

The Evolving Role of Diagnostic Genomics in Kidney Transplantation



Jacqueline Soraru¹, Aron Chakera^{2,3}, Nikky Isbel^{4,5,6}, Amali Mallawaarachichi^{7,8},
Natasha Rogers^{9,10,11}, Peter Trnka^{12,13}, Chirag Patel¹⁴ and Andrew J. Mallett^{15,16,17,18}

¹Department of Nephrology and Hypertension, Perth Children's Hospital, Perth, Western Australia, Australia; ²Department of Renal Medicine and Transplantation, Sir Charles Gairdner Hospital, Perth, Western Australia, Australia; ³Harry Perkins Institute of Medical Research, Perth, Western Australia, Australia; ⁴Department of Nephrology and Transplantation, Princess Alexandra Hospital, Brisbane, Queensland, Australia; ⁵Centre for Kidney Disease Research, University of Queensland, Brisbane, Queensland, Australia; ⁶Translational Research Institute, Brisbane, Queensland, Australia; ⁷Department of Renal Medicine, Royal Prince Alfred Hospital, Sydney, New South Wales, Australia; ⁸Garvan Institute of Medical Research, Sydney, New South Wales, Australia; ⁹Department of Renal Medicine and Transplantation, Westmead Hospital, Westmead, New South Wales, Australia; ¹⁰The Westmead Institute, Westmead, New South Wales, Australia; ¹¹Westmead Clinical School, Westmead, New South Wales, Australia; ¹²Child and Adolescent Renal Service, Children's Health Queensland, Brisbane, Queensland, Australia; ¹³Paediatrics and Child Health, School of Medicine, University of Queensland, St Lucia, Queensland, Australia; ¹⁴Genetic Health Queensland, Royal Brisbane Women's Hospital, Brisbane, Queensland, Australia; ¹⁵Faculty of Medicine, The University of Queensland, Herston, Queensland, Australia; ¹⁶Department of Renal Medicine, Townsville University Hospital, Douglas, Queensland, Australia; ¹⁷College of Medicine and Dentistry, James Cook University, Douglas, Queensland, Australia; and ¹⁸Institute for Molecular Bioscience, The University of Queensland, St Lucia, Queensland, Australia

Monogenic forms of heritable kidney disease account for a significant proportion of chronic kidney disease (CKD) across both pediatric and adult patient populations and up to 11% of patients under 40 years reaching end-stage kidney failure (KF) and awaiting kidney transplant. Diagnostic genomics in the field of nephrology is ever evolving and now plays an important role in assessment and management of kidney transplant recipients and their related donor pairs. Genomic testing can help identify the cause of KF in kidney transplant recipients and assist in prognostication around graft survival and rate of recurrence of primary kidney disease. If a gene variant has been identified in the recipient, at-risk related donors can be assessed for the same and excluded if affected. This paper aims to address the indications for genomic testing in the context for kidney transplantation, the technologies available for testing, the conditions and groups in which testing should be most often considered, and the role for the renal genetics multidisciplinary team in this process.

Kidney Int Rep (2022) 7, 1758–1771; <https://doi.org/10.1016/j.ekir.2022.05.019>

KEYWORDS: diagnostic genomics; genetic kidney disease; kidney transplant; whole exome sequencing; whole genome sequencing

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The role that diagnostic genomics plays within the practice of nephrology is evolving. End-stage KF or KF of undetermined etiology continues to comprise a significant minority of patients being assessed for kidney transplantation, with 5% of Australian patients with KF having an unknown primary kidney diagnosis.¹ Kidney biopsy has been the gold standard in diagnosing many kidney diseases, but it is often avoided in patients with advanced CKD due to increased complication rates or risk of misclassification of disease when performed at such a late stage. As a

result, many ascribed kidney diagnoses are incorrect or presumed.

Knowledge of underlying cause of KF is important in the context of kidney transplantation. It can provide some understanding of graft survival and recurrence rates of primary kidney disease and help identify and exclude at-risk related donors in the context of heritable disease. Known monogenic forms of kidney disease account for approximately 70% of pediatric and 10% of adult cases of CKD.²⁻⁷ In the last decade, the number of genes associated with genetic kidney disease (GKD) has grown significantly,^{5,8,9} as has the access to diagnostic genomic testing. Depending on the suspected monogenic kidney disease, diagnostic yield from genetic testing varies from 10% to 73%.¹⁰⁻²⁰ In a group of patients who reached KF of unknown cause before 40 years of age and were awaiting kidney

Correspondence: Andrew J. Mallett, Department of Renal Medicine, Townsville University Hospital, Douglas, Queensland 4814, Australia. E-mail: Andrew.mallett@health.qld.gov.au

Received 5 April 2022; revised 11 May 2022; accepted 16 May 2022; published online 25 May 2022

