Can We Personalize Treatment for Kidney Diseases?

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The idea of individualizing therapies to obtain optimal clinical results is not new but has only recently been applied to kidney diseases. Nonetheless, kidney disorders present a variety of opportunities to personalize medicine. Here, the heterogeneity of kidney disorders is reviewed to provide a rationale for pursuing personalized medicine. Data on adjusting therapy on the basis of pharmacogenetics/genomics and pharmacodynamics are summarized to demonstrate where the field is, and biomarker studies that reflect the future of personalized medicine are discussed. The goal of this review is to demonstrate that we can personalize therapy for kidney diseases but that considerable investment in new research will be required for personalized medicine to be routinely used in nephrology clinics.


Molecular heterogeneity is also found in primary and secondary glomerular diseases. Pediatric patients who had FSGS and displayed nephrotic-range proteinuria or renal insufficiency could be differentiated from patients who did not have nephrosis or renal insufficiency, respectively, on the basis of unique renal cortical RNA expression (3). Similarly, microarray analysis of laser-captured glomeruli from patients with classes III and IV lupus nephritis demonstrated four clusters of enriched RNA expression, including an IFN-α-inducible gene cluster and a cluster of fibrosis-related genes (4). Both of these studies were small, and neither correlated RNA expression patterns with outcomes in response to specific treatments.

Heterogeneity is also evident at the level of biomarkers that are commonly used to inform about clinical status. For example, a reduction in the level of complement components C3 and C4 is commonly used to corroborate activity of lupus nephritis; however, our own experience with the Ohio SLE Study (OSS) cohort (5) suggested that these are actually poor biomarkers of renal disease activity. The sensitivity/specificity of C3 and C4 for concurrent renal flare were 70/73% and 49/74%, respectively, and positive predictive values were 22 and 17%. The explanation for the lack of sensitivity and specificity becomes clear when C3 and C4 levels are plotted over the renal flare cycle for individual patients. OSS data for C3 levels that were followed prospectively from 33 moderate to severe renal flares in 28 patients are shown in Figure 1. The C3 level fell below the laboratory’s lower limit of normal (LLN) in 12 instances at flare, although likely reflective of their individual disease activity, would be difficult to interpret clinically outside the context of serial measurements. Finally, several flares were not associated with a decline in C3, and some even showed an increase in C3. Similar results were found for C4 (data not shown).

The individual variations in complement levels illustrate the
need for proteomics along with genomics in the developing discipline of personalized medicine. It is not likely that the complexity in the patterns of C3 levels shown in Figure 1 can be predicted simply by microarray analysis. For example, C3 and C4 are acute-phase reactants, and their production likely increases during the heightened inflammation that is associated with lupus nephritis flare; however, complement is also activated and consumed during lupus nephritis flares, thus lowering levels. In addition to these opposing effects on complement levels during disease flare, complement activation can theoretically be influenced by functional variants of complement regulatory proteins that have been previously shown to contribute to disease pathogenesis (6). Our data suggest that this in fact occurs, with an inefficient variant of the complement regulatory factor H being associated with lower C3 levels during lupus nephritis flare (Rovin and Birmingham, manuscript submitted). Finally, with respect to C4, gene copy number variation can substantially influence circulating plasma C4 levels (7).

**Approaches for Individualizing the Therapy of Kidney Diseases**

Therapy in the nephrology clinic may be personalized by using a pharmacogenetic/genomic/dynamic approach or a biomarker approach (Figure 2). Pharmacogenetics, pharmacogenomics, and pharamacodynamics have already been applied to some types of kidney disease in small studies. Although not conclusive, these studies provide preliminary evidence for the feasibility of personalizing medicine in kidney disease and an immediate avenue for further research. Conversely, biomarker discovery is under way for a variety of kidney diseases, but, presently, no biomarkers that can be used to tailor therapies have been validated. Thus, this approach will take longer to achieve clinical utility.

