Transplant International

ORIGINAL ARTICLE

Sequential genetic testing of living-related donors for inherited renal disease to promote informed choice and enhance safety of living donation

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SUMMARY

Living kidney donors (LKDs) with a family history of renal disease are at risk of kidney disease as compared to LKDs without such history suggesting that some LKDs may be pre-symptomatic for monogenic kidney disease. LKDs with related transplant candidates whose kidney disease was considered genetic in origin were selected for genetic testing. In each case, the transplant candidate was first tested to verify the genetic diagnosis. A genetic diagnosis was confirmed in 12 of 24 transplant candidates (ADPKD-*PKD1*: 6, ALPORT-*COL4A3*: 2, ALPORT-*COL4A5*: 1: nephronophthisis-*SDCCAG8*: 1; CAKUT-*HNF1B* and ADTKD-*MUC1*: 1 each) and 2 had variants of unknown significance (VUS) in phenotype-relevant genes. Focused genetic testing was then done in 20 of 34 LKDs. 12 LKDs screened negative for the familial variant and were permitted to donate; seven screened positive and were counseled against donation. One, the heterozygous carrier of a recessive disorder was also cleared. Six of seven LKDs with a family history of ADPKD were under 30 years and in 5, by excluding ADPKD, allowed donation to safely proceed. The inclusion of genetic testing clarified the diagnosis in recipient candidates, improving safety or informed decision-making in LKDs.

Transplant International 2021; 34: 2696–2705

Key words
Alport nephropathy, chromosomal microarray, genetic testing, multiplex ligation-dependent probe amplification, next-generation sequencing, polycystic disease

Received: 6 August 2021; Revision requested: 27 September 2021; Accepted: 28 September 2021; Published online: 3 November 2021

Introduction

Living donor kidney transplantation is the preferred option for the management of patients with end-stage kidney disease (ESKD) as it is associated with better life expectancy, improved quality of life, and shortened waiting times for transplant recipients. Although candidates accepted as kidney donors have a similar risk of kidney disease compared with an NHANES sample representative of the general US population, it has become clear that living kidney donors have an increased long-term risk of ESKD post-donation compared with a matched cohort of non-donors [1–3]. In a large retrospective study of US donors, the absolute risk of ESKD in donors was 0.03% at 15 years and 0.09% over their lifetime compared with 0.004% and 0.014% respectively

doi:10.1111/tri.14133

in matched controls [1]. A recent meta-analysis, which included studies with at least 10 years post-donation follow-up, reported cumulative incidence of ESKD in donors to be 1.1% [4].

The risk of ESKD in living kidney donors (LKD) is higher in African Americans and Latinx, men, obese donors, and donors biologically related to the recipient [1,5,6]. Among the biologically related donors, substantial variation is noted across different ancestries and recipient–donor relationships. An Asian donor with an identical twin recipient carries the maximum risk for ESKD at 259.4-fold compared with an unrelated donor. Asian donors additionally experience risk at 4.7-fold for full siblings and 3.5 times for offspring of a recipient as compared to unrelated donors, while black and white donors have 2.7-fold and 1.4-fold increased risk for offspring of the recipient respectively [5,7]. The strength of this biological ancestry-based relationship risk far exceeds that of other well-known risk factors for kidney disease, which strongly suggests the shared inheritance of genetic variants that increases lifetime risk of kidney disease. There are, in fact, published reports of donors presenting with the recipients' native kidney disease after donation emphasizing that some donor candidates may be pre-symptomatic for the familial disease at the time of nephrectomy [8–10].

Some monogenic diseases, such as autosomal dominant polycystic kidney disease (ADPKD), demonstrate age-related penetrance, and at the time they present to be considered as kidney donors, some family members may not yet manifest signs of ADPKD or other genetic disease that affects their related recipient. Given the increasing awareness that pre-symptomatic disease may contribute to increased risk of ESKD in related living donors, it is important to use all resources available to ascertain this risk. When testing the donor candidate for monogenic disease, the choice of test is driven by knowledge of the cause of the related recipient's kidney disease. However, the cause of kidney disease in transplant-eligible candidates is not always known or is sometimes misattributed to common conditions like diabetes and hypertension [11–13].

