SEMINARS IN
NEPHROLOGY

Focal and Segmental Glomerulosclerosis: Varying Biologic Mechanisms Underlie a Final Histopathologic End Point

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Focal and segmental glomerulosclerosis (FSGS) is a pathologic entity that is a common and increasing cause of end-stage renal disease. Typical manifestations include proteinuria, hypertension, worsening renal insufficiency, and, frequently, renal failure. The etiology, however, remains unknown in a majority of patients. There is an estimated recurrence rate of 30% to 40% in renal transplant patients, suggesting that the pathogenesis is not solely a result of intrinsic kidney disease. Although some of its characteristics have been reported, the precise identification of a circulating factor associated with FSGS has not been made. Remarkable progress has been made in recent years regarding biologic mechanisms surrounding FSGS and proteinuria. Insight into the pathogenesis of FSGS has been gained through the study of hereditary forms of FSGS and nephrotic syndromes. Mutations in cytoskeletal proteins that affect podocyte structure have been the target until recently. Here we review the current understanding of this glomerular disease and areas for future concentration.

KEYWORDS focal and segmental glomerulosclerosis, proteinuria, renal failure, circulating factor, nephrin, podocin, CD2AP, ?-actinin 4, TRPC6, podocyte

Focal and segmental glomerulosclerosis (FSGS) is a common cause of nephrotic syndrome in both adults and children that has a substantial risk for progression to end-stage renal disease (ESRD). Over the past 2 decades, the incidence of FSGS has been increasing. A review of data compiled during the 1970s and early 1980s showed that membranous nephropathy was previously the most common cause of nephrotic syndrome in adults.[1] Recently, a survey of data from 1,000 kidney biopsy examinations performed between 1995 and 1997 cited FSGS as the most common cause of nephrotic syndrome in adults, accounting for up to 35% of cases.[1] In addition to being the leading cause of idiopathic nephrotic syndrome in the United States, this disease accounts for 50% of cases in black adults, with a newly documented emergence of disease in both blacks and Hispanics over the past 20 years.[1] Black individuals tend to have a more aggressive form of the disease, with a 4-fold greater risk for progressing to ESRD than white or Asian individuals.[2] This trend of increasing incidence has been observed not only in large metropolitan areas, but also in small urban and rural communities.[3] With the growing number of affected individuals, a growing proportion of ESRD attributed to FSGS has been observed.[2]

The increased incidence of idiopathic FSGS suggests that both genetic and environmental factors may play an important role in the pathogenesis of this disease. There has been substantial progress over the past decade in defining the biologic basis of FSGS through the identification and understanding of genetic mutations associated with various familial forms. The etiology, however, remains unknown in a majority of cases. There is an estimated recurrence rate of 30% to 40% in renal transplant patients, suggesting that the pathogenesis is not solely a result of intrinsic kidney disease.[4] The histologic lesions are focal (discrete areas within the kidney of affected glomeruli) and segmental (portions of those glomeruli have tuft sclerosis) on light microscopy (Fig 1). Im-

