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See related article, "Calcimimetic Inhibits Late-Stage Cyst Growth in ADPKD," on pages 1527–1532.

The Promise of Well-Being: Stay in Shape with N(i)ck

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Just more than a decade ago, a report of a rare human genetic disorder initiated a new spurt of research that profoundly changed our understanding of the glomerular filtration barrier. In that landmark article,[1] mutations in the gene encoding the adhesion protein nephrin identified the cause of congenital nephrotic syndrome of the Finnish type. Subsequent studies localized nephrin to the podocyte slit diaphragm, a specialized

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cell junction that connects neighboring podocytes, the visceral epithelial cells of the glomerulus. These neighboring podocytes elaborate long, regularly spaced, interdigitated foot processes that enwrap the glomerular capillaries and form a 40-nm-wide filtration slit bridged by the slit diaphragm.

Further studies suggested that nephrin and associated proteins, in addition to their role as structural components of the slit diaphragm, act as signaling molecules to control podocyte development, cytoskeletal architecture, cell shape and viability, and the function of the glomerular filtration barrier.[2–4] Much insight into the signaling function of the slit diaphragm protein complex resulted from the identification of nephrin-interacting proteins,[5–9] yet the mechanisms that control ultrastructure and shape of the highly organized podocyte foot process remain poorly understood. This changed with the finding that nephrin binds Src homology 2 (SH2)/SH3 domain-containing Nck adaptor proteins, which in turn seems to control the podocyte cytoskeleton *in vivo*.[10,11]

The Nck family of adaptors (mammals carry two related *Nck* genes, *Nck1* and *Nck2*) functions in coupling phosphotyrosine signals to actin cytoskeletal reorganization. Each of these proteins contain multiple protein interaction domains: three SH3 domains that are known to interact with proline-rich binding partners such as the actin organizers WASP, SPIN90, and dynamin and an SH2 domain that mediates binding of transmembrane receptors and adhesion proteins through phosphotyrosine-dependent protein interactions.[12] Nephrin ectodomains recruit the Src family tyrosine kinase Fyn to phosphorylate the nephrin cytoplasmic tail, allowing recruitment of Nck and inducing localized Nck-driven actin polymerization.[13] Notably, selective gene deletion of *Nck1* and *Nck2* from podocytes in transgenic mice results in severe defects in the formation of podocyte processes and in congenital nephrotic syndrome.[10] Although the profound changes in cell shape and podocyte morphology in this model mirror the stereotypical reaction of podocytes to injury or damage,[14,15] the phenotype is clearly a developmental defect–mice are born without Nck1 or Nck2 in podocytes—and does not clarify the important question of whether slit diaphragm signaling and nephrin-mediated recruitment of Nck are involved in actively regulating podocyte cell shape and maintaining the ultrastructural and functional integrity of the glomerular filtration barrier *in vivo*.

In a remarkable article in this issue of *JASN*, Jones *et al.*[16] now show that inducible gene deletion of *Nck* in adult transgenic mice results in podocyte cell shape changes, massive proteinuria, and the development of glomerular scarring. Jones *et al.* use an inducible transgenic strategy that allows selective deletion of Nck expression in podocytes of adult mice. The authors carefully analyze the ultrastructural changes of podocytes in adult mice lacking Nck expression and definitively demonstrate that Nck1 and Nck2 maintain the shape and integrity of foot processes.