We hypothesized that unbiased comprehensive renal genetic screening of selected transplant recipients and their related donors would substantially enhance the identification of pre-symptomatic disease in donor candidates and, by extension, increase the likelihood of excluding disease in these candidates, who then can be more safely permitted to donate. A few years ago, we introduced genetic testing for LKD candidates whose related recipient was known to or considered to have a reasonable probability of monogenic kidney disease [14]. The objective of this retrospective study was to determine whether the testing program we introduced helped identify or confirm the cause of ESKD in transplant recipients and thereby improved the donor evaluation process by advancing donor safety or promoting informed choice.

Materials and methods

This retrospective study covered a seven-year period beginning in 2013. Study participants included renal transplant candidates referred to the Organ Transplant Center at the University of Iowa with known or suspected genetic renal disease and an asymptomatic relative who volunteered to be an LKD. Clinical and laboratory data were obtained from the medical record. Transplant recipient candidates were seen and evaluated by a genetic counselor and a nephrologist with expertise in genetic renal diseases and then screened with a targeted comprehensive renal gene panel, KidneySeq™ (IIHG, University of Iowa) by next-generation sequencing (NGS) as previously described [14,15]. A parallel workflow consisting of long-range PCR of the duplicated regions of *PKD1* was used for patients suspected to have polycystic kidney disease. In one case, a microdeletion in *PKD1* identified by NGS was confirmed by multiplex ligation-dependent probe amplification (MLPA) using kit P352 (MRC Holland, Amsterdam, the Netherlands). If a genetic etiology was identified by NGS, PCR amplification and Sanger sequencing of the identified "familial" variant were completed on donor DNA to determine the carrier status of the related living donor. In two patients, a chromosomal microarray (CMA) was used to identify the cause of ESKD. In some cases with an apparent tubulointerstitial phenotype, if genetic testing was negative, the patient's sample was additionally screened for *MUC1* variants (courtesy of Wake Forest University and the Broad Institute) by a probe extension assay following Mwo1 digestion of genomic DNA [16]. In all persons, identified single nucleotide variants and copy number variants (CNV) were classified by ACMG criteria [17,18].

The study was approved by the institutional review board (IRB no. 202011297) for human subject research.

Results

Transplant candidates

Twenty-four renal transplant candidates [mean age, 50.5 ± 16.8 years; range, 1–75 years; male, 14 (58.3%)]

with known or suspected genetic renal disease underwent genetic testing (Table 1). Prior to genetic testing, CKD etiology was reported as glomerular in 9 (37.5%), tubulointerstitial in 4 (16.7%), ciliopathy or cystic disease in 7 (29.2%), and congenital anomalies of the kidney and urinary tract (CAKUT) in 4 (16.7%). After genetic testing, of the nine recipients with suspected glomerular etiology, three were diagnosed with Alport's disease, one carried a variant of uncertain significance (VUS) in *ARHGAP24* (a gene associated with FSGS), and one recipient with C3 glomerulopathy carried a *CFH* VUS; the remaining 4 recipients tested negative. One of the four recipients with suspected tubulointerstitial disease had a pathogenic variant in *MUC1*; six of seven recipients with cystic disease carried pathogenic or likely pathogenic variants in *PKD1* and were diagnosed with ADPKD while the seventh was homozygous for a pathogenic variant in *SDCCAG8* and was diagnosed with autosomal recessive nephronophthisis; and of the four recipients with CAKUT, one carried a 17q12 deletion causing *HNF1B* nephropathy. In one waitlisted patient who had biopsy evidence of IgA nephropathy, the identification of microscopic hematuria in a related donor prompted further evaluation of the family history, which revealed a sibling with hearing loss but no kidney disease (Fig. 1). Genetic testing of the waitlisted candidate identified a likely pathogenic missense variant in *COL4A3* that substitutes a glycine residue at position 121 for a serine (c.361G>A, p.Gly121Ser) thereby disrupting the canonical G-X-Y repeat in the triple-helical domain of type IV collagen [19]. The related donor candidate carried the same variant and subsequently one of her daughters was also noted to have hematuria. Allele segregation in several family members with advanced chronic kidney disease (CKD) or microscopic hematuria strongly linked the variant to the renal phenotype.

