. All statistical analyses were performed with the SPSS software suite (version 22.0). The research design and statistical analysis was approved by University Hospitals/Case Western Reserve University Institutional Review Board.

RESULTS

A total of 34 patients were identified of which 16 met all inclusion criteria. Of the 18 excluded patients, 14 had either late-onset steroid resistance or steroid dependence, 2 patients were not treated with CNIs, and 2 patients' records were unavailable. Mean age at onset of SRNS was 6.9 years (1.7–13 years) and mean duration of follow-up was 6.6 years (range 0.6–17.6 years). Seven of the 16 children were male (43.8%). Nine of the children were African-American (56.2%), four children were Caucasian (25%), and three children were Hispanic (18.8%). All patients underwent biopsy soon after diagnosis of SRNS with eight patients having focal segmental glomerular sclerosis (50%), four with mesangial proliferative glomerulonephritis (25%), three with IgM nephropathy (18.8%), and one patient with minimal change disease (6.2%). The majority of patients were treated with cyclosporine A (10 patients, 62.5%) with only 2 patients (12.5%) receiving tacrolimus, whereas 4 patients (25%) were treated with both medications during the study period. Demographic and clinical features of the study cohort are summarized in **Table 1**.

Thirteen of the 16 patients (81.3%) achieved remission with CNI therapy. The three patients who failed to achieve initial remission all progressed to ESRD during the length of follow-up (7, 11 months, and ~5 years). Among patients achieving initial remission, relapses were common with a mean of 3.4 relapses (0.5 relapses/year). Complications that arose during the course of treatment included infections requiring admission (three patients), steroid-induced cataracts (one patient), venous/arterial thrombosis (three patients), and one patient who suffered posterior reversible encephalopathy syndrome. AKI was also very common with 13/16 patients (81.3%) having at least one episode of AKI. The mean number of AKI episodes was 2.1 ± 1.5 among all 16 patients in the study, which corresponds to

TABLE 1 | Study population baseline characteristics.

Gender	Male: 7 (44%) Female: 9 (56%)
Mean age at onset (years)	6.9 (1.7–13.9)
Histopathology	FSGS: 8 (50%) MPGN: 4 (25%) IgM nephropathy: 3 (19%) Minimal change: 1 (6%)
Ethnicity	African-American: 9 (56%) Caucasian: 4 (25%) Hispanic: 3 (19%)
Medication	Cyclosporine A: 10 (63%) Tacrolimus: 2 (12%) Both: 4 (25%)

a mean 0.7 ± 1.1 AKI episode per patient year of follow-up. When restricted to only those patients achieving remission with CNI therapy ($n = 13$) the mean number of AKI episodes per patient year was 0.34 ± 0.3 episodes. Reversibility to baseline creatinine was seen in 71% of AKI episodes with the remaining 29% adopting a new baseline.

A total of 6 out of 16 patients (37.5%) developed ESRD during the study period: 3/3 (100%) of CNI non-responders and 3/13 (23%) of CNI responders. **Table 2** summarizes the demographic and clinical features results based on renal outcome (ESRD vs. non-ESRD). The only two significant associations with ESRD in the study population were non-Caucasian race ($p = 0.024$), and the mean number of AKI episodes per patient year of follow-up ($p = 0.031$). No other significant associations with ESRD were identified in the study population with respect to age, gender, histopathology, medication, or number of remissions. Finally, eGFR was inversely associated with a higher mean number of AKI episodes ($p = 0.022$, 95% confidence interval $= -28.5$ to -2.5 mL/min/1.73 m^2). No other associations with lower final eGFR were identified. Kaplan–Meier renal survival analysis was performed and demonstrated survival rates of 87, 71, and 57% at 2, 5, and 10 years, respectively (see **Figure 1**). When CNI non-responders were eliminated from the statistical analysis, the only significant correlation was AKI episodes per patient year of follow-up with lower eGFR ($p = 0.03$). Kaplan–Meier renal survival analysis for CNI responders demonstrated renal survival rates of 88, 80, and 57% at 2, 5, and 10 years, respectively (see **Figure 2**).

TABLE 2 | Study demographics and results by renal outcome.

	Non-ESRD ($n = 10$)	ESRD ($n = 6$)	p-Value
Gender (% male)	4 (40%)	3 (50%)	0.696
Mean age at onset (years)	6.1 (1.7–13.2)	8.5 (1.7–13.9)	0.301
Histopathology	FSGS: 5 (50%) MPGN: 3 (30%) IgM nephropathy: 1 (10%) Minimal change: 1 (10%)	FSGS: 3 (50%) MPGN: 1 (17%) IgM nephropathy: 2 (33%)	0.828
Ethnicity	African-American: 6 (50%) Caucasian: 4 (40%)	African-American: 3 (50%) Hispanic: 3 (50%)	**0.024**
Medication	Cyclosporine A: 8 (80%) Tacrolimus: 1 (10%) Both: 1 (10%)	Cyclosporine A: 2 (33%) Tacrolimus: 1 (17%) Both medications: 3 (50%)	0.147
Mean follow-up duration (months)	92 (17–211)	61 (7–162)	0.278
Mean number of relapses	3.2	3.8	0.428
Mean number of AKI episodes	1.5	3.3	0.073
Mean number of AKI episodes (per patient year of follow-up)	0.27	1.5	**0.031**

Red bold text indicates statistical significance.

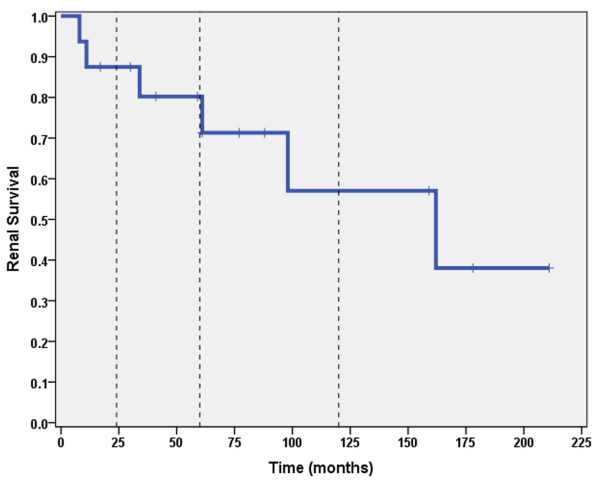

FIGURE 1 | Kaplan–Meier renal survival curve for all subjects. Vertical dashed lines represent time points of 2, 5, and 10 years for both CNI responders and CNI non-responders.

DISCUSSION

Steroid-resistant nephrotic syndrome remains a challenging clinical entity and many patients still struggle to achieve remission despite newer therapies. Failure to achieve remission with steroids was historically associated with rates of progression to ESRD >50% within a 5-year period (9, 10). Since the advent of CNIs (and other therapies), there has been a paucity of long-term outcome data regarding progression to ESRD. This study provides further evidence regarding the long-term outcomes of SRNS. A significant percentage (18.8%) failed to respond to CNIs, all of whom progressed to ESRD. Among those initially responding to CNIs, 23% (3/13) still progressed to ESRD. Although renal survival at 1 and 5 years was still fairly good (>80%), the 10-year renal survival of that group was 57%, demonstrating the importance of following these long-term clinical course.

A growing body of evidence in adult nephrology literature that suggests the recurrent episodes of AKI may be associated

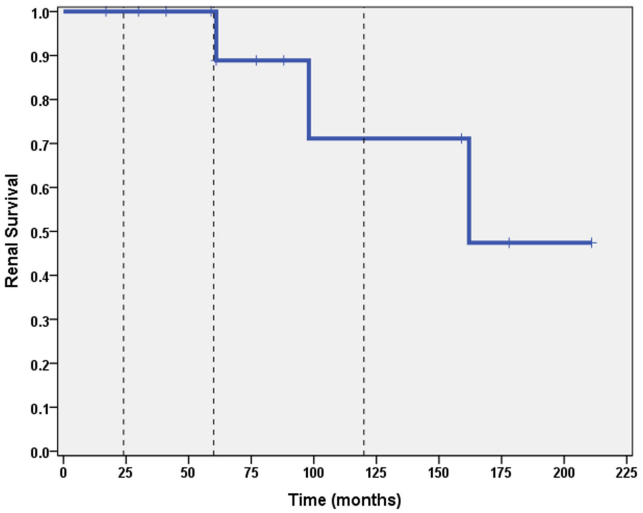

FIGURE 2 | Kaplan–Meier renal survival curve for calcineurin inhibitor responders. Vertical dashed lines represent time points of 2, 5, and 10 years for subjects who initially responded to CNI inhibitors.

with worse chronic kidney disease (CKD) (22–24). Several studies in pediatric ICU settings have demonstrated development of CKD after AKI in pediatric patients (25, 26). Given that patients with SRNS treated with CNIs, which may also be treated with angiotensin converting enzyme inhibitors or angiotensin receptor blockers, we hypothesized that this population is at high risk for developing AKI.

Our study did, in fact, confirm that AKI is a common occurrence among SRNS patients, with more than 80% of patients suffering an episode of AKI during their follow-up period and most patients having more than one episode of AKI. Exact etiology of these AKI episodes could not be determined retrospectively; however, the majority of AKI episodes occurred during time of relapse. Prior studies examining complications of nephrotic syndrome (not specifically SRNS) demonstrate that ~10% of all admissions for nephrotic syndrome are associated with AKI (27). Furthermore, we identified a significant association between AKI episodes and progression to ESRD. We hypothesize that these episodes of AKI lead to cumulative damage that increases the risk of progression to ESRD. This finding highlights the importance of rapidly identifying and managing even mild AKI in this population.

Several other studies have examined the outcome of SRNS but with important limitations. Otukesh et al. (15) examined 59 children with early steroid resistance and found a renal survival rate of 56% at 10 years; however, the study included both early and late steroid resistance and therapy with cyclophosphamide and mycophenolate mofetil in addition to cyclosporine A. Gellermann et al. (28) demonstrated effectiveness in achieving sustained remission using a combination of cyclosporine and mycophenolate mofetil in early SRNS secondary to FSGS with a 100% renal survival rate in their cohort. However, the study only included Caucasian children and at enrollment all patients were in remission via standard steroids, high dose methylprednisolone ± addition of cyclosporine if proteinuria did not improve. It is unclear how many of the given patients may have responded to the high dose steroids without the addition of cyclosporine and the inclusion of only a single ethnicity makes it difficult to extrapolate the results to other populations. Similarly, Hamasaki et al. (18) performed a prospective 5-year study examining SRNS and cyclosporine and found a renal survival rate of 94.3%. However, their study also was of a single ethnicity and the majority of the enrolled patients had minimal change disease with only 20% having FSGS. In addition, the shorter follow-up time compared to our study could have precluded identifying children who would still enter ESRD but at a later timepoint. Finally, in the largest series published to date, Zagury et al. (17) examined the outcomes of SRNS in their population within Brazil of 114 patients with early steroid resistance treated with cyclosporine A. They found a renal survival rate of 58.4% at 10 years and identified FSGS and cyclosporine resistance as predictors of progression to ESRD. These study, however, did not examine episodes of AKI.

