Prospects for Precision Medicine in Glomerulonephritis Treatment

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Prospects for Precision Medicine in Glomerulonephritis Treatment

Yulu Cherry Liu[1] and Justin Chun[2,3]

Abstract

Background: Glomerulonephritis (GN) consists of a group of kidney diseases that are categorized based on shared histopathological features. The current classifications for GN make it difficult to distinguish the individual variability in presentation, disease progression, and response to treatment. GN is a significant cause of end-stage renal disease (ESRD), and improved therapies are desperately needed because current immunosuppressive therapies sometimes lack efficacy and can lead to significant toxicities. In recent years, the combination of high-throughput genetic approaches and technological advances has identified important regulators contributing to GN.

Objectives: In this review, we summarize recent findings in podocyte biology and advances in experimental approaches that have opened the possibility of precision medicine in GN treatment. We provide an integrative basic science and clinical overview of new developments in GN research and the discovery of potential candidates for targeted therapies in GN.

Findings: Advances in podocyte biology have identified many candidates for therapeutic targets and potential biomarkers of glomerular disease. The goal of precision medicine in GN is now being pursued with recent technological improvements in genetics, accessibility of biologic and clinical information with tissue biobanks, high-throughput analysis of large-scale data sets, and new human model systems such as kidney organoids.

Conclusion: With advances in data collection, technologies, and experimental model systems, we now have vast tools available to pursue precision medicine in GN. We anticipate a growing number of studies integrating data from high-throughput analysis with the development of diagnostic tools and targeted therapies for GN in the near future.

Abrégé

Contexte: La glomérulonéphrite (GN) consiste en un groupe de troubles rénaux classés en fonction de caractéristiques histopathologiques communes. La classification actuelle de la GN illustre mal la variabilité individuelle dans la présentation, la progression de la maladie et la réponse au traitement. La GN est une cause importante d'évolution vers l'insuffisance rénale terminale (IRT). L'amélioration des traitements est absolument nécessaire, car les thérapies immunosuppressives actuelles manquent parfois d'efficacité et présentent une toxicité élevée. Dans les dernières années, la combinaison d'approches génétiques à haut débit aux avancées technologiques a permis d'identifier des régulateurs importants contribuant à la GN.

Objectifs: Cette revue constitue une synthèse des plus récentes découvertes en biologie des podocytes, de même que des avancées dans les approches expérimentales ayant ouvert la voie à la médecine de précision pour le traitement de la GN. L'étude donne un aperçu global des plus récents développements de la recherche sur la GN et discute de la découverte de possibles candidats pour l'élaboration de thérapies ciblées.

Résultats: Les avancées en biologie des podocytes ont permis d'identifier plusieurs candidats de cibles thérapeutiques et de possibles biomarqueurs des troubles glomérulaires. L'objectif de la médecine de précision pour la GN est poursuivi par la contribution des récents progrès technologiques en génétique, par l'accessibilité aux renseignements biologiques et cliniques obtenus grâce aux biobanques, par l'analyse à haut débit de grands volumes de données, et par l'entremise de nouveaux modèles humains tels que les organoïdes rénaux.

Conclusion: Forts des avancées en collecte de données, en technologie et dans les modèles expérimentaux, nous disposons désormais de plusieurs outils pour approfondir l'apport de la médecine de précision dans le traitement de la GN. Nous prévoyons, dans un avenir rapproché, qu'un nombre grandissant d'études intègreront les données provenant d'analyses à haut débit dans l'élaboration d'outils diagnostiques et de thérapies ciblées pour le traitement de la GN.

Keywords

glomerulonephritis, FSGS (focal segmental glomerulosclerosis), genomics, biomarker, cell biology

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What was known before

Recent discoveries in podocyte biology have made significant advancement in our understanding of the molecular defects for many glomerular diseases.

What this adds

Our review article highlights the ongoing efforts of implementing precision medicine for understanding the biology and treatment of glomerulonephritis.

Introduction

Glomerulonephritis (GN) is characterized by glomerular inflammation and classified based on histopathology and clinical presentation. It encompasses a spectrum of kidney diseases that collectively are the third leading cause of end-stage renal disease (ESRD).[1] The incidence of primary GN varies between 0.2 and 2.5 per 100 000 per year.[2] The risk of progression to ESRD is between 20% and 50%.[3] The pathogenesis of GN is complex with numerous factors that can trigger and contribute to the progression of glomerular injury. These include, but are not limited to, genetic predisposition, autoimmunity, malignancy, infections, and exposure to drugs.[1,4,5] GN can be categorized into renal disorders with the presence of proliferative changes (including IgA nephropathy [IgAN], lupus nephritis [LN], ANCA-associated vasculitis [AAV], idiopathic membranoproliferative GN [MPGN], antiglomerular basement membrane GN, and postinfectious GN) or absence of proliferative changes (including minimal change disease [MCD], focal and segmental glomerulosclerosis [FSGS], idiopathic membranous nephropathy [MGN], and steroid-responsive and steroid-resistant nephrotic syndrome).[6] The overarching goals in GN treatment are to improve quality of life for patients, prevent ESRD, and reduce burden to our health care system. However, treatment for glomerular diseases has been hampered by the nonspecific actions of immunosuppressive agents that often have no clear therapeutic target. Furthermore, immunosuppressive treatments for GN are sometimes ineffective and often lead to adverse drug toxicities including increased risk of opportunistic infections and irreversible side effects such as avascular necrosis. Given the abundant causes and heterogeneity of GNs, there is an urgent need for a better understanding of the pathogenesis of GNs at the molecular level, and to develop effective, targeted, and ideally personalized therapy for patients with GN.

In this review, we provide an overview of the (1) biology of podocytes, (2) treatments targeting the podocyte, (3) precision medicine in GN, (4) high-throughput technologies used for GN research, and (5) models used to study GN. Our goal here is to highlight examples of recent studies characterizing the mechanisms regulating glomerular disease, and how these discoveries may improve treatment of GN.

Biology of Podocytes

Glomeruli consist of many cell types, but much interest has been in podocyte biology as it is common to many forms of glomerular disease including MCD, FSGS, and MGN (see reviews[7,8]). Podocytes are highly specialized, terminally differentiated epithelial cells that are located adjacent to the glomerular capillaries, where they form part of the glomerular filtration barrier. Functionally, podocytes communicate with their environment and transmit cell signals through various intracellular pathways, such as receptor tyrosine kinases, G protein–coupled receptors, nuclear receptors, and integrins (see reviews[9-11]). Furthermore, as key components of the glomerular filtration barrier, podocytes adhere tightly to the glomerular basement membrane (GBM) to withstand the transcapillary filtration pressure.[12] The podocyte has a complex cellular architecture composed of a parachute-like cell body that attaches to the basement membrane through primary foot processes. Structurally, podocytes have microtubule-based cellular extensions called primary processes, and actin-based membrane extensions called foot processes (Figure 1). Podocyte foot process effacement, a hallmark feature of diseases leading to nephrotic syndrome, involves disruption of the actin cytoskeleton leading to compromise of the slit diaphragm and subsequent proteinuria.[12]

Our understanding of podocyte biology has emerged primarily from human genetic studies which have identified several genetic mutations that encode proteins important in podocyte structure and function. Proteins involved in podocyte biology and structure can be broadly categorized into regulators of (1) slit diaphragm, (2) cytoskeleton, or (3) involvement in GBM attachment (Figure 1). Many of these proteins are implicated in podocyte injury and nephrotic syndrome.

i. *Slit diaphragms* are the cell-to-cell junctions formed between adjacent podocytes, which are crucial for proper podocyte function.[12] Mutations in 2 key slit diaphragm proteins, nephrin (a transmembrane protein in the slit-diaphragm) and podocin (a cell junction protein), result in actin cytoskeleton

[1]Department of Cell Biology, Harvard Medical School, Boston, MA, USA
[2]Division of Nephrology, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA
[3]Division of Nephrology, Department of Medicine, University of Calgary, Alberta, Canada

Corresponding Author:
Justin Chun, Department of Medicine, Beth Israel Deaconess Medical Center, 99 Brookline Avenue, RN 320, Boston, MA 02215, USA.
Email: jchun2@bidmc.harvard.edu

