Unexpected Role of *TRPC6* Channel in Familial Nephrotic Syndrome: Does It Have Clinical Implications?

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Focal and segmental glomerulosclerosis (FSGS) is a leading cause of glomerulonephritis and chronic kidney disease (CKD) in children and young adults (1). As much as one fifth of the ESRD population carries this diagnosis, and the proportion of ESRD attributed to FSGS has increased more than 10-fold over the past two decades (2). The glomerular landmarks of FSGS can develop secondary to a variety of systemic conditions, including disorders that cause chronic hypoxemia, processes that augment glomerular blood flow, and diseases that reduce renal mass. The structural features of FSGS also develop in patients who lack recognized systemic diseases, so-called primary (or idiopathic) FSGS. Over the past three decades, the recognition of familial forms of FSGS has grown, and this information has been highlighted in recent years with characterization of several human genes that cause FSGS (3).

Most recently, a mutation in a member of the canonical transient receptor potential (TRP) family of proteins, *TRPC6*, was identified to cause FSGS in a large kindred of autosomal dominant FSGS (4). The mutation in *TRPC6*, in contrast to genes that have been implicated in the pathogenesis of FSGS, seems to perturb cytosolic calcium ($[Ca^{2+}]_i$) signaling. The purpose of this review is to examine what is known about *TRPC6* mutations in familial nephrotic syndrome and to explore how this new knowledge may be applied to the care of patients with glomerular diseases.

Familial Forms of FSGS

Exciting discoveries in the past 10 years have identified numerous genes that are important for human glomerular diseases. The study of Mendelian forms of FSGS has been the catalyst behind these discoveries, lending insight into the pathophysiologic processes that participate in this disease. Reports of familial forms of FSGS date back as far as 1956, with the observation of an autosomal recessive disease primarily within the Finnish population. The disease process is characterized by massive proteinuria *in utero*, with up to 20 to 30 g/d protein loss (5). Advances in gene mapping and molecular biology within the past decade helped localize the causative gene to chromosome 19q31.1 (6), with the subsequent identification of *NPHS1* (7). *NPHS1* encodes a gene product termed "nephrin," within which numerous mutations, including deletions, insertions, nonsense, missense, and splicing errors, have been described (8). Structurally, nephrin is composed of eight Ig C2 motifs, a fibronectin III–like domain, and a single transmembrane segment (Figure 1). Nephrin localizes to lipid "rafts" within the slit diaphragm of the podocyte (9–11). Lipid rafts are specialized microdomains of the plasma membrane with a unique lipid content and a concentrated assembly of signal transduction molecules (12). Localization to lipid rafts suggests that mutations of the nephrin gene likely affect its role in regulating podocyte signaling pathways (13,14).

Confirmation of the role that nephrin plays in the development of glomerular disease is demonstrated by mouse models for congenital nephrotic syndrome of the Finnish type (Finnish nephropathy). Targeted disruption of the nephrin gene in embryonic stem cells has resulted in mice homozygous for the mutant *NPHS1* allele, and the phenotype of these mice mirrors the human disease with the early onset of massive proteinuria and neonatal death (15). Additional mouse models that mimic recurrence of congenital nephrotic syndrome in humans after renal transplant have been developed (16,17). These models have been created by injection of mice with mAb directed toward the extracellular domain of nephrin (18). The biologic effects of the anti-mouse nephrin antibodies highlight the importance of nephrin and the slit diaphragm in the regulation of glomerular permselectivity (18).