Most waitlisted patients in this series with a positive genetic screen were diagnosed by comprehensive genetic testing by NGS with confirmatory Sanger sequencing as detailed previously [15], although in some instances, alternate techniques were employed to make the genetic diagnosis. For example, a heterozygous whole gene deletion of *HNF1B* as a cause of kidney disease in subject# 1-1 was confirmed by CMA following the identification of the 17q12 microdeletion in her son with intellectual disability (Fig. 2). It is now recognized that CNVs involving *HNF1B* account for about half of *HNF1β*-mediated disease and nearly all *HNF1B* gene deletions are secondary to 17q12 microdeletions [20]. In subject #8-1, *MUC1*-mediated autosomal dominant tubulointerstitial kidney disease (ADTKD) was diagnosed in a patient with CKD and bland urine without proteinuria prior to referral to the transplant center. Toxic gain-of-function *MUC1* disease occurs most commonly from a single nucleotide insertion of a cytosine in a string of cytosines within the coding region of *MUC1*, making reliable diagnosis by NGS problematic and therefore requiring the focused evaluation of *MUC1* when it is suspected to be the cause of disease [21]. In subject #22-1, a microdeletion in *PKD1* was identified by NGS testing and confirmed using an orthogonal technology called MLPA (Fig. 3).

Donor candidates

Thirty-four living-related donors (mean age: 33.9; range: 20–66; 74% <40 years) presented for genetic counseling and consideration of genetic testing (Table 2). Of these, 20 were children of transplant candidates and 11 were siblings. 20 of the 34 were screened for the familial genetic variant(s) identified after the recipient tested positive for a genetic renal disease. Seven potential donor candidates (30.5%) were carriers of a genetic variant associated with autosomal dominant disease and were advised against donation, while 12 did not carry the familial variant and were eligible to donate. In addition, a sibling of a patient with autosomal recessive nephronophthisis was a heterozygous carrier and was permitted to donate.

Discussion

We report our experience with testing of kidney waitlist candidates and their related LKDs for genetic renal disease in situations when a monogenic form of kidney disease was known or suspected in the waitlist candidate and an asymptomatic-related donor was considered to be at genetic risk. In this series, seven pre-symptomatic donor candidates related to four waitlisted persons were found to carry the same pathogenic or likely pathogenic variant as the recipient and were counseled about their own risk of disease and donation was avoided. Also, importantly, 13 at-risk donor candidates related to 11 waitlist persons screened negative for the familial variant, thus excluding, or significantly reducing risk of future disease and following counseling, these persons were permitted to donate. Six of seven donor candidates (siblings and children) who were at risk for ADPKD were under the age of 30, when ultrasonography has a negative predictive value of just about 90% [22]. In the remaining recipients, no genetic variant was identified

Table 1. Waitlist candidates who underwent genetic testing: showing demographics, presence or absence of family history, phenotypic class of presentation, genetic screen results, interpretation by ACMG criteria, and final genetic diagnosis.