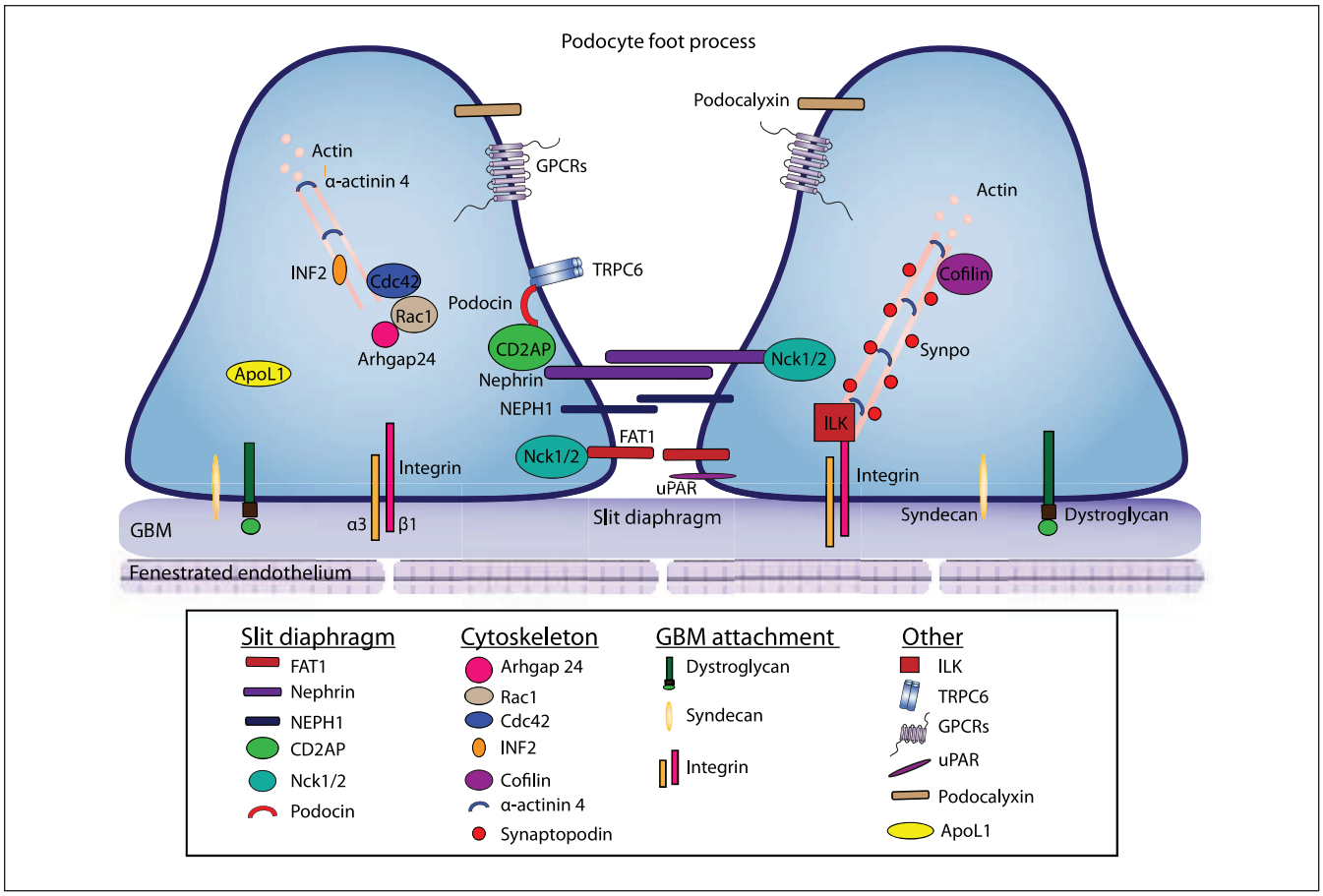

Figure 1. Schematic representation of podocyte foot processes.
Note. Two adjacent podocytes are connected by slit diaphragms, composed of nephrin and nephrin-like protein 1 (NEPH1). Podocyte foot process express integrins, dystroglycans, and syndecans, to form interactions with the glomerular basement membrane (GBM). α-actinin 4, inverted formin 2 (INF2), integrin-linked-kinase (ILK), and synaptopodin (synpo) interact with the actin network to regulate the podocyte foot process. Integrin α3, α3; β1 integrin, β1; transient receptor potential cation channel subfamily C member 6, TRPC6; CD2-associated protein, CD2AP; cytoplasmic protein Nck, Nck1/2; cell division control protein 42 homologue, Cdc24; Ras-related C3 botulinum toxin substrate 1, Rac1; Rho GTPase-activating protein 24, Arhgap 24; protocadherin fat 1, FAT1; urokinase plasminogen activator surface receptor, uPAR; G protein–coupled receptor, GPCR; ApoL1, apolipoprotein L1.

dysregulation and nephrotic syndrome.[13-15] A recent study by New et al showed that phosphorylation of nephrin is required to stabilize and restore podocyte morphology.[16] CD2AP (CD2-associated protein) is another gene that is mutated in human glomerular disease.[17] CD2AP, a scaffolding protein, NEPH1, a member of the nephrin-like protein family,[18,19] and protocadherin FAT1, an adherent junction protein [20,21] all interact with nephrin to form slit diaphragm junctions.[22] Nck1/2 are adaptor proteins that serve to link nephrin to the actin cytoskeleton of the cell.[23,24] Genetically, loss of NEPH1 in mice resulted in proteinuria and perinatal lethality,[25] whereas loss of FAT1 resulted in slit junction defects.[21] Loss of Nck1/2 in mouse podocytes resulted in proteinuria and defects in foot processes morphology.[26] Together, podocin, nephrin, and their associated proteins function to connect membrane junction proteins to the actin cytoskeleton of the cell. Mutations to any of these proteins can disrupt slit diaphragm's function and integrity, resulting in glomerular disease.

ii. *Regulators of the podocyte actin cytoskeleton* are important in podocyte function; disruption of components of the cytoskeleton can results in podocyte damage. For example, Rho GTPases (Cdc42, RhoA, and Rac1) have roles in maintaining the actin-based cytoskeleton in podocytes. Specifically, experiments suggest that Cdc42 is critical for the maintenance of the glomerular filtration barrier,[27,28] whereas the role of RhoA in maintenance of podocyte actin cytoskeleton is less clearly defined but may involve the stabilization of RhoA by the binding of synaptopodin, a proline-rich actin-associated protein that is involved in stress-fiber formation in podocytes.[27,29] Abnormal activation of Rac1 has been shown to lead to proteinuria with foot process effacement.[30] In addition, hyperactivation of Rac1 by 3 mutant forms of Rho-GDP dissociation

inhibitor-α was also shown to impair actin polymerization and slowed motility in podocytes.[31] The balance between RhoA and Rac1 signaling might be regulated in podocytes by Rho-GAP24 or Arhgap24.[32] Studies in the area of Rho-GTPase are still on-going, the relationship between RhoA, Rac1 and cytoskeletal integrity might be more dynamic than previously thought. For an in-depth review on Rho GTPase see Mouawad et al.[33] In addition, actin associated proteins are known to contribute to the development of FSGS. INF2, a formin family of actin-regulating protein plays a role in actin polymerization, and human mutations in INF2 are found in patients with FSGS.[34] Similarly, mutations in α-actinin 4, an actin-filament cross-linking protein, have also been identified in patients with FSGS.[35] The mouse model of α-actinin 4 recapitulates FSGS defects.[36] Synaptopodin is another actin-associated protein that is known to interact with α-actinin 4, and functions to regulate RhoA signalling.[27] Mice without synaptopodin have normal podocytes but exhibit slow recovery from chemically induced podocyte injury.[29] Other genes that likely have a regulatory role in the actin cytoskeleton are cofilin and anilin. Genetic deletion of known actin binding protein cofilin (actin-depolymerizing factor) resulted in proteinuria in mice, and mutations in anilin, an F-actin binding protein, were present in patients with FSGS. These studies illustrate the importance of cytoskeletal proteins in podocyte functions.[37,38] Working together, these proteins function to maintain actin-cytoskeleton structural integrity of podocyte foot processes, which is crucial to podocyte function.

iii. *Podocyte attachment* to the GBM is crucial for its function. Podocyte utilizes various anchor proteins to attach to the GBM. Podocytes express several integrin receptors, including the major cell-matrix adhesion receptor, integrin α3β1. In mice, loss of α3β1 weakens the podocyte-GBM interaction, and causes poor organization of laminin 511/521 into a functional GBM.[39-42] Other cell-matrix adhesion receptors expressed by podocytes include the integrins α2β1 and αvβ3, and type XVII collagen.[12,43] In addition, podocytes express α-dystroglycan, a transmembrane adhesion complex, that serves to attach podocytes to GBM. Researchers have found that dystroglycan levels are lowered in Minimal Change Disease.[44] Syndecan4, a heparin sulfate proteoglycan that functions in matrix adhesion through its interaction with extracellular matrix, is also involved in attachment of podocytes to GBM. In mice without Ndst1, the enzyme responsible for *N*-sulfation of heparan sulfate chains, there is a failure to assemble heparan sulfate glycosaminoglycan chains, resulting in disrupted localization of Syndecan4, and foot process effacement.[45,46] These studies highlight the importance of proper podocyte attachment to GBM in GN.

Last, other podocyte genes implicated in nephrotic syndrome have been identified; these include ApoL1,[47] ILK,[48] TRPC6,[49] podocalyxin,[41] GPCRs,[50] and suPAR[51,52] (Figure 1). Ongoing studies are dissecting the functions of these proteins. With improved understanding of their molecular mechanisms, we may be able to identify new therapeutic targets for precision medicine as well for biomarker discovery.