Steroid-resistant nephrotic syndrome is another human disorder that is characterized by autosomal recessive nephrotic syndrome. This disorder manifests between 3 mo and 5 yr of age, rapid progression to ESRD, and with few cases of recurrence after renal transplantation. The gene product is podocin (*NPHS2*), located on 1q25–31 (19). The structure of podocin is characterized by a single membrane domain forming a hairpin-like structure, with both N- and C-terminal domains in the cytosol. Podocin is expressed exclusively in podocytes (20), and specifically localizes to the base of the foot processes on either side of the slit diaphragm as seen on electron microscopy (21). Mice that lack the podocin gene product have a prominent renal phenotype that includes proteinuria and early death from renal failure (22). Podocin most likely functions in the structural organization of the slit diaphragm and regulation of its filtration function (20). It has been shown to interact *in vivo* with both nephrin and CD2-associated protein (CD2AP), a cytoplas-

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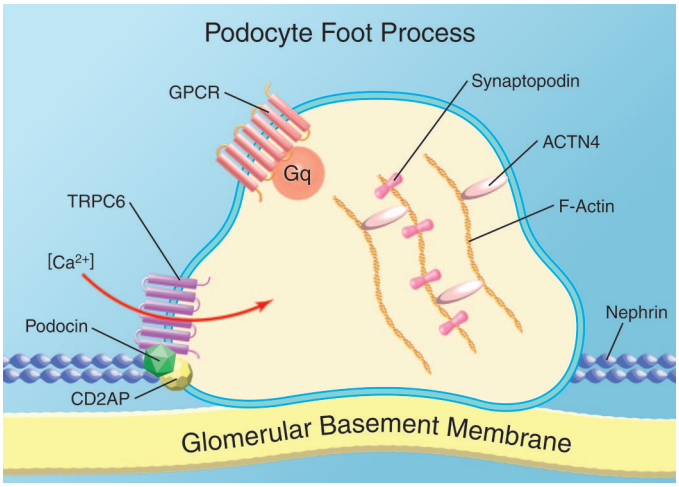

Figure 1. Proposed diagram of a podocyte foot process and various podocyte proteins with an important role in hereditary nephrotic syndromes. Transient receptor potential cation channel 6 (*TRPC6*) is thought to interact with CD2-associated protein (*CD2AP*), podocin and nephrin. *ACTN4*, α-actinin 4; F-actin, filamentous actin; GPCR, G protein–coupled receptor. Illustration by Josh Gramling—Gramling Medical Illustrations.

mic binding partner of nephrin (23). Podocin recruits nephrin to lipid rafts in the slit diaphragm (13,23,24). Recruitment of nephrin to lipid rafts, through the direct interaction of podocin with its cytoplasmic tail, is required for the proper initiation of nephrin signaling. Targeted disruptions of podocin inhibits both nephrin trafficking and nephrin-initiated signal transduction (24).

The nephrin-podocin complex seems crucial to nephrin signal transduction and maintenance of normal podocyte biology, but mutations that compromise the structural integrity of the podocyte also seem to result in foot process effacement and lesions that are characteristic of FSGS. Mutations in the α-actinin 4 gene (*ACTN4*), which localizes to chromosome 19q13, have been associated with autosomal dominant FSGS, characterized by adult-onset disease of variable severity and rate of progression to ESRD (25,26). *ACTN4* is one of four actinin genes, which encodes a 100-kD actin cross-linking protein. Although *ACTN4* is expressed in a wide range of tissues, it is very highly expressed in podocytes, functioning in the regulation of the podocyte cytoskeleton. Transgenic mice that express mutant *ACTN4* as well as *ACTN4*-deficient mice develop renal disease that resembles FSGS (27,28). Fractions of the mutant protein have been shown to form large aggregates within podocytes, ultimately compromising the function of the normal actin cytoskeleton, through both its abnormal function and its toxic accumulation (29).

The most recently reported disease-causing mutation for hereditary FSGS has been localized to chromosome 11q21-22, with the subsequent identification by Winn *et al.* (30) of transient receptor potential cation channel, subfamily C, member 6 (*TRPC6*) as the disease-causing gene. The missense mutation changes a highly conserved proline in the first ankyrin repeat of *TRPC6* to a glutamine at position 112 (P112Q). Additional work was reported by Reiser *et al.* (31), who corroborated findings implicating *TRPC6* in the pathogenesis of familial FSGS. Whereas previously reported mutations such as *NPHS1*, *NPHS2*, and *ACTN4* have emphasized the importance of cytoskeletal and structural proteins in glomerular diseases, *TRPC6*-related FSGS suggests an additional mechanism for renal disease pathogenesis. Knowledge of *TRPC6*-mediated calcium entry into cells may offer unique insights into therapeutic options for glomerular diseases.