Subject #	Sex/age/ethnicity	FH	Phenotypic class	Genetic screen	ACMG criteria [17,18]	Genetic diagnosis	Inheritance
1-1	F/49/EUR	Y	CAKUT	arr 17q12(31,802,146-33,287,364)x1	P: 1A, 2A	HNF1B nephropathy*	AD
2-1	M/55/EUR	Y	Cystic/ciliopathy	PKD1 p.Tyr2622Ter	LP: PVS1, PM2	ADPKD	AD
3-1	F/67/EUR	Y	Cystic/ciliopathy	PKD1 c.11488_11489insGCGACC	VUS: PM2, PM4, PP3	ADPKD	AD
4-1	M/43/EUR	Y	Glomerular	COL4A5 c.3657-9A>G	LP: PS3, PM2, PP5	XL Alport	XL
5-1	M/63/EUR	Y	Tubulointerstitial	Negative			
6-1	M/67/EUR	N	Glomerular	ARHGAP24 p.Gly493Arg	VUS: PM2, PP3	?FSGS-ARHGAP24	AD
7-1	M/59/EUR	N	Glomerular	Negative			
8-1	M/45/EUR	Y	Tubulointerstitial	MUC1 insC in VNTR	LP: PVS1, PS1, PM2, PP3	ADTKD	AD
9-1	M/28/EUR	N	Cystic/ciliopathy	PKD1 p.Glu2771Lys	P: PS1, PM1, PM2, PP3, PP5	ADPKD	AD
10-1	M/27/AFR	N	Glomerular	CFH p. p.Ser884Tyr	VUS: PM2, PP3	C3G	AD†
11-1	M/54/EUR	Y	Glomerular	Negative			
12-1	F/37/LAT	Y	Glomerular	Negative			
13-1	F/61/EUR	Y	Tubulointerstitial	Negative			
14-1	M/1/EUR	N	CAKUT	Negative			
15-1	F/59/EUR	Y	Glomerular	COL4A3 p.Gly934Arg	LP: PM1, PM2, PP1, PP3	Alport	AD
16-1	F/61/EUR	N	Glomerular	COL4A3 p.Gly121Ser	LP: PM1, PM2, PP3, PP5	Alport‡	AD
2-5	F/60/EUR	Y	Cystic/ciliopathy	PKD1 p.Tyr2622Ter	P: PVS1, PM1, PM2	ADPKD	AD
17-1	M/62/EUR	N	CAKUT	Negative			
18-1	M/58/EUR	N	Tubulointerstitial	Negative			
19-1	M/43/EA	Y	Glomerular	Negative			
20-1	F/75/EUR	Y	Cystic/ciliopathy	PKD1 p.Asp1165Gly	P: PM1, PM2, PP2, PP3	ADPKD	AD
21-1	F/66/EUR	Y	CAKUT	Negative			
22-1	M/39/EUR	Y	Cystic/ciliopathy	PKD1 chr 16: del2158468-2175817	P: PVS1, PM2	ADPKD	AD
23-1	F/34/EUR	N	Cystic/ciliopathy	Homozygous SDCCAG8 c.740+356C>T	LP: PM2, PP5(S)	Nephronophthisis§	AR

Ethnicity: AFR, African; EA, East Asian; EUR, European; LAT, Latino.

ACMG criteria: LP, likely pathogenic; P, pathogenic; VUS, variant of unknown significance.

Inheritance: AD, autosomal dominant; AR, autosomal recessive; XL, X linked recessive.

ADPKD: autosomal dominant polycystic kidney disease; ADTKD: autosomal dominant tubulointerstitial disease; CAKUT: congenital abnormality of kidney and urinary tract development; C3G= C3 glomerulopathy; FSGS= focal segmental glomerulosclerosis.

*Partial agenesis of pancreas present by MR.

†Incompletely penetrant.

‡IgA nephropathy by biopsy.

§Retinal dystrophy also present (Senior Loken Syndrome).