As with several of the published studies, our study had important limitations, including its retrospective, single center nature, and the small sample size. In addition, the majority of our patients in this study were treated with cyclosporine A, so data on tacrolimus were limited. Target CNI levels varied throughout the study follow-up period at the discretion of the treating physician. Trough levels were examined; however, they were highly variable due to numerous factors including improperly timed collection, patient non-compliance, and impact of illness. Due to this variation analysis of the impact of CNI trough levels was unable to be performed. Finally, although we identified an association between AKI and progression to ESRD, it is possible that the increased number of AKI episodes may reflect more severe baseline disease that would be more likely to progress to ESRD, rather than being a causative factor of ESRD. Severity of baseline disease could be further assessed by FSGS histopathology; however, these data were unavailable in our study due to many biopsies occurring prior to the development of the classification scheme used to identify FSGS histological variants. Further studies examining the role of FSGS histopathology, AKI, and CKD progression are needed to address this concern.

In conclusion, this study demonstrates that treatment with CNIs can be effective in achieving sustained remission for idiopathic SRNS. Although short-term prognosis (<5 years) was generally good, our study found that long-term renal survival was less favorable, with 43% of CNI-responsive patients progressing to ESRD at 10 years. These findings are consistent with previously published data, which suggest that, although newer therapies are slowing the rate of progression, close to 50% will still reach ESRD during childhood/early adulthood (16–18). Notably, our study found that increased episodes of AKI were associated with increased risk of ESRD. While we were unable to establish causality, these findings provide, for the first time, support for a relationship between repeated AKI and progression to ESRD in SRNS A larger, multi-center prospective study would be necessary to further define long-term outcomes of a diverse population of children with SRNS responsive to CNIs to and determine the role, if any, that episodes of AKI may play in the progression to ESRD. The possibility of long-term adverse effects of AKI episodes in patients with SRNS emphasizes the importance of AKI prevention

in this population. Future treatment guidelines should stress the importance of AKI prevention through avoidance of dehydration, nephrotoxic medications, and prompt treatment of infections and relapse episodes.

AUTHOR CONTRIBUTIONS

NB performed the initial chart review, data collection, statistical analysis, and manuscript authorship. KD assisted with the study design, identification of study participants, interpretation of study results, and manuscript authorship.

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Long-term Outcomes of Childhood Onset Nephrotic Syndrome

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There are limited studies on long-term outcomes of childhood onset nephrotic syndrome (NS). A majority of children with NS have steroid-sensitive nephrotic syndrome (SSNS). Steroid-resistant nephrotic syndrome (SRNS) is associated with a high risk of developing end-stage renal disease. Biomarkers and analysis of genetic mutations may provide new information for prognosis in SRNS. Frequently relapsing and steroid-dependent NS is associated with long-term complications, including dyslipidemia, cataracts, osteoporosis and fractures, obesity, impaired growth, and infertility. Long-term complications of SSNS are likely to be under-recognized. There remain many gaps in our knowledge of long-term outcomes of childhood NS, and further study is indicated.

Keywords: nephrotic syndrome, children, pediatric, outcomes, genetics, focal glomerulosclerosis, minimal-change disease

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INTRODUCTION

Nephrotic syndrome (NS) is characterized by proteinuria, edema, hypoalbuminemia, and hyperlipidemia. The seminal studies of childhood onset NS were performed in the 1970s, in the International Study of Kidney Disease in Children (ISKDC) (1, 2). The ISKDC studies established the most likely pathologic diagnoses of childhood NS, as biopsies were performed at presentation in all 127 patients. Overall, the most common diagnosis ($n = 95/127$) was minimal-change disease (MCD), which was present in 94% of the children with steroid-sensitive nephrotic syndrome (SSNS) (1). In steroid-resistant nephritic syndrome (SRNS), focal glomerulosclerosis (FSGS) was the most common histopathologic lesion (1). The ISKDC established that the majority of children with NS (80%) respond to corticosteroid treatment (1, 2). Thus, the ISKDC established the paradigm of treating all children presenting with NS with corticosteroids and only performing biopsies in steroid resistant patients (2).

Since the ISKDC, the majority of studies looking at outcomes classified patients by response to corticosteroids or other therapy and by NS relapse. The least severe course with SSNS is associated with few or no relapses. More complicated are those with SSNS with frequent relapses (FR = >2 relapses over a 6-month or >3 over a 12-month period) or steroid dependence (SD = relapses during treatment or within 2 weeks of stopping corticosteroids) (3). Some patients with initial steroid response develop SRNS (late non-responders) and others present with SRNS. Finally, there are the most severe cases of NS that are both corticosteroid and other treatment resistant. Long-term renal-related complications of childhood NS would include NS relapse in adulthood, hypertension, chronic kidney disease (CKD), and end-stage renal disease (ESRD). Long-term non-renal complications include complications of corticosteroids and other immunosuppressant medications, including effects on growth, bone health, fertility, and risk for malignancy. An additional consideration are psychosocial issues associated with NS. As with many chronic diseases, NS can represent a barrier to attaining educational degrees and employment and/or developing stable relationships/marriage. Indeed, a quality-of-life (QOL) survey of children with NS in the National Institutes of Health Focal

Segmental Glomerulosclerosis (NIH FSGS) clinical trial revealed that children with SRNS had poor QOL scores similar to children on dialysis (4).

Here, we will review the current data on long-term NS outcomes, potential new predictors of treatment and renal outcomes, and gaps in our knowledge that warrant further investigation.

OUTCOMES IN SRNS

It is clear that the worst outcomes occur in SRNS, with 34–64% progressing to ESRD within 10 years of diagnosis (5–8). The majority of SRNS patients are treated with second-line agents, such as calcineurin inhibitors and other immunosuppressant medications. There is a highly variable response, which may depend upon the population studied. For example, studies of calcineurin inhibitors report divergent response rates of between 25 and 75% in children with SRNS (9). This variability in response to calcineurin inhibitors likely reflects in part differences dependent on the underlying histopathology of the SRNS, with MCD more likely to respond than FSGS. Other factors, such as race or ethnicity of the population being studied, may also affect likelihood of response (10). In particular, African-American and Hispanic children with FSGS have poorer outcomes, with one study demonstrating 50% progress to ESRD within 3 years (11, 12). What is clear is that response to any therapy is a major prognostic factor (13). Abeyagunawardena et al. examined 10-year outcomes of 66 children with SRNS. Response to therapy was associated with 90% renal survival, while almost 50% with failure to respond to therapy had progressed to ESRD (13). Response to second-line immunosuppressant therapy and risk of progression may differ in children who initially respond to therapy, but then become non-responsive (late non-responders). Straatmann et al. demonstrated that up to 87% of late non-responders in a multicenter study initially responded to a calcineurin inhibitor (14). However, long term, 31% of the cohort became non-responsive, and had decreased renal function and increased progression to ESRD compared to those that remained treatment responsive (14).

BIOMARKERS TO PREDICT RESPONSE TO THERAPY

One of the largest areas of research has been the search for soluble factors that may be predictive of treatment response and may also serve as targets for future therapies. This interest was partly spurred due to studies showing that serum from patients with FSGS can induce proteinuria in rats and increase the glomerular permeability of isolated glomeruli (15). In addition, plasmapheresis has been able to induce remission in some patients with recurrent FSGS post transplant (16, 17).

One circulating factor that has been studied is the soluble urokinase plasminogen activator (suPAR). Wei et al. showed in a study that 94 children with FSGS and 70 adults and children with FSGS had elevated levels of suPAR when compared with healthy controls (18). Peng et al. then showed, in a group of 176 children with idiopathic NS followed for approximately 30 months, that suPAR levels were able to predict steroid resistance (19). However, these results have not been widely reproducible, questioning the role of suPAR to distinguish FSGS from other glomerular diseases (20–22). For example, Sinha et al. in a cross-sectional cohort study of 83 healthy pediatric controls versus 469 patients with NS, 138 of which had SSNS, was not able to show an association of suPAR levels with either SSNS versus SRNS. In the same study, suPAR levels were not significantly associated with the diagnosis of FSGS (22). This study demonstrated that suPAR had an inverse correlation with glomerular filtration rate, and some have proposed it may be a predictor of renal progression (22, 23).

Other investigators have looked to evidence of immune activation to predict response to immunosuppressive treatment. One study of 26 children with idiopathic SSNS that were followed for less than a year showed a differential pattern of regulatory Th1 and Th2 cells in patients in remission versus in relapse (24). Another study of 46 children with NS demonstrated that T-cell glucocorticoid receptor expression was higher in patients with NS responsive to steroid therapy and in patients with MCD, when compared to patients with FSGS (25). In one study of 17 patients with MCD and 22 with FSGS, urinary CD80 (present on antigen-presenting cells and podocytes) was able to distinguish MCD from FSGS. Another investigator determined that the urinary CD80 level associated with disease remission (26, 27). In a search for novel, yet unrecognized, markers of both steroid response and disease progression, investigators have used unbiased approaches to examine gene expression, the serum and urine proteome, and the metabolome (28–30). One group took serum from blood of 33 children with idiopathic SSNS at presentation, in remission while on medication, and in remission once medication was stopped. Evaluating serum peptide levels at all time points, they identified a novel marker, apolipoprotein AII. The levels of apolipoprotein AII correlated with degree of proteinuria at presentation and decreased with remission (31).

All these studies are limited by small numbers of patients and most are single-center studies. Poor reproducibility has plagued efforts to identify biomarkers in NS (32, 33). Thus, newly available large cohorts of NS patients, such as Podonet (34), NEPTUNE (35), and Cure GN that include well-characterized pediatric patient populations of NS are critical for discovery and validation of new biomarkers.

GENETIC FACTORS IN PREDICTING OUTCOME IN NS

One of the new factors that may predict response to therapy and renal outcomes are genetic variants of NS. Genetic mutations are most likely to be identified in congenital nephrotic syndrome (CNS). Mutations in NPHS1, NPHS2, LAMB2, and WT-1 were identified in two-thirds of a largely European cohort of 89 infants with NS under the age of 1 (36). The overall average age of ESRD was 5.6 years. While the numbers were small and many outcomes were not known, patients with NPHS1 mutations had ESRD at an average age of 4.6 years, whereas those with NPHS2 mutations had ESRD at an average age of 7.4 years (36). It is possible these differences could be explained by differences in clinical course

and management of congenital NS (e.g., nephrectomies), rather than the genetic basis of disease. However, it suggests that additional studies should address whether specific genetic mutations correlate with outcomes in early onset NS.

There are well over 40 genetic mutations associated with FSGS, and new mutations continue to be identified (37). A single-gene mutation may be identified in up to 29% of patients with SRNS onset prior to age 25 (38). Patients with genetic mutations are less likely to respond to immunosuppressant therapy and more likely to develop ESRD (39). One of the largest studies by Buscher et al. examined renal outcomes of at 10-year follow-up of 231 children in a European cohort with SRNS. For those presenting after 3 months of age, 58% children with SRNS associated with genetic mutations had progressed to ESRD, versus 29% with SRNS and no genetic mutation identified (39). Recently, genetic variants in *APOL1* were identified as risk factors for renal disease in people of African descent. Carrying two copies of *APOL1* coding variants (G1 and G2) is a risk factor for hypertensive nephropathy, lupus nephropathy, FSGS, and HIV nephropathy (40). Recent analysis of the FSGS Clinical Trial, a NIH supported randomized controlled trial comparing MMF to cyclosporine in children and young adults (41), demonstrated that 67–72% of 94 patients of African descent in the study harbored two copies of the *APOL1* risk variants, and they were more likely to progress to ESRD, with 40% reaching ESRD within approximately 8 years (42). Of note, APOL1 risk variants did not associate with response to therapy, although a small percentage responded to either therapy (41). Many genetic analyses, to date, have focused on largely European and homogeneous populations; the cohorts of NEPTUNE (35, 43) and CureGN provide an opportunity to understand the contribution of gene–gene and gene-environmental interactions in modulating long-term outcomes.