Biology of TRP Channels

The TRP channels have an extensive family of mammalian homologues (32). These channels have been implicated in diverse biologic functions such as cell growth, ion homeostasis, mechanosensation, and phospholipase C (PLC)-dependent calcium entry into cells. TRP channels were originally identified in the *Drosophila* visual system (33). It was observed that in *Drosophila* with the mutated TRP channel, blindness after intense and prolonged light exposure as a result of sustained Ca^{2+} entry occurred (34). There are common structural motifs in TRP channels. In addition to sequence homology, they are permeable to cations, especially calcium. The common feature among these channels is six transmembrane-spanning domains. The fifth and sixth transmembrane domains form tetramers that line the pore of the ion channel, and both the N- and C-termini are intracellular. Another frequent feature in some TRP channels is ankyrin binding repeats in the N-terminus.

The regulation and biologic functions of TRP channels have not yet been defined clearly. Hereafter, we concentrate on the TRPC family of proteins and, more specifically, *TRPC6*. The TRPC family of proteins is made of seven different proteins with four subfamilies (*TRPC1*, *TRPC3,6,7*, *TRPC4,5*, and *TRPC2*) based on sequence homology and operative similarities. The *TRPC3,6,7* subfamily are nearly 75% identical *via* sequence homology. The TRPC family of proteins is widely expressed in human tissues. It is known that most TRP channels are nonselective cation channels and permit Na^+ as well as Ca^{2+} entry into cells; the *TRPC3,6,7* subfamily has selectivity on the order of P_{Ca}/P_{Na} 1.5 to 6:1, with *TRPC6* being the most selective (32). *TRPC6* can be activated either *via* a G protein–coupled receptor (GPCR) pathway or by application of exogenous analogues such as diacylglycerol (DAG) and act independently of $[Ca^{2+}]_i$ store depletion (35). All members of the TRPC family have inositol 1,4,5 triphosphate receptor (IP3R) binding sites in the C-terminus, and calmodulin binds to this peptide sequence in a Ca^{2+}-dependent manner (36). As such, a mechanism for channel function that is coupled to intracellular calcium concentration can be inferred from these findings. *TRPC3,6,7* seem to co-assemble when heterologously expressed (37). It is now also believed that TRP channels form a signaling complex, or "signalplex," with other molecules such as the scaffolding protein *INAD*; in turn, *INAD* interacts with immunophilins (38). *TRPC6*-deficient mice display a higher contractility in tracheal and aortic rings after stimulation with agonists such as methacholine and phenylephrine (39). They also have a modest elevation in blood pressure; we speculate that this may be due to a vascular phenotype and may have an impact on the

human disease. In addition, constitutively active *TRPC3* ion channels are upregulated in *TRPC6*-deficient smooth muscle cells and do not replace *TRPC6* functionally, therefore indicating a nonredundant role (39). Given the previous emphasis on structural mutations as a cause for the glomerular pathology seen in FSGS, a new paradigm that features podocyte GPCR and downstream cellular signaling now can be added to the emerging mechanisms for this disorder.

PLC-Coupled Receptors on Podocytes

Vasoactive hormones are important regulators of glomerular ultrafiltration and may promote glomerular injury in disease states (40). As a result, the receptor systems and effector pathways that are activated by these hormones have been an intense area of investigation. The effects of vasoactive agents are typically mediated by binding to cell surface receptors that belong to the large superfamily of seven transmembrane-spanning receptor proteins (40,41). As shown in Table 1, podocytes express numerous heptahelical receptor systems (43–61). The majority of these receptors activate intracellular effector pathways (Table 1) by coupling to G proteins (41,62). An important exception may be the type 2 angiotensin II receptor (AT2). Cloning of the AT2 receptor (63,64) suggested that it belonged to a unique group of heptahelical receptors that do not couple to G proteins (64).