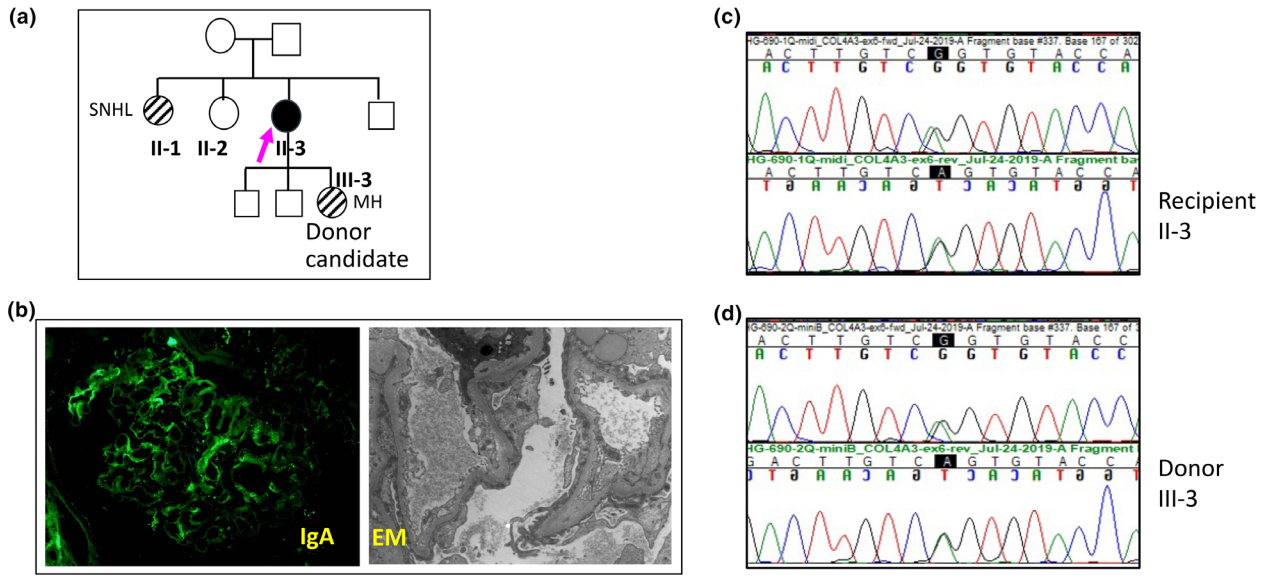

Figure 1 IgA nephropathy. (a) Pedigree, SNHL, sensorineural hearing loss; MH, microscopic hematuria, pink arrow indicates transplant candidate. (b) Left—immunofluorescence (IF) microscopy (40×): glomerulus with positive IgA mesangiocapillary staining. Right—transmission electron microscopy (10 000× direct magnification): glomerulus shows irregular thinning and thickening of the capillary basement membranes. There is vague increase in subendothelial laminar rarefactions, but without any overt evidence of lamellation or "basket weaving." Patchy, variable podocyte epithelial foot process effacement is also noted. In other fields (not depicted), immune-complex-mediated type electron-dense deposits which corresponded to the positive IgA by IF were confirmed, consistent with IgA nephropathy. (c and d) Sanger sequencing chromatograms showing the single nucleotide change, COL4A3 p.Gly121Ser in the recipient and the prospective donor.