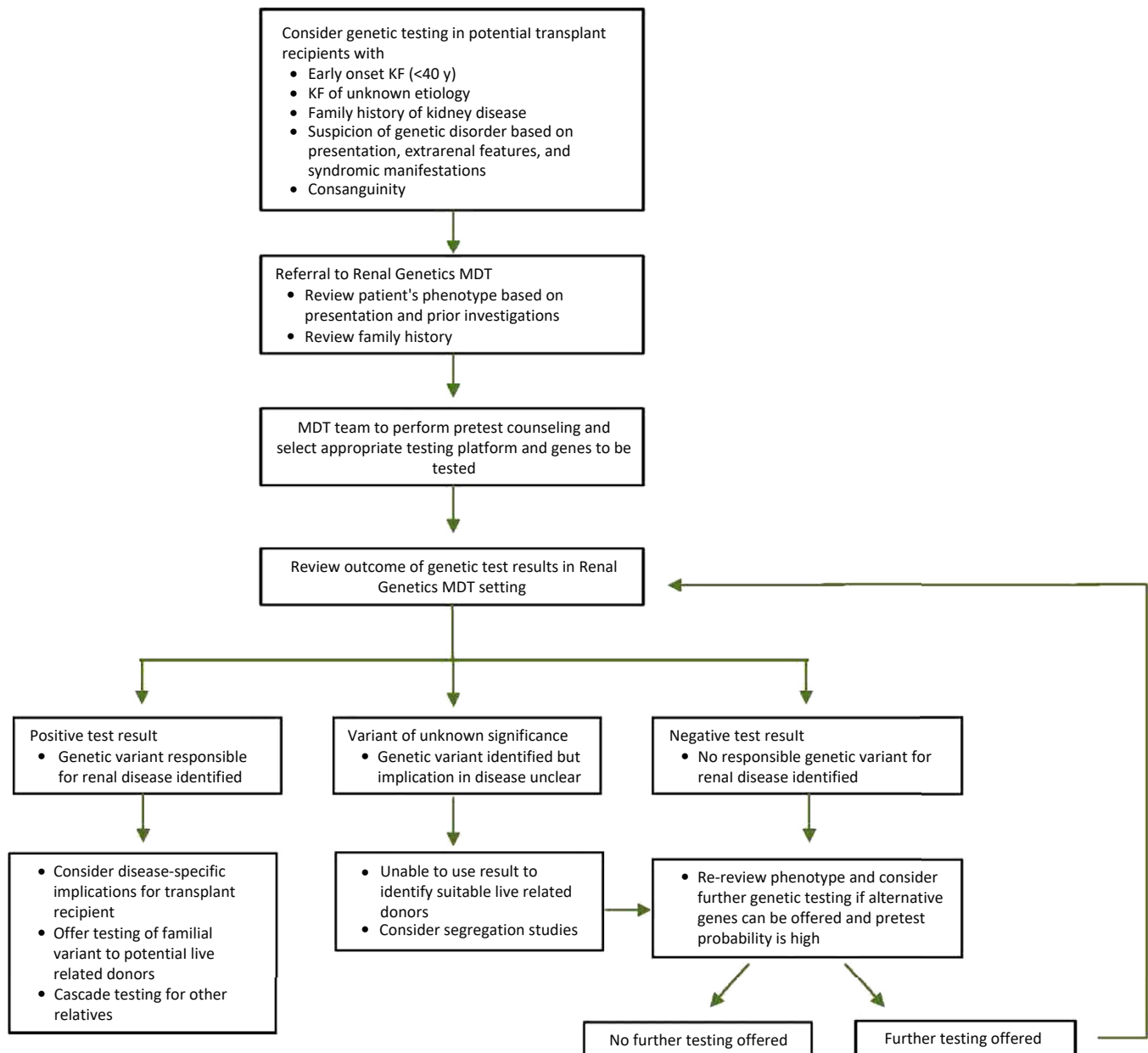


Figure 1. Suggested assessment of patients being considered for genetic testing around kidney transplantation. KF, kidney failure; MDT, multidisciplinary team.

transplantation, the diagnostic rate of pathogenic or likely pathogenic variants was 11%²¹ (Supplementary Table S1). With a lack of local guidelines, and great interest in the role of genomic testing around kidney transplantation, this paper aims to discuss the indications and limitations associated with testing and to make suggestions for testing in the context of GKD and kidney transplantation.

The Multidisciplinary Team

Clinical practice in the realm of kidney genetics, similarly to kidney transplantation, can be hard to navigate without a multidisciplinary team. There are practical challenges, including appropriate test selection, pretest

counseling, interpretation and delivery of results, and counseling and management of concerned family members. Multidisciplinary renal genetics clinics are currently becoming more readily available in Australia, the United Kingdom, Canada, and the United States of America²²⁻²⁶ and are important in streamlining a number of these processes and using the knowledge and experience of nephrologists, clinical geneticists, and genetic counselors to manage these complex patients. Each individual has a specific role to play within this collaborative team, with the typical role in Australian multidisciplinary teams as follows: The nephrologists primarily review the phenotype of the patient, trying to identify the condition or group of

conditions in which they fall. This is very important to select the genes or gene panels most likely to apply to the patient. The clinical geneticist provides appropriate pretest counseling and discussion around patterns of inheritance, the testing process, and diagnostic technologies. Once a genetic test result has been issued for a patient, discussion between the nephrologist and clinical geneticist is important in reaching a consensus on whether the finding is diagnostic or not. In the situation where a result is negative, discussions will be about additional testing if the chance that there is an underlying genetic condition is high. The genetic counselors assist in obtaining further information about family members and their result, often having completed a full genogram before review in the clinic. They educate on the role of the clinic and set expectations for the patients, rediscuss genetic principles discussed in the clinic, and provide easily accessible support throughout the process. The general workflow of testing in a renal genetics multidisciplinary team has been outlined in [Figure 1](#).

The first Australian multidisciplinary renal genetics clinic using this model was established in Brisbane in 2013, with initial outcomes reported previously.²⁷ Using this model, the national KidGen Collaborative was formed in 2016, with the goal being to provide definitive diagnoses to patients with GKD in renal genetics clinics across Australia (<http://www.kidgen.org.au>). Similar initiatives are underway internationally. It is recommended that, when available, families who require genetic testing in the context of renal transplantation are referred to or involve discussion with such appropriate kidney genetics centers for counseling and management.

