Molecular Diagnostics in Nephrology

By Abhay Vats

Recent advances in molecular diagnostics are making inroads in how we manage patients with kidney-related disorders. Techniques such as nucleic acid amplification and detection, genomic analysis, and advanced metabolomics have enabled development of several molecular diagnostic assays. These developments, in turn, affect the practice of various aspects of nephrology, such as management of acute kidney injury, chronic kidney disease (CKD) and its complications, as well as transplant nephrology.

Many of the ongoing advances in these fields will significantly change the way nephrology is practiced in the near future. Although molecular medicine advances encompass a wide variety of diseases and techniques, two of the major achievements in recent advances in diagnostics that have affected current clinical practice include 1) the way we approach genetic kidney diseases, and 2) transplant infections.

Genetics and genomics of kidney disease

In the past decade—especially since the sequencing of the entire human genome in 2003—the identification of genes that are mutated in specific disorders has entered an exciting era. Several approaches in the recent past have been used to identify genes associated with a large number of kidney diseases, such as linkage studies, gene expression analysis, and genomewide association studies (1). Combined effects of the genetic makeup of a person (genotype), the environment, and the gene–environment interactions are now thought to play a role in the risk of developing several kidney diseases, including CKD, and may also affect the ultimate clinical outcome. Molecular genetic diagnostics has made major contributions to clinical nephrology in the fields of nephrotic syndrome/focal segmental glomerulosclerosis (NS/FSGS), cystic kidney diseases, tubulopathies, and later, CKD.

Nephrotic syndrome

NS is a heterogeneous group of disorders characterized by heavy proteinuria, hypoalbuminemia, edema, and dyslipidemia. In the past decade, studies of familial cases of NS/FSGS have led to the identification of genes encoding proteins important for glomerular structure and function. There are several excellent reviews on various aspects of NS/FSGS genetics (2,3). Genetic tests are available for the targets followed by an asterisk below.

Briefly, structural elements of the slit diaphragm (nephrin*, podocin* and CD2AP) and actin cytoskeleton (α-actinin-4*) have been described in cases of NS/FSGS. The transcription factor WT1*, phospholipase Cε1 (PLCE1), the calcium channel protein TRPC6*, which localizes in membrane lipid complexes along with podocin, as well as laminin-B2, a structural component of the glomerular basement membrane, have also been implicated in causing NS/FSGS.

Several novel chromosomal loci have also been linked to various forms of NS/ FSGS and/or proteinuria, such as regions of chromosome 9p32, 11q24, and 13q22. The list of genes associated with this phenotype is likely to grow significantly in the years to come (4–6). Some of these genes (nephrin, podocin) cause autosomal recessive disorders with early childhood onset, while others (such as α-actinin-4*, TRPC6*) are dominantly inherited with generally adult onset of disease, incomplete penetrance, and variable expressivity. It has now been shown that a significant proportion of patients presenting with NS/FSGS in early childhood have an underlying Mendelian genetic cause, involving either nephrin. The genetic component of adult-onset disease is probably not as great as the pediatric-onset disease, but oligogenic and complex inheritance may play a bigger role in some situations (2). For example, certain haplotypes in the MYH9 gene that increase risk for FSGS and glomerular injury/NS have been found in some adult patients have been recently identified (1).

Cystic diseases of the kidney

Like the NS/FSGS syndromes, cystic diseases of the kidney (CDKs) are a heterogeneous group encompassing a large variety of diseases and clinical syndromes. During the past two to three decades, the inheritance patterns of many CDKs have been extensively studied, and several associated genes have been identified. There are now a large number of genes associated with CDKs, with at least nine genes mutated in hereditary cystic kidney disease (including NPHP1*) cause autosomal recessive polycystic kidney disease (e.g., Bardet-Biedl syndrome, at least three in Bardet-Biedl syndrome, at least three in McCyD (including UMOD*), and at least four in autosomal dominant polycystic kidney disease (including PKD1* and PKD2*) and recessive polycystic kidney disease (including PKHD1*)

This list, as with many other genetic kidney diseases, is destined to expand significantly in the near future. Despite the heterogeneity, all CDKs have one common morphologic feature—cysts in the kidney. Similar to the glomerular structure/function theme for NS/FSGS, most of the genetic cause of CDKs node to proteins that localize to the primary ciliium. Several excellent reviews on CDK genetics provide details of various genes involved in these ciliopathies and associated CDK syndromes or diseases (7–9).

