Translating genetic findings in hereditary nephrotic syndrome: the missing loops

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Hall G, Gbadegesin RA. Translating genetic findings in hereditary nephrotic syndrome: the missing loops. Am J Physiol Renal Physiol 309: F24–F28, 2015. First published March 25, 2015; doi:10.1152/ajprenal.00683.2014.—Nephrotic syndrome (NS) is a clinicopathological entity characterized by proteinuria, hypoalbuminemia, peripheral edema, and hyperlipidemia. It is the most common cause of glomerular disease in children and adults. Although the molecular pathogenesis of NS is not completely understood, data from the study of familial NS suggest that it is a “podocytopathy.” Virtually all of the genes mutated in hereditary NS localize to the podocyte or its secreted products and the slit diaphragm. Since the completion of human genome sequence and the advent of next generation sequencing, at least 29 causes of single-gene NS have been identified. However, these findings have not been matched by therapeutic advances owing to suboptimal in vitro and in vivo models for the study of human glomerular disease and podocyte injury phenotypes. Multidisciplinary collaboration between clinicians, geneticists, cell biologists, and molecular physiologists has the potential to overcome this barrier and thereby speed up the translation of genetic findings into improved patient care.

gene mutation; nephrotic syndrome; podocyte

Nephrotic Syndrome (NS) is a clinicopathological entity characterized by proteinuria, hypoalbuminemia, peripheral edema, and hyperlipidemia. It is the most common cause of glomerular disease in children and adults. The annual incidence of NS in the United States and Europe is estimated at 7/100,000. NS is a manifestation of various pathological changes in the kidney. In clinical practice, NS is classified based on the patient’s initial response to corticosteroid therapy. Classically, 80% of cases in the pediatric age group are steroid sensitive and are therefore classified as steroid-sensitive nephrotic syndrome (SSNS). The remaining 20% are called steroid-resistant nephrotic syndrome (SRNS). The SRNS variant of the disease is more prevalent in adults and is classically due to the pathological lesions of focal and segmental glomerulosclerosis (FSGS). FSGS is characterized by a rapid progression to end-stage kidney disease (ESKD), and it is the most common glomerular cause of ESKD. In 2005, FSGS accounted for 12% of ESKD cases in the United States, costing an estimated $3 billion in Medicare spending.

NS as a Podocytopathy

The molecular pathogenesis of NS is not completely understood; however, mounting evidence suggests that NS is due to defects in the glomerular filtration barrier (GFB). The GFB is composed of three layers: the specialized fenestrated endothelial cells and their overlying glycocalyx, the glomerular basement membrane (GBM), and glomerular visceral epithelial cells (i.e., podocytes) whose distal foot processes attach to the GBM. The functional integrity of the GFB depends on molecular cross talk between the three layers. While all three components are critical for the molecular sieving functions of the GFB, studies of familial NS/FSGS have revealed that the podocyte is the most important component of the GFB in that virtually all the genes mutated in hereditary SRNS localize to the podocyte or its secreted products and the slit diaphragm (SD) (Fig. 1) (1, 2, 8, 9, 25, 26, 31). These observations have given rise to the concept that most NS, especially FSGS, is due to an underlying podocytopathy. Podocytes are terminally differentiated cells consisting of a cell body, axon-like primary processes, and laterally radiating secondary foot processes that interdigitate with those of neighboring podocytes to form the highly specialized filamentous structure of the SD.

Genetics of NS

The completion of the human genome-sequencing project and advances in sequencing technology have accelerated the pace of gene discovery in Mendelian diseases. Insights into NS and other familial kidney diseases have greatly expanded in the era of next-generation sequencing (NGS) technologies. Classically, identification of single-gene causes of NS required the availability of large pedigrees with sufficient power to establish a disease locus, labor-intensive, fine-mapping strategies, and direct sequencing of genes in the refined locus. The first report of a single gene cause of NS appeared in the literature in 1998 when mutations in the nephrin gene (NPHS1) were reported as a cause of early-onset NS (24). Since then, integration of NGS technologies has led to the discovery of at least 29 genetic causes of NS, most of them within the last 10 years (Fig. 2).