Treatments Targeting Podocytes

Early podocyte injury may be reversible if the actin cytoskeleton can be repaired. However, sustained glomerular injury can result in permanent podocyte damage leading to podocyte hypertrophy, cell death, and eventual kidney failure.[53] Podocytopathies including membranous nephropathy, FSGS, and MCD have had variable responses to immunosuppressive therapy. Interestingly, the beneficial actions of immunosuppressive agents may be mediated by mechanisms not directly related to immunosuppression. Some mechanisms of podocyte injury include changes to the slit diaphragm structure and/or function, dysfunction of the actin cytoskeleton, podocyte interaction with the GBM, alterations in the transcriptional regulation of podocytes, calcium homeostasis dysregulation, upregulation of the innate immune system, and upregulation of cathepsin L–mediated proteolysis.[54] Given that podocyte injury leads to a number of GN, podocytes are attractive therapeutic targets for GN. For example, activation of integrins αvβ3 by the urokinase receptor (uPAR) causes foot process effacement, proteinuria, and FSGS in mice and humans. By blocking αvβ3 with an anti-β3 antibody or the small-molecule inhibitor cilengitide, it is possible to reduce proteinuria induced by uPAR.[51,52] Recently, Hayek et al showed that suPAR-activated αvβ3 integrin can bind to ApoL1 risk variants with higher affinity than wild-type ApoL1 causing more proteinuria in mice.[55] Future studies will be required to validate and further expand on these results to determine whether these identified targets are able to be therapeutic targets in human disease. Characterizations of the molecular mechanisms regulating podocyte actin cytoskeleton stabilization have provided insight to design more targeted therapies. Faul et al demonstrated that the antiproteinuric effect of cyclosporine A may not be fully explained by its immunosuppressive activity for the inhibition of nuclear factor of activated T cells (NFAT) signaling in T cells. Instead, cyclosporine appears to stabilize the actin cytoskeleton in podocytes by blocking the calcineurin-mediated dephosphorylation of synaptopodin.[56] Similarly, rituximab may stabilize the actin cytoskeleton and prevent podocyte apoptosis independent of its well characterized activity as a monoclonal antibody for CD20 on B lymphocytes. Fornoni et al found that rituximab treatment was associated with a lower incidence of posttransplant proteinuria and better preservation of glomerular filtration rate. Interestingly, when podocytes were induced by sera from patients with recurrent FSGS, rituximab or overexpression of sphingomyelin phosphodiesterase acid-like 3b (SMPDL-3b)

was able to prevent disruption of the actin cytoskeleton and apoptosis of podocytes.[57] The researchers concluded that rituximab may be able to be used in high-risk patients undergoing kidney transplant to prevent FSGS.

Clinically, rituximab has been shown to have good efficacy for MGN, idiopathic nephrotic syndrome, MCD, and FSGS.[58-61] Recently, Yu et al showed that abatacept (cytotoxic T lymphocyte associated antigen 4 immunoglobulin fusion protein), a costimulatory inhibitor that targets B7-1/CD80, reduced proteinuria in patients with FSGS.[62] These findings will need to be replicated in larger cohorts but suggest that therapies targeting podocytes may be feasible in the future with a better understanding of the molecular disruptions that lead to podocyte injury.

Precision Medicine in GN

The histopathological classification of GN has helped to categorize different GN with some features helpful for the diagnosis and prognostication of disease. For example, histopathological classification of ANCA[63] and IgAN (Oxford Classification[64]) has shown prognostic value in clinical settings, and may have potential for better predicting therapeutic outcomes. Nevertheless, our current histopathological classifications cannot accurately determine which immunosuppressive agents can best treat certain GN, making it difficult to select the appropriate type and duration of treatment. For decades, the main treatments for GN have been adopted from other fields of medicine and have remained largely unchanged. Technological advancements have improved the understanding of molecular mechanisms underlying glomerular disease, and provided opportunities to develop targeted therapies. It is possible that patients are genetically predisposed to have a better response to certain therapies. Some questions that have not been answered include "Can the dose and/or duration of immunosuppressive medications be determined prior to administration of immunosuppressive agents? "Is it possible to determine the best immunosuppressive agent for a particular patient?"

Precision medicine, combining modern technologies with molecular and genetic information to identify mechanisms of diseases for targeted treatment and disease prevention, may be coming of age in nephrology.[65] Precision medicine for GN research will likely be necessary due the heterogeneity of glomerular diseases. The goal for precision medicine is now being considered by many groups who have set up biobanks for the purpose of identifying biomarkers and pathways for potential therapeutic interventions. Several large tissue banks which have been established include the European Renal cDNA Bank (ERCB), the Nephrotic Syndrome Study Network (NEPTUNE), and the Clinical Phenotyping and Resource Biobank Core (C-PROBE).[65] In Canada, the Biobank for the Molecular Classification of Kidney Disease in Calgary, Alberta, has amassed thousands of patient samples.[66] In the Muruve lab, we demonstrated that correlating clinical outcome with RNA expression profiles from kidney biopsies obtained from patient affected with GN is a promising approach to identify biomarkers.[67,68] We anticipate that the biobanked samples will serve as an immense repository for research projects that can target specific conditions, and facilitate the recruitment of patient and samples that will be large enough to produce statistically meaningful data. It will be critical to have tissue banks with identical protocols to ensure reproducible handling, storage and molecular analysis of tissue (biopsy samples), and biological fluids (blood, urine, and saliva). Just as important, the tissue bank will need to be combined with large, well-defined patient databases using software tools for analyzing phenotypic information of patients (eg, PhenoTips[69]). The ability to test and validate the predictability of novel biomarkers highlights the importance of long-term longitudinal follow-up as a component of tissue banks.

Precision medicine is in its infancy in utilizing molecular and genetic information obtained from genome-wide association studies (GWAS), exome sequencing, and whole-genome sequencing with next-generation sequencing (NGS) and VAAST (Variant Annotation, Analysis, and Search Tool).[70] The ultimate goal for precision medicine in GN research will be to use small quantities of biological fluid for large-scale analytic tools. The availability of clinical data to correlate findings with experimental results from large data sets is permitting the analysis of specific individuals and paving the way for precision medicine. One excellent example for precision medicine in GN diagnosis and treatment is the discovery of PLA2R (phospholipase A2 receptor) as the antigen involved in the pathogenesis of membranous nephropathy. Salant's group identified PLA2R as the autoantigen in human membranous nephropathy.[71-73] Since its discovery in 2009, PLA2R is being used as a biomarker for membranous nephropathy. Currently, both immunofluorescence and ELISA (eg, EUROIMMUN Anti-PLA2R ELISA (IgG)) are available for testing the presence of PLA2R in clinics. For example, in Calgary, Alberta, we have used Mitogen Advanced Diagnostics Laboratory to analyze patient blood samples for the presence of anti-PLA2R (http://mitogen.ca/requisition-form/). Anti-PLA2R is helpful to categorize MGN as primary or secondary as autoantibodies are present in approximately 70% of patients with primary MGN.[73] Furthermore, there is evidence that anti-PLA2R antibodies may be used to monitor the activity of membranous nephropathy.[74] Future studies to characterize the mechanisms by which PLA2R leads to MGN will allow the development of more specific therapeutic approaches, including antibody inhibition therapy and immunoadsorption of circulating autoantibodies.[75] Other promising biomarkers such as circulating cell-free DNA (cfDNA) has been reported to be elevated in SLE.[76] cfDNAs are promising biomarkers for the diagnosis and classifications of GNs. In addition, microRNAs are known to play

multiple roles in kidney development, physiology, and pathology (see review[77]). A major goal for precision medicine will be to develop GN biomarker panels that readily available in clinics and hospitals to accurately diagnose and guide treatment for GN.

High-Throughput Technologies in GN Research

Since the completion of the human genome project on April 14, 2003, genome-based research has led to new insights into disease mechanisms and potential targeted treatments. New approaches to better understand GN involve large-scale research by genomics (studying the genome sequencing and analysis), transcriptomics (the study of RNA transcripts that are produced by the genome), proteomics (the large-scale study of proteins), and metabolomics (the study of chemical processes involving small metabolites). For an excellent overview of definitions, limitations, and integrative approaches in glomerular disease, please refer to Mariani et al's recent review.[78] In the next section, we will provide specific examples of recent studies that have utilized genomics, transcriptomics, proteomics, and metabolomics to study GN.

Genomics

Glomerular diseases have been studied using GWAS to compute hundreds of thousands of single-nucleotide polymorphisms (SNPs) in a genome to identify genetic associations in GN. Using GWAS, many genetic associations for MGN,[79] IgAN,[80-83] LN,[84] and FSGS [85] have been established. One example of how GWAS was used to identify causal variants for nondiabetic ESRD and FSGS in African American was demonstrated by the Pollak lab for the discovery of APOL1.[47] Initially, admixture mapping studies identified MYH9 as the gene associated with FSGS, HIV-associated nephropathy, nondiabetic ESRD, and hypertensive associated ESRD.[86,87] However, fine mapping and resequencing did not identify any causal variants for MYH9.[88,89] Instead, using conditional GWAS, the major source of genetic risk for nondiabetic ESRD and FSGS in African Americans was localized to APOL1.[47]

Recently, Hildebrandt's group applied a powerful approach using microfluidic multiplex PCR and NGS to detect a single-gene cause in about 30% of families with steroid-resistant nephrotic syndrome.[90] Using whole-exome sequencing and high-throughput exon sequencing, they were able to identify mutations in 3 nuclear pore genes that cause steroid-resistant nephrotic syndrome.[91] The work by Hildebrandt's group highlights the rapid changes in genomic tools available for sequencing patient samples and that the field is moving toward a more personalized approach to understand disease phenotype at the genetics level. Current genetic approaches have been instrumental in identifying risk alleles, genetic variants, or genetic modifiers of genes associated with GN. It is foreseeable in the near future that more researchers will use NGS and whole-genome sequencing to study GN as the costs of these approaches continue to decrease.