The precise role of GPCR in regulating podocyte function is incompletely understood. A large body of evidence, however, suggests that vasoactive hormones modulate glomerular filtration by modulating efferent and afferent arterial tone as well as altering the glomerular ultrafiltration coefficient K_f (40). A role for the podocyte in altering K_f has been proposed by several investigators (40,65,66). In this regard, the podocyte foot process contains a contractile apparatus (67) that may respond to vasoactive hormones (40,65,66). Although direct evidence for such a contractile response is lacking, it has been postulated that K_f may be regulated, in part, by modulating the size of the filtration slits through both calcium- and cAMP-dependent signaling pathways (40,65,66). As shown in Table 1, a large num-

Table 1. Heptahelical receptors that may modulate *TRPC6* function in podocytes[a]

Receptor	K_m	B_{max}[b]	Effector Pathway	References
Ang II				
AT1	≈3.0 nM	≈562 fmol/mg	cAMP, calcium, IP3	(42–46)
AT2	≈3.0 nM	≈196 fmol/mg	cAMP, calcium	(42–45)
Prostaglandin				
EP1	ND	ND	Calcium	(47)
EP4	ND	ND	cAMP	(47)
FP	ND	ND	Calcium	(47)
TP	ND	ND	Calcium	(47)
Endothelin				
ET1	≈0.4 nM	≈8.1×10^{10} sites/mg	Calcium, IP3	(48,49)
Leukotrienes				
LTC4	≈217 nM	≈4.5 pmol/mg	Cell proliferation	(50)
LTD4	ND†	ND	Cell proliferation, PKC	(51)
Bradykinin				
BK2	≈0.04 nM	≈77 fmol/mg	Calcium, IP3	(52,53)
ATP				
P2y2	ND	ND	Calcium, IP3	(54)
Histamine				
H1	ND	ND	Calcium, IP3	(55)
Adrenergic				
α1	ND	ND	Calcium	(56)
β4	ND	ND	cAMP	(56)
Thrombin	ND	ND	Calcium, IP3	(57)
Dopamine				
D1	ND	ND	cAMP	(58)
PTH	ND	ND	cAMP	(59,60)
Chemokines				
CCR4, 8, 9, 10	ND	ND	cAMP	(61)
CXCR1, 3, 4, 5	ND	ND	Calcium	(61)

[a]Ang II, angiotensin II; AT1, type 1 Ang II receptor; AT2, type 2 Ang II receptor; ≈, approximately; IP3, inositol 1,4,5 triphosphate; ND, not done; PKC, protein kinase C; PTH, parathyroid hormone.
[b]fmol or sites/mg protein.

ber of GPCR in podocytes are coupled to either cAMP or calcium signaling cascades; therefore, these GPCR systems have the potential to modulate podocyte contractility and may play a role in disease pathogenesis. Because many of these GPCR systems are Gq coupled they have the capacity to activate *TRPC6* (68). The K_m values suggests that GPCR on podocytes have a high affinity for ligand; therefore, the receptors are physiologically relevant binding sites. The B_{max} provides an index of receptor density that, in most receptor systems, correlates with the magnitude of physiologic response to agonist.

Vasoactive hormones, such as angiotensin II (Ang II), also have the capacity to modulate the surface charge of the podocyte and, in turn, alter glomerular permselectivity (69). This change in glomerular permselectivity may play a role in disease pathogenesis by enhancing protein excretion and progression of kidney injury in disease states (69,70). Indeed, GPCR systems expressed by podocytes are likely to play an important role in glomerular disease processes as discussed below.

Glomerular podocytes express numerous GPCR that are implicated in the pathogenesis of glomerular diseases, including receptors for Ang II (AT1 receptor), thromboxane, E-series prostaglandins (EP1 receptor), endothelin, and cysteinyl-leukotrienes (71–76). Although the signaling pathways that are activated by these GPCR are diverse, common to all of these receptor systems is activation of PLCβ through G proteins that belong to the Gq family (71,72,77–80). Although the precise mechanisms are incompletely understood, Gq-coupled receptors are potent activators of *TRPC6* (4,31,35,62,81). This activation seems to be mediated by stimulation of PLCβ and generation of the second messengers DAG and inositol 1,4,5-trisphosphate (IP3) (82). Both DAG analogs and IP3 enhance calcium conductance by *TRPC6* (4,31,35,62,81). Accumulating evidence suggests that the effects of IP3 on *TRPC6* activity may be mediated by binding to a complex that is composed of *TRPC6* and the IP3 receptor (68,83) and/or by IP3-stimulated release of calcium from the intracellular stores (68,84). This activation process causes redistribution of *TRPC6* from the intracellular pool to the plasma membrane and, in turn, produces a sustained increase in $[Ca^{2+}]_i$ levels (68,85).