Recently, two genes have been identified associated with treatment sensitive NS. Epithelial membrane protein 2 (EMP2) was identified in familial SSNS (44). While only a few patients were identified, the majority had no renal failure after over 20 years of follow-up (44). Overall, one can be optimistic that, in the future, identification of specific genetic mutations may help guide therapy and provide prognostic information to families.

LONG-TERM COMPLICATIONS OF STEROID-SENSITIVE NEPHROTIC SYNDROME ARE LIKELY UNDER-RECOGNIZED

There is increasing focus on a life-course approach to optimizing health outcomes, with recognition that exposures in childhood establish the risk for adult-onset disease (45). The vast majority of children will have SSNS and MCD, which is thought to have benign long-term outcomes. However, recent studies have suggested that other conditions that were thought to be benign, such as congenital solitary kidneys, are associated in increased risk of hypertension and ESRD in adulthood (46). The increased risk of ESRD required more than 30 years to manifest, and thus was unlikely to be detected without a concerted effort to study outcomes in these patients (46). Potential long-term complications of SSNS are likely to be under-recognized, as patients are often lost to follow-up.

OUTCOMES IN SSNS

There are a handful of studies looking at long-term outcomes of childhood onset SSNS (**Table 1**). All studies include either exclusively or mostly FR/SD NS patients.

RISK OF RELAPSE AS AN ADULT

An early study by Trompeter et al. suggested that only 5% of children will have persistent relapses as adults (47). However, several recent studies suggest that FRNS is common, and relapses may persist into adulthood. Esfahani et al. examined the course of NS of over 200 children from Iran with follow-up between 5 and 20 years (52). Eighty-three percent experienced relapses in childhood and over 50% required additional immunosuppressive treatment, likely indicating FR/SD NS. Less than half the cohort sustained a remission for more than 3 years. Similarly, Ishikura et al. performed 10-year follow-up of a randomized controlled trial of cyclosporine for FR/SDNS in Japan. Fifty percent continued to relapse and remain on immunosuppressives in adulthood (50).

TABLE 1 | Long-term complications of childhood SSNS.

	Complication	Reported prevalence	Comment
Renal	Relapses in adulthood	5–40% (47, 48)	Higher risk with early onset, more frequent relapses in childhood
	Decreased GFR/ESRD	<1% at 20-year follow-up (48)	
	Hypertension	6–46% (49, 50)	Highest in FRNS with adult relapses
Immunosuppression-related	Overweight/obesity	8–23% (50, 51)	
	Growth failure	8–16% (48, 50)	
	Osteoporosis	13–63% (49, 50)	Highest in FRNS with adult relapses
	Fractures	20% (51)	
	Cataracts	6–20% (49, 50)	Highest in FRNS with adult relapses
	Infertility	Up to 75–94% (49, 51)	Highest with cytotoxic therapy
	Malignancy	Unknown	
Psychosocial	Educational status and employment	<10% failure to complete high school and <45% unemployed (51)	Single-center study
	Marital status/stable relationships	<40% unmarried or not in stable relationship (51)	Single-center study

Fakhouri et al. studied 102/117 children admitted with SSNS in France using mailed questionnaires or phone calls to patients and/or their parents or attending physicians (48). More than 40% of the patients relapsed in adulthood (48). Younger age of NS onset, second-line immunosuppressant therapy, and more severe disease in childhood were associated with increased risk of relapse in adulthood. However, relapse rate in the first 6 months of disease was not predictive of adult relapses. Kabuki et al. also examined age of presentation effects on risk of relapse in 60 patients diagnosed with NS before age 10 in Japan with over 10 years follow-up (53). The majority (51/60) patients were relapse-free over 3 years. Early presentation (between age 1 and 3.9 years) was associated with more relapses and a longer interval between onset of NS and long-term remission. Conversely, older onset (after age 7 years) was associated with less active disease at 10 years follow-up.

Skrzypczyk et al. examined outcomes of 61/118 children with NS in Poland using a written questionnaire (51). Over 90% were SSNS and only 30% FRNS. However, 77% required second-line immunosuppressive therapy in addition to steroids. The patients had particularly benign courses in that all patients achieved remission and had normal renal function at the age of 18. Still 16% relapsed in adulthood, and risk of relapse as adult was associated with relapses as a child.

One of the longest follow-up studies was by Ruth et al. on 42 children with NS from Switzerland with a median follow-up of 22 years. Thirty-three percent of patients had relapses as adults. Number of relapses during childhood and adolescence and requirement for second-line therapy were risks for relapses in adulthood (54).

RENAL AND NON-RENAL COMPLICATIONS

Several of these studies examined long-term complications of immunosuppression. Ishikura et al. identified that on 10-year follow-up of exclusively FR/SDNS patients, a substantial number had short stature (13%), osteoporosis (13%), obesity (8%), cataracts (6%), and hypertension (6%) (50). Fakhouri et al. obtained information in their SSNS patients with 40% rate of relapse in adulthood (48). Of them, 7% had hypertension, and 1 out of the 102 patients had developed ESRD. The most common complications were likely long-term side effects of corticosteroids: 16% had short stature, 20% were overweight or obese, and 63% had osteoporosis.

One of the few direct assessments of long-term outcomes was performed by Kyrieleis et al. in 15 adult patients from the Netherlands with history of childhood onset FRNS who were still relapsing as adults (mean follow-up 24 years). Forty-six percent of patients had hypertension. Dual-energy X-ray absorptiometry identified osteoporosis in one-third of patients. Ophthalmologic examination identified cataracts in 3/15 (20%) of the patients. Six out of eight (75%) had abnormal semen examination with oligozoospermia, reduced sperm motility, and teratozoospermia.

The study of 61 patients with childhood NS from Poland by Skrzypczyk et al. demonstrated a substantial number of immunosuppressant-related complications. Sixteen percent were hypertensive, 23% were overweight, and 4.9% obese. Almost 20% had bone fractures and 8% had short stature (height <third percentile). Childlessness was more common (94%) in those treated with cytotoxic agents versus those not treated with cytotoxic agents. No increased risk of malignancy, infection, or cardiovascular events was detected.

Skrzypczyk et al. was one of the only studies to examine long-term psychosocial impact of NS as a chronic disease (51). In their follow-up study, 60.7% patients were in a steady relationship/married, 40.9% pursued higher education, and only 6.6% did not complete a high school (secondary) education. At the time of the study, 55.7% patients reported active employment.

Of note, none of studies that examined GFR observed a change (49, 51). The study with the longest follow-up (22 years) by Ruth et al. identified no effects on growth, obesity, or GFR, but did identify an effect on fertility (54). Only 8/42 patients had children, and cytotoxic therapy was associated with childlessness (54).

Nephrotic syndrome leads to endothelial dysfunction in children (55) and engenders a risk for artherosclerosis in adults (56, 57). In one study of 30 patients with childhood onset SSNS, dysregulation of lipids, including increased total cholesterol, low-density lipoprotein cholesterol, and homocysteine were persistent on follow-up at 4–15 years after completion of steroids (57).

GAPS IN OUR KNOWLEDGE OF LONG-TERM OUTCOMES OF SSNS

The majority of the above studies are retrospective chart reviews or utilized surveys, with the inherent biases of those types of study design. Virtually, all studies lack a control population, which is relevant for outcomes, such as obesity and hypertension, which may be common in the adult population. Often they represent a single-center experience and have small numbers. Even the largest studies report outcomes from countries with homogenous populations, with limitations in their generalizability to ethnically diverse populations. Finally, the average length of follow-up in most studies was approximately 10 years, well short of the 30-year follow-up required to identify effects of a solitary kidney on ESRD outcomes (46).

It seems possible that, as with other conditions previously thought to be entirely benign, SSNS may engender increased risk of hypertension, atherosclerosis, CKD, and ESRD in adulthood (**Table 2**). In addition, there are insufficient studies of patient-reported outcomes, including QOL assessments. There are limited data on implications of childhood NS on educational attainment, employment, and marital status. Better understanding of potential adverse consequences may allow for interventions targeting improvement of both patient-reported and psychosocial outcomes. Our lack of knowledge limits effective transition of children with NS to adult providers. Those with few or no relapses are likely not to follow-up or to follow-up rarely with a pediatric nephrologist as they approach adulthood. An adult nephrologist is unlikely to follow these patients, as the majority will not have renal impairment at entry into adulthood. So the major question becomes how and when to communicate the potential for long-term issues with adult providers. One study in Japan demonstrated that the majority of practitioners had no transition plan in place for children with SSNS (58).

TABLE 2 | Gaps in knowledge.

Gaps in knowledge	Opportunities to address
Life-course effects of childhood NS Are there risks of SSNS after 30 years? Are patients with childhood onset NS at higher risk of hypertension, CKD, and ESRD?	Registries of childhood onset nephrotic syndrome cNEPTUNE (pediatric cohort of incident nephrotic syndrome patients)
Genetic factors and biomarkers Can permeability factors or other biomarkers enable personalized approach to treatment? Can screening for genetic mutations or APOL1 variants in childhood onset NS stratify those children at highest risk for hypertension and renal disease in adulthood?	Multicenter collaborative cohorts of pediatric onset NS
Psychosocial impact and patient-reported outcomes Quality of life is significantly affected in childhood onset NS, particularly with severe disease. What is the effect on patient-reported outcomes? What is the long-term effect on educational status, employment and marital/stable relationships and are there interventions to improve outcomes?	Multicenter collaborative cohorts of pediatric onset NS
Transition into adulthood (1) When should children with NS receive counseling about potential risks in adulthood? (2) How will adult providers be made aware of potential for complications in adults with a history of childhood NS? (3) Are screenings for artherosclerosis, osteoporosis, cataracts, infertility, and/or malignancy indicated? (4) What is the optimal transition plan for children with SSNS as they enter adulthood?	Registries and multicenter collaborative cohorts of pediatric onset NS

Patient registries and follow-up of pediatric cohorts, such as cNEPTUNE (incident children with NS), may help provide answers to these questions.

CONCLUSION

Underlying renal pathology, genetic factors, and ethnicity likely modulate response to treatment and progression of ESKD. Well-characterized and prospectively followed cohorts provide an exciting opportunity to improve our understanding of the ability of biomarkers and genetics to predict outcomes. Many of the long-term complications of childhood SSNS can be attributed to immunosuppressant therapy. Long-term effects on endothelium and renal function are likely understudied. Long-term studies of childhood onset SSNS patients, perhaps by patient registries, are needed to understand the true risk of the disease to adult health.

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Changing the Paradigm for the Treatment and Development of New Therapies for FSGS

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Focal segmental glomerulosclerosis (FSGS) is a renal pathology finding that represents a constellation of rare kidney diseases, which manifest as proteinuria, edema nephrotic syndrome, hypertension, and increased risk for kidney failure. Therapeutic options for FSGS are reviewed displaying the expected efficacy from 25 to 69% depending on specific therapy, patient characteristics, cost, and common side effects. This variability in treatment response is likely caused, in part, by the heterogeneity in the etiology and active molecular mechanisms of FSGS. Clinical trials in FSGS have been scant in number and slow to recruit, which may stem, in part, from reliance on classic clinical trial design paradigms. Traditional clinical trial designs based on the "learn and confirm" paradigm may not be appropriate for rare diseases, such as FSGS. Future drug development and testing will require novel approaches to trial designs that have the capacity to enrich study populations and adapt the trial in a planned way to gain efficiencies in trial completion timelines. A clinical trial simulation is provided that compares a classical and more modern design to determine the maximum tolerated dose in FSGS.