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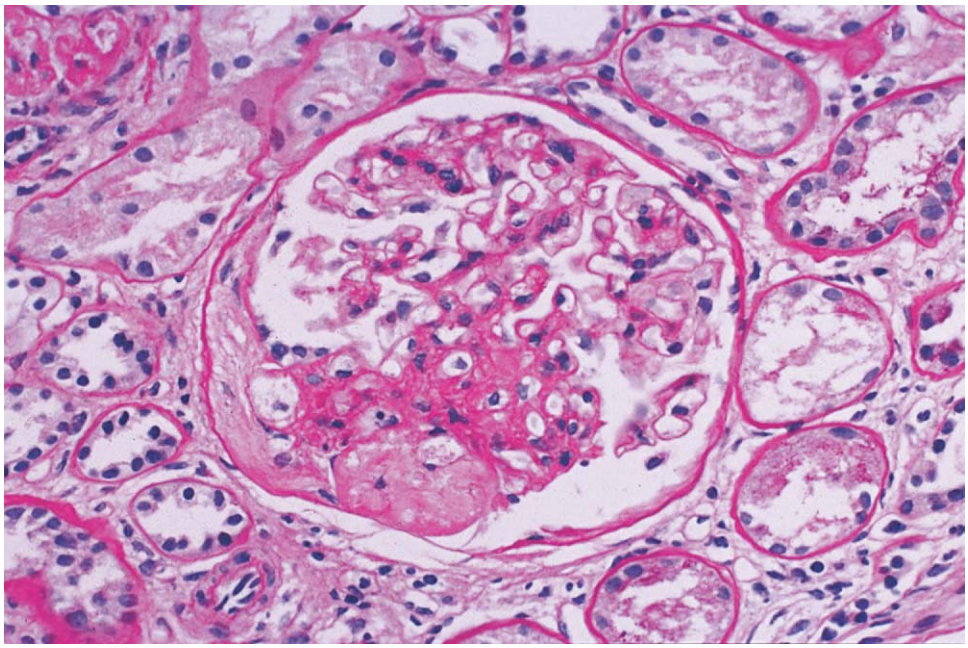

Figure 1 Light micrograph of a renal biopsy specimen from an individual with FSGS. The micrograph shows a glomerulus with typical segmental sclerotic lesions. Periodic acid-Schiff stain (magnification, 325×).

munofluorescence occasionally shows C3 and immunoglobulin (Ig)M immune deposits. Diffuse epithelial cell foot process effacement similar to minimal change disease is evident on electron microscopy. There are several histologic variants that are distinct from the classic form including collapsing, tip, perihilar, and cellular variants. Each of these variants is associated with both primary and secondary forms, however, the factors responsible for these light microscopic changes are not understood. The histologic lesions of FSGS can be associated with various conditions such as morbid obesity, intravenous heroin use, chronic lithium therapy, and viral infections such as human immunodeficiency virus and hepatitis C.

The clinical hallmarks of FSGS include proteinuria, nephrotic syndrome, and, frequently, the progressive loss of renal function. The degree of proteinuria may vary from mild to nephrotic range, as does the age at presentation, which occurs anywhere from infancy to late adulthood. The primary treatment for FSGS is steroid therapy; however, the response rates are inconsistent. It is estimated that approximately 50% of patients with persistent nephrotic range proteinuria reach ESRD after 10 years of follow-up evaluation.[5,6] These phenotypic differences show the heterogeneity of this disease process, and support the existence of varying biologic mechanisms causing the final histopathologic end point. Because patient response rates to treatment markedly vary, it will become increasingly important to distinguish the underlying distinct disease processes. Only as we progress in our understanding of the pathogenetic mechanisms of FSGS will we be able to provide more effective patient care.

Circulating Factor

Is FSGS a systemic disease of abnormal lymphocyte function resulting in the secretion of a circulating chemical mediator that is toxic to an immunologically innocent glomerular membrane as initially proposed in the 1970s,[7] or is it an intrinsic kidney disease caused by abnormalities in the podocyte cytoskeleton as suggested by the discovery of several novel mutated podocyte proteins? The heterogeneity of this disease process suggests that the answer is both. The circulating factor theory first was proposed in the 1970s after Hoyer et al[8] observed the recurrence of nephrotic proteinuria in renal transplant patients who were diagnosed initially with FSGS. Support for the idea of a pathogenetic circulating factor came from studies by Zimmerman et al,[9] who showed proteinuria in rats that received injections of serum from patients with recurrent focal glomerular sclerosis after renal transplantation. Proteinuria was not observed in rats that received serum injections from patients without recurrence of FSGS after transplant.[9] It was not until 2 decades later that further progress was made delineating the role of a circulating factor in the pathogenesis of idiopathic nephrotic syndrome when Dantal et al[10] showed that the removal of serum proteins by adsorption to protein A Sepharose (Amersham Biosciences Corp., Piscataway, New Jersey) led to remission of proteinuria in patients with recurrence of nephrotic syndrome after transplantation. Similar findings have been reported with the incubation of isolated normal rat glomeruli in medium containing serum or plasma from patients with recurrent FSGS. These experiments caused an increase in albumin permeability.[11] This capacity to increase albumin permeability was reduced by plasmapheresis.[12] Shortly thereafter, it became standard practice to treat patients with recurrent FSGS in renal allografts with plasmapheresis resulting in decreased proteinuria and prolonged allograft survival.[13-18] Despite acknowledgment within the nephrology community that removal of circulating factor through plasmapheresis results in improved patient outcomes, optimal treatment regimens have not been defined through prospective controlled studies.[19]

