**2007 Young Investigator Award: TRP’ing into a New Era for Glomerular Disease**

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**ABSTRACT**

FSGS is a pathologic lesion that frequently causes the nephrotic syndrome and ensuing renal failure. The cause remains unknown in the majority of individuals; however, in the past two decades, rare familial forms have been identified. It has been suggested that known genetic causes of the hereditary form of this disease account for upwards of 18% of cases. Mutations in five genes have been found to cause inherited nephrotic syndromes and FSGS. In this article, I discuss the phenotypic characteristics of hereditary FSGS and the transient receptor potential cation channel 6 (TRPC6) protein, which is the genetic impetus for an autosomal dominant form of FSGS. The TRP channels have been implicated in varied biologic functions such as mechanosensation, ion homeostasis, cell growth, and phospholipase C-dependent calcium entry into cells. The mutated ion channel causes an increase in calcium transients. Current evidence also suggests that blocking TRPC6 channels may be of therapeutic benefit in idiopathic FSGS, a disease with a generally poor prognosis. Preliminary experiments reveal that the commonly used immunosuppressive agent FK-506 can inhibit TRPC6 activity in vivo. This creates the intriguing possibility that blocking TRPC6 channels within the podocyte may translate into long-lasting clinical benefits in patients with FSGS.

The diagnosis of FSGS requires the presence of glomerular sclerosis and tuft collapse in discrete areas of the kidney. Segmental hyalinosis, glomerular deposits that are positive for IgM and/or C3 by immunofluorescence microscopy as well as epithelial cell foot process effacement by electron microscopy, are often seen but not required to make the diagnosis (Figure 1). The clinical hallmarks include proteinuria, nephrotic syndrome, and frequently the progressive loss of renal function. A recent review of available data suggested that FSGS is also a considerable cause of ESRD, accounting for up to 20% of dialysis patients. Studies performed at several institutions documented an increased incidence of FSGS in biopsies of adult patients, and it is the leading cause of idiopathic nephrotic syndrome among black individuals.

Conventionally, FSGS has been defined as primary (idiopathic), secondary, or familial. Although the idiopathic form of FSGS is most common, secondary FSGS can occur in association with HIV infection, heroin use, sickle cell disease, obesity, and reflux nephropathy. Autosomal dominant and recessive forms of FSGS have been described as well as those associated with congenital syndromes. It is now thought that perhaps up to 18% of cases of FSGS are due to familial disease. Through the use of advanced molecular genetic cloning techniques, several mutations have been found in key podocyte proteins, including nephrin (NPHS1), podocin (NPHS2), α-actinin-4 (ACTN4), CD2-associated protein (CD2AP), and phospholipase C-ε1 (PLCE1).

The clinical phenotype of FSGS is varied. We have identified more than 100 families including 2000 individuals and collected 880 DNA samples in our hereditary kidney disease database. Individuals with apparent autosomal recessive FSGS seem to have more aggressive disease, present at a younger age, and have more proteinuria on presentation. We also found that recessive disease, black ethnicity, and a high degree of proteinuria correlated with worse kidney survival. Interestingly, of the 41 individuals who underwent 48 kidney transplant procedures, only one patient had recurrence of FSGS in the renal allograft. This leads to an important clue about the pathogenesis of this disease. Idiopathic FSGS frequently recurs; however, hereditary FSGS does not, indicating that inherited disease has a kidney-specific cause. Through whole-genome linkage analysis, fine-mapping, and candidate gene screening, a mutated gene was localized to chromosome 11q21–22 and subsequently identified as the transient receptor potential cation channel, subfamily C, member 6 (TRPC6) gene in a large family from New Zealand.

this particular subset of families, affected individuals present in their third or fourth decade of life with high-grade proteinuria. Sixty percent of these individuals progress to ESRD within 10 yr. The original missense mutation changed a highly conserved proline in the first ankyrin repeat of TRPC6 to a glutamine at amino acid 112 (P112Q). Subsequently, Pollak and colleagues identified TRPC6 mutations in five other unrelated families of diverse ethnic origin. In each family, inheritance was consistent with an autosomal dominant pattern and the observed amino acid substitutions occurred in highly conserved residues throughout evolution. Two mutations predicted amino acid substitutions in the N-terminal intracellular domain of TRPC6, two predicted amino acid substitutions in the C-terminal intracellular domain, and one encoded a premature stop codon near the C terminus. In all families, TRPC6 variant and disease inheritance segregated.