Transcriptomics

Transcriptomics studies can use a single cell or tissue to generate high-throughput RNA expression profiles.[92] Transcriptomics, a widely used high-throughput technology,[93] is being used to study glomerular diseases including IgAN,[94,95] FSGS,[96] and lupus nephritis.[97-99] Recently, Banchereau et al performed personalized immunomonitoring using the blood transcriptome to identify molecular networks that enable stratification of lupus nephritis patients into 7 groups.[99] Transcriptomic analysis performed using glomeruli dissected from patient biopsy samples have also been used to validate previous experimental data from mouse model of lupus nephritis.[100,101] In the study by Hodgin et al, researchers used traditional microarray to analyze biopsies from patients with subtypes of FSGS. They found that genes involved in slit diaphragm function were differentially upregulated, and further showed different molecular signature involved in different glomerular injuries.[96] Similarly, Peterson et al performed cDNA microarray analysis on biopsies from patients with lupus nephritis. From the transcriptome profiling, 4 clusters of genes were identified that represent lupus nephritis molecular signatures.[97] For ANCA-associated vasculitis (AAV), Brix et al used microarray analyses of renal biopsy samples from patients with AAV crescentic GN. Interestingly, the CC chemokine ligand 18 was found to be upregulated in patients with AAV crescentic GN and proposed to be biomarker for disease activity and relapse.[102] Overall, these studies illustrate the strength of using transcriptomics to characterize molecular defects in GN. It is now possible to use small samples from kidney biopsies to perform transcriptomic analysis as Drop-seq has the capability to profiling thousands of individual cells by separating them into nanoliter sized aqueous droplets.[92] One can envision that kidney biopsies obtained from biobanks will be used to study every type of GN in the future.

Proteomics

Proteomics has been the most extensively researched "omics" in GN. Many researchers use proteomic tools to identify biomarkers for the diagnosis of glomerular diseases without the need for an invasive tissue biopsy. Numerous studies have implemented different protocols for mass spectrometric analysis such as capillary electrophoresis, liquid chromatography (LC-MS), and matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS) using various biological fluids.[103]

Using urine, serum, or tissue, many groups have employed proteomic techniques for biomarker discovery in GNs including ANCA-associated vasculitis, IgAN, MN, MCD, and FSGS which has been recently reviewed by L'Imperio et al.[103] The latest technologies in proteomics that have been applied to GN research are MALDI-MSI, laser capture microdissection (LCM), and SOMAscan. MALDI-MSI enables the analysis of protein expression data with tissue molecular images in formalin-fixed paraffin-embedded tissue which allows identification of tissue morphology.[104] LCM allows the selective isolation of glomeruli from kidney tissue to avoid contamination by protein or genetic material from interstitium or tubules.[105] LCM of kidney tissue has been successfully used for MPGN, amyloidosis, cryoglobulinemic GN, fibrillary GN, and immunotactoid GN.[106,107] Jain et al used LCM and MS on kidney biopsy of a 58-year-old asymptomatic woman with MPGN suspected secondary to monoclonal gammopathy but an unclear diagnosis as there was a mismatch between the patient's serum monoclonal protein (IgG kappa) and immunofluorescence staining pattern (nonspecific IgM, absence of light chain restriction). By using LCM and MS performed on the kidney biopsy tissue, deposits of monoclonal IgG kappa were detected which helped to make the diagnosis of monoclonal gammopathy-associated MPGN. Sethi et al used LCM followed by MS to compare and distinguish the constituents of the deposits from patients with amyloidosis, fibrillary GN, and immunotactoid GN to cryoglobulinemic GN. Remarkably, by using advanced proteomic methods, these researchers were able to identify different protein signatures for each disease. For example, serum amyloid P and apolipoprotein E were present in large spectra numbers in amyloidosis but not in cryoglobulinemic GN, and Ig gamma-1 chain C region in immunotactoid glomerulopathy compared with fibrillary and cryoglobulinemic GN.[107] LCM combined with different MS protocols will serve as an important platform to identify molecular differences between forms of GN and help make diagnoses for challenging cases.

SOMAscan is a new technology by SomaLogic that uses a novel targeted proteomic technique based on aptamer technology, which can currently measure 1310 protein analytes from blood.[108] This new technology allows the detection of hundreds of molecules from small quantities of substrate. In 2010, Gold et al applied SOMAscan as proteomics biomarker discovery technology to a clinical study of chronic kidney disease (CKD) and identified 2 known CKD biomarkers (cystatin C and β2-microglobulin) and an additional 58 potential CKD biomarkers.[109] These potential CKD biomarkers have yet to be validated, but SOMAscan appears to be a promising proteomic technique that could be applied to biomarker identification in glomerular disease.

Metabolomics

A metabolomics approach to study GN has gained scientific interest with recent studies showing promising results, particularly in diagnosis. The use of metabolite composition in the urine as a diagnostic tool is an attractive noninvasive alternative to renal biopsy. For example, solid-phase microextraction-chromatography-mass spectrometry (SPME-GC-MS) was used by Wang et al to study urinary volatile organic compounds, as a marker for pathological changes in the kidney.[110] Five urinary metabolites (tartronic acid, carbamic acid, sulfide, allyl methyl, hydrogen azide, and benzeneethanamine,N-[(pentafluorophenyl)methylene]-.beta.,4-bis[(trimethylsilyl) oxy]-), were identified and significantly increased in IgAN compared with MPGN patients. These results suggest urinary metabolites may be used as biomarkers to differentiate between different forms of GN. With the generation of large "omic" datasets, an integrative approach (data sharing and team work) will be the key to fully utilize this information not only for precision medicine in GN but for understanding molecular signatures of diseases as well.[78]

Models in GN Research

Experimental Animal Models

Animal models, primarily rat and mouse models, have been extensively used to improve our understanding of GN. Detailed information on animal models in kidney disease and GN can be found in other reviews.[111,112] Some advantages of rodent models include their small size making it economical to house animals and the ability to generate genetically defined strains making it possible to design spontaneous disease models. We have included a table for examples of available animal models that have been used for most types of GN with some of their strengths and limitations (Table 1).

The investigations using animal models are well illustrated by animal models for Heymann nephritis, ddY mouse, and the anti-GBM mouse model for MGN, IgAN, and anti-GBM respectively. The Heymann nephritis model has improved our understanding of membranous nephropathy by immunizing rats with tubular brush border antigens, but the target antigen in the human glomerulus is different from megalin-involved Heymann nephritis.[116] Megalin is not expressed in human podocytes and therefore not pathogenic in humans. Instead, PLA2R appears to be a major target but is not expressed in rodents or rabbits.[111] Transgenic animal models can circumvent the problem of genes with the caveat of potentially altering the genome. Next, there have been many models used to replicate aspects of IgAN, but each model only partially models the disease. For example, the ddY mouse model has increased circulating IgA and IgA mesangial deposits but only minimal proteinuria and no hematuria.[120] In comparison, the Thy.1 nephritis model which requires an injection of an antibody to Thy.1 (antigen found on mesangial cells) can

Table 1. Animal Models of GN.

GN	Example model	Description of model	Strengths	Limitations	References
MCD	PAN nephrosis	Aminonucleoside causing direct cellular toxicity	Rapid onset of nephrotic range proteinuria with classic morphological features of MCD	Requires repeated injections to induce damage Proteinuria only partially glucocorticoid sensitive	113,114
FSGS	Adriamycin nephropathy	Adriamycin causing direct cellular toxicity	Highly reproducible and robust A single intravenous dose is enough to induce disease	Strain-specific renal injury No clear clinical counterpart Mechanism not well defined Adriamycin has batch variability and narrow therapeutic window	115
MGN	Heymann nephritis (Passive)	Nephrotoxic, antiserum injected into a rat/ mouse to elicit immune complex formation	Best characterized rat model, nearly identical pathology to human MGN, relative short onset and progression of disease	Megalin is not pathogenic in humans as it is not expressed in human podocytes The dominant immunoglobulin deposited in human MGN is IgG4	116,117-119
IGAN	ddY mouse	Elevated levels of circulating IgA and IgA mesangial deposits	Spontaneous development Severe glomerular IgA deposition	Does not fully represent human disease Mild proteinuria with no hematuria	120
LN/SLE	Spontaneous SLE Induced	NZB/W F1 mouse model with lupus-phenotype Pristane-induced model	Spontaneous development Mimics autoimmune phenotypes in human.	Female mice are more affected in some models Pristane can induce other adverse effects	121 122
AAV	MPO AAV	Immunization of MPO knockout mice with mouse MPO	Antibody mediated model	Partially models disease. Difficulty in modeling all organs affected Milder disease with fewer crescents	123
Anti-GBM	Anti-GBM disease	Macrophage proliferation in Bowman's space with expression of the proliferating cell nuclear antigen	Resembles the human clinical disease	Variation in susceptibility among different mice and rat strains	124
CRGN	Crescentic	Injection of antibody to whole rabbit glomeruli	Forms crescents	Other pathogenic factors can cause anti-GBM disease	125
MSPGN	Thy.1 nephritis	Injection of anti-Thy 1 antibody	Single injection of antibody	Partially models human MsPGN	126,127

Note. GN = Glomerulonephritis; MCD = minimal change disease; PAN = puromycin aminonucleoside; FSGS = focal segmental glomerulosclerosis; MGN = membranous glomerulopathy; IgAN = IgA nephropathy; LN = lupus Nephritis; SLE = systematic lupus erythematosus; MPO = myeloperoxidase; AAV = ANCA-associated vasculitis; GBM = *glomerular basement membrane; CrGN = crescentic GN; MsPGN = mesangial proliferative GN*.

result in mesangiolysis and mesangial cell proliferation but does not lead to IgA deposits. Last, the many animal models for anti-GBM have established the importance of anti-GBM in the pathophysiology of disease providing the rationale for using immunosuppression and plasma exchange, but limited information has been obtained with respect to the mechanisms of injury following induction with limited efficacy of therapies introduced after induction.