Indications, Risks, and Benefits of Genetic Testing

There are a number of clinical factors that should prompt consideration of genetic testing to determine cause of primary kidney disease. Examples of these include strong family history, early onset of disease, syndromic presentation, or extrarenal manifestations of a known genetic renal disease. In such situations where testing is offered and a positive result occurs, one is able to provide a diagnostic label to the patient's kidney disease, which will benefit planning before kidney transplant, cascade testing in their relatives (including live related donors), and reproductive planning for the proband and their relatives. In addition, in future, there may be a role for pharmacogenetics in mainstream practice around transplantation.²⁸⁻³⁰

A situation in which genetic testing should be considered in a potential kidney transplant recipient is when the etiology of the recipient's kidney disease is

unclear. In this situation, the recipient should be phenotyped, with the genomic test using either exome sequencing (ES) or genome sequencing (GS). Using these testing platforms, analysis of a panel disease-specific genes or all monogenic kidney disease genes as is described in PanelApp Australia should be undertaken.³¹ Knowledge of the underlying cause of KF is important in management of a patient peritransplant as the primary kidney disease may affect graft survival by risk of recurrence or rejection. For example, genetic testing for steroid-resistant nephrotic syndrome (SRNS) assists in prognosticating around risk of relapse after transplant and providing meaningful information regarding other manifestations such as malignancy, already an important post-transplant issue, which may be heightened in the case of potential *WT1* mutations.

In addition, a diagnosis of heritable kidney disease may guide potential live related donors due to their chance of being affected by the same condition. In some instances, there is a clear family history of kidney disease but no known pathogenic variant, and so the potential recipient is the first in the family to be tested based on phenotype, with a positive result allowing cascade testing within the family. Although kidney donors generally have good outcomes, they are at increased risk of developing CKD, KF, and hypertension.⁵⁻⁷ This risk may be further increased if the donor and recipient are genetically related,³²⁻³⁴ and in this situation, the cause of the recipient's primary kidney disease should be identified wherever possible. Kidney Disease: Improving Global Outcomes guidelines suggest that for recipient kidney diseases with a high rate of identifying pathogenic variants, such as atypical hemolytic uremic syndrome, Alport syndrome, and focal segmental glomerular sclerosis, genetic testing of related recipient and donor pairs is undertaken.³⁵ In the situation of a condition such as Fabry disease, it would be important to test potential related female donors, as alpha-galactosidase A levels may not sufficiently exclude a diagnosis of Fabry disease in these at-risk individuals. There have been cases of females donating a kidney to relatives but who subsequently find out that they also have Fabry disease.³⁶ Fabry disease is 1 of the 6 described renal phenotypes on the American College of Medical Genetics secondary findings list which requires reporting if a pathogenic variant is incidentally detected.³⁷

There are benefits in testing potential live related donors, regardless of positive or negative outcome. In the event of a positive gene test result, the affected relative has been diagnosed at an earlier stage in their disease and may result in earlier multidisciplinary care and potential access to and greater benefit from therapies. This is particularly important in conditions such

as Fabry disease where there is specific enzyme replacement therapy or chaperone therapy. In the event of a negative gene test result, there would be an expected slightly increase in the number of transplants from living related donors, with confidence in accepting younger donors. This is particularly important in families with autosomal dominant polycystic kidney disease (ADPKD), as diagnosis of the condition cannot be ruled out based on imaging alone in young potential live related donors.

Unrelated donors do not require genetic testing unless they have their own family history of kidney disease or a mild phenotype that otherwise does not exclude them from donation (e.g., microscopic hematuria without proteinuria).³⁸

Types of Testing Platforms

The aim of diagnostic genetic testing is to identify the causative pathogenic genetic variant for a disease in an individual patient. The vast variation within the human genome makes this task and the interpretation of results very difficult. The human genome consists of ~3 billion DNA nucleotides, with up to 20 million nucleotide variants being present in a given individual.³⁹ It is the role of the diagnostic laboratory staff and clinical geneticist to determine which variations in the human genome are related to disease using a number of testing platforms, bioinformatics tools, and databases.

Chromosomal Microarray

Many genomic disorders are based on copy number variations, which are a gain or loss of germline DNA ranging from 1 kilobase to several megabases in size. These are a common feature of the human genome^{40,41} and are easily detectable at the chromosomal level using chromosomal microarray. This is a most often used and effective first-line test in patients with congenital anomalies of the kidney and urinary tract.^{42,43} One study of 522 children with renal hypoplasia identified copy number variations that were pathogenic for a known genetic disorder in 55 of 380 (14.5%) cases with isolated urinary tract malformations.⁴³ Chromosomal microarray is also the first-line test when considering *HNF1β*-related disorders as a cause for congenital kidney disease. The 17q12 deletion syndrome (spanning the *HNF1β* gene) is a more common cause for structural and functional abnormalities of the kidney and urinary tract, including cystic and tubulointerstitial kidney phenotypes, in combination with maturity-onset diabetes of the young type 5 and neurodevelopmental or neuropsychiatric disorders.⁴⁴ Chromosomal microarray is also the first-line test when considering patients with kidney disease and other clinical manifestations that may support a syndromic diagnosis for their GKD.

Multiplex Ligation-Dependent Probe Amplification

Multiplex ligation-dependent probe amplification is an assay used to detect small copy number variations of genomic DNA sequences and is particularly useful in detection of exon duplication and deletion, which would not be detectable on a chromosome microarray. It has the advantage of speed and high throughput compared with conventional methods, including higher sensitivity, as compared with exome- or genome-based structural variant analysis. This method is particularly helpful in the situation of *PKD1-TSC2* contiguous gene deletion syndrome,^{45,46} especially as mosaicism is sometimes present in this condition.⁴⁶

Sanger Sequencing

Sanger sequencing has high value in detecting single-nucleotide variants and small insertions or deletions (<5–10 base pairs), and as such, it remains the gold standard for molecular diagnosis when only 1 specific single-gene disorder is suspected. For example, it is useful in the diagnosis of Fabry disease.^{47,48} In addition, Sanger sequencing can confirm massively parallel sequencing findings or sequence-specific regions that were not attainable with massively parallel sequencing.