The TRP channels are implicated in a variety of biologic functions, such as mechanosensitization, ion homeostasis, cell growth, and PLC-dependent calcium entry into cells. The TRP ion channels are a large class of proteins in diverse mammalian species united by a common primary structure that contains six membrane-spanning domain polypeptide subunits, with both carboxy and amino termini located intracellularly. All of the TRP channels assemble as tetramers that line the pore of the ion channel. The TRPC family is expressed in a wide variety of human tissues and can be divided into four subfamilies (TRPC1; TRPC 4,5; TRPC3,6,7; and TRPC2) on the basis of sequence homology and functional similarities. Studies have shown that TRPC1 channels co-localize with the autosomal recessive polycystic kidney disease protein PKD2. The second TRPC subfamily comprises TRPC4 and TRPC5, which both contain a carboxy terminal PDZ-binding motif not present in other TRP. PDZ domains are peptide-binding domains that organize membrane proteins, particularly at cell–cell junctions, including neuronal synapses. Less information is available about the TRPC2 subfamily, but it seems to be a pseudogene in humans. TRPC2-deficient mice, however, exhibit abnormal mating behavior, and data have shown that this channel may have a role in pheromone signaling.

The TRPC3, TRPC6, and TRPC7 subfamily is approximately 75% identical and forms a cationic nonselective channel that shows both inwardly and outwardly rectifying cation currents. TRPC6 is the most selective of the TRP channels; the TRPC3,6,7 subfamily has selectivity on the order of P_{Ca}/P_{Na} 1.5 to 6:1. The TRPC3,6,7 subfamily seems to co-assemble when expressed heterologously. TRPC6 is a 100-kD protein with intracellular N- and C-termini; the fifth and sixth transmembrane domains form tetramers. In contradistinction to classical signal transduction pathways that involve calcium trafficking, TRP channels can be
activated independent of intracellular calcium concentration or membrane depolarization. TRPC6 channels have been shown to be activated in response to PLC stimulation. The GPCR pathway involves ligand binding to membrane receptors, activation of PLC, the generation of inositol 1,4,5 triphosphate with binding to its receptor, and release of intracellular calcium from the endoplasmic reticulum. Recent studies using positional cloning have identified mutations in PLCE1 as causing early-onset nephrotic syndrome with ESRD. Kidney histology of affected individuals shows diffuse mesangial sclerosis, and immunofluorescence reveals an arrest in normal glomerular development. Importantly, two children with truncating mutations in PLCE1 responded to treatment with corticosteroids or cyclosporin A, indicating that molecular causes of nephrotic syndrome may at least be modifiable.