**Therapy Based on Genetic Variants in Drug Metabolic Pathways**

**Cyclophosphamide.** Cyclophosphamide (CYC) is used in a variety of severe glomerular diseases, including lupus nephritis and pauci-immune vasculitis. To be effective, CYC must be converted to its active metabolite, phosphoramide mustard, and the rate-limiting step of this pathway is mediated by the cytochrome P450 enzyme system, including the specific enzymes CYP2B6 and CYP2C19 (8). Cytochrome P450 enzymes are highly polymorphic. In CYP2B6 allele 5 (CYP2B6*5), a cysteine replaces the arginine at position 487, and this decreases enzymatic activity. CYP2C19 allele 2 (CYP2C19*2) is an alternative splice variant that leads to an inactive enzyme (8,9). The frequency of CYP2B6*5 is as high as 11% in white and black populations, 3% in Hispanic populations, and 0.3 to 5% in Asian populations (10–12). CYP2C19*2 is even more frequent at 15 to 17% in white and black patients and up to 30% in Asian patients (12–14). Patients with these CYP450 variants may be

![Figure 1. C3 levels measured prospectively during moderate to severe lupus nephritis flare cycles. Each line represents an individual, independent renal flare cycle. Baseline data were an average of two to three measurements taken ≥6 mo outside any (renal or nonrenal) flare activity. The other points on the x axis indicate months before or after the clinical diagnosis of renal flare. (Top) All flare cycles in which C3 fell at flare, compared with baseline. (Bottom) All flare cycles in which C3 did not drop at the time of renal flare, compared with baseline.](image1)

![Figure 2. Approaches to individualize the therapies of kidney diseases. (Top) Pharmacogenetic, pharmacogenomic, and pharmacodynamic approaches. (Bottom) Biomarker approaches. It is likely that both approaches will be used in combination to generate personalized medicine panels to individualize therapies.](image2)

Modify Therapy to Account for:

- Genetic variants of metabolic pathways that activate or inactivate drug
- Genetic or acquired variants of drug uptake and transport mechanisms
- Genetic or acquired variants of drug targets

Modify Therapy Using Biomarkers that:

- Predict impending disease flare/activity
- Predict disease severity/prognosis
- Predict response to therapy
poor metabolizers of CYC and are thus at risk for undertreatment when CYC is used for their glomerulonephritis. This hypothesis was tested in a cohort of 62 patients who had lupus nephritis and were treated with monthly pulse CYC and stratified by genotype for outcome (15). Patients who were homozygous for CYP2B6*5 or CYP2C19*2 had a significantly greater risk for not achieving complete remission and for developing end-stage kidney disease compared with patients who were not homozygous for these alleles. It is interesting that heterozygotes did not have an intermediate response, and the reason for this is not clear; however, only seven patients were homozygous for CYP2B6*5 or CYP2C19*2, so it is difficult to draw firm conclusions. Nonetheless, these data are provocative and suggest that cytochrome P450 genetic variants that are important in CYC activation should be studied in a larger population of renal patients who need CYC therapy. Of note, a chip is available for clinical laboratories to genotype cytochrome P450 enzymes, but this has not achieved widespread use to date.

Azathioprine and Mycophenolate Mofetil. Both azathioprine (AZA) and mycophenolate mofetil (MMF) have important roles in treating or preventing a variety of kidney disorders, such as lupus nephritis, maintenance therapy for vasculitis, and renal allograft rejection. The active metabolite of AZA is 6-mercaptopurine (16). 6-Mercaptopurine is metabolized into toxic 6-thioguanine nucleotides unless inactivated by thiopurine methyl transferase (TPMT). Although most white patients have wild-type TPMT, 10% have a heterozygous defect in the enzyme that causes a moderate decrease in activity, and 0.3% have a homozygous defect that results in very low activity (17). To avoid multisystem toxicity from AZA, it is recommended that dosing be modified by TPMT genotype, a test that is available clinically but not widely used (18).