Clinical Translation of Genetic Findings and Barriers to Translation

Identification of novel NS genes has significantly improved our understanding of NS disease mechanisms and led to improved patient outcomes. For example, the discovery of mutations in nephrin has laid the groundwork for understanding the molecular structure of the podocyte SD. Before the identification of nephrin, transmission electron microscopy (TEM) studies revealed that the SD is made up of a “zipper-like” array of regularly spaced cross bridges between interdigitating podocyte foot process (43). Despite the advances in our understand-
The cross bridges are formed by homophilic interactions of nephrin (24).

Studies of familial nephrotic syndromes have shown that most of the genes mutated in hereditary NS serve critical functions in the maintenance of the podocyte actin cytoskeleton and signaling in the SD (2, 4, 5, 13, 16, 21, 35, 44). The knowledge gained from these studies and careful phenotyping data have led to the development of a framework for genetic testing of NS that may have relevance in clinical practice (17). Furthermore, it is now known that most cases of genetic NS are resistant to immunosuppression; it is therefore justifiable to modify the intensity and duration of immunosuppression in individuals with monogenic NS (17, 18). This shift in provider practice, informed by basic research discovery, has the potential to spare many patients the untoward side effects associated with prolonged courses of high-dose corticosteroid and other more potent immunosuppressive agents. Also, establishing genetic diagnoses can be helpful in streamlining pre- and post-kidney transplant management and prognostication.

Identification of podocyte dysfunction as central to the pathogenesis of NS has led to the reevaluation of the mechanisms of action of existing therapies for NS. For example, Ransom et al. (32) demonstrated that glucocorticoids prevent or reverse purine aminonucleoside-induced podocyte injury via direct stabilizing effects on the podocyte actin cytoskeleton and upregulation of Rho-GTPase activity. Similarly, Faul et al. and
other investigators (3, 11, 39) described the role of cyclosporine A as a direct inhibitor of cathepsin-L-mediated synaptopodin degradation, and Fornoni et al. (12) reported that rituximab ameliorates podocyte actin cytoskeletal dysfunction and apoptosis via inhibition of sphingomyelin phosphodiesterase acid-like 3b depletion and downregulation of acid sphingomyelinase activity. Furthermore, Canaud et al. (7) described a potential mechanism for sirolimus-induced proteinuria via the mTORC2-dependent inhibition of AKT2 phosphorylation in podocytes. Most recently, Hall et al. (22) demonstrated that phosphodiesterase-V inhibitors ameliorated AKT2 phosphorylation in podocytes. Because of its intrinsic and potentially modifiable enzymatic activity, the value of TRPC6 conductance in podocytes (42). Because of its intrinsic and potentially modifiable enzymatic activity, the value of TRPC6 conductance in podocytes (42).

Animal models for NS. With respect to in vitro modeling, most researchers in this field rely almost exclusively on primary and immortalized podocyte culture for biochemical and molecular genetic analyses (33, 34). Although these mouse- and human-derived immortalized podocyte models have been of benefit, they lack essential podocyte characteristics that may be of importance in achieving a full understanding of the mechanisms of podocyte injury. For instance, conditionally immortalized podocytes do not form SD cross bridges in vitro. Given that a number of the gene mutations relevant to FSGS occur in SD proteins, these models may not allow us to appreciate the global alterations in podocyte structure and signaling caused by such mutations. In addition, molecular alterations such as targeted gene knockdown and exogenous promoter-driven gene overexpression studies in these models may also be problematic as such broad disruptions in the biology of a protein may introduce unintended physiological irregularities due to altered protein localization and stoichiometry, and cytotoxicity. One promising alternative to these strategies may be found in induced pluripotent stem cells (iPS). Directed differentiation of iPS cells into glomerular podocytes has been reported as a potential patient-specific stem cell-based therapy for CKD (37). While there have been no successful clinical applications of this strategy so far in the treatment of kidney disease, this approach may hold promise for the study of hereditary SRNS/FSGS. iPS cells derived from affected patients may provide disease-specific model systems with representative gene and protein expression profiles relevant to the pathogenesis of the disease. This could potentially improve our understanding of disease mechanisms and the evaluation of therapeutic strategies.