Practical considerations for clinical nephrologists

There are currently more than 30 genes associated with CDKs and over 15 genes with NS/FSGS that encompass autosomal dominant and recessive as well as X-linked inheritance. Most of these diseases are monogenic with Mendelian inheritance, but there are examples of oligogenic inheritance, such as three mutations in two genes for both CDKs and NS/FSGS (2,9). The variable expressivity and the broad spectrum of symptoms associated with various kidney diseases suggest that genetic modifiers are important in determining the outcome of a large number of CDKs and possibly NS/FSGS. Finally, new genes are constantly being discovered and mutations in many different genes can produce similar phenotypes, often with substantial overlap of symptoms.

This significant genetic heterogeneity has made molecular diagnostics particularly difficult for practicing nephrologists because the clinical differential diagnosis does not always help in pointing to the right gene to analyze. Nephrologists need to be aware of the various developments in molecular diagnostics and be able to identify appropriate patients as well as tests. Many genetic tests (including those identified in the previous section by *) are now commercially available, and the initial restrictions of insurance reimbursements have gradually been addressed and removed. An NCBI website called GeneTests (http://www.ncbi.nlm.nih.gov/sites/GeneTests) lists the laboratories where the tests for various genes related to kidney diseases can be performed.

Molecular diagnostics and transplant infections

Infectious diseases and their complications are a major contributor to morbidity and mortality associated with long-term outcomes of renal transplantation. Recipients of renal and other solid organ as well as bone marrow transplants are uniquely predisposed to develop infections caused by a variety of pathogens in addition to bacteria, viruses, and fungi. The identification and management of viral infections has been aided most significantly from advances in molecular diagnostics, especially related to nucleic acid amplification technologies such as real-time PCR over the last decade (10–12).

Transplant recipients can acquire viral infections from a variety of sources, including the donor, reactivation of latent virus, or from the community. Human herpes viruses, i.e., cytomegalovirus (CMV), Epstein–Barr virus (EBV), and HHV6 are some of the most common in the infectious etiology of a variety of infections. The polyoma BK virus is particularly important to nephrologists because it remains latent in renal tubular cells after primary infection. The virus becomes reactivated in kidney transplant recipients causing an opportunistic infection leading to both acute and chronic allograft dysfunction (13). Respiratory viral illnesses due to influenza, respiratory syncytial virus, parainfluenza, and human metapneumovirus may affect all types of transplant recipients, but may be particularly troublesome in the very young and old (12).

Less common viruses affecting transplant recipients include adenoviruses and parvovirus B19. Recent reports of donor-derived infections have attracted a lot of attention both in medical literature as well as the lay media. Although donor-derived infectious diseases appear to complicate less than 1 percent of all transplant procedures, when a transmission occurs, significant morbidity and mortality can result, as has been recently reported for transmission of HIV, hepatitis C virus, and West Nile virus (WNV) from organ donors to recipients. These reports have highlighted the importance and clinical impact of this complication of renal and other solid organ transplantation. Molecular diagnostic assays based on nucleic acid amplification such as PCR are now commercially available, and others are now available for a large variety of these infections. Many of them (e.g., respiratory pathogens) can be ordered as a panel or a battery of rapid molecular tests.

Among the problems with the emerging field of viral molecular diagnostics are a lack of standardized diagnostic criteria, a lack of knowledge about the viruses, such as HIV and HCV), high cost, and the possibility of false positives (due to contamination) or false negatives (due to viral mutations or testing procedural problems)

In addition, health care providers must often wait anywhere from one day to one week to obtain test results, depending on the laboratory used and organism being tested, even though the testing procedure by itself takes less than two to three hours, thus potentially leading to a delay in diagnosis and initiation of appropriate therapy.