How the *TRPC6^{P112Q}* Mutation Causes FSGS

Cytoskeletal and structural proteins have previously been recognized as important in hereditary proteinuric kidney diseases. The implication of a calcium channel in the pathogenesis of FSGS suggests an altogether different cause of glomerular disease pathogenesis. Expression of *TRPC6* has been determined to be ubiquitous in the kidney, including podocytes and endothelial and tubular cells (4,86). Expression of *TRPC6* in glomeruli is particularly noteworthy as abnormal podocyte function seems to be a final common pathway in a variety of proteinuric kidney diseases (87).

In addition, the *TRPC6^{P112Q}* mutation causes markedly increased and prolonged calcium influx into cells; biotinylation experiments show an altered subcellular localization of the mutant *TRPC6* protein in cells that are transfected with either the wild-type or mutant protein (4). The relative distribution of *TRPC6^{P112Q}* protein in the plasma membrane is significantly greater than wild-type protein. Increased calcium transients as a result of mutations in *TRPC6* are in accordance with reports by others (31).

The importance of Ang II as a mediator of kidney injury was enumerated above. The P112Q mutation does indeed affect Ang II–dependent calcium signaling. This mutation causes higher peak intracellular Ca^{2+} changes in *TRPC6^{P112Q}* transfected cells in response to stimulation by Ang II. It is interesting that when the analogous mutation in *TRPC3* is introduced in HEK 293 cells, the same increase in *TRPC3*-mediated calcium entry is observed after Ang II stimulation.

Additional work by others has substantiated the above findings. Recently, Reiser *et al.* also demonstrated evidence that *TRPC6* is an important component of the slit diaphragm (31). These authors also found evidence of mutations in *TRPC6* in five families with familial FSGS. They likewise found evidence that *TRPC6* is expressed throughout the kidney and specifically in podocytes in the kidney. Immunogold labeling revealed *TRPC6* in the major and minor foot processes of the podocyte. *TRPC6* co-localized with CD2AP, nephrin, and podocin in cultured mouse podocytes and co-immunoprecipitated with nephrin and podocin. *TRPC6* seemed to be upregulated in 2-d-old nephrin-deficient mice. Electrophysiology studies confirmed augmented calcium influx in two of five families. That mutations in either the N- or the C-terminus of the *TRPC6* protein cause the same functional changes (an increase in calcium transients) suggests that multiple mechanisms involving *TRPC6* abnormalities exist, such as dysregulation of the ion channel, or altered interaction with other slit-diaphragm proteins, with the supposition that this results in disrupted glomerular cell function or causes apoptosis.

It is unclear how mutations in *TRPC6* cause FSGS. One could posit many different possibilities. Calcium as a second messenger is a potent effector of cellular functions such as contraction, volume regulation, immunologic responses, cell migration, and proliferation. It is known that intracellular calcium concentrations are tightly regulated. Exaggerated calcium signaling conferred by the *TRPC6^{P112Q}* mutation may disrupt glomerular cell function or may cause apoptosis (31). Injurious signals that are triggered by Ang II and are known to promote kidney injury and proteinuria may be amplified by the mutant protein. Podocytes are known to be highly dynamic as they adjust to glomerular filtration pressures. Perhaps *TRPC6* is unable to guide needed protein chaperones such as nephrin or podocin in sealing the filtration barrier in response to mechanical forces (31,88,89). Another plausible mechanism is that the enhanced and sustained increases in intracellular calcium induced by the *TRPC6* mutation activate the calcium-dependent phosphatase calcineurin. Calcineurin is linked directly to induction of apoptosis through dephosphorylation of the protein BAD (90,91).