This is interesting for a number of reasons. In response to injury, podocytes undergo a dramatic change in morphology—termed foot process effacement—resulting from alterations in cytoskeletal and intercellular junctional architecture. Effacement results from simplification of foot processes, namely loss of foot process shape and retraction, widening, and shortening of the processes of each podocyte. The frequency of filtration slits is also reduced, giving the appearance of a continuous cytoplasmic sheet covering the glomerular basement membrane.[15]

Effacement is not specific to one disease but rather is synonymous with podocyte injury of many forms, providing a fluid and reversible process that directly correlates with the development of proteinuria both in human disease and in experimental models. Thus, it is interesting that *Nck* gene deletion and, accordingly, loss of nephrin-dependent Nck recruitment exactly mirror this phenotype. The glomerular morphology of gene-deleted mice undergo the widely known continuum from mild foot process widening to focal areas of fusion and the development of extensive effacement that mirrors minimal-change disease in humans; however, these changes obviously precede the loss of podocytes and the development of focal areas of glomerulosclerosis,[16] which is consistent with FSGS in humans, raising the hypothesis that minimal-change disease and FSGS in many cases are two extremes of an ellipse.

Nephrin and associated proteins are conserved through evolution and play important roles in patterning of the eye in *Drosophila melanogaster* or synapse targeting in *Caenorhabditis elegans*. All of these changes require defined cytoskeletal rearrangements; therefore, it will be important to determine whether nephrin-induced Nck activity is also required in these events. As Jones *et al.*[16] clearly demonstrate, nephrin phosphorylation is maintained in the adult glomerulus supporting the importance of signaling at the slit diaphragm for regulation of podocyte cell shape, cytoskeletal architecture, and cell viability.

It is worth noting that these new findings have the same limitations as the original report concerning a developmental role of nephrin-Nck signaling. Although Jones *et al.* clearly demonstrate that *Nck1* and *Nck2* are essential for developing and maintaining the complex ultrastructure of podocyte secondary processes, both articles lack direct proof that Nck proteins exclusively act through the nephrin/slit diaphragm axis *in vivo*. In fact, this has to be addressed in future studies, for instance using knock-in mouse models of nephrin with mutated tyrosine residues. Despite this limitation, Jones *et al.*[16] undoubtedly demonstrate the importance of Nck in controlling the ultrastructure of podocyte foot processes in maintaining the integrity of the glomerular filtration barrier.

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DISCLOSURES

None.

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See related article, "Nck Proteins Maintain the Adult Glomerular Filtration Barrier," on pages 1533–1543.

Glomerular Filtration: Still Sympathetic to Endothelin's Influence?

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Methodologic and analytic advances in molecular genetics identify many disease-predisposing variants in the human genome. One of the challenges presented by these studies is to define the mechanisms by which a given genetic variant affects a pathophysiologic disorder. This inevitable quandary confronts the study of Chen *et al.*[1] in this issue of *JASN*. The authors previously reported that a genetic polymorphism of chromatogranin A (CHGA), a soluble protein released from secretory granules of chromaffin cells and sympathetic nerves, predicts hypertensive ESRD in black patients. Using population genetic techniques, they further showed that the *CHGA* locus is a *trans*-QTL for endothelin-1 secretion and that CHGA stimulates endothelin release from endothelial cells. In view of the major role for endothelin postulated in the pathogenesis of a variety of cardiovascular and renal diseases, the authors questioned whether endothelin secretion is the mechanistic link between CHGA and renal dysfunction.

Using cultured human umbilical vein endothelial cells, Chen *et al.*[1] now show that CHGA increases release of endothelin along with other peptides typically stored in Weibel-Palade bodies, including von Willebrand factor and angiopoietin 2. CHGA also increases endothelin secretion from mouse glomerular endothelial cells, and co-culture of CHGA-exposed glomerular endothelial cells with mesangial cells potentiates secretion of TGF-β1. In humans, plasma CHGA correlates positively with plasma endothelin and negatively with GFR. A promoter haplotype for *CHGA* predicts the level of GFR in individuals with GFRs in the normal range, whereas *CHGA* haplotypes in the 3′ untranslated region associate with GFR in individuals with progressive renal disease. The conclusion that CHGA triggers exocytotic release of endothelin from the glomerular endothelium and that this, in turn, regulates the level of GFR in normal and declining renal function offers some novel implications. These include genetic modulation of GFR in normal and diseased kidneys under the potential influence of the sympathochromaffin protein, the utility of circulating

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