Keywords: FSGS, nephrotic syndrome, therapy, adverse effects, clinical trials

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INTRODUCTION

Focal segmental glomerulosclerosis (FSGS) manifests with proteinuria, hypertension, and in the worse cases progresses to kidney failure. FSGS is a renal pathology finding that represents a constellation of rare kidney diseases and results in a significant public health burden accounting for 5% of adults and 12% of children with incident end stage kidney disease (ESKD) in the US annually (1). Broadly, FSGS describes a kidney scarring pattern that occurs in a focal and segmental pattern, but does not describe the underlying pathophysiology. FSGS is often classified as primary or secondary (e.g., following systemic illnesses IgA nephropathy, post-infectious glomerulonephritis). Genetic causes of FSGS may represent a distinct type that does not fit well into this classification. In general, the distinction between primary, secondary, and genetic causes of FSGS have classically driven therapeutic decision-making and clinical trial design. However, emerging precise molecular mechanisms may represent distinct endophenotypes of FSGS, which may also vary temporally by disease initiation, maintenance, or progression.

Despite the significant patient and health burden, there is a paucity of therapeutic options for those with FSGS. Therapies available include immunosuppression, renin–angiotensin–aldosterone blockade, lipid lowering agents, and other blood pressure lowering agents as necessary. Unfortunately, the available immunosuppression therapies have a significant toxicity profile that may be dose limiting. Side effects, such as those altering physical appearance (e.g., alopecia, hirsutism, and weight gain) or physical function (e.g., weakness, tremor, and infertility), may contribute to poor adherence. These decisions are further complicated by those with monogenetic forms of FSGS, who may respond to immunosuppression therapy, but at very low rates (2).

A number of underlying biological mechanisms, multiple causes of FSGS, and side effect profiles contribute to the present day challenge of identifying effective and acceptable treatments. Globally, research teams are seeking a better understanding of the underlying biological mechanisms of subgroups of patients with FSGS that may provide targets for future therapy (3). This paper will provide a summary of commonly used therapies for FSGS and present strategies for successful clinical trial design to support the testing of novel agents.

CURRENT THERAPIES FOR FSGS

A majority of therapies for FSGS (**Table 1**) have been either tested in phase 2 trials, but not used for registration (product labeling) or utilized without significant evidence as a treatment of last resort (3–7). The published estimates of efficacy vary widely from 25 to 69% across agents. Recent evidence supports that certain patient populations have a lower likelihood of responding to therapy. For instance, individuals with high risk APOL1 genotypes, found in individuals of African ancestry, have a higher likelihood of unfavorable outcomes (continued proteinuria, ESKD) (8–11). Furthermore, efficacy may also be predicted by response to prior therapy, such as glucocorticoids. Differences in such patient characteristics have important implications in study interpretation and trial design.

TABLE 1 | Commonly used therapies for FSGS in 2015.

	Proteinuria remission (%)	Cost (1)	Monitoring (2)
Corticosteroids	25–59	$	+
Calcineurin inhibitors			
Cyclosporine	46–69	$$$	++
Tacrolimus	[a]	$$$	++
Mycophenolate	33	$$	++
Cyclophosphamide	27–55[b]	$	+++
ACTH	29[a]	$$$$$	+
Rituximab	38[b]	$$$$	+

(1) Cost comparison of these agents is based on a course of therapy, with monthly costs ranging from the approximately $30 ($) to $46,000 ($$$$$).

(2) Monitoring frequency is labeled for frequency to screen for side effects, drug level, and therapeutic effects. Increasing number of + are used to show increasing frequency of standard lab monitoring.

[a]The expected response to calcineurin inhibitors is approximately the same. Publications that have compared the two show similar efficacy but a worse adverse event profile with cyclosporine.

[b]Results are reported from steroid resistant and steroid sensitive patients.

The initial selection of an appropriate therapeutic regimen by the treating physician is related to the anticipated likelihood of disease control (proteinuria resolution, preservation of kidney function) and the safety profile of the therapeutic agent. In the absence of more precise biomarkers, the subsequent tailoring of therapeutic regimens for patients are driven by disease characteristics (treatment response), side effect profile, or cost/convenience factors. **Table 2** summarizes the common side effects that may influence differential treatment selection.

CURRENT CLINICAL TRIAL DESIGNS AND STATISTICAL CONSIDERATIONS FOR RARE DISEASES

Since the Orphan Drug Act was passed in 1983, an increased number of drugs and biologics have been approved for rare diseases in the US (12). An orphan drug is defined as one targeted toward rare diseases (prevalence <200,000 persons) or disease with greater prevalence but for which the cost of drug development is expected to not be recoverable from US sales (13). In Europe, rare diseases are defined as life-threatening or chronically debilitating conditions that affect ≤5 in 10,000 people in the EU (Official Journal of the European Union 2009/C 151/02).

Although there is an ethical imperative to hold clinical trials in rare diseases to rigorous ethical and scientific standards, analyses of rare vs. non-rare clinical trials indicate that there are differences in design characteristics. Trials in orphan drugs are more likely to be smaller, non-randomized, lack blinding, and use disease response instead of progression or survival endpoints (14–16).

CHANGING THE PARADIGM

The utilization of traditional clinical trial designs may not be appropriate for rare diseases. Fortunately, a myriad of design and analysis options (see **Table 3**) exist in the clinical trials and statistical literature that allow for the study of drugs in rare diseases, such as FSGS, that meet stringent effectiveness and safety standards. A clinical trial development program of studies in FSGS should result in evidence that provides confidence for

TABLE 2 | Common side effects reported with treatment of FSGS.

Medication	Common side effects
Corticosteroids	Weight gain, hyperglycemia, hypertension, osteopenia, mood changes, weakness
Calcineurin inhibitors[a]	
Cyclosporine	Hypertension, gingival hyperplasia, hypertrichosis, infection
Tacrolimus	Hypertension, infection, tremor
Mycophenolate	Nausea/diarrhea, leukopenia, teratogenic, infection
Cyclophosphamide	Nausea, leukopenia, infection, alopecia, teratogenic
ACTH	Weight gain, hypertension, rash, acne, hypertrichosis, mood changes, weakness
Rituximab	Infusion reaction, infection, leucopenia

[a]Calcineurin inhibitors have an uncommon side effect of nephrotoxicity, which may influence clinicians and patients about initial use or duration of therapy.

TABLE 3 | Brief glossary of clinical trials terms.

Term	Brief description
3 + 3 trial design	A conventional and popular phase 1 dose escalation design that estimates the MTD[a] by sequentially studying cohorts of size 3
Adaptive design	A clinical study design that uses accumulating data to decide how to modify aspects of the study as it continues, without undermining the validity and integrity of the trial (17)
Bayesian methods	Bayesian methods use prior information on the differences between treatments before the trial is completed, and update this information based on data obtained from the trial. The difference between treatments is not a single fixed parameter in the Bayesian approach; rather, a distribution of potential values characterizes treatment differences
Continual reassessment method (CRM)	CRM is an adaptive dose-finding study design that uses Bayesian methods to estimate the MTD. It frequently results in fewer adverse events and more accurately estimates the MTD
Crossover design	A clinical trial design in which participants receive a sequence of different treatments, resulting in within-subject comparisons that generally reduced the required sample size. This design is in contrast to the parallel-group design where participants receive only one protocol-specified treatment
Dose limiting toxicity (DLT)	Severe but (ideally) reversible adverse events that occur within a generally short protocol-defined period
Frequentist methods	A framework of statistical inference that is generally taught in most introductory statistical courses, that treats the difference between treatments as an unknown and fixed parameter. Clinical trial results are considered from the perspective of multiple independent repetitions of the experiment which sometimes cause difficulties in the interpretation of results
"Learn and confirm" clinical trial paradigm	An alternative to the traditional "phased" approach to drug development (i.e., phase 1, 2, and 3). The goal of the learning phase is to assess the relationship between the dose and administration of a new drug and its expected efficacy and safety. The goal of the confirming phase is to capitalize on the more complete information obtained in the learning phase to efficiently study the risk-benefit of the new agent
Maximum tolerated dose (MTD)	The highest dose of a drug or treatment that does not cause unacceptable side effects (18)
N-of-1 design	Single-subject clinical trial that has the goal of determining the best intervention for an individual patient based on objective criteria
Seamless trial designs	Clinical trial designs that address, within a single trial, objectives that are normally achieved through separate trials

MTD, maximum tolerated dose.

patients, regulators, investigators, and clinicians, given the current therapeutic options and knowledge base.

Classical drug development employs a "learn and confirm" paradigm over a series of steps. The drug development pipeline begins with discovery with preclinical *in vitro* and *in vivo* animal experiments and toxicology studies. Phase 1, phase 2, and proof-of-concept studies in human participants follow in the exploratory phase, with (generally) two adequate and well-controlled phase 3 studies conducted in the full development confirmatory phase. After approval, post-marketing phase 4 studies are often conducted to provide additional characterization of the efficacy and safety in a broader patient population. Generally, each step involves a separate study during the human studies stage.

This classical drug development process is often not feasible in rare diseases. In this setting multistage designs, particularly adaptive designs, and seamless phase 1/2 or phase 2/3 trials may be used to maximize information while minimizing the strain on the available patient population [based on Orloff et al. (61)] (**Figure 1**). Adaptive designs refer to studies that include "a prospectively planned opportunity to modify one or more specified aspects of the study design and hypotheses based on analysis of study data (usually interim data)" (19). Adaptive trials have been a recent topic in the clinical trials literature, with seminal papers in 2006 (17, 20–22); however, the concept has been around since the 1970s in medical trials with adaptive randomization and sequential designs (23). In addition, Bayesian methods offer an alternative statistical approach to inference (relative to the frequentist or classical approach) that treats probability as a measure of the degree of personal belief instead of the frequency achieved in long-run repetitions of an experiment. Bayesian methods have gained acceptance since the early 2000s, with use of the phase 1 continual reassessment method (CRM; Bayesian in everything but name) (24), and Bayesian stopping rules in phase 2 trials (25). The literature on trial design in rare diseases or small sample size situations is redolent with suggestions for use of Bayesian methods (26–29).

These novel designs have been successfully used in a variety of therapeutic areas, including rare and common diseases. A modification of the time-to-event CRM approach (TITE-CRM) was used in a phase 1 study of continuous MKC-1 in patients with advanced or metastatic solid malignancies (30). The study identified a maximum tolerated dose by studying 24 patients, and concluded that the adaptive Bayesian design allowed for a more efficient dose escalation and also allowed for late toxicities. Kaufmann et al. (31) designed a multicenter adaptive two-stage phase 2 trial of CoQ10 in amyotrophic lateral sclerosis (ALS) to identify an appropriate dose in stage 1 and then compare the selected dose against placebo for futility in stage 2. They concluded their adaptive design avoided the need for a much larger conventional phase 3 trial. Finally, an adaptive seamless phase 2/3 randomized trial of dulaglutide combined with metformin in type 2 diabetes patients efficiently (32) explored a large number of doses and selected two doses for head-to-head comparison with sitagliptin; both dulaglutide doses demonstrated superior glycemic control (33).