A potentially key observation in these studies is that the *TRPC6* mutations cause sustained increases in $[Ca^{2+}]_i$ levels only after agonist stimulation without appreciably altering basal $[Ca^{2+}]_i$ levels (4). The dependence on agonist stimulation for increased $[Ca^{2+}]_i$ levels indicates that therapies that target the relevant GPCR systems may be useful for slowing the development of renal disease in predisposed individuals. Al-

ternatively, strategies that target final common signaling cascades such as *TRPC6* might be an attractive strategy for reducing podocyte injury induced by activation of multiple GPCR systems.

These findings, taken together with previously published studies highlight the importance of $[Ca^{2+}]_i$ in podocyte biology. The ability of the podocyte to regulate precisely $[Ca^{2+}]_i$ levels seems to play a central role in glomerular disease processes. Because ion channels are generally amenable to pharmacologic manipulation, these studies raise the exciting possibility that manipulating $[Ca^{2+}]_i$ levels by targeting *TRPC6* may be a useful strategy for treating not only familial disease but also patients with other primary and secondary forms of FSGS.

Native and Mutated *TRPC6* in Primary FSGS and Other Chronic Glomerular Diseases

The association between *TRPC6* mutations and FSGS will improve our understanding of the pathogenesis of familial forms of glomerulonephritis, but this discovery also advances two hypotheses that may pertain to patients with other forms of progressive glomerular diseases: (*1*) That adaptation of native *TRPC6* channels underlies effective therapies for many progressive glomerular diseases and (*2*) that the cell-signaling cascade that governs $[Ca^{2+}]_i$, including activity of *TRPC6* channels, is critical to maintain the health of the podocyte in CKD.

Because some *TRPC6* mutations result in FSGS and exaggerated responses in intracellular calcium, it is attractive to consider that the *TRPC6* itself serves as the ideal therapeutic target. Electrophysiologic studies define *TRPC6* channels as nonselective for cations with relatively low selectivity for Ca^{2+} over Na^+ (81). As previously discussed, TRPC6 activity is stimulated by analogues of DAG, enhanced intracellular calcium levels, and/or activation of a complex of the IP3 receptor and *TRPC6* (4,31,35,68,81,83,92). It is interesting that DAG analogues increase calcium conductance by TRPC6 independent of protein kinase C activity, a common target of DAG (81). Additional knowledge of the TRPC6 activation site or its mode of activation by binding of the inositol 1,4,5-trisphosphate receptor may provide new targets to inactivate the channel (93). The enthusiasm to develop specific *TRPC6* antagonists must be balanced, however, by a few observations. Although the receptor-activated calcium transients may be dramatically enhanced in some *TRPC6* mutants, the TRPC channels admit relatively small amounts of calcium per channel (94). In addition, because members of the *TRPC3*, *6*, and *7* families share electrophysiologic characteristics, the gene products may serve some redundant roles (95). Therefore, any pharmacologic agent that is effective for blocking *TRPC6* may also need to block *TRPC3* and *TRPC7*, which are very similar in function to *TRPC6*. Regardless, development of specific pharmacologic inhibitors for *TRPC6* will be eagerly anticipated.

Normal function of the podocyte *TRPC6* channel may be responsible for at least part of the renoprotective effects of medications that block the renin-angiotensin system. Benefits of angiotensin-converting enzyme inhibitors (ACEi) and AT1 receptor blockers (ARB) are that they reduce proteinuria and limit progression of renal disease not only in FSGS but also in chronic glomerular diseases such as diabetic nephropathy and IgA nephropathy (92,96–99). The ACEi also reduce protein excretion and potentially limit progression to ESRD in renal diseases that manifest with less dramatic proteinuria, ostensibly when the podocyte is less disrupted, including hypertensive nephrosclerosis and autosomal dominant polycystic disease (100,101). As detailed above, *TRPC6* channels and their activation participate in both the amplitude and the duration of $[Ca^{2+}]_i$ responses in the podocyte after agonist activation of the AT1 receptor. Effective blockade of this response with ACEi and ARB has been the most effective and consistent pharmacologic interventions to limit progression of many forms of CKD.

