tions as a result of the widely known function of the viral protein Nef in downregulating cell surface receptors such as CD4 on T cells.

This issue with Nef function in renal cells *versus* leukocytes is of central importance to the hypothesis being tested, because this group previously showed that Nef causes STAT3 dysregulation in podocytes. The authors speculate this failure of Tg26 mice to recreate the widely known Nef-related CD4 downregulation is attributable to expression levels, suggesting there is sufficient Nef to activate STAT3 in the kidney but insufficient Nef to downregulate CD4 in T cells; however, a previous comparison of expression levels between kidney and lymphoid organs in Tg26 heterozygous mice is similar.[6] Moreover, the protein trafficking functions of Nef (CD4 downregulation) are separable from its function in mediating signal transduction events such as STAT3 activation, and ongoing transgenic work by the Jolicoeur laboratory is segregating the renal disease–causing effects of Nef through leukocytes *versus* renal cells[7,8]; however, this segregation is not attributable to levels of transgene expression. Nef has a dauntingly complex array of functions in host cells,[9] and exactly how Nef orchestrates pathogenesis in rodent models, let alone humans, is far from established definitively.

As Feng *et al.* demonstrate, creating compound transgenic mice is a sophisticated genetic approach to testing developmental and pathogenesis paradigms *in vivo* but with equally sophisticated challenges in design and execution. The strongest conclusion from this study is the overall role of STAT3 in the pathogenesis of HIVAN; however, there are issues in definitively attributing this to Nef alone in renal cells—the basis of their hypothesis—because Tg26 mice express Nef and many other HIV-1 proteins in other cell types. In light of their observations, the authors propose STAT3 should be a druggable target for HIVAN. Small molecule inhibitors for STAT3 are currently being development for cancer therapy and seem to have both antiproliferative and immunomodulatory properties.[10] Thus, this is a logical next step in which the Tg26 mouse will be a good small animal model for testing, although the specifics of drug action, such as cell targets, would remain unclear.

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DISCLOSURES

None.

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See related article, "Reduction of Stat3 Activity Attenuates HIV-Induced Kidney Injury," on pages 2138–2146.

Surprising Results following Conditional Podocyte Inactivation

Martin R. Pollak
Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts

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The genetic contribution to steroid-resistant nephrotic syndrome and related disorders of the podocyte has been widely appreciated only in the past decade. In fact, for at least 50 yr, nephrosis has been observed occasionally in multiple members of the same family. In a 1957 study, Farquhar *et al.*[1] performed

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Correspondence: Dr. Martin Pollak, Brigham and Women's Hospital, 77 Avenue Louis Pasteur, Boston, MA 02115-5727. Phone: 617-525-5840; Fax: 617-525-5841; E-mail: mpollak@rics.bwh.harvard.edu

a detailed electron microscopic analysis in kidneys from four siblings all with manifestations of nephrosis, noting a loss of the normal intricate glomerular epithelial cell architecture. This intricate and delicate—and perhaps even beautiful—glomerular epithelial cell structure has become the focus of intense investigation, accelerated in part by the discovery of genes underlying monogenic forms of podocyte disease.

In 1995, Fuchshuber *et al.*[2] in Antignac's group[3] identified a locus for what they termed steroid-resistant nephrotic syndrome on chromosome 1. Five years later, these investigators identified the responsible gene, NPHS2, encoding a novel transmembrane protein they named podocin.

Since the positional cloning of NPHS2, understanding of the role of podocin in glomerular function has grown tremendously, as has our understanding of the role of variation in NPHS2 in human disease. We have learned that podocin is an integral membrane protein that localizes to the glomerular slit diaphragm and interacts with nephrin, the protein mutated in congenital nephrotic syndrome of the Finnish type.[4,5] We know that podocin facilitates nephrin-mediated cell signaling, and mutations in podocin disrupt nephrin trafficking to the cell membrane.[6,7] Worm biology has also contributed to our understanding: Podocin shares certain structural and functional characteristics with the homologous touch-sensitive *Caenorhabditis elegans* protein MEC-2, binding cholesterol and regulating associated ion channels.[8]

Of the various genes that when mutated lead to human phenotypes, NPHS2-associated disease gives rise to the widest spectrum of clinical disease. Although we typically think of the nephrin gene NPHS1 as *the* congenital nephrotic syndrome gene, NPHS2 mutations are also a frequent cause of this neonatal syndrome.[9] At the other end of the spectrum, mutations in both NPHS2 alleles can also cause a clinical presentation much later in life as adult-onset FSGS.[10]

Understanding the *in vivo* role of podocin in podocyte function and disease is clinically important: NPHS2 mutations are responsible for a large fraction of steroid-resistant nephrotic syndrome and FSGS.[10] Several years ago, Antignac's group[11] developed a mouse model lacking expression of the podocin gene NPHS2. These mice develop severe nephrosis before birth and massive podocyte foot process effacement, as well as mesangial sclerosis and vascular lesions of variable severity. Much of this variability depends on genetic background. These investigators went on to develop a mouse with a genetically engineered R138Q point mutation in podocin to model one of the most common disease-causing human mutations. The homozygous mice died at an early age, showing mislocalization of podocin as well as nephrin. Although these mice also express severe nephrosis, mesangiolysis, and mesangial sclerosis, the kidneys show a different pattern of altered gene expression than did the knockout mice.[12]

In this issue of *JASN*, Mollet *et al.*[13] report a new mouse model of podocin-mediated disease. This model takes nice advantage of Cre/Lox technology that allows targeted inactivation of a gene of interest. Using a Cre transgenic mouse in which the podocin promoter drives expression of Cre recombinase in a tamoxifen-inducible, podocyte-specific manner, Mollet *et al.* inactivate podocin in podocytes. When administered to 6-wk-old transgenic mice, tamoxifen led to the inactivation of podocin in approximately 70% of podocytes. This in turn led to massive albuminuria, hyperlipidemia, slowly progressive loss of glomerular filtration, and eventually death from kidney failure.