Study Population

A common approach to the testing of novel agents for FSGS or other conditions will select a patient sample that has demonstrated

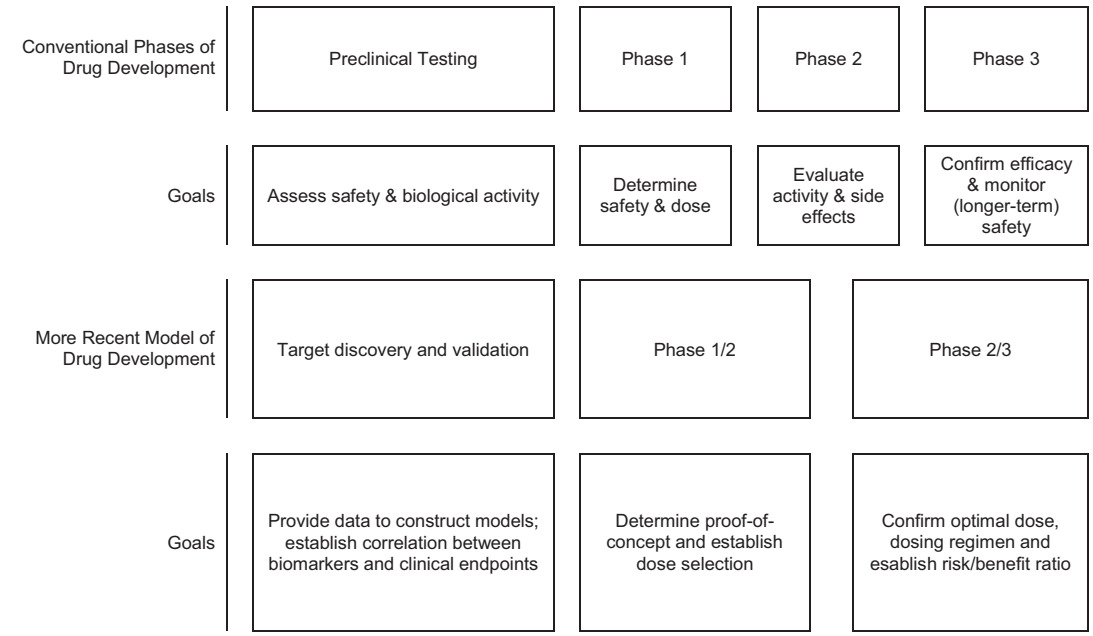

FIGURE 1 | **Models of drug development**.

resistance to standard therapies, positioning the novel agent as a salvage therapy. Depending on the investigational drug target, this approach may doom the agent to failure as the patients on study may have already entered a late or irreversible phase of the disease. FSGS targeted therapies may be best suited to patients in early or mid-disease where the potential for the drug to demonstrate activity against a molecular target can be shown. Enrichment designs represent a unique opportunity in FSGS by selecting patients who are more likely to respond to therapy, based on specific biomarkers. Such designs reduce sample size by reducing patient heterogeneity, improving the chance of successful enrollment. The development of biomarkers and targeted agents targeted agents in earlier phases of drug development in FSGS should help in assessing whether this strategy would be advantageous in later confirmatory stages (34).

Finally, FSGS affects patients of all ages. As the implications of uncontrolled FSGS resulting in kidney failure are similar across the lifespan, drug development strategies should include children in every setting where drug safety has not shown specific additional risk in immature preclinical testing.

Endpoints

The goal of FSGS therapies is the normalization of urinary protein excretion [measured urine protein/creatinine ratio (UP/C)], preservation of kidney function (3), restoration of the patient health, and avoidance of adverse events (3). Efficacy endpoints currently being used in many FSGS trials include the proportion of participants who achieve complete remission (UP/C <0.3 g/g and preserved kidney function) and partial remission (50% UP/C reduction and <3 g/g and preserved kidney function). These endpoints have been justified based on two retrospective observational studies demonstrating the relationship between remission status and long-term renal survival (35, 36). The timing for achieving remission is generally assessed at 3–6 months after initiation of study medication. Characterizing response as a binary outcome generally leads to larger sample sizes (37). Smaller clinical trials may be achievable in the confirmatory stages of development in FSGS, for similar Type I and Type II error rates, if UP/C is analyzed as a continuous outcome. For example, multiple measures of UP/C can be collected with analyses based on longitudinal methods [see, for example, the approach of Greene et al. (38) for eGFR endpoints]. Research is needed to better understand the differences in UP/C that are clinically meaningful and would result in changes in clinical practice.

Learning and Confirmatory Phase Goals and General Considerations

During the learning phase of drug development in FSGS, we investigate the correct dose range by investigating preliminary efficacy and ensuring that the drug meets minimal requirements for dose-limiting toxicity and tolerance. In the confirmatory phase, the goal is to obtain sufficient efficacy and safety information in well-controlled trials to support its acceptance. The control group may be placebo (superiority trials) or an active control (superiority, non-inferiority, or equivalence trials). The highest levels of medical evidence are achieved through the use of randomization, blinding, and concurrent controls, and there should be strong rationale for *not* using these features in FSGS trials.

Crossover designs and N-of-1 trials have been suggested for rare diseases (27) because they reduce sample size by allowing for within-subject treatment comparisons. In FSGS confirmatory trials, where the endpoints may be measured at 6 months or later,

there are design implications of period effects where the disease is not stable over the time course of the two treatment periods.

The impact of design and analysis decisions should be fully evaluated. Frequentists calculate sample size based on the hypothesis testing framework that specifies the type I error, power, and expected treatment difference. The Bayesian approach does not employ a strict need to calculate sample size because the goal is to update prior beliefs about the null hypothesis with the data. In past FSGS trials, the traditional, equal-allocation, fixed sample-size design was most commonly used. Clinical trials simulations can be used for future trials to assess the tradeoffs between frequentist and Bayesian options, assessing characteristics of the design for specific agents and phases, such as probability of stopping for futility incorrectly and sample size options to achieve a certain decision criterion.

Adaptive Designs

An adaptive design is defined as "a clinical study design that uses accumulating data to decide how to modify aspects of the study as it continues, without undermining the validity and integrity of the trial" (17). An adaptive design requires access to accumulating data at multiple stages of the trial (20). Adaptation rules applied at each interim analysis may affect: how a subject will be allocated to the available arms of the trial, how many subjects will be sampled at the next stage, when to stop the trial for efficacy, harm or futility, and other decision features of the trial (20). An important prerequisite for an adaptive trial is that the accumulating information on the end point can be assessed quickly enough to trigger the adaptive decisions relative to the enrollment rate. FSGS trials typically meet this criterion because the prevalence of the disease results in slow enrollment and normalization of urine protein excretion can be observed as early as 2–3 months depending on the agent under investigation.

In early phases of FSGS development, the following adaptive approaches may be most useful:

- *Adaptive dose finding* is used in early phase clinical development to identify the minimum effective dose and/or the maximum tolerable dose, which is used to determine the dose level for the next phase clinical trials. In particular, the continual re-assessment method allows assessment of dose-limiting toxicity in small cohorts at a given dose (one to four patients commonly), updating the dose-toxicity curve, and treating additional cohorts until a pre-specified level of certainty is achieved (39).
- *Drop-the-loser or play-the-winner design* allows for dropping an inferior treatment group or maintaining a superior treatment group. A drop-the-losers design also allows adding additional arms. These designs would be useful in FSGS when there are uncertainties regarding the dose levels for a new agent. These two approaches can be considered types of adaptive randomization methods, where the probability of being assigned to a dropped arm is 0 (see below).
- *Adaptive seamless 1/2 trial designs* address within a single trial objectives that are normally achieved through separate trials in phase 1 and phase 2 of clinical development.

Adaptive designs that may be more useful during later stage drug development in FSGS include

- *Sequential designs* allow for prematurely stopping a trial due to safety, futility, or efficacy (benefit) with options of additional adaptations based on results of interim analysis. Types of sequential designs include group sequential designs that employ repeated significance testing at pre-specified interim analysis times and boundaries approach where the amount of information and treatment effect size are assessed multiple times during the study (40–42). With these designs, the final sample size is unknown at the trial initiation, but sample sizes are generally smaller than a classical fixed sample size approach (40).
- *Sample size re-estimation design* allows for sample size adjustment based on interim analyses. When limited information is available *a priori* on the variance of the outcome (e.g., variance of change from baseline to month 3 in UP/C) or the estimated treatment effect (e.g., proportion of participants who achieve complete remission), sample size re-estimation is attractive. A common method is to calculate the conditional power that a treatment difference will be observed at the end of the trial, given the current information. Hybrid methods are often used where the trial may continue with the original sample size if the conditional power if sufficient, may be stopped for futility if the increase is sample size is too great, or may be enlarged if neither of the other conditions is met (43).
- *Adaptive randomization design* allows modification of randomization schedules based on varied or unequal probabilities of treatment. It is a type of allocation rule that determines how new patients are assigned to treatments dynamically (20). One such type of adaptive randomization is response-adaptive randomization where the allocation probabilities are unbalanced to provide a greater likelihood that treatments having more favorable outcomes are assigned.
- *Adaptive seamless 2/3 trial design* addresses within single trial objectives that are normally achieved through separate trials in phase 2b and phase 3 of clinical development.

Bayesian Methods

The difference between treatments is assumed to be an unknown and fixed parameter in the frequentist framework, whereas the treatment difference is not a single fixed parameter in the Bayesian approach. It is characterized by a distribution of potential values (26, 44). Bayesian methods use prior information on the differences between treatments before the trial is completed, and update this information based on data obtained from the trial producing a posterior distribution that can then subsequently be used as the prior for the next interim analysis or stage of a trial. This approach is attractive in the context of seamless phase 1/2 and phase 2/3 trials where smooth transitions between stages can occur as we learn more about treatment differences as the data accumulate (45, 46). However, there are many examples of adaptive designs that use both Bayesian (47) and frequentist approaches (47–49).

A key stumbling block in Bayesian methods is elicitation of the prior distribution of the treatment difference and the degree of

certainty and subjectivity (27). Information on the prior distribution for the unknown treatment effect can be determined from data from the literature (e.g., a single drug approved for another indication, characteristics for a class of similar agents) or from expert knowledge (28). The biases that may result from expert opinion have been widely discussed and heuristics have been developed to minimize these biases (50–52). Hampson et al. (28) provide an example of elicitation of priors for a clinical trial in very rare diseases. Interestingly, the eliciting of prior beliefs may provide benefits for frequentist clinical trials in considering the magnitude of effect for determining sample size and assessing the level of evidence needed to convince the clinical community to change practice (53).

Both the use of adaptive design methods and the Bayesian approach in clinical trials is consistent with the FDA's *Clinical Path Initiative* (54) that was originally developed to deal with the problem of increased trial spending without a resulting increase in the success rate of new drug approvals. The FDA advocated for advancing innovative trial designs, capitalizing on use of prior experience, or accumulating information in a trial. Dr. Woodcock, then Acting Deputy Commissioner for Operations at the FDA, emphasized two points: (1) there should be scientific evidence that a drug or biological works and (2) there should be a degree of certainty about the prediction that the new product works. She notes that the law does not tell us exactly what the degree of certainty should be, but a level of evidence from the current development that depends somewhat upon the prior knowledge base – whether mechanistic or more general. Thus, the extension of these ideas from common diseases to rarer diseases, such as FSGS, seems a natural step in clinical trials methodology. As with conventional clinical trials designs, the new proposed paradigms do not compromise the ethical imperative to protect human subjects according to such guidelines as the Belmont Report and the Declaration of Helsinki.