Differences between species limit our ability to extrapolate findings in animal models to physiological and disease conditions in the human body. Many genes and proteins that are expressed and regulated are often very different between species even when comparing mice with rats or humans with other primates. For example, results from rat models often cannot be replicated in mice.[111] Also, mouse models are particularly difficult to use for experiments modeling GN as many mouse strains are resistant to glomerulosclerosis and immune-mediated mechanisms.[111] Furthermore, mice often have alternate genotypes with additional copies of genes (eg, renin) or the absence of genes (eg, APOL1).[111,128] Bearing this in mind, we and other have resorted to modeling glomerular disease using human inducible pluripotent stem cells (iPSC). Human stem cells have the advantage of having the appropriate genes and regulatory proteins animal models may lack increasing the probability of determining mechanisms regulating human biological processes and disease.

Kidney Organoids

Gene editing in human stem cells and kidney organoids ("mini-kidneys") are invaluable tools to further develop the field of precision medicine in GN. With limited samples to process from precious kidney biopsy samples, one may envision recreating a model from individual patients using stem

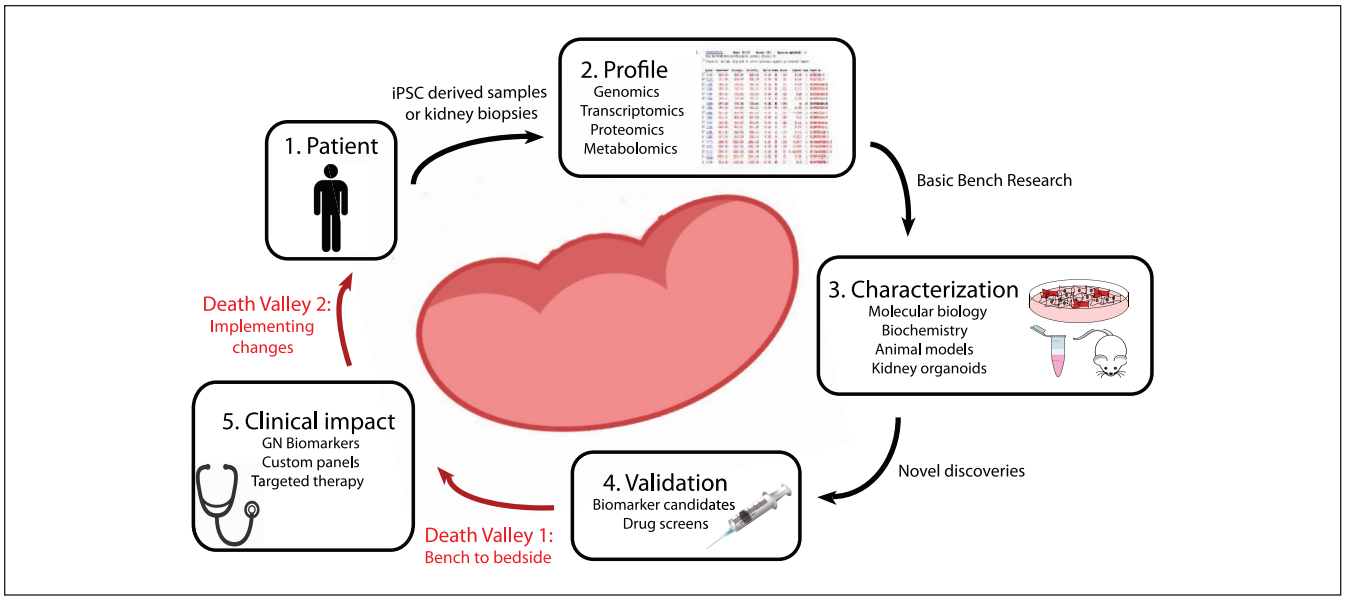

Figure 2. Overview of the patient-oriented, precision medicine research scheme.
Note. In precision medicine, utilizing integrative biological approaches to advance bedside discoveries, promote translational research, increase clinical output, and improve patient care. (1) Patient care will start with a detailed analysis of the patient's clinical, biochemical, and molecular information obtained from clinical history, blood work, and tissue derived samples. (2) Genomic, transcriptomic, proteomic, and/or metabolomic profiling of patient samples from a collection of samples from patients with GN to identify and classify novel signatures that are unique to each type of GN. (3) Characterization of pathways and understanding the molecular mechanisms of potential targets unique to GN using a combination of molecular cell biology, biochemistry, animal models, and kidney organoids. (4) Validation of targets that can be used as biomarkers for specific GN and the development of pharmacologic compounds identified from drugs screens. (5) Improvements to patient care with biomarkers that can expedite the diagnosis of GN, custom DNA/RNA/protein panels for GN for prognostication and used to guide customized, targeted therapies. Death Valley 1: Challenge of translating targets identified from basic science research into clinically relevant diagnostic, prognostic, and therapeutic tools. Death Valley 2: Challenge of implementing changes in health care to make new diagnostic and therapeutic tools available for patient care.

cells to better study disease and to serve as a platform for drug testing. Several groups have how established protocols to generate nephron progenitor cells and kidney organoids from human induced pluripotent stem cells.[129-131] Recently, gene editing by the clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 system has demonstrated that podocyte genes can be knocked out in nephron progenitor cells (NPC)[131] and kidney organoids.[132] For proof of concept, Li et al used the CRISPR-Cas9 to knockout nephrin (NPHS1) in cultured NPC and were able to successfully deliver gene targeting constructs to efficiently create NPHS1 knockout nephron organoids.[131] Freedman et al demonstrated that CRISPR-Cas9 knockout of PKD1/PKD2 and podocalyxin in human stem cells and kidney organoids may be a useful approach to model polycystic kidney disease and glomerular disease, respectively. Impressively, knockout of the polycystic kidney disease genes PKD1 or PKD2 induced cyst-like structures from kidney tubules,[132] while podocalyxin knockout using CRISPR-Cas9 in stem cells and derived podocytes resulted in defective microvilli assembly and failed junctional migration.[133] Together, these studies demonstrate the ability to effectively delete genes in kidney organoids, which can result in the expected features of glomerular and tubular kidney diseases. It is foreseeable in the future that nephron progenitor cells and kidney organoids will be further enhanced to generate knockout and knockin disease models of GN using CRISPR-Cas9. Such human models will be promising platforms for disease-modeling, diagnostics and drug screening.

Future Outlook

The current classification of GN based on histopathology reflects our limited understanding of the basic molecular mechanisms that lead to disease. Not surprisingly, current treatments for GN remain nonspecific with approaches relying almost exclusively on immunosuppression with limited knowledge of the molecular targets. Immunosuppression remains the mainstay treatment for GN which has been effective for immune-mediated diseases but less effective for monogenic diseases and often leads to complications related to medication toxicities. Nonetheless, recent studies using genetic and cell biology have identified molecular defects and regulators of podocyte injury in GN, opening the possibility of developing specific or targeted therapies. In addition, new technologies in genome sequencing combined with large scale proteomics and sequencing databases will lead to the discovery of new genes to contribute to our understanding of podocyte function. We anticipate that in the near future, there will be validated biomarkers to useful to distinguish between different causes of GN. The advances in

biotechnology are rapidly paving the way for translation research and personalized medicine which will certainly contribute to the development of more effective and safer therapies. With precision medicine for GN treatment on the horizon, we must be mindful of the challenges in bridging basic research to clinical practices, referred to as "Death Valleys" by the Canadian Institutes for Health Research (http://www.cihr-irsc.gc.ca/e/44000.html). Death Valley 1 is the challenges in translation of basic research, basic podocyte biology, to clinic in the form of new therapy for patients (Figure 2). Valley 2 refers to the difficulty in implementing changes in health care decision making, hence utilizing the newly developed therapeutics for GN patients.[134,135] It is likely that the cost associated with new tests and technologies will be a major barrier for implementing new diagnostic tests and treatments. Both Valleys need to be overcome to truly improve patient care and quality of life.

Ethics Approval and Consent to Participate

No ethics approval or consent to participate was required for this publication.

Consent for Publication

All authors have read and approved the final version of this manuscript.

Availability of Data and Materials

No primary data is presented in this publication.

