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**Fishing for New Glomerular Disease-Related Genes**

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Identification of new disease-related genes contributing to vari-
sous forms of glomerular pathobiology is a critical step leading to the development of novel treatments and therapies for kidney-
related disorders. Significant progress toward this end has been
realized by contributions from the Matrix Biology group at the Karolinska Institutet, led by Karl Tryggvason. Several years ago, gene-profiling methods were used to identify genes that show sig-
nificantly elevated levels of expression in the renal corpuscle. A series of ensuing publications validated these findings with in vitro and in vivo methods, combined with extensive data-mining ef-
forts to describe highly expressed transcripts and protein-protein
interactions occurring in the glomerulus. The end result was the creation of GlomBase and GlomNet. GlomBase identified over 300 novel transcripts having elevated glomerular expression but with ill-defined function. The need for an efficient, reliable, and relevant method to evaluate these candidate genes in determining their potential for interrogating glomerular disease is crucial. One such relevant animal model that has already proven invaluable in providing data for functional studies in a number of organ systems is the zebrafish (*Danio rerio*). A systematic investigation into the role these genes may play in glomerular development and disease is currently underway. A recent article originating from the Tryggvason group identified the epithelial polarity gene, *crumbs* (*crb2b*), as having a critical role in maintaining the integrity of the podocyte slit diaphragm. This article highlighted the usefulness of the ze-
brafish in obtaining functional data when interrogating a spec-
ific gene. In the current issue of *JASN*, Nishibori et al. report on another one of the more than 300 genes originally identified by this group.\(^7\)

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sion, University of Miami Miller School of Medicine, 1580 NW 10th Avenue, Batchelor Building, 6th Floor, Suite 631 Miami, FL 33136. Phone: 305-243-8551; Fax: 305-243-2309; E-mail: smangos@med.miami.edu

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Glucocorticoid-induced transcript 1, GLCCI1, is expressed in the glomerulus of the mouse kidney at an elevated level, according to their GlomBase and GlomChip data. In the current article, this group confirms the original data, first with an in vitro analysis, followed by immunohistochemistry. Their findings clearly show GLCCI1 expression in the glomerulus, and more precisely, using immunogold EM, in the foot processes of podocytes. Zebrafish gclci1 shares considerable sequence identity with both mouse and human GLCCI1, and Nishibori et al. demonstrate that gclci1 expression becomes elevated at the precise time that the zebrafish slt diaphragm matures.

According to previous studies, the glomerulus of the zebrafish pronephros begins to mature and starts to filter the embryonic blood at approximately 48 h postfertilization. Functional studies using zebrafish were also performed, and disrupting the expression of gclci1 using antisense oligonucleotide morpholinos (MO) leads to embryos that did not develop.9–12 Another interesting point of this study lies in new questions that arise from it. What does the discovery of Glucocorticoid-induced transcript 1 mean for the clinical understanding of steroid-sensitive and resistant glomerular disease? Is there a future link to be discovered that connects minimal change disease (MCD) with the downregulation of GLCCI1 in patients? What are the protein interactors and precise modus operandi of Glucocorticoid-induced transcript 1? The fact that we now raise these questions on the heels of these novel mechanistic studies in fish clearly shows that we have to pay increasing attention to, and give credit for, the use of this organism when studying human-related processes of kidney disease or novel glomerular disease-related genes. With the refinement of functional screening methods in zebrafish, the list of these glomerular disease-related genes is on the verge of exploding, and GLCCI1 joins the likes of coflin-1, lat3, crb2b, CAR, and tcp6.

These experiments represent a major step forward in streamlining functional studies when using the zebrafish to study glomerular or podocyte-specific disease-related genes. Early work in this area relied on the labor-intensive and time-consuming process of injecting fluorescently conjugated dextrans into the blood supply of the embryos under investigation (which could include embryos that were genetically mutated, previously injected with MOs or mRNA, or treated with chemicals/toxins/small molecules), followed by histologic examination of the pronephric tubules for evidence of a filtration barrier breach.

Work by several groups has made strides in improving and streamlining these functional assays, including such strategies as using real-time monitoring of fluorescent dye clearance from the blood stream of injected embryos. With the relative ease in generating transgenic zebrafish lines, even more convenient models are in the pipeline. These new models, like the one presented by Nishibori et al., promise the elimination of the necessity for fluorescently labeled dextran injections. The community eagerly awaits the development of an ELISA-based screening model, where fish urine can almost instantaneously be checked for signs of proteinuria.

Nishibori et al. use a relatively inexpensive and quick technique to determine whether the embryonic glomerular filtration apparatus is intact. Proof of concept for using such a strategy was demonstrated by comparing the fish water (containing the fish urine) from nephrin-MO-injected embryos. Nephrin MO treatment has previously been shown to cause severe morphologic and functional damage to the kidneys of zebrafish embryos. Albeit not as severe as the nephrin morphants, gclci1 morphants show evidence of proteinuria using this protein gel system. The filtration barrier of the embryonic glomerulus should exclude proteins larger than 70 kD, but proteinuric fish excrete detectable levels of proteins in the range of 150 kD. When fish urine was analyzed using mass spectrometry sequencing, it was found to contain mostly Vitellogenin and Vitellogenin-like proteins. As explained by the authors, Vitellogenin is a nutrient transport protein found in the yolk and blood of embryos as well as in the serum of adult fish.

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