Considerable advances have been made in further charac-

terizing the identity of the permeability factor. Focusing on the sera of patients with posttransplant recurrence and using biochemical manipulations on plasmapheresis fluid have helped to determine several characteristics of the permeability factor. It consists of a highly glycated hydrophobic protein with an anionic charge and an apparent molecular weight of less than 30 kd,[20,21] however, the precise identification of the circulating factor associated with FSGS and with posttransplant recurrence has not been made. Current evidence also suggests that there may be more than 1 circulating factor. IgG eluates from the serum of individuals with collapsing glomerulopathy have been shown to produce podocyte damage and proteinuria in rats.[22] These factors remain in the circulation when the serum of patients is adsorbed onto protein A Sepharose, raising the possibility that individuals with collapsing glomerulopathy have more than 1 circulating factor.[22]

Buffalo/Mna rats have been shown to develop lesions spontaneously that mimic the histologic pattern seen in human FSGS.[23,24] More interestingly, the Buffalo/Mna rats have shown a recurrence of proteinuria after transplantation with subsequent remission of lesions in Buffalo/Mna kidneys transplanted into normal hosts.[25] This relevant animal model may prove useful for further elucidating the mechanisms of idiopathic FSGS and primary nephrotic syndrome. It also may play a role in unmasking the identity of the elusive circulating factor(s). Further research undoubtedly will continue in this area, and also proceed to address the questions of its origin and relationship to the development of proteinuria and glomerular sclerosis.

Genetics

The study of Mendelian forms of FSGS have provided some very useful insights into the pathophysiologic mechanisms of this disease. Congenital nephrotic syndrome of the Finnish type (or Finnish nephropathy), an autosomal-recessive disease characterized by massive proteinuria in utero, initially was described in 1956 by Hallman et al.[26] The disease exists predominantly in Finland, but has been described elsewhere in Europe and North America (especially among Mennonites in Lancaster County, PA[27]), and is characterized by severe nephrosis with up to 20 to 30 g/d of proteinuria. Infants typically die of nephrotic complications unless treated with nephrectomy and renal transplantation. Decades after the initial description, a genome-wide linkage analysis localized the causative gene to chromosome 19q13.1.[28] *NPHS1* was discovered to be the disease-causing mutation.[29] The *NPHS1* gene, containing 29 exons spanning 26 kb, encodes a gene product termed *nephrin*. Numerous mutations, including deletions, insertions, nonsense, missense, and splicing errors have been described.[30] This 185-kd protein contains 8 Ig C2 motifs, a fibronectin III–like domain, and a single transmembrane segment. Nephrin localizes to signaling domains known as lipid rafts within the slit diaphragm (SD) of the podocyte.[31-33] It has been shown to play a role in regulating signaling pathways.[34,35]

Individuals with heterozygous *NPHS1* mutations appear to have no phenotype other than prenatal proteinuria and increased α-fetoprotein levels that are detected in utero.[36] In Finland, 2 mutations termed *Fin major* (deletion at nucleotides 121 and 122 leading to a frameshift) and *Fin minor* (premature stop codon at amino acid 1109) cause 95% of the observed disease. The identification of these mutations has made screening for these 2 alleles in carriers a cost-effective and sensitive test for early diagnosis. Twenty percent to 25% of affected children with congenital nephrotic syndrome of the Finnish type (CNF) can go on to have a recurrence of less severe proteinuria after renal transplant.[37] Antiglomerular and antinephrin antibodies were shown in a high percentage of these patients.[38]