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References

1. Hricik DE, Chung-Park M, Sedor JR. Glomerulonephritis. *N Engl J Med*. 1998;339(13):888-899.
2. McGrogan A, Franssen CF, de Vries CS. The incidence of primary glomerulonephritis worldwide: a systematic review of the literature. *Nephrol Dial Transplant*. 2011;26(2):414-430.
3. Barbour S, Beaulieu M, Gill J, Djurdjev O, Reich H, Levin A. An overview of the British Columbia Glomerulonephritis network and registry: integrating knowledge generation and translation within a single framework. *BMC Nephrol*. 2013;14:236.
4. Lau KK, Wyatt RJ. Glomerulonephritis. *Adolesc Med Clin*. 2005;16(1):67-85.
5. Floege J. Primary glomerulonephritis: a review of important recent discoveries. *Kidney Res Clin Pract*. 2013;32(3):103-110.
6. Kidney Disease: Improving Global Outcomes (KDIGO) CKD-MBD Work Group. Kidney Disease: Improving Global Outcomes (KDIGO): glomerulonephritis work group. *Kidney Int*. 2012;2:139-274.
7. Barisoni L, Schnaper HW, Kopp JB. Advances in the biology and genetics of the podocytopathies: implications for diagnosis and therapy. *Arch Pathol Lab Med*. 2009;133(2):201-216.
8. Kwoh C, Shannon MB, Miner JH, Shaw A. Pathogenesis of nonimmune glomerulopathies. *Annu Rev Pathol*. 2006;1:349-374.
9. Greka A, Mundel P. Cell biology and pathology of podocytes. *Annu Rev Physiol*. 2012;74:299-323.
10. Khurana S, Bruggeman LA, Kao HY. Nuclear hormone receptors in podocytes. *Cell Biosci*. 2012;2(1):33.
11. Reiser J, Sever S, Faul C. Signal transduction in podocytes—spotlight on receptor tyrosine kinases. *Nat Rev Nephrol*. 2014;10(2):104-115.
12. Sachs N, Sonnenberg A. Cell-matrix adhesion of podocytes in physiology and disease. *Nat Rev Nephrol*. 2013;9(4):200-210.
13. Kestila M, Lenkkeri U, Männikkö M, et al. Positionally cloned gene for a novel glomerular protein—nephrin—is mutated in congenital nephrotic syndrome. *Mol Cell*. 1998;1(4):575-582.
14. Boute N, Gribouval O, Roselli S, et al. NPHS2, encoding the glomerular protein podocin, is mutated in autosomal recessive steroid-resistant nephrotic syndrome. *Nat Genet*. 2000;24(4):349-354.
15. Ruotsalainen V, Ljungberg P, Wartiovaara J, et al. Nephrin is specifically located at the slit diaphragm of glomerular podocytes. *Proc Natl Acad Sci U S A*. 1999;96(14):7962-7967.
16. New LA, Martin CE, Scott RP, et al. Nephrin tyrosine phosphorylation is required to stabilize and restore podocyte foot process architecture. *J Am Soc Nephrol*. 2016;27(8):2422-2435.
17. Kim JM, Wu H, Green G, et al. CD2-associated protein haploinsufficiency is linked to glomerular disease susceptibility. *Science*. 2003;300(5623):1298-1300.
18. Barletta GM, Kovari IA, Verma RK, Kerjaschki D, Holzman LB. Nephrin and Neph1 co-localize at the podocyte foot process intercellular junction and form cis hetero-oligomers. *J Biol Chem*. 2003;278(21):19266-19271.
19. Liu G, Kaw B, Kurfis J, et al. Neph1 and nephrin interaction in the slit diaphragm is an important determinant of glomerular permeability. *J Clin Invest*. 2003;112(2):209-221.
20. Fukasawa H, Bornheimer S, Kudlicka K, Farquhar MG. Slit diaphragms contain tight junction proteins. *J Am Soc Nephrol*. 2009;20(7):1491-1503.
21. Ciani L, Patel A, Allen ND, ffrench-Constant C. Mice lacking the giant protocadherin mFAT1 exhibit renal slit junction abnormalities and a partially penetrant cyclopia and anophthalmia phenotype. *Mol Cell Biol*. 2003;23(10):3575-3582.
22. Shih NY, Li J, Karpitskii V, et al. Congenital nephrotic syndrome in mice lacking CD2-associated protein. *Science*. 1999;286(5438):312-315
23. Jones N, Blasutig IM, Eremina V, et al. Nck adaptor proteins link nephrin to the actin cytoskeleton of kidney podocytes. *Nature*. 2006;440(7085):818-823

24. Verma R, Kovari I, Soofi A, Nihalani D, Patrie K, Holzman LB. Nephrin ectodomain engagement results in Src kinase activation, nephrin phosphorylation, Nck recruitment, and actin polymerization. *J Clin Invest*. 2006;116(5):1346-1359.
25. Donoviel DB, Freed DD, Vogel H, et al. Proteinuria and perinatal lethality in mice lacking NEPH1, a novel protein with homology to NEPHRIN. *Mol Cell Biol*. 2001;21(14):4829-4836.
26. Jones N, New LA, Fortino MA, et al. Nck proteins maintain the adult glomerular filtration barrier. *J Am Soc Nephrol*. 2009;20(7):1533-1543.
27. Asanuma K, Yanagida-Asanuma E, Faul C, Tomino Y, Kim K, Mundel P. Synaptopodin orchestrates actin organization and cell motility via regulation of RhoA signalling. *Nat Cell Biol*. 2006;8(5):485-491.
28. Yanagida-Asanuma E, Asanuma K, Kim K, et al. Synaptopodin protects against proteinuria by disrupting Cdc42:IRSp53:Mena signaling complexes in kidney podocytes. *Am J Pathol*. 2007;171(2):415-427.
29. Asanuma K, Kim K, Oh J, et al. Synaptopodin regulates the actin-bundling activity of alpha-actinin in an isoform-specific manner. *J Clin Invest*. 2005;115(5):1188-1198.
30. Yu H, Suleiman H, Kim AH, et al. Rac1 activation in podocytes induces rapid foot process effacement and proteinuria. *Mol Cell Biol*. 2013;33(23):4755-4764.
31. Auguste D, Maier M, Baldwin C, et al. Disease-causing mutations of RhoGDIα induce Rac1 hyperactivation in podocytes. *Small GTPases*. 2016;7(2):107-121.
32. Akilesh S, Suleiman H, Yu H, et al. Arhgap24 inactivates Rac1 in mouse podocytes, and a mutant form is associated with familial focal segmental glomerulosclerosis. *J Clin Invest*. 2011;121(10):4127-4137.
33. Mouawad F, Tsui H, Takano T. Role of Rho-GTPases and their regulatory proteins in glomerular podocyte function. *Can J Physiol Pharmacol*. 2013;91(10):773-782.
34. Brown EJ, Schlöndorff JS, Becker DJ, et al. Mutations in the formin gene INF2 cause focal segmental glomerulosclerosis. *Nat Genet*. 2010;42(1):72-76.
35. Kaplan JM, Kim SH, North KN, et al. Mutations in ACTN4, encoding alpha-actinin-4, cause familial focal segmental glomerulosclerosis. *Nat Genet*. 2000;24(3):251-256.
36. Michaud JL, Lemieux LI, Dubé M, Vanderhyden BC, Robertson SJ, Kennedy CR. Focal and segmental glomerulosclerosis in mice with podocyte-specific expression of mutant alpha-actinin-4. *J Am Soc Nephrol*. 2003;14(5):1200-1211.
37. Garg P, Verma R, Cook L, et al. Actin-depolymerizing factor cofilin-1 is necessary in maintaining mature podocyte architecture. *J Biol Chem*. 2010;285(29):22676-22688.
38. Gbadegesin RA, Hall G, Adeyemo A, et al. Mutations in the gene that encodes the F-actin binding protein anillin cause FSGS. *J Am Soc Nephrol*. 2014;25(9):1991-2002.
39. `Chen YM, Liapis H. Focal segmental glomerulosclerosis: molecular genetics and targeted therapies. *BMC Nephrol*. 2015;16:101.
40. Kamaly N, He JC, Ausiello DA, Farokhzad OC. Nanomedicines for renal disease: current status and future applications. *Nat Rev Nephrol*. 2016;12(12):738-753.
41. Schmieder S, Nagai M, Orlando RA, Takeda T, Farquhar MG. Podocalyxin activates RhoA and induces actin reorganization through NHERF1 and Ezrin in MDCK cells. *J Am Soc Nephrol*. 2004;15(9):2289-2298.
42. Pozzi A, Jarad G, Moeckel GW, et al. Beta1 integrin expression by podocytes is required to maintain glomerular structural integrity. *Dev Biol*. 2008;316(2):288-301.
43. Pozzi A, Zent R. Integrins in kidney disease. *J Am Soc Nephrol*. 2013;24(7):1034-1039.
44. Regele HM, Fillipovic E, Langer B, et al. Glomerular expression of dystroglycans is reduced in minimal change nephrosis but not in focal segmental glomerulosclerosis. *J Am Soc Nephrol*. 2000;11(3):403-412.
45. Sugar T, Wassenhove-McCarthy DJ, Esko JD, van Kuppevelt TH, Holzman L, McCarthy KJ. Podocyte-specific deletion of NDST1, a key enzyme in the sulfation of heparan sulfate glycosaminoglycans, leads to abnormalities in podocyte organization in vivo. *Kidney Int*. 2014;85(2):307-318.
46. Sugar T, Wassenhove-McCarthy DJ, Orr AW, Green J, van Kuppevelt TH, McCarthy KJ. N-sulfation of heparan sulfate is critical for syndecan-4-mediated podocyte cell-matrix interactions. 2016;310(10):F1123-F1135
47. Genovese G, Friedman DJ, Ross MD, et al. Association of trypanolytic ApoL1 variants with kidney disease in African Americans. *Science*. 2010;329(5993):841-885
48. Dai C, Stolz DB, Bastacky SI, et al. Essential role of integrin-linked kinase in podocyte biology: bridging the integrin and slit diaphragm signaling. *J Am Soc Nephrol*. 2006;17(8):2164-2175.
49. Reiser J, Polu KR, Möller CC, et al. TRPC6 is a glomerular slit diaphragm-associated channel required for normal renal function. *Nat Genet*. 2005;37(7):739-744.
50. Schroeter C, Benzing T, Brinkkötter P, Rinschen M. Deep mapping of the podocyte proteome unravels altered protein dynamics during differentiation. *Faseb J*. 2017;31(1)(suppl):1006.1.
51. Wei C, El Hindi S, Li J, et al. Circulating urokinase receptor as a cause of focal segmental glomerulosclerosis. *Nat Med*. 2011;17(8):952-960.
52. Wei C, Möller CC, Altintas MM, et al. Modification of kidney barrier function by the urokinase receptor. *Nat Med*. 2008;14(1):55-63.
53. Reiser J, Sever S. Podocyte biology and pathogenesis of kidney disease. *Annu Rev Med*. 2013;64:357-366.
54. Greka A. Human genetics of nephrotic syndrome and the quest for precision medicine. *Curr Opin Nephrol Hypertens*. 2016;25(2):138-143.
55. Hayek SS, Koh KH, Grams ME, et al. A tripartite complex of suPAR, APOL1 risk variants and αvβ3 integrin on podocytes mediates chronic kidney disease. *Nat Med*. 2017;23(8):945-953.
56. Faul C, Donnelly M, Merscher-Gomez S, et al. The actin cytoskeleton of kidney podocytes is a direct target of the antiproteinuric effect of cyclosporine A. *Nat Med*. 2008;14(9):931-938.
57. Fornoni A, Sageshima J, Wei C, et al. Rituximab targets podocytes in recurrent focal segmental glomerulosclerosis. *Sci Transl Med*. 2011;3(85):85ra46
58. Kerjaschki D, Neale TJ. Molecular mechanisms of glomerular injury in rat experimental membranous nephropathy (Heymann nephritis). *J Am Soc Nephrol*. 1996;7(12):2518-2526.