Activity of *TRPC6* may also pertain to glucocorticoid therapy, long considered the first effective line of therapy in idiopathic FSGS. When used either intravenously or orally, steroids have pleiotropic effects (102,103). Glucocorticoids bind to cytoplasmic receptors that translocate to the nucleus, where the transcription of certain genes is modified, including genes for cytokines, chemokines, eicosanoids, and other pathways that participate in the inflammatory response (104). As addressed above, a number of eicosanoid products activate GPCR on podocytes and are capable of subsequently increasing *TRPC6* activity. Steroids also exert responses at the level of the glomerulus by modifying podocyte metabolism. Glucocorticoids promote podocyte growth and migration in culture and may increase expression of the glucocorticoid receptor (105,106). Recently, dexamethasone was demonstrated to protect podocytes in culture when they were treated with puromycin, an exposure that causes apoptosis of glomerular epithelial cells and causes nephrotic syndrome in experimental animals (107,108). Although the importance of *TRPC6* for the steroid response has not been characterized, intracellular calcium influx is widely known to be an important step in apoptosis in the injured renal cell (109).

Nearly 60% of patients who have primary FSGS and receive glucocorticoid therapy for as long as 6 months will be resistant to therapy, will continue to exhibit proteinuria, and will carry a disproportionate risk to develop ESRD (110). These at-risk patients warrant other medical interventions. The North American FSGS Trial, performed in a population of patients with steroid-resistant FSGS, suggested that a 6-month course of cyclosporine (CsA) combined with prednisone will reduce the amount of proteinuria and preserve kidney function (compared with prednisone alone) (111). Clinical trials that were composed of smaller patient groups also suggested that tacrolimus (FK506) may have similar clinical effect to CsA, to increase the likelihood of achieving clinical remission in patients who have FSGS and are resistant to steroid therapy (112). The modes of action of FK506 and CsA require that the drugs bind to the intracellular immunophilins FKBP-12 and cyclophilin, respectively, and that the immunophilin complex interact and inhibit the $[Ca^{2+}]_i$-dependent phosphatase calcineurin (113). Effectiveness of these medications to suppress the immune system and to induce remission in FSGS is traditionally attributed to reduc-

tion of calcineurin activity and the resulting decrease in cytokine promoter activity (113,114). Alternatively, effectiveness of calcineurin inhibitors in FSGS may rely on modification of native TRP channels in the podocyte. The immunophilin targets for CsA and FK506 are capable of binding with *INAD*:TRP complexes (described above). Therefore, the clinical benefit of calcineurin inhibitors in patients with steroid-resistant FSGS may be mediated by interactions with the immunophilin FKBP-12 and *TRPC6* (93).

The especially poor prognosis of patients with steroid-resistant FSGS has driven clinical investigators to explore other effective treatments. The National Institutes of Health is currently enrolling these high-risk patients in a trial in that will compare outcomes in groups that are treated with a CsA-based regimen (similar to that used in the North American FSGS Trial outlined above) or with high-dose pulse oral steroids and mycophenolate (68). As a component of the ancillary studies of this trial, genotyping for genetic mutations associated with FSGS, including *TRPC6*, may provide an opportunity to determine the prevalence of known genetic mutations that are associated with steroid-resistant idiopathic FSGS.