Mouse models for CNF have been generated with targeted disruption of the nephrin gene in embryonic stem cells.[39] Mice homozygous for the mutant *NPHS1* allele mimic human disease with the immediate development of massive proteinuria and neonatal death.[39] Injection of mice with monoclonal antibodies to nephrin such as monoclonal antibody 5-1-6, produce massive amounts of proteinuria.[40] These antimouse nephrin antibodies are directed toward the extracellular domain of nephrin, highlighting the importance of nephrin and the SD in the regulation of glomerular permselectivity.[40] In addition, mutant *NPHS1* mouse lines, generated by the random integration of a promoterless reporter gene into an intron, promoter, or in-frame into an exon (gene trapping), also show features consistent with CNF.[41] Morphologically, these mice lack SDs and show fibrotic glomeruli with cystic tubular lesions. Interestingly, they do not show prominent changes in the morphogenesis of the developing collecting ducts, suggesting that nephrin plays a functional rather than developmental role.[41]

Steroid-resistant nephrotic syndrome is another autosomal-recessive form of familial nephrotic syndrome. The causative mutations are in the gene *NPHS2*, which is located on 1q25-q31.[42] The disease is characterized by steroid-resistant idiopathic nephrotic syndrome, with onset between 3 months and 5 years of age, rapid progression to ESRD, and few or isolated cases of recurrence after renal transplantation. Histologically, minimal glomerular changes were observed on early biopsy examination, with FSGS changes present at later stages. The gene product, podocin, is an integral 383-amino acid membrane protein of approximately 42 kd and was found to be expressed exclusively in podocytes.[43] Podocin is part of the stomatin protein family, and similar to other stomatin family proteins it has a single-membrane domain forming a hairpin-like structure, with both N- and C-terminal domains in the cytosol.[44] By using electron microscopy, podocin has been localized to the base of the foot processes on either side of the SD.[44] It accumulates in an oligomeric form of lipid rafts in the SD and has been shown to interact in vivo with both nephrin and CD2-associated protein (*CD2AP*), a cytoplasmic binding partner of nephrin.[45] *CD2AP* haploinsufficiency appears to cause a susceptibility to FSGS in human beings as in mice.[46] Although the precise glomerular function of these podocyte proteins remain unknown, podocin has been shown to bind to the cytoplasmic

Table 1 Known Genes for Hereditary Nephrotic Syndromes and FSGS

Disease	Locus	Inheritance	Gene/Protein	References
Finnish nephropathy	19q13.1	AR	*NPHS1*/nephrin	28,29
Steroid-resistant nephrotic syndrome	1q25-32	AR	*NPHS2*/podocin	42,43
FSGS1	19q13	AD	*ACTN4*/α-actinin–4	48,49
FSGS2	11q21-22	AD	*TRPC6*/transient receptor potential cation channel 6	51,52
FSGS3	6p12	?	*CD2-AP*/CD2-associated protein	45,46

AR, autosomal recessive; AD, autosomal dominant.

tail of nephrin and enhance its signaling ability through mitogen-activated protein kinases.[31]

Podocin mutations now have been reported widely in both familial autosomal-recessive disease and in individuals with sporadic adult-onset FSGS. The numerous *NPHS2*-mutated alleles, which have been described in different ethnicities spanning Europe, North Africa, and Asia, have considerable heterogeneity in their biologic effect. Individuals with podocin mutations have FSGS that has variable pathologic changes, severity, and age of onset. In light of the phenotypic heterogeneity produced by mutations within the *NPHS2* gene, one must ask the question of whether screening individuals for podocin mutations has any important implications for the clinical and therapeutic approach to patients with nephrotic syndrome? Analysis from the largest cohort of patients with *NPHS2* mutations originating mainly from France and North African countries has shown that patients with 2 pathogenic *NPHS2* mutations present with early onset steroid-resistant nephrotic syndrome and a very low incidence of posttransplantation recurrence.[47] However, individuals with double-heterozygous *NPHS2* mutations sometimes account for atypical cases with mild, late-onset disease and recurrence after renal transplantation.[47] Although evidence to date suggests that *NPHS2*-associated disease will be steroid resistant, no prospective controlled trials have been undertaken to evaluate this question specifically. There is anecdotal evidence that suggests treatment with chemotherapy or immunotherapy may delay the progression of ESRD in individuals with hereditary FSGS.[48] In addition, steroid-sensitive populations with sporadic disease have not been tested widely for podocin abnormalities. Further knowledge regarding the frequency of podocin mutations will need to be verified before initiating tailored therapy regimens.