59. Cohen CD, Calvaresi N, Armelloni S, et al. CD20-positive infiltrates in human membranous glomerulonephritis. *J Nephrol*. 2005;18(3):328-333.
60. Ravani P, Ponticelli A, Siciliano C, et al. Rituximab is a safe and effective long-term treatment for children with steroid and calcineurin inhibitor-dependent idiopathic nephrotic syndrome. *Kidney Int*. 2013;84(5):1025-1033.
61. Kronbichler A, Bruchfeld A. Rituximab in adult minimal change disease and focal segmental glomerulosclerosis. *Nephron Clin Pract*. 2014;128(3-4):277-282.
62. Yu CC, Fornoni A, Weins A, et al. Abatacept in B7-1-positive proteinuric kidney disease. *N Engl J Med*. 2013;369(25):2416-2423.
63. Berden AE, Ferrario F, Hagen EC, et al. Histopathologic classification of ANCA-associated glomerulonephritis. *J Am Soc Nephrol*. 2010;21(10):1628-1636.
64. Trimarchi H, Barratt J, Cattran DC, et al. Oxford Classification of IgA nephropathy 2016: an update from the IgA Nephropathy Classification Working Group. *Kidney Int*. 2017;91(5):1014-1021.
65. Wyatt CM, Schlondorff D. Precision medicine comes of age in nephrology: identification of novel biomarkers and therapeutic targets for chronic kidney disease. *Kidney Int*. 2016;89(4):734-737.
66. Muruve DA, Mann MC, Chapman K, et al. The biobank for the molecular classification of kidney disease: research translation and precision medicine in nephrology. *BMC Nephrol*. 2017;18(1):252.
67. Chun J, Chung H, Wang X, et al. NLRP3 localizes to the tubular epithelium in human kidney and correlates with outcome in IgA nephropathy. *Sci Rep*. 2016;6:24667.
68. Vilaysane A, Chun J, Seamone ME, et al. The NLRP3 inflammasome promotes renal inflammation and contributes to CKD. *J Am Soc Nephrol*. 2010;21(10):1732-1744.
69. Girdea M, Dumitriu S, Fiume M, et al. PhenoTips: patient phenotyping software for clinical and research use. *Hum Mutat*. 2013;34(8):1057-1065.
70. Yandell M, Huff C, Hu H, et al. A probabilistic disease-gene finder for personal genomes. *Genome Res*. 2011;21(9):1529-1542.
71. Beck LH, Jr. Monoclonal anti-PLA2R and recurrent membranous nephropathy: another piece of the puzzle. *J Am Soc Nephrol*. 2012;23(12):1911-1913.
72. Beck LH, Jr., Salant DJ. Membranous nephropathy: from models to man. *J Clin Invest*. 2014;124(6):2307-2314.
73. Beck LH, Jr., Bonegio RGB, Lambeau G, et al. M-type phospholipase A2 receptor as target antigen in idiopathic membranous nephropathy. *N Engl J Med*. 2009;361(1):11-21.
74. Schlumberger W, Hornig N, Lange S, et al. Differential diagnosis of membranous nephropathy with autoantibodies to phospholipase A2 receptor 1. *Autoimmun Rev*. 2014;13(2):108-113.
75. Ronco P, Floege J. Ten-year advances in immunopathology of glomerulonephritis: translated into patients' care or lost in translation? *Semin Immunopathol*. 2014;36(4):377-339.
76. Zhang S, Lu X, Shu X, et al. Elevated plasma cfDNA may be associated with active lupus nephritis and partially attributed to abnormal regulation of neutrophil extracellular traps (NETs) in patients with systemic lupus erythematosus. *Intern Med*. 2014;53(24):2763-2771.
77. Trionfini P, Benigni A, Remuzzi G. MicroRNAs in kidney physiology and disease. *Nat Rev Nephrol*. 2015;11(1):23-33.
78. Mariani LH, Pendergraft WF, III, Kretzler M. Defining glomerular disease in mechanistic terms: implementing an integrative biology approach in nephrology. *Clin J Am Soc Nephrol*. 2016;11(11):2054-2060.
79. Coenen MJ, Hofstra JM, Debiec H, et al. Phospholipase A2 receptor (PLA2R1) sequence variants in idiopathic membranous nephropathy. *J Am Soc Nephrol*. 2013;24(4):677-683.
80. Feehally J, Farrall M, Boland A, et al. HLA has strongest association with IgA nephropathy in genome-wide analysis. *J Am Soc Nephrol*. 2010;21(10):1791-1797.
81. Gharavi AG, Kiryluk K, Choi M, et al. Genome-wide association study identifies susceptibility loci for IgA nephropathy. *Nat Genet*. 2011;43(4):321-327.
82. Yu XQ, Li M, Zhang H, et al. A genome-wide association study in Han Chinese identifies multiple susceptibility loci for IgA nephropathy. *Nat Genet*. 2011;44(2):178-182.
83. Kiryluk K, Li Y, Scolari F, et al. Discovery of new risk loci for IgA nephropathy implicates genes involved in immunity against intestinal pathogens. *Nat Genet*. 2014;46(11):1187-1196.
84. Chung SA, Brown EE, Williams AH, et al. Lupus nephritis susceptibility loci in women with systemic lupus erythematosus. *J Am Soc Nephrol*. 2014;25(12):2859-2870.
85. Genovese G, Tonna SJ, Knob AU, et al. A risk allele for focal segmental glomerulosclerosis in African Americans is located within a region containing APOL1 and MYH9. *Kidney Int*. 2010;78(7):698-704.
86. Kao WH, Klag MJ, Meoni LA, et al. MYH9 is associated with nondiabetic end-stage renal disease in African Americans. *Nat Genet*. 2008;40(10):1185-1192.
87. Kopp JB, Smith MW, Nelson GW, et al. MYH9 is a major-effect risk gene for focal segmental glomerulosclerosis. *Nat Genet*. 2008;40(10):1175-1184.
88. Friedman DJ, Pollak MR. Genetics of kidney failure and the evolving story of APOL1. *J Clin Invest*. 2011;121(9):3367-3374.
89. Nelson GW, Freedman BI, Bowden DW, et al. Dense mapping of MYH9 localizes the strongest kidney disease associations to the region of introns 13 to 15. *Hum Mol Genet*. 2010;19(9):1805-1815.
90. Sadowski CE, Lovric S, Ashraf S, et al. A single-gene cause in 29.5% of cases of steroid-resistant nephrotic syndrome. *J Am Soc Nephrol*. 2015;26(6):1279-1289.
91. Braun DA, Sadowski CE, Kohl S, et al. Mutations in nuclear pore genes NUP93, NUP205 and XPO5 cause steroid-resistant nephrotic syndrome. *Nat Genet*. 2016;48(4):457-465.
92. Macosko EZ, Basu A, Satija R, et al. Highly parallel genome-wide expression profiling of individual cells using nanoliter droplets. *Cell*. 2015;161(5):1202-1214.
93. Pesce F, Pathan S, Schena FP. From -omics to personalized medicine in nephrology: integration is the key. *Nephrol Dial Transplant*. 2013;28(1):24-28.
94. Cox SN, Sallustio F, Serino G, et al. Activated innate immunity and the involvement of CX3CR1-fractalkine in promoting hematuria in patients with IgA nephropathy. *Kidney Int*. 2012;82(5):548-560.