Massively Parallel Sequencing

Massively parallel sequencing, otherwise known as high throughput sequencing, involves simultaneously sequencing multiple DNA segments at the same time. It is a more modern sequencing technology that allows sequencing in a time-efficient and cost-effective manner, particularly for large-scale genomic investigation, as compared with other sequencing methods, such as Sanger sequencing.

ES examines coding regions (exons) only, whereas GS examines coding and noncoding regions (introns and exons). These tests can be undertaken with an engineered targeted panel approach (i.e., only a selective panel of genes are sequenced and analyzed) or as virtual panels on a backbone of ES or GS (i.e., all human genes are sequenced and only those listed on the panel are virtually analyzed). An additional benefit is that for both ES and GS, there is the ability to return to and reanalyze the data and reassess genes previously not reviewed, without having to repeat the test. In the future, advances in these platforms, such as short-read and long-read technology, may be useful for those with previously uninformative genomic sequencing.^{49–51}

These testing platforms are increasingly becoming a useful tool in diagnosis of kidney disease in the context of panel testing according to clear clinical phenotype. An example of its use is in the case of Alport syndrome, where a patient with the applicable phenotype would only need to be tested for variants in the genes of importance, *COL4A3*, *COL4A4*, and *COL4A5*. Genomic

testing in this case has been found to yield a diagnosis in 83% of patients with familial hematuria.⁵²

In a North American pediatric cohort of 79 consanguineous or familial cases of suspected nephronophthisis, ES identified a causative mutation(s) in 63% of families. The suspected diagnosis of nephronophthisis was confirmed in most of these cases, but in 36% of families, a different molecular diagnosis, or phenocopy disorder, such as tubulopathy, Alport syndrome, or congenital anomalies of the kidney and urinary tract, was identified.¹⁷ Another example is genomic testing for nephrotic syndrome, for which one would examine a panel of genes associated with the condition (*NPHS1*, *NPHS2*, *WT1*, *TRPC6*, etc.).

There are still limitations with using such testing platforms. In the context of targeted panel testing, the yield is related to the genes chosen on the panel, with restriction of the number of genes reducing the time and cost it takes to test; however, the panel may need frequent review and update as new genes involved in disease are identified or previously implicated genes are found to have weaker associations. Increasing the number of genes examined increases diagnostic sensitivity, albeit with diminishing diagnostic gains, but can also increase the number of variants of uncertain significance (VUS) and thus complicate clinical follow-up. ES and GS, as compared with targeted panel testing with probes specifically designed to capture genes of interest, have a lower per-base coverage, meaning that some pathogenic variants can be poorly covered across some testing platforms.^{53,54} For example, the sites corresponding to approximately 50% of reported pathogenic variants in *WT1* (responsible for Denys-Drash or Frasier syndromes) have been found to be poorly covered in 3 ES capture kits.⁵⁵ As with all tests, sequencing methodologies have their specific sets of limitations, including reduced coverage in some gene regions of interest, or challenges in sequencing homologous or repetitive regions of the genome. Therefore, it is crucial that clinicians consider whether their gene/genes of interest are adequately examined by their test of choice.

Considerations Around Testing

Interpretation of Results

There are caveats to genetic testing which are applicable to testing in anticipation of kidney transplant. The first consideration is the way in which genetic test results are reported. A positive test result occurs when (i) a variant has been identified that is classified as pathogenic or likely pathogenic according to the American College of Medical Genetics variant classification criteria⁵⁶ and is within a gene that has a clear evidence-based relationship to a kidney phenotype and

(ii) the number of variants identified in the gene matches the mode of inheritance of the condition (i.e., 2 variants on separate alleles for autosomal recessive disorders) (Figure 2). If the patient's clinical phenotype matches the kidney phenotype associated with the gene in which the pathogenic or likely pathogenic variant has been identified, the condition associated with the gene is the likely diagnosis.

A negative test result means no reportable gene variants have been identified. Importantly, this does not mean that the patient's kidney disease is not genetic, and one should not provide reassurance that a form of GKD has been excluded in the patient. This outcome could be the result of a number of factors. First, owing to limitations in the testing technology's ability to detect the disease-causing variant, for example, intronic or regulatory region variants. Second, there may be an undiscovered gene or mechanism involved in the kidney disease. Third, the disease-causing gene may not have been analyzed due to incorrect panel selection and/or misphenotyping. This reinforces the importance of the initial and thorough clinical assessment. Further genetic investigations, potentially in the research context, may need to be pursued, where available, to identify the underlying cause of the GKD.

A VUS is a variant in a gene where there is insufficient evidence to classify it as either pathogenic, likely pathogenic, likely benign, or benign (Table 1). This is an uninformative result for the patient and cannot be interpreted as the cause of the patient's kidney disease. A VUS may be highly suspicious if the patient's clinical phenotype matches the kidney phenotype associated with the gene in which a pathogenic or likely pathogenic variant is usually identified and the number of variants identified in the gene matches the mode of inheritance of the condition. In this situation, further clarification might be obtained by first re-reviewing the patient's clinical phenotype, sometimes with nongenetic investigations (e.g., imaging, biopsy), but caution must be taken not to overcall implication of a VUS in the disease. Second, clarification can be obtained with segregation studies with targeted testing of the VUS in affected and unaffected family members. Third, diagnostic genomics staff can review the VUS in population databases, such as gnomAD or ClinVar, to determine population frequency in healthy controls. Last, diagnostic genomics staff can perform *in silico* predictions for pathogenicity or arrange functional studies of the variant in model systems through research collaborations. In many instances, these additional steps may not be sufficient to change the variant's classification, but reappraisal of the VUS every 2 to 3 years is recommended with the benefit of