Future directions

Several kidney diseases, including CKD, are currently being investigated for the role of genetic—and epigenetic—factors in their causation and outcome (14,15). In the future, the rapidly evolving field of genomics, including genomewide association studies and epigenomics, could identify several genetic determinants (such as mutations, SNPs, or copy number variants) of key renal disorders and transform medical diagnosis and treatment, moving toward “personalized medicine.”

Outcomes in the CKD population may therefore be improved by establishing individual genetic or epigenetic profiles, thus enabling physicians to design an individualized therapeutic strategy. Personalized medicine based on a more individualized therapy could be applied in, for example, pharmacotherapy, dialysis therapy, and nutritional and lifestyle modifications. But the vision of individualized treatment based on a patient’s genetic makeup and other biological markers needs further work and statistical and clinical validation (11,14).

Another area poised for major advance in the near future is point-of-care diagnostics. Rapid molecular diagnostics based on...
novel nucleic acid amplification methods such as Loop-mediated isothermal AMPlification (LAMP) and innovative detection technologies like optical sensing or nucleic acid lateral flow devices (NALF) have gained significant attention in recent years due to advantages of assay speed, potential for point-of-care availability, and low cost (16,17).

Microfluidic systems such as lab-on-a-chip, or BioMEMS offer a great advantage by integrating a large number of assay handling steps into a single device, making the system independent of user intervention and, thereby, less prone to contamination. Miniaturization and integration of assay steps into a lab-on-a-chip type device is being developed for several pathogens, as well as SNP's and mutations, to speed up assay time and make assays available onsite at competitive costs. However, the emerging technologies and approaches still require significant simplification and clinical validation.

Because meaningful assays for infectious diseases need to include a range of pathogens, multiplexed functionality is often required and will need to be implemented in future point-of-care assays (18,19). It is possible in the not too distant future that several of the opportunistic viral infections as well as key mutations or SNPs may be detected with the sensitivity and specificity required and will need to be implemented as well as SNPs and mutations, to speed up assay time and make assays available in future point-of-care assays (18,19). It is possible in the not too distant future that several of the opportunistic viral infections and several of the opportunistic viral infections as well as key mutations or SNPs may be detected with the sensitivity and specificity of PCR or sequencing while the patient is waiting in the outpatient or outlying clinics due to disadvantages of PCR tests.

Abhay Vats, MD, is associate professor of pediatrics in the division of pediatric nephrology and congenital anomalies of kidneys: evidence of locus on chromosome 13q. Kidney Int 2003; 64:17–24.

Frankly, we’re flattered!

But it takes more than just imitation to become America’s APD leader.

For the past 15 years, when clinicians have prescribed home care for their PD patients, chances are they’ve entrusted them to HomeChoice. Not only has it been the only pump-based cycle for over a decade, it’s the only APD cycle with a history of proven efficacy and unparalleled 24/7 patient and technical support.

We’re dedicated to renewing that trust, each and every night – without compromise.

For details on why HomeChoice is America’s APD leader, visit www.apdleader.com

Caution: Federal (U.S.) law restricts this device to sale by or on the order of a physician or other licensed practitioner. For safe and proper use of this device, refer to the device instructions.

For more information, please visit: www.apdleader.com

Baxter Healthcare Corporation
Hwy. 101 and Centennial Blvd.
McGaw Park, IL 60085, USA
1-888-736-2543
www.apdleader.com

For the past 15 years, when clinicians have prescribed home care for their PD patients, chances are they’ve entrusted them to HomeChoice. Not only has it been the only pump-based cycle for over a decade, it’s the only APD cycle with a history of proven efficacy and unparalleled 24/7 patient and technical support.

We’re dedicated to renewing that trust, each and every night – without compromise.

For details on why HomeChoice is America’s APD leader, visit www.apdleader.com

Caution: Federal (U.S.) law restricts this device to sale by or on the order of a physician or other licensed practitioner. For safe and proper use of this device, refer to the device instructions.