Similarly, MMF is metabolized to the inactive mycophenolic acid (MPA) glucuronide by uridine diphosphate glucuronosyltransferases (e.g., UGT1A9). UGT1A9 has several promoter polymorphisms that affect its activity and can thus increase or decrease exposure to MPA (19,20). For example, the single-nucleotide polymorphisms (SNPs) T-275A and C-215T in the UGT1A9 promoter are associated with higher hepatic expression of UGT1A9, which correlates with decreased exposure to MPA in renal allograft recipients (21). The lower exposure to MPA may be due, in part, to impaired enterohepatic recirculation of MPA, which significantly contributes to total MPA exposure in vivo (21). It is interesting that these promoter SNPs were found to be significant predictors of acute rejection in renal allograft recipients who received a fixed dosage of MMF in combination with tacrolimus (22). Genotyping for these polymorphisms may be useful in titrating MMF dosage to maintain efficacy.

Calcineurin Inhibitors. Cyclosporin A (CsA) and tacrolimus are used most commonly to prevent solid-organ allograft rejection but also have an expanding role in the treatment of glomerular diseases, including membranous nephropathy and FSGS. These calcineurin inhibitors (CNIs) both are metabolized by the same pathways including the cytochrome P450 enzymes CYP3A4 and CYP3A5 and the p-glycoprotein pump encoded by the multidrug resistance 1 (MDR1) gene. The genes that control these proteins have a number of SNPs, and some seem to influence the pharmacokinetics of the CNIs. These polymorphisms and their effects have been reviewed recently (23). The effect of these SNPs is more pronounced for tacrolimus than for CsA. Furthermore, allograft rejection does not seem to be associated with CYP3A4, CYP3A5, or MDR1 variants, but CNI toxicities may be associated (24–27). Thus, despite extensive pharmacogenetic data for the CNI metabolic pathways, the applicability of these SNPs to clinical care remains unclear.

Therapy Based on Drug Target Variants

The Angiotensin-Converting Enzyme Gene Polymorphism. The renin-angiotensin-aldosterone system is the target of angiotensin-converting enzyme inhibitors (ACEI) and angiotensin receptor blockers (ARBs). The ACE gene contains an insertion (I)/deletion (D) polymorphism of 287 DNA bases located in intron 16 (28). The D allele is associated with increased ACE activity and high circulating angiotensin levels compared with the I allele. This polymorphism has been postulated to affect the efficacy of ACEIs and ARBs on BP control, renal protection, and proteinuria; however, despite a large number of studies, there is no consensus, on the basis of an individual’s ACE genotype, on whether ACEI or ARB treatment is preferential or should be modified. Literature can be found showing that the response to ACEIs or ARBs is not dependent on the polymorphism (28,29), is dependent on the II genotype (30,31), or is dependent on the DD genotype (32–34). It should be pointed out that differences in salt intake, underlying kidney disease, race/ethnicity, and initial level of renal function often make the interpretation and direct comparison between studies difficult.

Perhaps a better way to elucidate the role of the ACE gene polymorphism in choosing renoprotective therapy is to compare directly the effect of ACEIs and ARBs as a function of genotype. This has been done in a few small studies. In patients with type 2 diabetes and nephropathy, the ACE I/D genotype did not influence urinary albumin excretion after treatment with ACEI or ARB (35); however, the ARB caused a significant reduction in urine TGF-β, a potentially pathogenic cytokine in diabetic nephropathy (DN), but only in II individuals. Similarly, progression to end-stage renal failure in IgA nephropathy was slowed or prevented more effectively by an ARB than an ACEI, primarily as a result of the effect of the ARB in II individuals (36). This is counterintuitive, because one might have expected ARBs and ACEIs to be equally effective in II individuals, and ARBs more effective in DD individuals.