For similar reasons, whole animal modeling of NS has been equally challenging. For decades, investigators have relied upon mice for human disease modeling as 99% of mouse genes have a homolog within the human genome (28). Despite this high degree of similarity, mice are often resistant to glomerular injury and fail to recapitulate human phenotypes. In addition, mouse colonies are difficult and costly to establish and maintain. More recently, focus has shifted to the use of zebrafish derived immortalized podocyte models have been of benefit, genetic analyses (33, 34). Although these mouse- and human- derived immortalized podocyte models have been of benefit, they lack essential podocyte characteristics that may be of importance in achieving a full understanding of the mechanisms of podocyte injury. For instance, conditionally immortalized podocytes do not form SD cross bridges in vitro. Given that a number of the gene mutations relevant to FSGS occur in SD proteins, these models may not allow us to appreciate the global alterations in podocyte structure and signaling caused by such mutations. In addition, molecular alterations such as targeted gene knockdown and exogenous promoter-driven gene overexpression studies in these models may also be problematic as such broad disruptions in the biology of a protein may introduce unintended physiological irregularities due to altered protein localization and stoichiometry, and cytotoxicity. One promising alternative to these strategies may be found in induced pluripotent stem cells (iPS). Directed differentiation of iPS cells into glomerular podocytes has been reported as a potential patient-specific stem cell-based therapy for CKD (37). While there have been no successful clinical applications of this strategy so far in the treatment of kidney disease, this approach may hold promise for the study of hereditary SRNS/FSGS. iPS cells derived from affected patients may provide disease-specific model systems with representative gene and protein expression profiles relevant to the pathogenesis of the disease. This could potentially improve our understanding of disease mechanisms and the evaluation of therapeutic strategies.

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of disease-related changes occurring within pronephric structures (40, 41). Furthermore, zebrafish colonies are less expensive to maintain and different reports have demonstrated that genetic manipulation of genes causing human NS in zebrafish produces phenotypes that are very similar to human disease (40, 41). Therefore, this model holds promise for drug discovery and testing. However, the main limitation of this model system is that it is not amenable to longitudinal study. Development of methods that facilitate gene manipulation in adult fish will go a long way in overcoming these barriers. An emerging technology that holds promise in this regard is the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas gene-editing system. Recently, Irion et al. (23) reported the successful use of CRISPR/Cas to target and repair a truncating mutation of \( alb \) \((alb^{bb})\), which produces hydropigmentation in zebrafish. Through coinjection of circularized wild-type \( alb \) DNA with the CRISPR/Cas system in zebrafish larvae at the one-cell stage, the authors report roughly 10% germ line transmission of the repaired allele to the next generation (23). Such advances in the use of CRISPR/Cas technology may soon enable us to expand the utility of zebrafish in the modeling of human glomerular diseases and to develop and test novel therapeutic tools for hereditary care.

**Conclusion**

In conclusion, genetic studies in the last two decades using forward genetics and NGS has significantly improved our understanding of the pathogenesis of NS. Unfortunately, there is a mismatch between the knowledge and translation to bedside. This is due, in part, to the lack of suitable in vitro and in vivo models for NS. Collaboration between clinicians, geneticists, cell biologists, and molecular physiologists has the potential to overcome this barrier and thereby speed up the translation of genetic findings into improved patient care.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**

Author contributions: G.H. and R.A.G. provided conception and design of research; G.H. and R.A.G. performed experiments; G.H. and R.A.G. analyzed data; G.H. and R.A.G. interpreted results of experiments; G.H. and R.A.G. prepared Figs.; G.H. and R.A.G. drafted manuscript; G.H. and R.A.G. edited and revised manuscript; G.H. and R.A.G. approved final version of manuscript.

**REFERENCES**


Nephrotic Syndrome, Gene Mutations, Clinical Translation