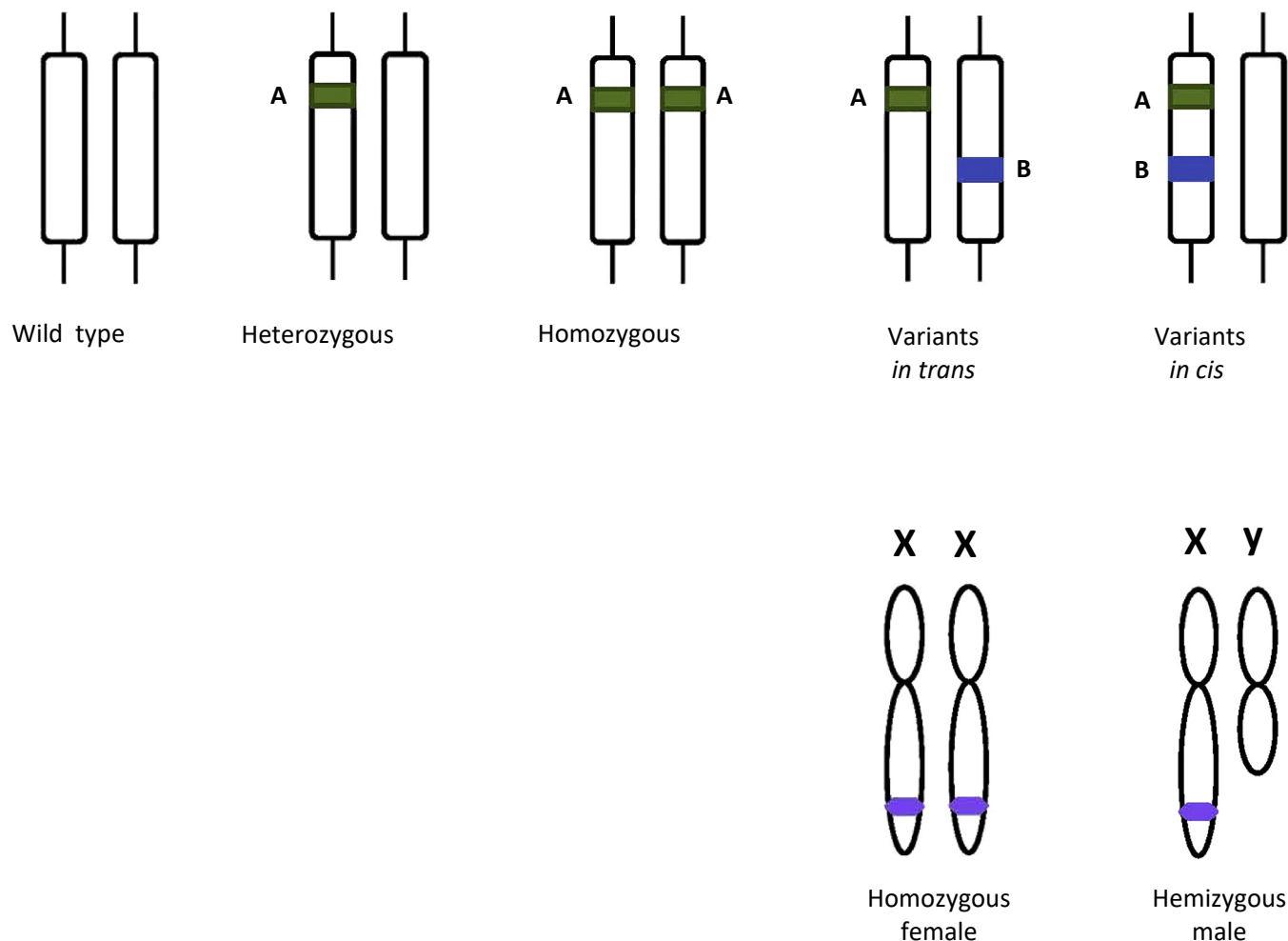


Figure 2. Illustration of zygosity at a single locus.

additional knowledge and tools. It is important to note that predictive testing for a VUS cannot be offered in potential live-related donors as it cannot be confidently linked with the disease.

In addition, most KF cases are related to diabetes, hypertension, or autoimmune conditions, and it is unlikely an underlying monogenic cause be identified in these situations. Similarly, many kidney diseases are polygenic, and so a single pathogenic gene variant may not carry the same weight with regard to diagnosis, and Mendelian inheritance patterns may not apply. Recently, genome-wide association studies have discovered thousands of genetic variants associated with the disease, with polygenic risk scores aggregating the individual effects of these variants and correlating them with disease risk. Unfortunately, this is not well established in KF and yet to enter mainstream clinical practice.

Predictive Testing of Potential Related Living Donors

After a potential renal transplant recipient has undergone genetic testing with a causative gene variant identified, apparently unaffected relatives who are considering kidney donation can be offered testing for

the same gene variant. The process of offering testing for a familial gene variant in an apparently unaffected individual is called predictive genetic testing and aims to clarify their risk of kidney disease, which can be a significant health revelation, and potentially determine their suitability to proceed with kidney donation. Many studies have indicated that predictive testing carries significant anxiety for many people,⁵⁷⁻⁵⁹ and as such it is important that they undergo proper pretest and post-test counseling.

A positive predictive test outcome means that the individual has the identified familial gene variant, and although previously considered a potential donor, it can now be referred early for surveillance, or started on therapies that may change disease course. In the same instance, this individual no longer has the opportunity to donate to their relative which may have a potentially negative psychological impact on them, the recipient, and their relationship.

