Clinical Applications of Genetic Discoveries in Kidney Transplantation: a Review

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Abstract
Growth in knowledge of the genetics of kidney disease has revealed that significant percentages of patients with diverse types of nephropathy have causative mutations. Genetic testing is poised to play an increasing role in the care of patients with kidney disease. The role of genetic testing in kidney transplantation is not well established. This review will explore the ways in which genetic testing may be applied to improve the care of kidney transplant recipients and donors.

Introduction
The genetics of kidney disease has undergone explosive growth in recent years due to significant advances in sequencing technology and genetics methodology (1,2). Recent data suggest that significant percentages of patients with nephropathy have causative monogenic variants underlying their disease (3,4). The role of genetic testing in the clinical practice of kidney transplantation is relatively undefined. However, given the growth of the field, genetic testing is poised to play a larger role in the evaluation and management of both transplant recipients and donors. This review will explore the ways in which genetic testing may improve care in kidney transplantation.

Genetics of Kidney Disease
Knowledge of the genetic basis of kidney disease has expanded dramatically in recent years, driven by rapid advances in genetic technologies. The cost of sequencing the entire human genome has dropped dramatically from an estimated $100 million in 2001 to approximately $1000 in 2017 (5) due to the development of next-generation sequencing, a collection of techniques that involve the rapid, parallel sequencing of DNA fragments followed by computational analysis to assemble the source sequence. Whole-exome sequencing (WES) is a comprehensive mode of testing by next-generation sequencing that involves sequencing only the exome, the approximately 2% of the genome which encompasses most mutations known to cause human disease. WES now costs as little as approximately $1000 or less in clinical settings, which compares favorably with many other tests and procedures used routinely in transplantation. WES offers the advantage of allowing for the analysis of an unlimited number of genes at the time of testing, or in the future when subsequent genes of interest may be discovered (6).

To date, mutations in >500 genes have been implicated in causing various forms of kidney disease (3,7). These mutations, although individually rare, are in aggregate relatively common among patients with kidney disease. Several recent papers have demonstrated that a significant proportion of both pediatric and adult patients with nephropathy have causative genetic variants. Mallet et al. (8) used a series of targeted gene panels to evaluate 135 families with familial renal disease; genetic diagnoses were made in 58 families (43%) across a range of diagnoses. Lata et al. (9) selected 92 patients out of 344 referred to Columbia University for evaluation of noncystic kidney disease. The authors identified diagnostic mutations in 24% of the patients using WES and evaluation of 287 genes associated with nephropathy. A follow-up study examined a larger cohort of >3000 mostly adult patients with a diverse array of kidney diseases, who were not necessarily selected for characteristics likely to suggest genetic disease. Nonetheless, diagnostic mutations were identified in 9% of the patients overall, including 24% of those with congenital or cystic diseases and 17% of those with unknown cause. Similarly, Mann et al. (10) examined a cohort of 102 pediatric transplant recipients. Using WES, a genetic cause for nephropathy was identified in >30% of the patients. Connaughton et al. performed WES on 138 adults from 114 families in a multicenter study in Ireland. Overall, a genetic diagnosis was made in 37% of patients (4). The rate of diagnosis was 15% even in patients who had neither extrarenal manifestations nor family history of nephropathy. Among patients with unknown etiology of kidney disease, the diagnostic rate was 47%. Collectively these recent data show that monogenic causes of kidney disease are relatively common. The data also identify subsets of patients enriched for causative genetic mutations, such as those with unknown cause of kidney disease and those with family history.
Role of Genetic Testing in Kidney Transplantation

Genetic testing has three main applications in clinical kidney transplantation: the risk assessment of donors, disease characterization of recipients, and improving drug selection and dosing for recipients using pharmacogenomic data.

Living Donors

A central challenge of living donor kidney transplantation involves estimating the postnephrectomy risk of the donor with regard to subsequently developing kidney disease, hypertension, or other diseases (11). Recent large-scale studies have shown that although donors have generally excellent outcomes, nephrectomy may confer a several-fold increase in risk of ESKD (11–13). Those studies suggest that risks may be higher for donors who are related to their recipients (14,15), which implies that genetic factors are relevant. The use of genetic data is likely to improve estimates of risk to donors who are related to recipients or who otherwise have family history of nephropathy.