The TRPC6P112Q mutation augments intracellular calcium influx into the podocyte, leading to FSGS through unclear mechanisms. My colleagues and I hypothesized that this results in disrupted glomerular cell function or causes apoptosis. Interestingly, stimulation by angiotensin II (AngII) also causes higher peak intracellular Ca²⁺ changes in TRPC6P112Q-transfected cells. AngII, acting through its AT1 receptor, plays a critical role in the generation of proteinuria and progression of kidney injury. One possible mechanism for podocyte dysfunction is that increased intracellular calcium may modify the contractile structure of podocyte foot processes, resulting in an alteration of the ultrafiltration coefficient. Another potential mechanism for the association between TRPC6 and familial FSGS is that an alteration in the intracellular calcium concentration causes podocyte apoptosis through a variety of mechanisms, resulting in glomerulosclerosis. There are data that show that podocyte number is a critical determinant for the development of glomerulosclerosis and that a decrease in podocyte number leads to progressive renal failure. Wiggins and colleagues showed that a single injection of puromycin aminonucleoside, a podocyte toxin, caused a marked decrease in podocyte number in rats and subsequent glomerulosclerosis. Human studies also showed that a decrease in podocyte number in Pima Indians with type 2 diabetes correlated with progression of diabetic nephropathy. An increase in intracellular calcium may cause loss of podocytes through apoptosis, detachment, or lack of proliferation. Apoptosis may result from an ability of mutant TRPC6 to augment the deleterious effects of AngII. The mechanisms of podocyte detachment remain unknown but likely involve the abnormal function of specific integrins such as α3β1 integrin.

Lack of proliferation may be another process by which FSGS occurs. Although podocytes are terminally differentiated cells, proliferation is a prerequisite for normal glomerular formation. Proliferation is governed at the level of the cell cycle through regulatory proteins. One may speculate that during glomerulogenesis, an abnormally high intracellular calcium concentration causes an alteration in the ratio of cyclins and CDK to CDK inhibitors and therefore limits normal podocyte proliferation, causing glomerulosclerosis.

Two of the five families with TRPC6 mutations in the Pollak cohort had an associated 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 slit diaphragm proteins. The exploration for interactions of TRPC6 with the other known causes of hereditary FSGS and nephrotic syndromes as well as alterations in cellular signaling will be an area of intense interest. Benzing and colleagues demonstrated that mechanosensory abnormality protein and podocin bind cholesterol and that this binding regulates TRPC6 channel complexes. Altered mechanosensation may cause abnormal podocyte contractile function. TRPC6 is a sensor of mechanically and osmotically induced membrane stretch independent of PLC activation.

Glomerulosclerosis is also the final common pathway for a variety of kidney diseases, such as hypertension and diabetes. Abnormalities in the highly specialized glomerular podocyte, such as foot process effacement and slit diaphragm alterations, are common to all forms of nephrotic syndrome. Important advances in recent years have helped us to understand better podocyte structure and function as well as protein interactions at the slit diaphragm. Instead of merely being cytoskeletal structural proteins, nephrin, podocin, and CD2AP are involved in cellular signaling (Figure 3). Targeted disruptions of podocin inhibit both nephrin trafficking and nephrin-initiated signal transduction.

What remains unanswered is whether treatment of individuals with hereditary nephrotic syndromes and idiopathic FSGS should be customized on the basis of their specific genetic mutations. We know that the vast majority of individuals with podocin mutations do not respond to steroids. No prospective, randomized, controlled trials have been undertaken to examine this question specifically. There is, however, anecdotal evidence suggesting that treatment with chemotherapy or immunotherapy delays progression of end-stage kidney disease in individuals with hereditary FSGS. We believe that targeting TRPC channels may have therapeutic implications in FSGS because TRPC6 seems to cause FSGS through increases in calcium transients. We have seen abnormal trafficking of the TRPC6P112Q protein to the plasma membrane, and this may be related to the cellular effects. There will be inherent difficulties in developing a TRPC6 “blocker” in that TRPC3, 6, and 7 share 75% sequence homology. The larger question is how we use these newly found data regarding all of the gene defects that cause FSGS and hereditary nephrotic syndromes.

Finally, it is unclear whether all individuals with hereditary nephrotic syndromes should be screened for mutations in the known genes and even whether they should be treated. We also do not know whether or how single-nucleotide polymorphisms in these genes affect kidney function. By understanding the genotype and phenotype correlations, pharmacogenomics may allow us to increase drug effectiveness and attenuate drug toxicity.
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REFERENCES

11. Freedman BL, Spray BJ, Tuttle AB, Buckalew...