Autosomal-dominant FSGS is typically a disease of adults, with variable age of onset, severity, and progression to ESRD. At present there are 2 known disease-causing genes. Through linkage analysis, the first reported locus for inherited autosomal-dominant FSGS mapped to chromosome 19q13,[49] with the subsequent identification of α-actinin 4 (*ACTN4*).[50] *ACTN4* is 1 of 4 actinin genes that encodes a 100-kd actin cross-linking protein. It is expressed in a wide range of tissues; however, it appears to be expressed very highly in podocytes. Mutated α-actinin–4 binds filamentous actin more strongly in vitro than wild-type, thus postulating a role for α-actinin–4 in the regulation of the podocyte cytoskeleton.[50] Mouse models deficient for this gene show progressive proteinuria, and glomerular disease, with podocyte foot process effacement on electron microscopy.[51] The exact function of α-actinin–4 in the kidney requires further investigation. Mice with homozygous deletions for *ACTN4* show an increased amount of cell motility, showing that this protein likely plays a functional role in the regulation of cell movement.[51] For unclear reasons, despite the widespread expression of α-actinin–4, the disease phenotype appears to be limited to the kidneys in both mice and human beings.

The most recently reported disease-causing mutation for hereditary FSGS has provided striking new insight into the genetic heterogeneity and pathogenesis of nephrotic syndrome. The character of disease in this particular subset of families is particularly aggressive with affected individuals presenting in their third or fourth decade with high-grade proteinuria. Sixty percent of these individuals progress to ESRD within 10 years. A genomic screen performed on a New Zealand kindred mapped the locus of the disease to chromosome 11q21 to 22.[52] After examination of 42 candidate genes, transient receptor potential cation channel 6 (*TRPC6*) emerged as the causative gene with a missense mutation in exon 2.[53] Previously reported mutations in familial disease such as *NPHS1*, *NPHS2*, and *ACTN4* have emphasized the importance of cytoskeletal and structural proteins in proteinuric kidney diseases. Table 1 lists the current podocyte proteins thought to cause human glomerular disease. Figure 2 shows our current understanding of the molecular composition of the podocyte foot process. The SD, a porous filter structure, consists of nephrin molecules from adjacent foot processes. The nephrin molecules form a zipper-like ultrafilter structure creating the main size-selective filter barrier in the kidney.[54] The intracellular protein, *CD2AP*, connects the cytoplasmic domain of nephrin to the cytoskeleton.[55] Podocin also localizes to the SD and associates via its C-terminus with *CD2AP* and nephrin.[44,45] Podocin likely acts as a scaffolding protein, serving in the structural organization of the SD and the regulation of its filtration function.[44,45] α-actinin–4 stabilizes the actin-cytoskeleton by cross-linking actin filaments. Mutations in nephrin, podocin, *CD2AP* (in mice),[55] and α-actinin–4 lead to proteinuria and nephrotic syndrome. The implication of a calcium channel in the pathogenesis of FSGS suggests an altogether different mechanism for glomerular disease pathogenesis. *TRPC6* encodes the transient receptor potential cation channel 6, and is part of the TRP channel family of proteins. The TRP channels have been implicated in diverse biologic functions such as cell