EXAMPLE

One context where using adaptive and Bayesian methods in clinical trials can benefit advances in FSGS therapies is in dose-finding trials. The methods that are used for dose-finding trials in nonrare diseases could be costly and imprecise. We can reduce the sample size needed for estimating the maximum tolerated dose while maintaining precision by using the CRM first presented by O'Quigley et al. (55). We compare the most common method for dose-finding clinical trials (the 3 + 3 design) to the CRM using simulations run under reasonable settings for FSGS.

The two main goals of dose-finding clinical trials are to put as many subjects as possible in the dose closest to the maximum tolerated dose (MTD) and to estimate the MTD as accurately as possible. The MTD is defined by the National Cancer Institute as "the highest dose of a drug or treatment that does not cause unacceptable side effects" (18). Unacceptable side effects are determined by the proportion of subjects in the population who would have a dose limiting toxicity (DLT). In our FSGS example, we consider a >30% decrease in eGFR to identify a DLT and 30% of the study population having DLTs at a dose defines the MTD. Generally, to obtain the best estimates of the dose–response relationship, we would want to allocate patients equally to all doses to gain as much information as possible. However, this is contrary to the goal of treating as many patients with the currently estimated MTD. Therefore, we compare the 3 + 3 and CRM methods on how often each selects the correct MTD dose and how many subjects are allocated to the MTD dose to evaluate the ability of these methods to meet both goals simultaneously.

Methods

As described by Storer (56), the 3 + 3 method assigns the first three subjects in the lowest dose group. If no subjects have a DLT, then the next three subjects receive the next highest dose. If one subject out of the original three has a DLT, then the next three subjects are allocated to the same dose. If two or more subjects have DLTs, then the trial is stopped and the MTD is selected to be the next lowest dose. If the first dose has more than one DLT, then it is selected as the MTD. Because many drugs assessed in FSGS trials have been developed for other indications, we limit the number of doses studied to four in this example; thus, the maximum sample size is 24 with the 3 + 3 design.

The CRM is an adaptive and Bayesian method for dose-finding trials in which we use prior information combined with collected data to help find the MTD (Bayesian) and give future subjects doses based on estimates from collected and prior information (adaptive). After each subject is enrolled and treated, the outcome information is combined with prior information and previous outcomes to update the dose–response curve from which the MTD is estimated and given to the next subject. The sample size in CRM can be fixed or variable. To provide a fairer comparison with the 3 + 3 design, we used an early stopping rule to select the estimated MTD as the dose which is assigned seven times in the trial (which limits the maximum sample size to 24). Stopping rules of this kind were first used by Korn et al. (57) and further discussed by O'Quigley (58).

For both 3 + 3 and CRM designs, we ran 10,000 trial simulations for four different true DLT probability scenarios. For each scenario, we compared the proportions of dose selected, the proportion of doses assigned, and the average and SD of sample sizes for both designs. We chose to always include the MTD of interest as one of the doses in our simulations for ease of comparison of trial types. The simulations were run using R and the package dfcrm created by Cheung (59, 60).

Results

In Scenario 1, the CRM selects the correct dose about 62% of time where the 3 + 3 design identifies the MTD just under 35% of the time (**Table 4**). The 3 + 3 design also chooses dose 1 in this scenario 50% of time, meaning that half of the time we would expect the 3 + 3 to identify the estimated MTD to be a dose where no DLTs would happen. There are two doses in Scenario 2 in which the true DLT proportion is 30%, which are identified by the CRM 72% of the time and by the 3 + 3 design <50% of the time. Scenario 3 and Scenario 4 show the CRM is doing a better job at selecting the correct dose by approximately 10% over the 3 + 3 design. In Scenario 4, the 3 + 3 design again selects a dose as the estimated MTD where no DLTs would occur nearly 40% of the time.

TABLE 4 | Percentage of time true DLT dose is selected by design in 10,000 simulated trials.

Dose scenario	Design	Dose			
		1	2	3	4
Scenario 1: (0.00, 0.30, 0.40, 0.60)	3 + 3	50.3	**34.6**	14.6	0.6
	CRM	10.9	**62.2**	24.1	2.8
Scenario 2: (0.05, 0.05, 0.30, 0.30)	3 + 3	5.0	48.6	**26.5**	**19.9**
	CRM	6.1	21.5	**46.7**	**25.7**
Scenario 3: (0.05, 0.10, 0.15, 0.30)	3 + 3	11.7	16.5	41.3	**30.5**
	CRM	7.1	12.9	37.6	**42.4**
Scenario 4: (0.00, 0.25, 0.30, 0.45)	3 + 3	39.7	30.6	**25.0**	4.6
	CRM	5.9	47.8	**33.9**	12.4

The bolded value is the true DLT dose.

TABLE 5 | Mean (SD) sample size by design in 10,000 simulated trials.

Dose scenario	Design	
	3 + 3	CRM
Scenario 1: (0.00, 0.30, 0.40, 0.60)	10.1 (3.2)	12.0 (1.7)
Scenario 2: (0.05, 0.05, 0.30, 0.30)	13.3 (3.5)	11.8 (2.0)
Scenario 3: (0.05, 0.10, 0.15, 0.30)	14.4 (3.7)	11.9 (2.2)
Scenario 4: (0.00, 0.25, 0.30, 0.45)	11.3 (3.7)	12.1 (1.7)

The CRM tends to have about 12 subjects needed for the trial with SDs around 2 for all scenarios investigated (**Table 5**). On the other hand, the 3 + 3 design shows greater fluctuations in mean sample size (10.1–14.4) with larger SDs (approximately 3.5 for each simulation) than the CRM designs.

Our small simulation study shows the advantages of a Bayesian adaptive approach (CRM) over a more traditional clinical trial dose-finding design (3 + 3) in terms of assigning as many patients to the MTD as possible and selecting the correct MTD. In the long term, using the CRM over the 3 + 3 design could lead to enormous financial and time savings by increasing the probability that we move to further phases of clinical trials with the correct dose. Although there is no clear winner between the trial types for sample size under all scenarios investigated (**Table 5**), the stability of the average sample size and smaller SD when using the CRM would allow investigators to be more confident in expected sample size needed in the planning stages of the trial. Using the CRM may also decrease the concern of running a trial which uses a sample size closer to the maximum number of 24 subjects.

CONCLUSION

Focal segmental glomerulosclerosis therapies are challenging based on incomplete efficacy and safety information, leading to the inability to define the right agent for the right patient. Novel agents that are based on molecular profiling are emerging which will benefit from an enriched trial eligibility approach. While enrichment may improve signal, trials will need to be designed for feasibility in FSGS endophenotypes defined by molecular profiling and target-relevant biomarkers. Rational application of more modern clinical trials designs, that have found increasing acceptance in the pharmaceutical, regulatory, and academic environments, increases the chance of successful studies that evidence of safe and effective therapies in rare diseases, such as FSGS.

AUTHOR CONTRIBUTIONS

All authors give final approval to publish this work and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. CS, JJ, DS, SM, JT, and DG: conception and design of the work, drafting the work, and revising it critically for important intellectual content.

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The evidence-based approach to adult-onset idiopathic nephrotic syndrome

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Adult-onset nephrotic syndrome (NS) differs from its pediatric counterpart in several important ways. Most importantly, NS in adults is more etiologically heterogeneous compared to children, and thus treatment approaches rely heavily on the histological diagnosis provided by renal biopsy. The evidence-based approach to treatment of adult NS has been critically examined by the Kidney Disease Improving Global Outcomes (KDIGO) guidelines in glomerulonephritis, published in 2012. Here, we examine the strengths and limits of those guidelines and review recent work that expands the evidence-based approach.

Keywords: nephrotic syndrome, minimal-change disease, focal segmental glomerulosclerosis, KDIGO guidelines, clinical practice guidelines

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Introduction

The field of nephrology carries the dubious distinction of consistently producing the fewest number of randomized controlled trials among the internal medicine specialties (1, 2). Within nephrology, glomerular diseases have been particularly neglected, especially the primary glomerular diseases, including idiopathic nephrotic syndrome (NS) (3, 4). Consequently, high-quality clinical evidence to guide treatment decisions in NS has been sparse, and nephrologists have long navigated these murky waters according to principles of physiology and insights gleaned from observational or uncontrolled studies.

With these limitations in mind, a Kidney Disease Improving Global Outcomes (KDIGO) working group attempted to synthesize the available evidence into a series of disease-specific clinical practice guidelines for glomerulonephritis, published in 2012 (5). These guidelines were pioneering in that nothing so comprehensive with respect to glomerular diseases had ever been produced by one of the field's major organizations. Other systematic reviews have examined specific diseases or problems in NS [particularly noteworthy are Cochrane reviews covering corticosteroid use, immunosuppression, and lipid-lowering therapy (6–8)], but none claimed broad consensus to establish practice guidelines. Clinical practice guidelines serve several important purposes to clinicians and researchers (9). By critically synthesizing and grading the quality of the evidence base, they help define not only what is optimal but also the breadth of what is reasonable. By defining the limits of current evidence, they highlight those areas where evidence is lacking to justify adoption or broad endorsement of specific practices.

Using the KDIGO guidelines as a foundation, we here examine the evidence-based approach to adult-onset NS, limiting the discussion to minimal-change disease (MCD) and focal segmental glomerulosclerosis (FSGS). Strikingly, of the recommendations offered in the KDIGO guidelines, over 75% were considered to be based on low- or very low-quality evidence

(grade C and D, respectively). In particular, for adult MCD and adult FSGS, a total of 23 statements were made of which only 2 (8.7%) were level 1 recommendations, both based on grade C evidence. No statement was supported by grade A evidence, and only one suggestion was graded B (moderate quality). Of the remaining 20 statements, 17 were classified as suggestions based on low- or very low-quality evidence (2C or 2D), and three statements (13%) lacked evidence to be graded at all. This is a sobering picture of the limits of evidence that define the "state of the art" in the clinical management of adult NS.

Comparing Adult-Onset to Pediatric Nephrotic Syndrome

Adult-onset NS differs from its pediatric counterpart in several important ways. The prospective International Study of Kidney Diseases in Children showed that in children aged 1–16 presenting with NS, there was a 95% likelihood of responding to a course of glucocorticoids by 16 weeks (10). This finding justified the recommendation that kidney biopsy not be routinely done immediately in nephrotic children, but rather deferred to those not responding to (or relapsing after) an empiric course of corticosteroids. Importantly, most children presenting with NS will have MCD, although the incidence of other diagnoses appears to be increasing (particularly FSGS) and these account for most treatment-resistant cases (11, 12). In adults, NS comprises a much more diverse group of diseases, with considerable demographic variation, including both primary and secondary conditions (13, 14). For this reason, early kidney biopsy is critical to properly categorize the disease and direct the subsequent clinical approach in adults. Among primary or idiopathic diseases, the most common remain FSGS, membranous nephropathy, and MCD. Because this Frontiers research topic is primarily concerned with pediatric NS, we will focus on MCD and FSGS, which also cause the majority of pediatric NS and where comparisons may be most fruitful.