A negative predictive test outcome means that the patient does not carry the familial gene variant and can be considered for donation. It also carries complex

Table 1. Definitions

Term	Definition
Allele	One of two, or more, versions of the same gene
Biallelic	Pertaining to both alleles of a single gene
Chromosomal microarray	Technology used to identify translocations, copy number variants, and chromosomal aneuploidies
Exome	The part of the genome that consists of exons
Exome sequencing	Technology that can identify single-nucleotide variants, insertions, or deletions within coding regions of the genome
Gene variant	A change in the DNA nucleotide sequence of a particular gene
Genome	The complete set of genetic material in an organism
Genome sequencing	Technology that can identify single-nucleotide variants, insertions, and deletions within coding and noncoding regions of the genome
Genotype	The genetic constitution of an individual
Monogenic	Involving or controlled by a single gene
Multiplex ligation-dependent probe amplification	Technology used to identify copy number variations, point mutation, or DNA methylation abnormalities in specific genes of interest
Panel testing	Identification of genetic variants in a specific set of curated genes
Phenotype	A set of observable characteristics in an individual arising from the interaction of their genotype with their environment
Polygenic	Involving or controlled by multiple genes
Proband	The individual serving as the starting point of genetic investigation in a family
Sanger sequencing	Technology that provides targeted sequencing in identifying single-nucleotide variants and insertions and deletions < 10 base pairs in length
Segregation analysis	Technique used to determine if a gene underlies the distribution of a given phenotypic trait

psychological impacts, with survivor guilt a common experience in family members with a negative test result. This describes the situation in which a patient tests negative for the familial variant associated with a disease in their family, and they feel guilt toward those who have tested positive or are affected. There is limited information around predictive testing in kidney disease, but studies performed with patients being tested for Huntington's disease reveal that 10% of those who receive a negative predictive result have difficulty coping with their gene status based on assessments of psychological well-being.⁶⁰

There are other nonmedical implications to predictive genetic testing, such as impact on the ability to obtain income or life insurance. It is important that patients are aware of these issues and have the opportunity to make informed decisions. Therefore, predictive genetic testing should only be performed with appropriate pretest and post-test genetic counseling by a clinical geneticist or genetic counselor.

Common GKD

ADPKD

ADPKD is the most common genetic condition that results in KF. In approximately 78% of phenotypical

cases, there is a pathogenic variant in the *PKD1* gene, with approximately 15% having a pathogenic variant in the *PKD2* gene.⁶¹ Direct *PKD* gene sequencing has a higher cost and longer turnaround time. Pseudogenes are also problematic in genetic testing of ADPKD as *PKD1* bears 97.7% similarity in sequence to 6 pseudogenes.⁶² GS has been found to efficiently circumvent these challenges,⁶² with this now being revealed in clinical contexts.⁶³

The genotype of patients with ADPKD is predictive of the clinical course. Compared with patients with *PKD2*, those with *PKD1* gene mutations progress to KF on average 20 years earlier and die at a younger age.⁶⁴ Importantly, up to 18% of affected individuals might experience significant intrafamilial disease variability⁶⁵ compared with that anticipated by their genotype and/or family history.

The diagnosis of ADPKD requires an age-specific renal phenotype and a 50% risk of inheritance based on a positive family history. Currently, ultrasound is used first line to assess for the renal phenotype. In all patients (both *PKD1* and *PKD2*), the overall sensitivity, specificity, and accuracy of ultrasound for diagnosis is 97%, 100%, and 98%, respectively.⁶⁶ In families with a *PKD1* or *PKD2* mutation, there is variable emergence of renal cysts by age 40 years, constituting a clinical diagnosis of ADPKD, though phenocopy and atypical (non-*PKD1/PKD2*) forms of ADPKD can confound this along with a sometimes erroneous reassurance of excluding ADPKD at younger ages based on an apparent absence of renal cysts. Consequently, the current recommendation for ultrasound-based exclusion of a diagnosis of ADPKD in an at-risk individual with an affected first-degree family member is the absence of kidney cysts at 40 years of age or older.⁶⁶ More recently, diagnostic criteria for magnetic resonance imaging have been established for those aged 16 to 40 years. The presence of >10 renal cysts in patients in this age group is sufficient for diagnosis of ADPKD in an at-risk individual, with 100% positive predictive value and sensitivity.⁶⁷ Conversely, a total of <10 renal cysts in the same patient population can be considered sufficient for disease exclusion, with a negative predictive value of 100% and a specificity of 98.3%.⁶⁷ For potential living kidney donors in this age range, a more conservative criterion of <5 renal cysts on magnetic resonance imaging for disease exclusion has been suggested.⁶⁷ Diagnostic criteria are not yet established for CT.⁶⁸

Failure to confirm or exclude a diagnosis of ADPKD and atypical forms of cystic kidney disease^{69,70} has implications for both donors and recipients in the context of kidney transplantation. First, genetic testing in the recipient can be performed to confirm the type of

cystic kidney disease. In such situations, the affected recipient would undergo genetic testing of typical and atypical ADPKD genes, and if a mutation is found, then a related donor would undergo targeted testing for the identified causative familial variant. If that variant is identified, the potential donor would be excluded from proceeding to kidney donation.⁷¹ The primary application of genetic testing for ADPKD in at-risk but seemingly unaffected relative is not recommended unless an established disease-causative variant has previously been identified in an affected family member. It is recommended that if there are no other suitable donor options, related donors over the age of 40 years undergo renal ultrasound, and those between 18 and 40 years of age undergo magnetic resonance imaging screening, and if cystic kidney disease is unable to be confidently excluded, the potential donors are screened for an identified familial ADPKD-causative variant to determine their eligibility to donate.