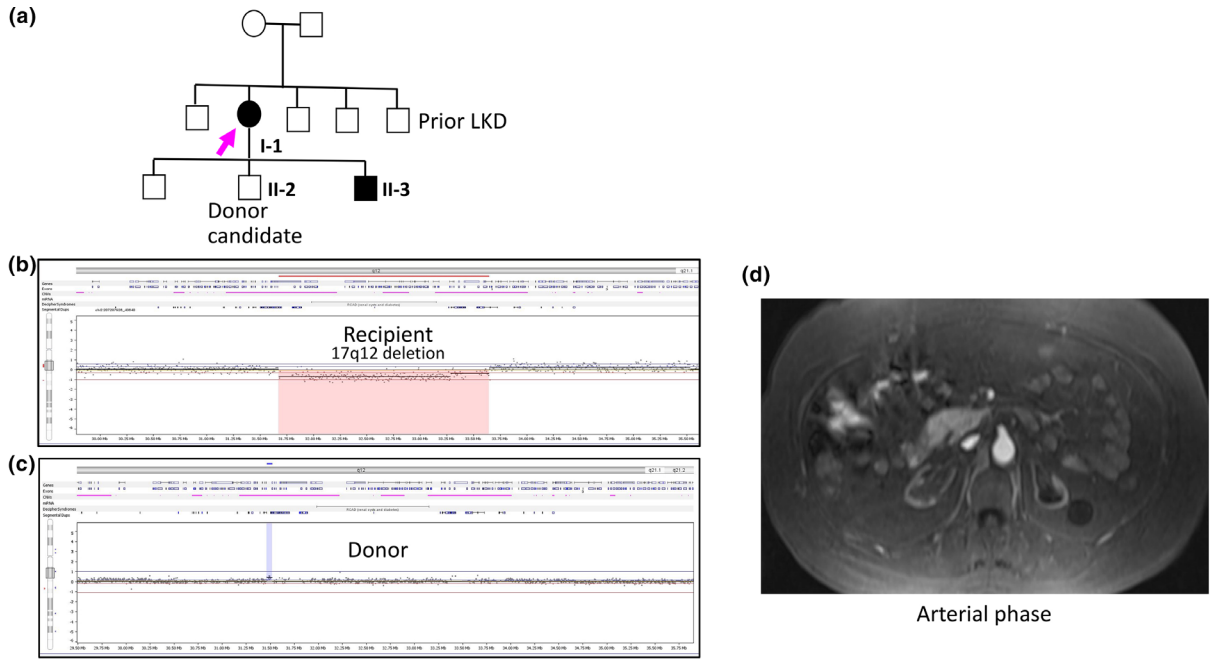

Figure 2 HNF1B CNV. (a) Pedigree of affected proband (I-1) including her previous related LKD and her two sons, one of which was affected (II-3) and positive for the same recurrent 17q12 microdeletion and the other unaffected son (II-2) who was under consideration as a LKD. (b) CMA data of the proband exhibiting the recurrent 17q12 microdeletion. (c) CMA data of the proband's unaffected son (II-2) showing no evidence of the recurrent microdeletion. (d) Contrast-enhanced arterial phase axial MR image of the proband demonstrates normal pancreatic head but absence of pancreatic parenchyma in the expected locations of the body and tail.