The prevalence of genetic causes of NS is unsurprisingly different between pediatric and adult populations. An increasing number of monogenetic defects have been found to cause neonatal or childhood NS, most of which affect genes critical to podocyte function, follow autosomal recessive transmission, and histologically produce FSGS (15, 16). The precise role for genetic screening remains ill-defined. The KDIGO guidelines did not endorse screening, stating that

> Routine evaluation for genetic mutations is not recommended in this guideline due to the variable availability of genetic testing, significant cost, low to absent prevalence observed in some populations, and the lack of systematic studies of treatment response and prognosis relative to specific genetic polymorphisms (5)

Reconsideration of such claims should be prompted by the pace of discovery and technological improvements in genetic diagnosis, precipitously falling costs, expanding availability, and progress in understanding the clinical consequences of mutations.

Genetic variants may predict with high accuracy which patients will have steroid-resistant disease, potentially allowing a clinician to spare them from toxic empiric therapy (17). Emerging research suggests that there may also be a genetic signal for steroid-*sensitive* disease (18). Certain mutations also predict freedom from relapse following transplantation (15). Mutations are increasingly being found in older patients with NS. A recent publication from the SRNS Study Group showed that a single-gene cause of steroid-resistant NS could be identified in nearly a third of affected families, including >10% where disease onset was between 13 and 18 years of age (19). Santín and colleagues likewise identified a high proportion of mutations in Spanish patients with primary NS, including 14% among adults (median age of onset 33 years), and proposed a screening algorithm based on age of onset and whether disease was familial or sporadic (20). Such algorithms will need to be validated and adjusted based on local demographics, testing availability, and reimbursement practices, but with the advancements in speed and affordability of next-generation sequencing there is already reasonable justification for screening selected patients in the clinic.

Adult Minimal-Change Disease

Adult MCD shares many similarities to the pediatric form, although time to remission appears prolonged and acute kidney injury more common, whereas the risk of relapse may be less (21–23). The largest published series of adult MCD included 340 Chinese patients (24). Only 9.7% were steroid resistant, the remainder reached remission in a median of 10 weeks. During follow-up, 42% of responders relapsed, and 27% became frequent relapsers or steroid dependent (FR/SD). Certain groups may have higher morbidity. In a series of 95 adults seen at our referral center, almost all treated with steroids, 25% presented with acute kidney injury (25). Mean time to remission was 13 weeks, but one-quarter were steroid-resistant and 73% of responders relapsed, with 41% of responders frequently relapsing.

The KDIGO guidelines for adult MCD are presented in **Box 1**. Several recommendations deserve discussion. Like in pediatric NS, corticosteroids are recommended as first-line therapy (graded 1C). Clinicians will recognize that the dose of corticosteroids is high enough to cause significant morbidity, such as Cushings syndrome, bone loss, and hyperglycemia, especially over the time suggested (minimum 4 weeks, maximum 16 weeks, with a slow taper up to 6 months). Is it possible to achieve results with less? Direct evidence from adults is largely lacking, but at least three recent randomized controlled studies in children showed that extended courses of steroids did not prevent the development of FR/SD disease, just delayed its recognition (26–28). This prompted a revision to a Cochrane systemic review, concluding that the benefit of prolonged courses of steroids was likely overestimated by earlier studies and that it seems there is no benefit of increasing the duration of prednisone beyond 2–3 months (6). Should these pediatric studies influence practice in adults? Here, it is worth noting that the KDIGO guidelines are based on low-quality evidence, and indeed their "recommendation is based *largely on extrapolation from RCTs in children*" (p. 177, emphasis ours).

A separate issue concerns the recommendations for alternative agents to corticosteroids. KDIGO suggests alternative agents in patients with relative contraindications or intolerance

BOX 1 | KDIGO guidelines for minimal-change disease (MCD) in adults.

5.1: *Treatment of initial episode of adult MCD*
5.1.1: We recommend that corticosteroids be given for initial treatment of nephrotic syndrome. (*1C*)
5.1.2: We suggest prednisone or prednisolone be given at a daily single dose of 1 mg/kg (maximum 80 mg) or alternate-day single dose of 2 mg/kg (maximum 120 mg). (*2C*)
5.1.3: We suggest the initial high dose of corticosteroids, if tolerated, be maintained for a minimum period of 4 weeks if complete remission is achieved, and for a maximum period of 16 weeks if complete remission is not achieved. (*2C*)
5.1.4: In patients who remit, we suggest that corticosteroids be tapered slowly over a total period of up to 6 months after achieving remission. (*2D*)
5.1.5: For patients with relative contraindications or intolerance to high-dose corticosteroids (e.g., uncontrolled diabetes, psychiatric conditions, severe osteoporosis), we suggest oral cyclophosphamide or CNIs as discussed in frequently relapsing MCD. (*2D*)
5.1.6: We suggest using the same initial dose and duration of corticosteroids for infrequent relapses as in Recommendations 5.1.2, 5.1.3, and 5.1.4. (*2D*)

5.2: *FR/SD MCD*
5.2.1: We suggest oral cyclophosphamide 2–2.5 mg/kg/day for 8 weeks. (*2C*)
5.2.2: We suggest CNI (cyclosporine 3–5 mg/kg/day or tacrolimus 0.05–0.1 mg/kg/day in divided doses) for 1–2 years for FR/SD MCD patients who have relapsed despite cyclophosphamide, or for people who wish to preserve their fertility. (*2C*)
5.2.3: We suggest MMF 500–1000 mg twice daily for 1–2 years for patients who are intolerant of corticosteroids, cyclophosphamide, and CNIs. (*2D*)

5.3: *Corticosteroid-resistant MCD*
5.3.1: Re-evalulate patients who are corticosteroid resistant for other causes of nephrotic syndrome. (*Not Graded*)

5.4: *Supportive therapy*
5.4.1: We suggest that MCD patients who have AKI be treated with renal replacement therapy as indicated, but together with corticosteroids, as for a first episode of MCD. (*2D*)
5.4.2: We suggest that, for the initial episode of nephrotic syndrome associated with MCD, statins not be used to treat hyperlipidemia, and ACE-I or ARBs not be used in normotensive patients to lower proteinuria. (*2D*)

FR, frequently relapsing; SD, steroid dependent; CNI, calcineurin inhibitor; MMF, mycophenolate mofetil; AKI, acute kidney injury.

to high-dose corticosteroids (2D), or for patients with FR/SD disease (2C/2D). Three agents are named: cyclophosphamide and calcineurin inhibitors (CNIs) receive slightly stronger endorsement, followed by mycophenolate mofetil (MMF). Several points here deserve mention. One consideration is why these agents are considered second-line behind steroids. There have been only two controlled trials of steroids in adult MCD, only one placebo-controlled (the other comparator was no treatment), involving, respectively, 28 and 31 subjects (29). Steroids have never been directly compared against alternate agents for initial treatment, and observational data suggests similar (if not better) frequencies of response to alternate agents (21, 22, 25). The strength of recommendation supporting steroids rests largely on the totality of evidence showing that steroids are effective (especially in children), but NOT on evidence showing that other agents are ineffective or that they are inferior to steroids. The reason steroids have been studied more is that they have been used more, largely for historical and circumstantial reasons (availability, affordability, prescriber familiarity, etc.). There is certainly sufficient equipoise to justify a randomized trial in adults comparing an alternative (and one hopes better tolerated) agent to corticosteroids, though only time will tell if the nephrology community deems this a sufficient priority to pursue.

Recommendation 5.1.5 to use alternate agents for patients with contraindications or intolerance to steroids is graded as 2D, a suggestion based on low-quality evidence. It should be recognized that the weakness of the evidence relates to the choice of replacement, not to the premise of withholding steroids in the first place – there need be very little "evidence" to justify NOT giving a medication which is poorly tolerated or contraindicated! In adults, relative contraindications to steroid use are more common (e.g., diabetes, osteoporosis), as is steroid intolerance, and in clinical decision-making individual patient considerations must trump broad practice guidelines (30). Another nuance not specifically considered in the guidelines, but reflected by the weak grading of evidence, concerns the long-term sequelae of various therapeutic choices. The justification for rating cyclophosphamide and CNIs as nearly equivalent in the guidelines is based on observational studies and a few small randomized trials comparing the two [summarized in the guidelines and elsewhere (29)]. These studies have no information on potential long-term toxicities of the drugs, for example, late malignancies from cyclophosphamide or nephrotoxicity from CNIs. Obtaining such evidence is certainly feasible; for instance, it was recently shown in a carefully followed cohort of membranous nephropathy (another common cause of idiopathic NS in adults) that treatment with cyclophosphamide was associated with a more than threefold increased risk of subsequently developing cancer (31).

The anti-CD20 monoclonal antibody rituximab is not mentioned in the KDIGO guidelines on adult MCD, except in an appeal for further research. In the years since the guidelines were published, such research increasingly supports the efficacy of rituximab in both children and adults, particularly for steroid sensitive and SD/FR disease. The Rituximab for Childhood-onset Refractory Nephrotic Syndrome Study in Japan was a multicenter, double-blind, randomized trial of rituximab vs. placebo in 52 children with FR/SD NS (32). Median relapse-free survival was 267 days in the rituximab group compared to 101 days in the placebo group ($P < 0.0001$). The rituximab group had significantly fewer relapses, and required significantly less steroids. In Italy, Ravani and colleagues carried out a multicenter, open-label, randomized non-inferiority trial of rituximab vs. tapering steroid therapy in 30 children with SD NS on high-dose prednisone (33). The differences in relapse were dramatic; median time to relapse was 18 months for the rituximab group, whereas 14/15 children in the control group relapsed within 4 months. The rituximab group received much lower cumulative doses of prednisone. Observational studies bolster the results of these randomized studies.

Recent reports from large case series in children, and several series of adults with MCD, have consistently found that rituximab is associated with prolonged remissions and allows reduction or cessation of steroids or other immunosuppressants in SD/FR patients (34–38). In sum, these data support a role for rituximab in the care of patients with SD/FR disease. Where rituximab should rank among the various other immunosuppression choices may be clarified by future head-to-head clinical trials and careful cost-benefit analyses.

Adult Idiopathic FSGS

The KDIGO guidelines for adult FSGS are presented in **Box 2**. Many of the treatment recommendations regarding steroid use are analogous to those for MCD and recapitulate the issues discussed earlier. Some additional issues specific to FSGS are worth exploring further.

The first two recommendations, 6.1.1 and 6.1.2, are not graded and present a small paradox. The clinician is urged to perform a thorough evaluation to exclude secondary forms of FSGS, but what this evaluation should entail is left undefined except that it should *not* include genetic testing. Following these non-graded recommendations is a relatively strongly graded (1C) recommendation that corticosteroids or immunosuppression be considered *only* for "idiopathic FSGS with clinical features of nephrotic syndrome." It is not specified which particular clinical features of NS are sufficient to justify therapy.

The guidelines are necessarily vague because of the inherent limitations in our classification of FSGS. It should be clear that the purpose of these recommendations is to direct steroids and immunosuppression to those patients with the highest likelihood of response (idiopathic FSGS), while excluding those for whom such therapy is ineffective (most cases of secondary FSGS). However, establishing a clear diagnosis of idiopathic FSGS – and by extension, deciding whom to treat with steroids/immunosuppression – is not trivial. There is no evidence-based approach for ruling out secondary FSGS. The list of conditions that may produce FSGS lesions on biopsy is long and diverse (39). In the rationale, the KDIGO guidelines state, "idiopathic FSGS is defined by exclusion of *any other identifiable cause* of secondary FSGS" (p. 181, emphasis ours), but this definition is problematic. Obesity, diabetes, and hypertension all may cause secondary FSGS, but these are common conditions and none of them exclude the possibility of a separate or superimposed "idiopathic" FSGS. Such issues present real, practical challenges to the clinician attempting to apply the guidelines in deciding whether to treat a patient with nephrotic-range proteinuria and a biopsy showing FSGS lesions.