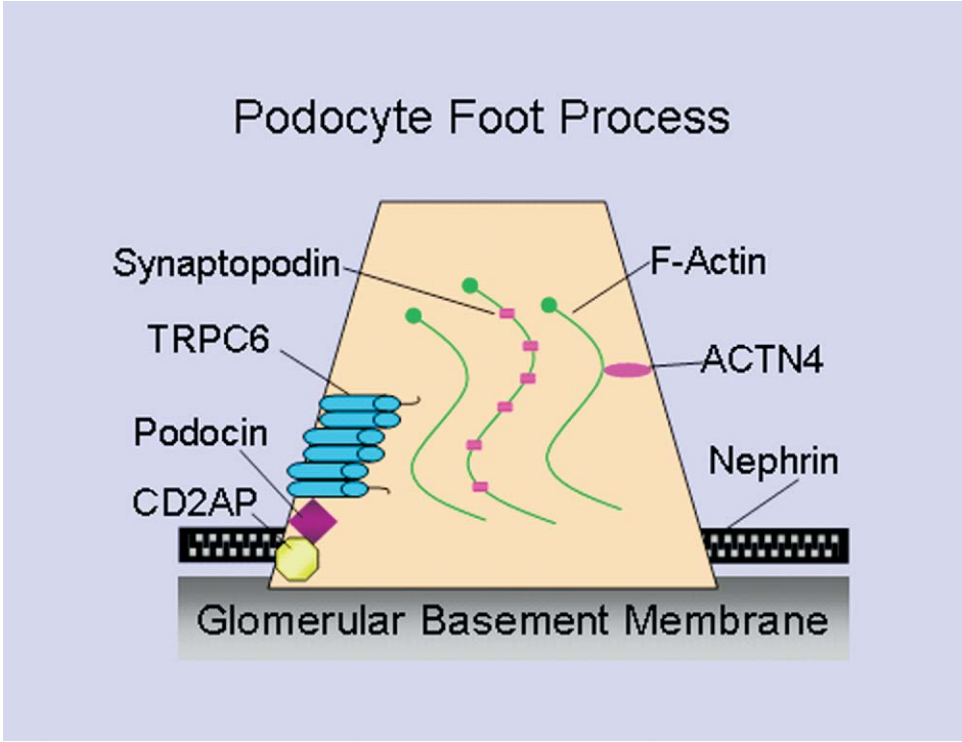

Figure 2 Proposed diagram of a podocyte foot process and the interaction between various podocyte proteins with an important role in hereditary nephrotic syndromes. TRPC6, transient receptor potential cation channel 6; ACTN4, α-actinin–4; CD2-AP, CD2-associated protein; F-ACTIN, filamentous actin.

growth, ion homeostasis, mechanosensation, and phospholipase C (PLC)-dependent calcium entry into cells. The P112Q *TRPC6* mutation causes a gain of function, as evidenced by exaggerated calcium influx into cells, with the supposition that this results in disrupted glomerular cell function or causes apoptosis.[53] Additional work has corroborated findings implicating *TRPC6* in the pathogenesis of autosomal-dominant FSGS.[56] Of the additional *TRPC6* mutations that have been reported in 5 other families, 2 were associated with an increase in calcium influx. This suggests that multiple mechanisms involving *TRPC6* abnormalities exist, which may result in dysregulation of the ion channel or altered interaction with other SD proteins.[53] The search to discover potential interactions of *TRPC6* with other known causes of hereditary FSGS and nephrotic syndromes will be an area of active interest in the future.

Glomerular dysfunction is the final common pathway for a variety of proteinuric kidney diseases such as diabetes mellitus and systemic lupus erythematosus. The glomerular podocyte serves as the final barrier to protein loss. Abnormalities in these specialized cells, such as foot process effacement and molecular reorganization of the SD, are common to all forms of nephrotic syndrome. A significant amount of progress has been made in recent years in helping to define podocyte structure and the multiple interactions of proteins associated with this glomerular epithelial cell. These studies highlight the heterogeneous disease process of FSGS. Numerous podocyte proteins have been identified such as nephrin, podocin, α-actinin, and, most recently, *TRPC6* through the study of inherited podocytopathies. Understanding the multiple interactions of these proteins will provide insight into the mechanisms of podocyte injury leading to glomerular disease. Further progress also will be made in identifying the elusive circulating factor, and the role of the immune system in causing podocyte injury. As more is learned regarding the pathogenesis of glomerular disease, targeted treatment strategies can be developed to impact the enormous and growing morbidity and mortality from ESRD.

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