The Organ Procurement and Transplantation Network (OPTN) policy for the medical evaluation of living kidney donors states that “hospitals must develop and comply with a written protocol for polycystic kidney disease or other inherited renal disease as indicated by family history” (16). Similarly, the Kidney Disease Improving Global Outcomes guidelines (17) state that “transplant programs should have a strategy for evaluating for inherited kidney disease in donor candidates when there is a family history of kidney failure and the recipient’s cause of kidney failure is uncertain.” Further, when the donor is related to the recipient, the cause of recipient ESKD should be determined whenever possible. The Kidney Disease Improving Global Outcomes (KDIGO) guidelines enumerate several specific diseases which, when present in the family history, should trigger consideration of genetic testing of donors due to high rates of causative mutations, including FSGS, atypical hemolytic uremic syndrome, Alport disease, sickle cell trait, and autosomal dominant tubulointerstitial kidney disease. Because patients with nephropathy of a variety of etiologies are increasingly recognized to have high rates of causative genetic mutations (see above), compliance with the OPTN and KDIGO policies imply that genetic testing should be commonplace. The University of Iowa recently published a description of their experience with a program of genetic testing in donors with family history of kidney disease using a targeted panel of nephropathy genes (18). Several cases were described in which genetic testing allowed for selection of donors confirmed to lack disease-causing alleles present in affected family members. In addition, two recent papers have urged consideration of genetic testing in prospective donors with family history of potentially monogenic forms of ESKD as described in the KDIGO guidelines (19,20).

Two situations in which genetics affect donor suitability and which are commonly encountered merit additional discussion: donors with family history of cystic kidney disease, and donors of African ancestry who may have high-risk APOL1 alleles.

Cystic Kidney Disease

Autosomal dominant polycystic kidney disease (ADPKD) is the most common genetic form of kidney disease, affecting an estimated 1:500 to 1:1000 individuals. Patients with cystic kidney disease represent approximately 12% of recipients in the United States, and 18% of living donor recipients (21). Thus, prospective donors with family members with cystic disease are frequently encountered. Most ADPKD is caused by a mutation in either the polycystic kidney disease 1 (PKD1) or PKD2 gene. The genotype is predictive of the clinical course and can be used to exclude disease presence on clinical grounds. For example, in families with a PKD1 mutation, the absence of any renal cyst by the age of 30 provides almost complete certainty of disease exclusion (22,23). However, in families with a PKD2 mutation or an unknown genotype, ADPKD cannot be excluded by imaging until the age of 40. Because most patients with ADPKD still do not have genetic testing at the time of ESKD, our institution’s clinical practice is thus to screen all prospective familial donors with negative imaging under the age of 40 with genetic testing. Genetic testing (generally done by WES or Sanger sequencing) requires identification of the PKD mutation in the recipient, followed by testing for the familial mutation in the donor.

Multiple genes besides PKD1 and PKD2 can cause a cystic phenotype mimicking ADPKD (24); thus ADPKD may be misdiagnosed and imaging criteria for excluding the presence of disease in prospective donors may be unreliable. Therefore, it is important to have a relatively low threshold to order genetic testing when the affected patient does not conform to the classic ADPKD presentation of bilaterally uniformly enlarged, massively cystic kidneys (23,25).

APOL1 Nephropathy

APOL1 is the gene encoding apoL1. Recently, two risk alleles for nephropathy, termed G1 and G2, were identified in populations of African ancestry (26). Patients who are homozygous for the risk alleles have substantially increased risk of developing ESKD (27) from a variety of different causes including HIV-associated nephropathy and hypertension. The risk seems to be associated with ApoL1 expression in the kidney itself rather than in other tissues. For example, several papers have reported that homozygous APOL1 risk alleles in donors are associated with worse allograft survival (28–30), whereas the APOL1 genotype of the recipient does not seem to affect allograft outcomes (31). APOL1 risk alleles likely explain at least in part why African American donors exhibit higher rates of subsequent development of ESKD (14,15). The mechanism by which APOL1 risk alleles increase the incidence of ESKD is not known, and most patients with two risk alleles never develop ESKD. However, a recent study (32) of 136 African American donors revealed that donors with a high-risk genotype (two risk alleles) had lower eGFR both pre- and postdonation, and demonstrated a faster rate of eGFR decline postdonation compared with donors with zero or one risk allele. A larger study (called APOL1 Long-term Kidney Transplantation Outcomes Network; APOLLO) of the effects of APOL1 genetics on kidney donors (and recipients) has started enrolling patients and should yield more precise data on the issue (33). At present, due to the lack of precise data on the implications of ApoL1 genotypes, there is debate as to whether donors of recent African ancestry should undergo APOL1 genotyping. Several expert groups have recommended educating prospective donors about APOL1 alleles and offering testing to those who choose to pursue it (19,20,34,35). A recent survey
of transplant centers in the United States revealed that about half of centers offer APOL1 testing to black donors (36). Further, Julian et al. (37) have proposed that, with regard to deceased donors, Apol1 genotype should replace race in the calculation of Kidney Donor Risk Index, which predicts longevity of the allograft and is used in organ allocation decisions. This interesting suggestion seeks a more granular assessment of organ quality from African American donors, but it would require rapid genotyping of deceased donors and will require further research before widespread implementation.