The Ig Fc Receptor Polymorphism. Rituximab, a monoclonal anti-CD20 (B cell) antibody, has been undergoing testing as a novel therapeutic agent for a variety of kidney diseases, including lupus nephritis, ANCA-associated vasculitis, and membranous nephropathy. One potential mechanism of action of rituximab is B cell death via antibody-dependent cell-mediated cytotoxicity. Rituximab binds to CD20 on B cells and brings these B cells in contact with NK cells and macrophages by binding to their surface Fc receptors. The amino acid at position 158 of Ig Fc receptor (FcγRIIIa) is polymorphic, and
when it is a valine, the binding affinity for IgG1 (rituximab’s isotype) is increased 10-fold. The frequency of 158V is relatively high (25 to 30% in our OSS cohort), which suggests that the polymorphism may significantly influence the efficacy of rituximab. In support of this, one small study showed that patients who had systemic lupus erythematosus (SLE) and were homozygous for the low-affinity FcγRIIIa allele needed a 10-fold higher serum rituximab level to achieve the same degree of B cell depletion as patients who were homozygous and heterozygous for 158V (17). Unfortunately, treatment outcome has not yet been related to Fc receptor genotype in kidney disease, and a second, small study of patients with SLE showed that B cell depletion correlated with the low-affinity FcγRIII genotype (37). Thus, the role of the Fc receptor polymorphism in the efficacy of rituximab therapy remains to be determined in a large study before recommendations can be made concerning the utility of FcγRIIIa genotyping before starting rituximab.

**Pharmacodynamic Approaches: In Vitro Peripheral Blood Mononuclear Cell Responsiveness as a Predictor of Immunosuppressive Efficacy.** In an interesting series of studies, peripheral blood mononuclear cells were cultured from patients who had renal disease and were to be treated with an immunosuppressant, and the cells were given a mitogenic stimulus (38–42). Lymphocyte proliferation in the absence or presence of varying concentrations of the intended immunosuppressant was measured, allowing a determination of lymphocyte drug sensitivity. This estimate of drug sensitivity was correlated to clinical outcomes. For example, in minimal-change disease, eight patients with high sensitivity to CsA in vitro experienced complete remission significantly sooner than six low-sensitivity patients (40). Similarly, in patients who had FSGS and were treated with corticosteroids, there was a significant correlation between improvement in creatinine clearance and proteinuria at 3 and 6 mo (n = 13) and percentage inhibition of lymphocyte proliferation by corticosteroids in vitro (41). Finally, in a group of 51 renal allograft recipients who were treated with corticosteroids, AZA, and CsA, allograft survival was highest in patients whose lymphocytes were highly sensitive to CsA in vitro (42). Although it is not likely that this technique could be made cost-effective or efficient enough for clinical use, it is conceivable that susceptibility to in vitro immunosuppression could be determined by a gene signature or protein biomarker profile of peripheral blood mononuclear cells, and this could be used clinically to decide on an appropriate immunosuppressive regimen.

**Therapy Based on Biomarkers**

Biomarker discovery for kidney diseases is currently an active area of investigation and will have a direct impact on personalized medicine. For example, biomarkers that predict impending disease activity could be used to initiate treatment early, which could minimize the initial insult, allow a reduction in duration and intensity of therapy, improve outcomes, and lessen chronic renal injury. Biomarkers that predict response to therapy could be used to choose the most appropriate regimen for an individual patient. Biomarkers that reflect disease severity could be used to adjust the intensity of therapy. The use of proteomic techniques has facilitated biomarker discovery in kidney diseases, as illustrated by recent work in DN and lupus nephritis.

**Predicting the Development of DN.** Protein profiling was done on urine samples from patients with type 2 diabetes using mass spectrometry (SELDI-TOF MS) before patients had any evidence of kidney involvement (43). Patients were followed prospectively, and 10 yr after the urine samples had been collected, half of the patients had developed DN. Found was a 12-protein urine signature that differentiated patients who were destined to develop nephropathy in 10 yr from those who were destined to remain stable, with a sensitivity of 71%, a specificity of 76%, and a positive predictive value of 74%. The limiting factor of this study, in terms of validation and clinical applicability, is that none of the 12 proteins that compose the DN signature has yet been identified.

In a slightly different approach, we examined urine that was collected from patients who had type 1 diabetes within 3 yr of onset of microalbuminuria but normal renal function (44). Patients were followed for 10 to 12 yr and divided into those who remained stable and those who experienced an early but progressive decline in kidney function. Liquid chromatography–matrix-assisted laser desorption/ionization TOF MS was used to analyze the urine proteome. The goal was to identify biomarkers that would predict who with type 1 diabetes would progress quickly to chronic kidney disease and who would not. Three peptides derived from collagen types IV and V and tenascin-X were decreased in the urine of patients with declining renal function and seemed to reflect protection from progression. Conversely, fragments of inositol pentakisphosphate 2-kinase, zona occludens 3, and FAT tumor suppressor 2 increased in the urine of progressors and were significantly associated with an early decline in kidney function.