Utility of Genetic Typing of Patients with CKD

Discoveries in the past few years have successfully identified *TRPC6* and other genes that impart renal risk. Despite these advances, recognition of genes that are responsible for the majority of cases of ESRD remains relatively elusive. This likely results from the fact that renal failure is a polygenic disease that manifests only after specific combinations of environmental exposures (115). In addition, ESRD is the final end point of a wide variety of illnesses, and it is naïve to anticipate that a single causative human gene can be identified. The clinical utility of a mythical "ESRD gene" may also be limited by comorbid conditions that are associated with all stages of CKD. The National Kidney Foundation proposed four categories of risk factors when considering adverse outcomes in CKD: (*1*) Factors that increase susceptibility to CKD; (*2*) factors that initiate kidney damage; (*3*) factors that promote progression of CKD; and (*4*) factors that predispose to complications of ESRD (116). If information from new "renal failure" genes such as *TRPC6* will influence clinical management of patients with CKD and not be limited to those with FSGS, then it may be more realistic to apply this new information to one of these risk categories. Because the penetrance of FSGS in individuals with the *TRPC6* mutation is relatively high in the published descriptions to date, it may be that polymorphisms of *TRPC6* act as susceptibility or initiation factors for renal disease. However, additional genetic data need to be gathered in the human FSGS population.

The heterogeneous clinical outcome of patients with primary FSGS suggests that knowledge of a genetic basis of renal disease, including *TRPC6* status, could have clinical utility. Baseline criteria of patients who reach a diagnosis of primary FSGS, including race, degree of proteinuria, histologic presentation, and baseline GFR, can be used to determine those who are at risk to develop ESRD (117,118). Responses to treatment, most importantly, complete or partial regression of proteinuria, also correlate with long-term renal prognosis (118). Because only part of the risk for attaining ESRD seems explainable by these baseline and treatment variables, characterization of *TRPC6* (and perhaps other causative genes) may aid early identification of at-risk patients with FSGS. Previous knowledge of *TRPC6* status may also permit tailoring of initial medication choices in primary FSGS. Status of podocin mutations may be able to predict responsiveness to conventional steroid treatment, but it is not yet clear whether *TRPC6* will carry similar implications (119). After attainment of ESRD, however, patients who have *TRPC6* mutations and receive a renal allograft are less likely to develop recurrent FSGS. Therefore, *TRPC6* characterization may be helpful in preoperative screening of potential kidney transplant recipients and also in potential living-related donors in families that carry the disease.

The clinical responses in other chronic glomerular diseases are similarly varied, and genetic factors such as *TRPC6* may explain the inconsistent outcomes. Genotyping may prove to be valuable in this large population of patients. For example, the status of ACE gene polymorphisms has been proposed as a way to determine whether a patient with early diabetes may be at disproportionate risk to develop diabetic nephropathy (120). A clinician might use information regarding *TRPC6* status to consider relatively innocuous interventions such as lifestyle modifications or more aggressive management such as early ACEi or ARB therapy. Because many *TRPC6* mutations described thus far are associated with exaggerated cytosolic calcium responses, particularly to AT1 receptor activation, these patients may also be considered for regimens that include combination therapy with ACEi and ARB (92). These patients might also be considered for antagonists for other podocyte GPCR when they are developed in the future.

Conclusion

Over the past 10 years, familial nephrotic syndromes have shed significant insight into normal podocyte homeostasis. Mutations involving the podocyte proteins nephrin, podocin, CD2AP, and α-actinin 4 have highlighted the importance of the maintenance of podocyte structure in the pathogenesis of glomerular disease. With the surprise implication of *TRPC6* in familial nephrotic syndrome, aberrant calcium signaling and the role of GPCR in regulating podocyte function will now be at the forefront of investigation for developing targeted therapies against FSGS, a disease that has been notoriously difficult to treat. Precise regulation of podocyte $[Ca^{2+}]_i$ suggests that perhaps targeting *TRPC6* agonists or even direct manipulation of *TRPC6* channels themselves will prove to be an effective strategy for treating glomerular diseases. Although knowledge regarding the function of podocyte proteins and their role in maintaining normal cell-signaling cascades will undoubtedly progress, further understanding in the clinical arena regarding the polygenic influences and risk factors that are involved in determining rates of disease progression and response to therapy will need to be ascertained in a standardized manner to permit tailoring of therapy in individuals. Since the initial descriptions of familial nephrotic syndrome made decades ago,

considerable progress has been made regarding the importance of normal podocyte function on a molecular level. Optimistically, within the next decade, we will see a translation of this knowledge, imparting long-lasting clinical and therapeutic implications.

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