Of note, evidence exists for the presence of other susceptibility alleles for kidney disease in addition to Apol1, particularly for diabetic nephropathy (38) and for IgA nephropathy (39). Before consideration of incorporation of these susceptibility alleles into clinical practice, additional research to define the magnitude (and hopefully mechanisms) of the risks will be required, as is ongoing for Apol1 alleles.

**Recipients**

The genetic testing of prospective kidney transplant recipients is useful in many cases. As discussed above, in patients with a living related donor, identification of causative mutations in the recipient is used to guide subsequent testing and interpretation of donor genotypes. This approach aligns with the KDIGO recommendation to determine the cause of ESKD in recipients with living related donors whenever possible.

Genetic diagnoses may be psychologically beneficial to patients seeking transplant, many of whom have no clear explanation for ESKD. Genetic diagnoses are useful to family members of the patient and help facilitate their early evaluation and treatment. Unlike renal biopsies, genetic data can be informative even after ESKD has developed. Indeed, in their program Schrezenmeier et al. (40) have reported genetic testing on patients who are waitlisted for a transplant under the age of 40 with unknown cause of nephropathy. Using targeted gene panel testing, they identified pathogenic mutations in 15 of 81 patients (19%).

Genetic testing in transplant synergizes with overall trends in health care toward the provision of more individualized care (41). Recipients are expected to experience more tailored post-transplant care due to genetic testing. For example, the finding of a mutation in a type IV collagen gene in a patient with FSGS would reduce concerns about recurrent disease after transplantation and may result in reduced follow-up testing. Genetic diagnoses often point to the likelihood of disease in other organ systems and prompt referral and evaluation, for example to an audiologist in a patient with collagen mutations.

Some medical centers are beginning large-scale WES of patients across their health care system (42), motivated by the expectation that ≥3% may be found to have one of approximately 60 actionable mutations identified by the American College of Medical Genetics and Genomics (43). This collection of highly penetrant alleles conveys significant risk for a variety of cardiac, metabolic, and oncologic diseases which may benefit from early discovery and management. Knowledge of the presence of these alleles is relevant to post-transplant care of affected recipients.

Another benefit of increased genetic testing on transplant recipients is that it will yield rich sources of data for future research. When coupled with detailed phenotypic and clinical data available for patients receiving a transplant, investigation into the genetic basis of both kidney disease and transplant outcomes is possible. iGeneTRAIN is a consortium encompassing genetic data on patients who have received a transplant from ≥45 genetic studies (44,45). Studies from this data set promises to advance the understanding of not only genetic causes of kidney disease, but also genetic determinants of rejection and allograft survival. For example, a growing body of research from the iGeneTRAIN and others suggest that allelic mismatches outside of HLA genes between kidney donors and recipients can have important implications for allograft outcomes (46–48). The larger availability of WES data on donor and recipient pairs will advance these studies.

**Pharmacogenomics**

Another growing area for the use of genetics in recipients of a kidney transplant is pharmacogenomics, the study of how genes affect how a patient responds to medications. The two transplant medications for which there is clear pharmacogenomics data are tacrolimus and azathioprine. The Clinical Pharmacogenetics Implementation Consortium has guideline recommendations for both drugs and is an excellent reference for practitioners (49,50).

Tacrolimus is the most commonly prescribed immuno-suppressant medication postkidney transplant (51) and variations in the gene CYP3A5 are well known to affect tacrolimus levels in patients after receiving a transplant. CYP3A5*1 is the functional allele, with loss-of-function variants including *3, *6, and *7. Based on the alleles a patient carries, the patient can be classified into three phenotypes: extensive metabolizer, intermediate metabolizer, and poor metabolizer (49). A meta-analysis found an association between CYP3A5*1 expressers and acute rejection but did not find an association with acute or chronic nephrotoxicity (52). Strategies that incorporate pharmacogenetic data into tacrolimus dose selection have been developed and shown to shorten the time to target tacrolimus levels in new transplant patients (53).

Azathioprine was approved by the Food and Drug Administration in 1968 and has a range of uses including kidney transplantation and autoimmune diseases. The gene product of TPMT, thiopurine s-methyltransferase (TPMT), allows azathioprine to be degraded from a toxic to a non-toxic metabolite. Similar to CYP3A5, patients can carry a functional TPMT allele (*1) or loss-of-function alleles (*2, *3A, *3B, *3C, *4), leading to normal or high TPMT activity, intermediate activity, or deficient activity (50). Knowledge of TPMT alleles (and thus TPMT activity) can be used to tailor azathioprine dose to avoid toxicity. Studies have demonstrated the cost benefit of TPMT testing in patients with irritable bowel disease and rheumatologic disorders (54,55).