SRNS

SRNS accounts for 15% of childhood cases of nephrotic syndrome and 40% of adult-onset cases of nephrotic syndrome.⁷² In the last 20 years, >39 genes have been identified as involved in the pathogenesis of SRNS,⁷³ with both autosomal dominant and recessive inheritance patterns. Furthermore, 85% of SRNS presenting clinically before 3 months of age and 66% of cases presenting before 1 year of age can be explained by biallelic/recessive mutations in 1 of the following 4 genes: *NPHS1*, *NPHS2*, *LAMB2*, or *WT1*.⁷⁴ Other studies have reflected the high incidence of monogenetic causes for SRNS,⁷⁵⁻⁷⁷ with 29.5% of all all-comers in a worldwide cohort of patients who presented with SRNS before age 25 years having an identifiable mutation.⁷⁸ It is recommended that those who have presented with SRNS or phenotypes compatible with such a diagnosis, particularly at younger ages of onset, are offered genetic testing before renal transplantation.

Familial Hematuria and COL4A-Related Nephropathy

Benign familial hematuria, now most often known as thin basement membrane disease, is characterized by the presence of recurrent and/or persistent microhematuria, often though not exclusively first detected in childhood or adolescence. The diagnosis of this condition has been best traditionally defined by histopathology revealing a thinned glomerular basement membrane on electron microscopy examination of a kidney biopsy. Clinically, it is also inferred as a diagnosis by the presence of isolated recurrent or persistent microhematuria without other lower urinary tract pathology or significant proteinuria. Both thin basement membrane disease and Alport syndrome develop as a

result of pathogenic variants in the *COL4A3*, *COL4A4*, and *COL4A5* genes. Most cases (85%) of Alport syndrome are inherited in an X-linked pattern and are as a result of *COL4A5* gene variants.⁷⁹ The autosomal dominant and autosomal recessive cases are due to monoallelic or biallelic mutations in *COL4A3* and *COL4A4*, respectively.⁷⁹ In the case of X-linked Alport syndrome, the previous belief that females are more mildly affected than males is being appropriately challenged, and there is a broad and broadening phenotype spectrum in affected females, which includes KF.⁸⁰

Genetic testing for a potential transplant recipient with familial hematuria should be considered if there are extrarenal manifestations making a diagnosis of Alport syndrome more likely. This would be useful for the affected recipient in confirming the diagnosis and for potential at-risk related family members to identify suitable potential kidney donors. Such testing can also ensure early intervention and follow-up for both kidney and other organ involvement. We would recommend genetic testing for any potential kidney transplant recipients with a diagnosis of thin basement membrane disease or Alport syndrome and cascade testing for any related donor deemed to be at risk for the familial gene variant to determine diagnostic clarity and donor suitability. In addition, in some parts of the world, such as French Polynesia, the population prevalence of Alport syndrome has been noted to be higher than anticipated. Genetic and clinical studies have provided an understanding of the basis of this,⁸¹ and such information is important to consider as part of both recipient and donor assessment within a kidney transplantation setting, particularly with a globalized community.

Fabry Disease

Fabry disease, an X-linked lysosomal storage disorder, is caused by the deficiency of alpha-galactosidase A enzyme and the progressive intracellular accumulation of globotriaosylceramide. It has multisystemic manifestations, including hypertrophic cardiomyopathy, dysrhythmias, valvular insufficiency, kidney disease, gastrointestinal dysmotility, hypohidrosis, acroparesthesias, and cerebrovascular accidents. The Fabry Registry has revealed that KF occurs in 14% of males and 2% of females with the condition, with a median age of commencement of kidney replacement therapy at 38 years in both groups.⁸²

Those with the clinical phenotype usually undergo testing of alpha-galactosidase A levels as a first-line diagnostic test, with deficiency being definitive in the diagnosis of hemizygous males. Enzyme levels may not be as reliable in diagnosing heterozygous females.

This is mainly driven by random X inactivation and is the reason many females require genotyping to confirm the diagnosis.⁸³ Other emerging nongenetic tests, such as Lyso-GB3 testing, can also assist among suspected female cases and in circumstances of diagnostic uncertainty. We recommend genetic testing in both males and females who have an unclear etiology of kidney disease and have features concerning for Fabry disease is considered before transplantation.

Considerations for Particular Groups

APOL1 Risk Alleles

Variants in this gene are more often identified in populations with African genetic ancestry, with at least 30% of African Americans carrying 1 risk allele.^{84,85} These risk variants have been linked to the increased rates of CKD and KF found in these populations, with the mechanism by which this occurs being unknown.

Studies have indicated that African Americans with 2 risk variants (G1/G1, G2/G2, or G1/G2) are at 10.5-fold (95% CI 6.0–18.4) greater risk of having focal segmental glomerular sclerosis-associated KF, 7.3-fold (95% CI 5.6–9.5) greater risk of hypertensive KF, and 7.5-fold greater risk of HIV-associated nephropathy (HIVAN) KF, as compared with patients with 1 risk allele.⁸⁶ *APOL1* risk alleles have subsequently been found to be involved in increased risk of CKD⁸⁷ and sickle cell kidney disease.⁸⁸

The effect of high-risk genotypes in kidney transplant recipients is unclear. One study evaluated 119 African Americans and found that 49% carried high-risk *APOL1* alleles, with no differences in graft survival at 5 years after adjusting for kidney type.⁸⁹ Another study evaluated 2 large prospective cohorts and has revealed a strong correlation between the number of recipient risk alleles and death-censored allograft loss, independent of donor *APOL1* genotype and recipient ancestry.⁹⁰ Furthermore, recipient *APOL1* genotype was associated with clinical and subclinical T-cell-mediated rejection of the graft.⁹⁰

Another consideration is when the donor carries a high-risk *APOL1* genotype. Data have suggested that patients who received a kidney from these donors have worse graft survival outcomes.^{91,92} These recipients are more likely to develop focal segmental glomerular sclerosis with earlier allograft failure and subsequent KF in the donor.⁹³ In 1 study, 11% of donors with *APOL1* high-risk genotypes developed KF ($P = 0.02$) and more developed CKD stage 3 or higher ($P < 0.01$) as compared with low-risk genotype donors.⁸⁴ The APOLLO study will be aiming to confirm whether the presence of high-risk *APOL1* genotypes in deceased donors is associated with death-censored kidney