Although the potential biomarkers identified in these investigations need independent verification and validation, both studies illustrate that the urine proteome can be used to predict future kidney events. These investigations also suggest that in complex diseases, it is unlikely that a single biomarker will be sufficient to model a specific aspect of the disease and that biomarker panels will be required. Nonetheless, the ability to know, at the time of diagnosis of diabetes, who is destined to develop DN will be critically important in terms of BP control, renoprotective measures, and glycemic control. As more specific therapies to prevent DN are developed, this information can be used to apply these therapies to patients who are likely to realize the most benefit. This will provide not only individualized therapy but also cost-effective use of what will likely be very expensive drugs.

**Predicting Lupus Nephritis Flare.** We and others have been actively engaged in modeling lupus nephritis flares in an effort to identify biomarkers that forecast impending flare (reviewed in reference [45]). The rationale for this work is that if treatment is started before renal flare is diagnosed clinically, then the flare may be more responsive, requiring a smaller cumulative dosage and shortened duration of immunosuppression. Furthermore, earlier treatment of lupus nephritis flare has
been equated with better overall preservation of renal function and decreased chronic renal parenchymal injury (46–48). To discover biomarkers that forecast future events in SLE, we used a longitudinal proteomic approach. In the OSS, urine samples from individual flare cycles were obtained at baseline (disease stability), before flare, at the time of flare, and after treatment and analyzed by SELDI-TOF MS (49). This allowed comparison of the same proteins/peptides throughout an individual’s flare cycle. Proteins that were expressed differentially between baseline urine and preflare urine were considered potential biomarkers of impending lupus renal flare. In this way, we identified several candidate biomarkers as potential forecasters (49). The most intriguing of these is hepcidin, an iron regulatory peptide hormone mostly made by the liver (50,51); however, hepcidin is regulated by cytokines such as TNF and IL-6, both of which have been implicated in SLE (52–55). Hepcidin may therefore reflect the intrarenal cytokine milieu in lupus.

We found that the 20–amino acid isoform of hepcidin increased approximately 4 mo before flare was declared clinically and slowly returned to baseline after treatment (49). Furthermore, immunohistochemical analysis showed that hepcidin was expressed by infiltrating interstitial leukocytes in SLE kidney biopsies. The role, if any, of hepcidin in the pathogenesis of lupus nephritis remains to be determined and its value as a forecaster of impending flare validated in an independent lupus nephritis cohort. Nonetheless, these data demonstrate the power of longitudinal proteomics in identifying novel biomarkers of kidney disease that can potentially be used to guide therapy.

Perspective
The interindividual variability of kidney diseases that seem phenotypically and pathologically similar is readily apparent at the molecular and biochemical levels. Pharmacogenetics/genomics/dynamics provide a basis for individualizing drug treatments; however, with respect to renal therapeutics, these studies have been small and underpowered. Larger, prospective trials in ethnically diverse populations will be needed, and disease outcomes after tailoring therapy on the basis of genetics have to be defined. The informed use of novel biomarkers, derived from longitudinal observations of individuals, will facilitate the development of mathematical and statistical models that predict disease activity and outcomes. These models can form the basis for individually titrating therapies. An important caveat is that these tests must be cost effective and applied to the correct patient populations. A relevant example of this concern is the VKORC1 enzyme polymorphism that affects warfarin resistance. This SNP accounts for 25% of dosage variability in European Americans (56,57) and costs more than seven times as much as an international normalized ratio (INR). Although gene testing could guide initial dosing, following the INR, which would have to be done anyway, is probably more cost-effective for most people. The genetic test may be useful in identifying warfarin-resistant patients, who need a different form of anticoagulation, from noncompliant patients, for example.

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Disclosures
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