Several additional genes with variant alleles are relevant to the metabolism of medications commonly taken by patients who have received a transplant including NUDT15 (azathioprine), CYP2C19 (clopidogrel and voriconazole), CYP2D6 (codeine, oxycodone, tramadol, tricyclic
antidepressants), CYP2C19 (tricyclic antidepressants), and HLA-B (allopurinol). Multigene tests are available to simultaneously type multiple pharmacogenetic loci for similar cost to traditional typing of the TMPT gene alone (56). Unfortunately, insurance coverage may not yet be available for multigene tests in many instances.

Implementation of Genetic Testing in a Clinical Kidney Transplantation Program and Future Research

Genetic testing in the evaluation of kidney disease is a rapidly developing field. As discussed above, available guidelines from the OPTN and KIDIGO imply that genetic testing in renal transplant should become more widespread, particularly in the evaluation of living donors with family history of nephropathy. There is so far only scant literature on how renal transplant centers are incorporating genetic testing into clinical practice (18,40).

The implementation of genetic testing in transplant centers requires attention to several evolving issues including reimbursements for genetic testing and privacy concerns (e.g., sharing genetic results from a recipient with a prospective related donor). Informed consent for genetic testing ideally allows for future use of the data in research. Further, the interpretation of genetic data requires significant expertise from both geneticists and nephrologists. The list of nephropathy genes is evolving, as is the list of causative mutations. Complicating matters further, some mutations previously identified as causative may be reconsidered in light of subsequent reports (57). The clinical significance of identified mutations is not always unambiguous. For example, in autosomal recessive Alport syndrome, the significance of heterozygous mutations in family members of the affected individual requires integration of clinical, familial, and genetic factors (58,59).

Despite these difficulties, given the high rates of genetic causes of nephropathy (3,4,10), genetic testing of patients receiving transplants is expected to offer clinical benefit. The clearest clinical benefit is to donors related to recipients, where genetic testing may be able to rule out presence of a causative allele in the donor (18). Thus, at our center, we are beginning to apply WES to all approved recipients who do not have an obvious secondary cause of ESKD (e.g., diabetic nephropathy or drug toxicity) and who have prospective living related donors. For recipients found to have causative mutations in a set of evaluated nephropathy genes, prospective related donors are screened for the identified mutation. Our goal is to identify donors who may harbor alleles that put them at unacceptable risk for developing nephropathy. Costs for genetic testing are generally a small fraction of the total cost of recipient and donor evaluations and are commonly covered by insurance. Genetic counseling is provided by members of the clinical genetics team and/or the transplant nephrologist. In addition, recipients are characterized by a pharmacogenomics gene chip, which identifies alleles relevant to the metabolism of several drugs used in patients who have received a transplant, including tacrolimus and azathioprine. Pharmacogenetic data are incorporated into the electronic health record and are automatically recalled when relevant medications are prescribed in the system.

Research is needed to quantify the positive and negative effects of genetic testing on donors and recipients. Important research questions include determining the fraction of donors and recipients whose clinical care was meaningfully affected by genetic data, characterizing the cost of genetic testing in monetary terms as well as in disruptions to the pretransplant evaluation, and delineating of the appropriate criteria to identify patients likely to benefit from testing. The benefits of pharmacogenetics have not been well established regarding clinical kidney transplant outcomes such as rejection, graft survival, episodes of drug toxicity, and hospitalization rates. However, as pharmacogenetic testing becomes cheaper and more accessible, it will facilitate the performance of the necessary studies.

The genetics of kidney disease will continue to evolve as knowledge grows and genotyping technology becomes more powerful. The kidney transplant community will need to determine the best ways to incorporate these advances into improving the care of transplant donors and recipients.

Author Contributions
E. Marin conceptualized the review; all authors wrote the original draft, and reviewed and edited the manuscript.

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References
16. Organ Procurements and Transplantation Network: Organ pro-
11. Lentine KL, Lam NN, Segev DL: Risks of living kidney donation:
22. Pei Y, Watnick T: Diagnosis and screening of autosomal dominant
28: 2749–2755, 2017
AQ, Henderson ML, Snyder JJ, Segev DL: Quantifying post-
JAMA
Science
329: 841–845, 2010
329: 13–23, 2018
30: 22–28, 2020
72: 302–308, 2018
29: 192–193, 2010
20: 140–152, 2010
Lancet 393: 919–935, 2019