Table 2. General recommendations for genetic testing in the context of kidney transplantation

General recommendations	
• Thorough assessment of renal phenotype in potential kidney transplant recipients is an important first step toward genetic diagnosis	
• Thorough family medical history	
• Affected transplant recipient to undergo genetic testing first	
• Selection of known disease-associated genes that match phenotype	
• Involvement of renal genetics multidisciplinary team for test guidance, pretest counseling, test interpretation, and/or delivery of results, and cascade screening of relatives	
• If pathogenic or likely pathogenic variant identified, can test potential live related donors for the same variant after genetic counseling	

transplant survival primarily, including the association of high-risk donor genotypes on recipient renal function and proteinuria post-transplant, and donor kidney outcomes.⁹⁴

At present, there is a debate about whether potential donors with African ancestry should undergo genotyping for *APOL1* risk alleles. A recent survey of transplant centers in the United States of America indicated that approximately half offer testing to African American donors,⁹⁵ with some centers' clinical decisions based on the outcome of these tests.⁹⁶ In these situations, testing is mostly being used in live donor situations with donors harboring high-risk alleles being excluded. It is estimated that up to 13% of African American donors would be excluded in

Table 3. Disease-specific recommendations for genetic testing

ADPKD	
At-risk donors over the age of 40 years	• Can be considered suitable if they have a normal renal tract ultrasound
At-risk donors under the age of 40 years	• If aged 18–40 years, can exclude ADPKD if there are <5 kidney cysts on MRI
	• If donor's MRI is equivocal, test the kidney transplant recipient for a panel of typical and atypical ADPKD genes
	• If a variant is identified, testing potential related donors is recommended
SRNS	
	• Test recipients with phenotypes compatible with SRNS, especially those who had a diagnosis very early in life
	• If variant identified, testing potential related donors is recommended
Familial hematuria	
	• Test transplant recipient, to differentiate between Alport syndrome and TBMD
	• If a variant is identified, testing potential related donors is recommended
Fabry disease	
	• Test male and female kidney transplant recipients if cause of KF unclear and features consistent with Fabry disease
	• Test potential related female donors as α -galactosidase A levels may not exclude disease

ADPKD, autosomal dominant polycystic kidney disease; KF, kidney failure; MRI, magnetic resonance imaging; SRNS, steroid-resistant nephrotic syndrome; TBMD, thin basement membrane disease.

this situation, reducing the availability of kidney donors in these populations⁹⁷ but safeguarding donor health.

Although awaiting ongoing studies and registries such as the APOLLO to clarify the effect of *APOL1* risk alleles on clinical outcomes, the recommendation is to consider *APOL1* genotyping in the situation of a live donor with African American ancestry and/or a recipient with African American ancestry. The genetic testing provides additional information that can be used to counsel the donor regarding risk of KF later in life, but also the recipient regarding increased risk of graft failure.

CFHR5

CFHR5 mutations have been identified as a cause of monogenic kidney disease and specific forms of heritable C3 glomerulonephritis. Although rare in the general population, *CFHR5*-related nephropathy is endemic in Greek Cypriot populations and those of Greek Cypriot heritage. One study has identified 91 cases across 16 families,⁹⁸ with an autosomal dominant inheritance pattern of a heterozygous exon 2 and 3 duplication identifiable on multiplex ligation-dependent probe amplification but not as easily with other sequencing approaches. Patients usually presented with microscopic hematuria or synpharyngitic hematuria before the age of 30 years, suggesting some degree of phenocopy with IgA nephropathy. Interestingly, males were more likely than females to progress to CKD and KF (80% vs. 20% respectively)⁹⁸ though *CFHR5* is an autosomal gene. In patients with Greek Cypriot heritage, and unknown cause of KF or a glomerulopathy of unclear cause, it would be important to consider *CFHR5* gene mutations resulting in C3GN before transplantation. If a variant in this gene was identified, potential live related donors could be screened for the same variant, especially given the emerging phenotypic variability in affected individuals.

The Need for Guidelines

There are no uniform guidelines for genetic testing around kidney transplantation, and given renal genetics is an evolving field, this is an area that requires focus and development in the future. Future studies are needed to evaluate the short- and long-term effects of both primary and secondary genetic findings on medical care, treatment decisions, transplantation eligibility, donor eligibility, and graft survival. Studies such as APOLLO will contribute significantly to this space. Further studies are also needed to investigate ethical issues that may arise from genetic testing around transplantation, including rate of live donation, delays in time to transplantation, and health care

utilization. All these factors will affect the suggestions for testing around kidney transplantation and recommendations to do so. Some initial general (Table 2) and condition-specific (Table 3) recommendations are suggested.

Summary

Although the accumulation of knowledge and clinical experience to date in the area of kidney genetics has evolved in the past decades and is now rapidly accelerating, there remain significant opportunities to realize benefits for patients and their families. Kidney transplantation is one such complimentary area of practice, with great promise to both increase access to living related kidney transplantation safely and to inform multidisciplinary care within affected families. There are some challenges to consider with the ever-evolving field of kidney genetics, including an incomplete understanding of how certain gene variants are related to disease (as is the case with the *APOL1* gene variants), inequity in access to a kidney genetics service in some parts of the world, and the cost of tests to the patient when funding is not covered. Furthermore, genetic testing may shed light on the primary kidney disease in recipients and help clarify suitable donors but can delay the time to transplant due to the testing process.

DISCLOSURE

All the authors declared no competing interests.

AUTHOR CONTRIBUTIONS

JS and AJM conceived this work and principally drafted the manuscript. All co-authors contributed to the drafting, editing, and revision of the manuscript and have approved the final version.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Table S1. Recent studies applying genetic testing in kidney disease of unknown cause

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