These concerns highlight the limitations of classifying FSGS as "idiopathic" or "secondary." At the heart of the matter is determining whether a patient's cause of FSGS may be susceptible to steroids or immunosuppression, given the overwhelming evidence that achieving partial or complete remission with therapy dramatically improves prognosis (40–44). Identifying the circulating factor or factors responsible for idiopathic FSGS remains a yet unrealized hope, but one that when achieved should greatly assist in determining which patients deserve a trial of immunotherapy (as well as chipping away at the inelegant term, "idiopathic.") In the meantime, what evidence is available to help determine whom to treat?

A potentially evolving role for genetic testing was discussed previously, and in a future of personalized medicine may become a routine part of the diagnostic workup. Some additional points may be helpful. Deegens and colleagues showed that the degree of foot process effacement, measured by electron microscopy, was a sensitive and specific test to differentiate idiopathic from secondary FSGS (45). In fact, foot process effacement seen in post-reperfusion biopsies of transplanted kidneys diagnosed recurrent FSGS with high specificity (46). By light microscopy, the perihilar variant of FSGS is most characteristic of hyperfiltration injury, but does not rule out idiopathic FSGS (47). Among clinical features, normal serum albumin and lack of edema despite nephrotic-range

BOX 2 | KDIGO guidelines for idiopathic focal segmental glomerulosclerosis (FSGS) in adults.

6.1: *Initial evaluation of FSGS*
6.1.1: Undertake thorough evaluation to exclude secondary forms of FSGS. (*Not Graded*)
6.1.2: Do not routinely perform genetic testing. (*Not Graded*)

6.2: *Initial treatment of FSGS*
6.2.1: We recommend that corticosteroid and immunosuppressive therapy be considered only in idiopathic FSGS associated with clinical features of the nephrotic syndrome. (*1C*)
6.2.2: We suggest prednisone be given at a daily single dose of 1 mg/kg (maximum 80 mg) or alternate-day dose of 2 mg/kg (maximum 120 mg). (*2C*)
6.2.3: We suggest the initial high dose of corticosteroids be given for a minimum of 4 weeks; continue high-dose corticosteroids up to a maximum of 16 weeks, as tolerated, or until complete remission has been achieved, whichever is earlier. (*2D*)
6.2.4: We suggest corticosteroids be tapered slowly over a period of 6 months after achieving complete remission. (*2D*)
6.2.5: We suggest CNIs be considered as first-line therapy for patients with relative contraindications or intolerance to high-dose corticosteroids (e.g., uncontrolled diabetes, psychiatric conditions, severe osteoporosis). (*2D*)

6.3: *Treatment for relapse*
6.3.1: We suggest that a relapse of nephrotic syndrome is treated as per the recommendations for relapsing MCD in adults (see Chapters 5.1 and 5.2). (*2D*)

6.4: *Treatment for steroid-resistant FSGS*
6.4.1: For steroid-resistant FSGS, we suggest that cyclosporine at 3–5 mg/kg/day in divided doses be given for at least 4–6 months. (*2B*)
6.4.2: If there is a partial or complete remission, we suggest continuing cyclosporine treatment for at least 12 months, followed by a slow taper. (*2D*)
6.4.3: We suggest that patients with steroid-resistant FSGS, who do not tolerate cyclosporine, be treated with a combination of mycophenolate mofetil and high-dose dexamethasone. (*2C*)

CNI, calcineurin inhibitors.

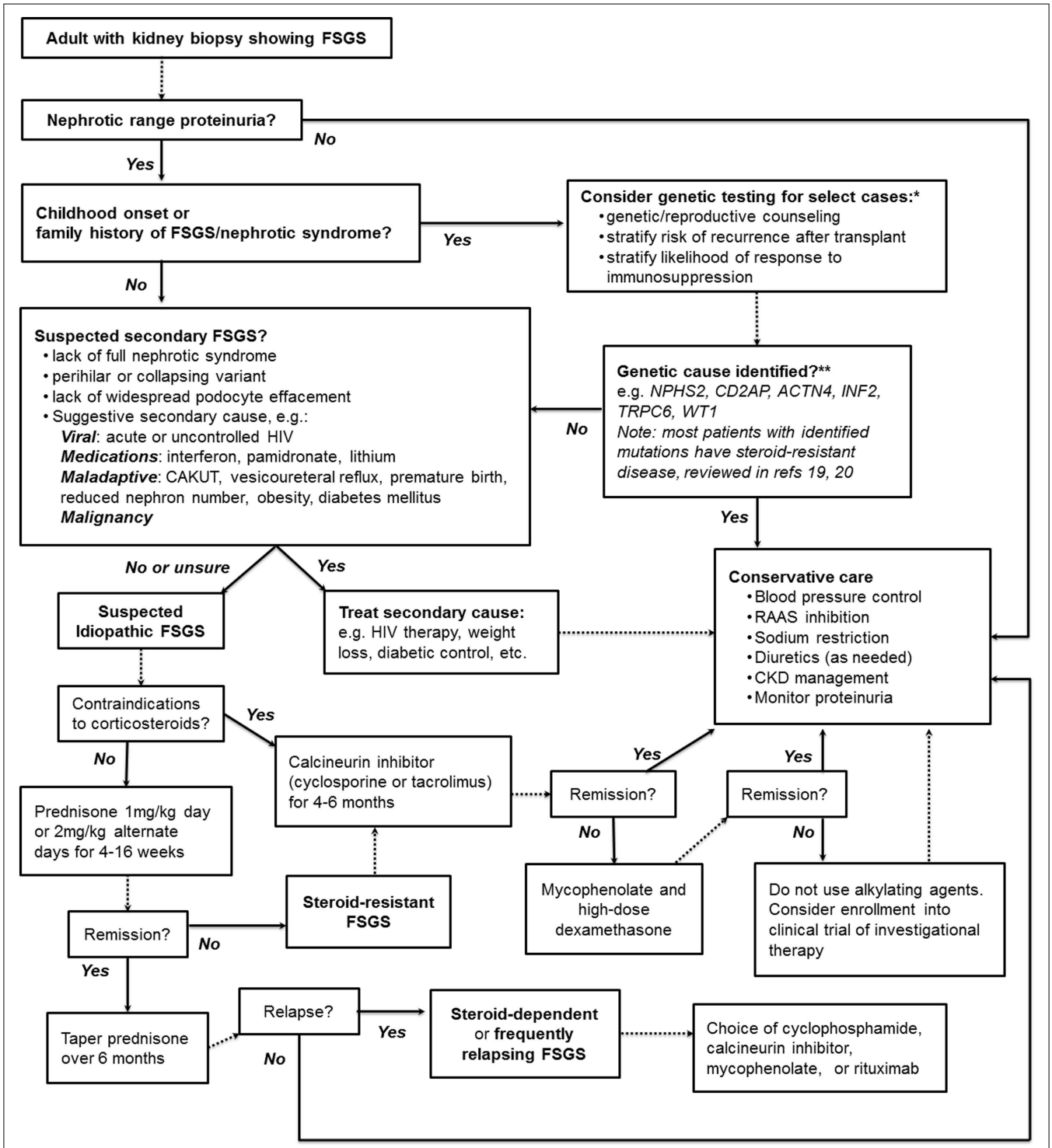

FIGURE 1 | A suggested algorithm for the clinical approach to adult FSGS. *Note that KDIGO guidelines explicitly recommend against genetic testing. **Highlighted are some of the monogenic causes of FSGS more likely to be found in adults with genetic disease; see text and highlighted references for further discussion. *NPHS2*, Podocin; *CD2AP*, CD2-associated protein; *ACTN4*, α-actinin-4; *INF2*, inverted formin-2; *TRPC6*, transient receptor potential channel 6; *WT1*, Wilms tumor protein.

proteinuria are common findings in secondary FSGS, but rare in idiopathic FSGS (48). In the absence of nephrotic-range proteinuria, immunosuppression is generally unnecessary since subnephrotic patients have excellent long-term renal prognosis with kidney survival rates exceeding 90% at 5–10 years on conservative therapy alone (43, 49, 50).

The treatment of steroid-resistant FSGS remains particularly challenging. KDIGO suggests cyclosporine with a 2B recommendation. Tacrolimus, the other commonly used CNI, is not formally recommended. The rationale cites the lack of randomized trials, but also states that limited observational data suggests tacrolimus may be an alternative to cyclosporine. Additional data published after the KDIGO guidelines support this notion. A small, single-center study of adults with idiopathic FSGS compared intravenous monthly cyclophosphamide to tacrolimus for 6 months, with both groups receiving steroids (51). There were no significant differences between the groups, but both groups had improved proteinuria and serum albumin with stable GFR. Tacrolimus was also compared to cyclophosphamide in a multicenter randomized trial of 131 children with steroid-resistant NS (mostly MCD and FSGS) (52). Tacrolimus showed remarkably higher rates of complete or partial remission (82.5 vs. 45.9%, $P < 0.001$), with shorter time to remission and fewer serious infections. The superior likelihood of remission was maintained in the subgroup with FSGS (HR 2.54, 95% CI 1.09–5.93, $P = 0.03$). In an uncontrolled trial of 44 adults with steroid-resistant FSGS treated with tacrolimus for 24 weeks, 52.3% of patients achieved complete or partial remission (53).

For steroid-resistant patients intolerant to cyclosporine, KDIGO recommends only combined MMF and high-dose dexamethasone. This is based on one large randomized trial which reported that this combination had similar efficacy to cyclosporine, but the limitations of this trial have been reviewed in detail (54). No other agents receive formal recommendations; in our view, this is largely appropriate for practice guidelines given the limited evidence concerning other agents. Of note, no formal recommendation is made for or against cytotoxics, although they are explicitly discouraged in the KDIGO guidelines for steroid-resistant NS in children (5). Rituximab has shown potential for steroid-sensitive FSGS in case reports and small series, but it appears largely ineffective for steroid-resistant disease (55–57). A recent small series examined adrenocorticotropic hormone gel in 24 patients with idiopathic FSGS and found that 7 (29%) experienced remission with therapy, including 5/15 (33%) who were steroid-resistant.

Figure 1 presents our suggested summary algorithm for the diagnostic and therapeutic approach to an adult with FSGS on kidney biopsy, which largely follows the KDIGO guidelines. Such an algorithm, like the practice guidelines themselves, should not supplant clinical judgment.

Conclusion

The KDIGO clinical practice guidelines have played a pioneering role in providing evidence-based consensus recommendations for treating glomerular diseases, including adult idiopathic NS. While the recommendations are necessarily limited by the quality of evidence underlying them, they serve an excellent starting point for a discussion of the evidence-based approach to NS. New research adds to, but rarely supplants, prior evidence, which is why venerable "tried and true" interventions such as corticosteroids continue to play such a prominent role in the recommendations. As with all clinical practice guidelines, a critical appreciation of their limitations and an eye toward emerging lines of evidence are necessary to most effectively apply their lessons to individual patients.

Author Contributions

PC drafted the manuscript. JR conceptualized the work and critically revised it.

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