We now know with certainty that podocin is not only essential for the development of normal glomerular architecture but also critical for maintaining normal glomerular, podocyte, and slit diaphragm function. The absence of overt vascular and mesangial lesions in this model also suggests that podocin has different, albeit related, functions in development and in adult physiology. Despite that this model leads to expression of Cre in 70% of podocytes (and a 50% reduction in podocin expression after 1 wk), these mice progress rapidly to overt kidney failure. This raises new questions about podocyte–podocyte and glomerular–glomerular communication: Why does loss of 50% of podocin lead to a much more severe phenotype than, say, losing 50% of nephrons from nephrectomy?

Too many knockout models fail to answer the question asked. Like the old joke about the grasshopper (the distinguished professor demonstrates that the grasshopper's legs are required for hearing, because once its legs are cut off, it fails to jump in response to a loud noise), absence of a gene from the beginning of development (from a knockout experiment) may not accurately answer questions of mature physiology. The study by Mollet *et al.* illustrates the power of current mouse genetic tools to address such questions.

DISCLOSURES

None.

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See related article, "Podocin Inactivation in Mature Kidneys Causes Focal Segmental Glomerulosclerosis and Nephrotic Syndrome," on pages 2181–2189.

More Evidence that Cystatin C Predicts Mortality Better than Creatinine

Peter P. Reese and Harold I. Feldman

Department of Medicine, Renal Division, Center for Clinical Epidemiology and Biostatistics, and Leonard Davis Institute, University of Pennsylvania, Philadelphia, Pennsylvania

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The main clinical benefit of estimating renal function in the general population is to identify and treat patients at risk for developing ESRD, cardiovascular events, and death.[1] Unfortunately, the most widely used equations to estimate GFR are limited, in part, by their reliance on serum creatinine as the filtration marker (estimated GFR [eGFR]). The Modification of Diet in Renal Disease (MDRD) equation, for example, overestimates renal function among individuals with low muscle mass and underestimates it among those with GFR >60 ml/min per 1.73 m^2.[2–4] The use of serum cystatin C, a cysteine protease inhibitor that is freely filtered by the glomerulus, has potential advantages over creatinine as a filtration marker in that its production is not dependent on muscle mass.[2] As a result, cystatin C offers opportunities to estimate GFR more accurately than creatinine-based equations and additionally may predict worsening kidney function even when the GFR is actually near the normal range.[5]

Beyond the relationship to measured GFR, cystatin-C–derived eGFR (ecGFR) was a better predictor of mortality than creatinine-based estimates in a study of elderly individuals[6]; however, the ability to generalize these findings to a younger and broader set of individuals is unknown. In this issue of *JASN*, Astor *et al.*[7] extend previous observations and demonstrate convincingly that ecGFR predicts mortality more accurately than the MDRD equation in a sample from the general population in the United States. If replicated, then these findings should spur trials to determine whether the use of cystatin C improves patient outcomes by identifying those with an elevated risk for death, both within and outside the setting of diagnosed chronic kidney disease (CKD).

Astor *et al.*[7] focus on the relationship of ecGFR to mortality, but it is instructive to examine the challenges of using creatinine-based equations to assess renal function—the original reason for interest in cystatin C as a diagnostic test. The accuracy of a creatinine-derived eGFR and its value for detecting progressive kidney disease depends on the patient's level of renal function and associated level of proteinuria.[1] Patients with an eGFR <30 ml/min per 1.73 m^2 using MDRD are at substantial risk for later developing ESRD, but among patients with less severely diminished eGFR (45 to 90 ml/min per 1.73 m^2), the prognostic value of an eGFR is limited, particularly when albuminuria is absent. For instance, follow-up of individuals who participated in the Multiple Risk Factor Intervention Trial (MRFIT) revealed that a subset with eGFR <60 (mean 55 ml/min per 1.73 m^2) have only a 5.6% absolute risk for ESRD over 25 yr. In comparison, among participants with eGFR 60 to 75 ml/min per 1.73 m^2, approximately 5.7% of those with 1+ proteinuria on urine dipstick evaluation and 17.7% of those with 2+ proteinuria develop ESRD.[8] The creatinine-based eGFR alone, therefore, has limited utility in predicting which patients with mildly diminished eGFR and no proteinuria will experience renal deterioration in the future.[9]

There are theoretical reasons to believe that cystatin C is a superior filtration marker compared with creatinine,[10] however, a comparison by Stevens *et al.*[11] of the performance characteristics of the MDRD equation with a cystatin C–based estimate in a large pooled population of patients with established

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Correspondence: Dr. Peter P. Reese, Renal Electrolyte and Hypertension Division, Department of Medicine, University of Pennsylvania School of Medicine, 1 Founders, 3400 Spruce Street, Philadelphia, PA 19104. Phone: 215-662-7934; Fax: 215-615-0349; E-mail: peter.reese@uphs.upenn.edu