95. Cox SN, Sallustio F, Serino G, et al. Altered modulation of WNT-beta-catenin and PI3K/Akt pathways in IgA nephropathy. *Kidney Int*. 2010;78(4):396-407.
96. Hodgin JB, Borczuk AC, Nasr SH, et al. A molecular profile of focal segmental glomerulosclerosis from formalin-fixed, paraffin-embedded tissue. *Am J Pathol*. 2010;177(4):1674-1686.
97. Peterson KS, Huang JF, Zhu J, et al. Characterization of heterogeneity in the molecular pathogenesis of lupus nephritis from transcriptional profiles of laser-captured glomeruli. *J Clin Invest*. 2004;113(12):1722-1733.
98. Anders HJ, Kretzler M. Glomerular disease: personalized immunomonitoring in lupus and lupus nephritis. *Nat Rev Nephrol*. 2016;12(6):320-321.
99. Banchereau R, Hong S, Cantarel B, et al. Personalized immunomonitoring uncovers molecular networks that stratify lupus patients. *Cell*. 2016;165(3):551-565.
100. Bethunaickan R, Berthier CC, Zhang W, Kretzler M, Davidson A. Comparative transcriptional profiling of 3 murine models of SLE nephritis reveals both unique and shared regulatory networks. *PLoS One*. 2013;8(10):e77489.
101. Almaani S, Meara A, Rovin BH. Update on lupus nephritis. *Clin J Am Soc Nephrol*. 2017;12:825-835.
102. Brix SR, Stege G, Disteldorf E, et al. CC chemokine ligand 18 in ANCA-associated crescentic GN. *J Am Soc Nephrol*. 2015;26(9):2105-2117.
103. L'Imperio V, Smith A, Chinello C, Pagni F, Magni F. Proteomics and glomerulonephritis: a complementary approach in renal pathology for the identification of chronic kidney disease related markers. *Proteomics Clin Appl*. 2016;10(4):371-383.
104. De Sio G, Smith AJ, Galli M, et al. A MALDI-Mass Spectrometry Imaging method applicable to different formalin-fixed paraffin-embedded human tissues. *Mol Biosyst*. 2015;11(6):1507-1514.
105. Woroniecki RP, Bottinger EP. Laser capture microdissection of kidney tissue. *Methods Mol Biol*. 2009;466:73-82.
106. Jain D, Green JA, Bastacky S, Theis JD, Sethi S. Membranoproliferative glomerulonephritis: the role for laser microdissection and mass spectrometry. *Am J Kidney Dis*. 2014;63(2):324-328.
107. Sethi S, Theis JD, Vrana JA, et al. Laser microdissection and proteomic analysis of amyloidosis, cryoglobulinemic GN, fibrillary GN, and immunotactoid glomerulopathy. *Clin J Am Soc Nephrol*. 2013;8(6):915-921.
108. Willis JC, Lord GM. Immune biomarkers: the promises and pitfalls of personalized medicine. *Nat Rev Immunol*. 2015;15(5):323-329.
109. Gold L, Ayers D, Bertino J, et al. Aptamer-based multiplexed proteomic technology for biomarker discovery. *PLoS One*. 2010;5(12):e15004.
110. Wang C, Feng Y, Wang M, et al. Volatile organic metabolites identify patients with mesangial proliferative glomerulonephritis, IgA nephropathy and normal controls. *Sci Rep*. 2015;5:14744.
111. Becker GJ, Hewitson TD. Animal models of chronic kidney disease: useful but not perfect. *Nephrol Dial Transplant*. 2013;28(10):2432-2438.
112. Durvasula RV, Shankland SJ. Models of glomerulonephritis. *Methods Mol Med*. 2003;86:47-66.
113. Ryan GB, Karnovsky MJ. An ultrastructural study of the mechanisms of proteinuria in aminonucleoside nephrosis. *Kidney Int*. 1975;8(4):219-232.
114. Messina A, Davies DJ, Dillane PC, Ryan GB. Glomerular epithelial abnormalities associated with the onset of proteinuria in aminonucleoside nephrosis. *Am J Pathol*. 1987;126(2):220-229.
115. Bertani T, Poggi A, Pozzoni R, et al. Adriamycin-induced nephrotic syndrome in rats: sequence of pathologic events. *Lab Invest*. 1982;46(1):16-23.
116. Farquhar MG, Saito A, Kerjaschki D, Orlando RA. The Heymann nephritis antigenic complex: megalin (gp330) and RAP. *J Am Soc Nephrol*. 1995;6(1):35-47.
117. Heymann W, Hackel DB, Harwood S, Wilson SG, Hunter JL. Production of nephrotic syndrome in rats by Freund's adjuvants and rat kidney suspensions. *Proc Soc Exp Biol Med*. 1959;100(4):660-664.
118. Edgington TS, Glassock RJ, Dixon FJ. Autologous immune complex nephritis induced with renal tubular antigen. I. Identification and isolation of the pathogenetic antigen. *J Exp Med*. 1968;127(3):555-572.
119. Salant DJ, Darby C, Couser WG. Experimental membranous glomerulonephritis in rats. Quantitative studies of glomerular immune deposit formation in isolated glomeruli and whole animals. *J Clin Invest*. 1980;66(1):71-81.
120. Imai H, Nakamoto Y, Asakura K, Miki K, Yasuda T, Miura AB. Spontaneous glomerular IgA deposition in ddY mice: an animal model of IgA nephritis. *Kidney Int*. 1985;27(5):756-761.
121. Helyer BJ, Howie JB. Renal disease associated with positive lupus erythematosus tests in a cross-bred strain of mice. *Nature*. 1963;197:197.
122. Satoh M, Richards HB, Shaheen VM, et al. Widespread susceptibility among inbred mouse strains to the induction of lupus autoantibodies by pristane. *Clin Exp Immunol*. 2000;121(2):399-405.
123. Xiao H, Heeringa P, Hu P, et al. Antineutrophil cytoplasmic autoantibodies specific for myeloperoxidase cause glomerulonephritis and vasculitis in mice. *J Clin Invest*. 2002;110(7):955-963.
124. Lan HY, Nikolic-Paterson DJ, Mu W, Atkins RC. Local macrophage proliferation in the pathogenesis of glomerular crescent formation in rat anti-glomerular basement membrane (GBM) glomerulonephritis. *Clin Exp Immunol*. 1997;110(2):233-240.
125. Ophascharoensuk V, Pippin JW, Gordon KL, Shankland SJ, Couser WG, Johnson RJ. Role of intrinsic renal cells versus infiltrating cells in glomerular crescent formation. *Kidney Int*. 1998;54(2):416-425.
126. Bagchus WM, et al. Glomerulonephritis induced by monoclonal anti-Thy 1.1 antibodies. A sequential histological and ultrastructural study in the rat. *Lab Invest*. 1986;55(6):680-687.
127. Ishizaki M, Masuda Y, Fukuda Y, Sugisaki Y, Yamanaka N, Masugi Y. Experimental mesangioproliferative glomerulonephritis in rats induced by intravenous administration of anti-thymocyte serum. *Acta Pathol Jpn*. 1986;36(8):1191-1203.
128. Lugli EB, Pouliot M, Portela Mdel P, Loomis MR, Raper J. Characterization of primate trypanosome lytic factors. *Mol Biochem Parasitol*. 2004;138(1):9-20.
129. Morizane R, Lam AQ, Freedman BS, Kishi S, Valerius MT, Bonventre JV. Nephron organoids ed from human plu-

ripotent stem cells model kidney development and injury. *Nat Biotechnol*. 2015;33(11):1193-1200.
130. Takasato M, Er PX, Chiu HS, et al. Kidney organoids from human iPS cells contain multiple lineages and model human nephrogenesis. *Nature*. 2015;526(7574):564-568
131. Li Z, Araoka T, Wu J, et al. 3D culture supports long-term expansion of mouse and human nephrogenic progenitors. *Cell Stem Cell*. 2016;19(4):516-529.
132. Freedman BS, Brooks CR, Lam AQ, et al. Modelling kidney disease with CRISPR-mutant kidney organoids derived from human pluripotent epiblast spheroids. *Nat Commun*. 2015;6:8715.
133. Kim YK, Refaeli I, Brooks CR, et al. Gene-edited human kidney organoids reveal mechanisms of disease in podocyte development. *Stem Cells*. 2017;35:2366-2378.
134. Farragher JF, Elliott MJ, Silver SA, Lichner Z, Tsampalieros A. Translational research in kidney transplantation and the role of patient engagement. *Can J Kidney Health Dis*. 2015;2:42.
135. Canadian Institutes of Health Research. *Canada's Strategy for Patient-Oriented Research: improving health outcomes through evidence-informed care*. Canadian Institutes of Health Research. www.cihr-irsc.gc.ca/e/44000.html. Published 2011. Accessed January 13, 2018.