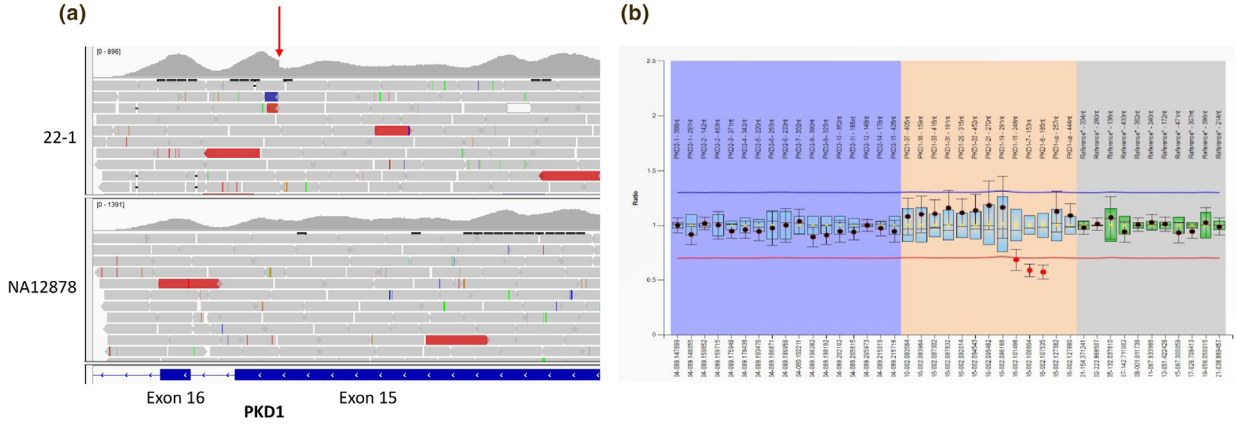

Figure 3 PKD1 microdeletion. (a) Integrative genomic viewer (IGV) data tracks of a portion of the PKD1 gene sequence data for subject # 22-1 and NA12878 control. Red arrow indicates the approximate breakpoint in exon 15. The number by the data tracks indicates the number of reads aligned to the region depicted: 893 reads for subject 22-1 and 1391 reads for the control sample (NA12878); this difference is indicative of a heterozygous deletion across the region and was observed to extend from exon 2 to exon 15. (b) MLPA confirmation of PKD1 deletion in subject 22-1: Ratio chart of the results for subject 22-1 analyzed with P352 MLPA kit. *X*-axis, map view locations, *Y*-axis, results ratios for PKD2, PKD1 and several reference probes in chromosomes 1, 2, 5, 7, 8, 11, 13, 15, 17, 19, and 21. Blue (1.3) and red (0.7) lines ratio indicate threshold for copy gain and loss, respectively. The ratios for the probes corresponding to exons 7, 6, and 15 showed allele loss.

despite comprehensive testing, significantly reducing the probability of known genetic renal disease. This information was used to counsel donors who were then able to make their decision with more confidence.

Genetic testing of waitlist candidates and living-related donors for monogenic renal disease is generally not part of the typical donor evaluation process. Sometimes, the cause of the kidney disease in the recipient candidate is not known and is not readily ascertained, as the referral to the transplant center generally comes from a dialysis provider rather than the nephrology practice that cared for the patient. In addition, a renal biopsy may not have been done because of its limited value in advanced renal disease. The transplant candidates' cause of ESKD may be gleaned from CMS 2728, which determines Medicare entitlement and often has inaccuracies in the noted primary cause of renal failure [23,24]. It is important to note that the Scientific Registry of Transplant Recipients (SRTR) 2019 annual data report lists 17.6% of transplant waitlist candidates with 'other' as the etiology of ESKD [25].

There are several reasons to determine the transplant candidates' cause of ESKD. First, for a disease like focal and segmental glomerulosclerosis (FSGS), determining that it is monogenic in etiology would indicate a substantially lower likelihood of disease recurrence post-transplant [26,27]. Second, for some genetic diseases, appropriate perioperative and post-transplant management may be necessary to avoid early graft loss. For example, with complement-mediated hemolytic uremic syndrome (HUS) perioperative and post-operative use of eculizumab can successfully prevent disease recurrence [28,29]. For ESKD secondary to primary hyperoxaluria, a combined liver-kidney transplant rather than a kidney transplant alone provides the best chance for kidney allograft survival [30]. Third, confirmation of certain genetic diseases may help in recognizing additional manifestations of the identified disease. For example, the enigmatic and erratic tacrolimus drug levels in subject #I-1 were eventually attributed to exocrine pancreatic insufficiency secondary to partial agenesis of the pancreas, part of the syndromic manifestations of *HNF1B*-mediated disease (Fig. 2). Fourth, identifying the cause of kidney failure in the waitlisted candidate is necessary to determine whether the living donor needs additional testing, as we demonstrate in this series [31]. The most recent living donor guidelines from KDIGO state that when the intended recipient is genetically related to the donor candidate, the cause of the intended recipient's kidney failure should be determined whenever possible [32].

It is difficult to complete an accurate donor risk assessment for a familial disease without genetic testing. While donor evaluation at most transplant centers typically includes an extensive medical, surgical, and psychosocial history, physical examination, laboratory testing, and diagnostic imaging, genetic testing is not routinely included. In our clinical practice at the University of Iowa, we have been testing selected living donors for inherited renal disease when the cause of

Table 2. Outcome of genetic testing in related donor candidates: showing demographics, donor relationship to waitlist candidate, genetic screen results, and impact of genetic screen results.

Subject no	Sex/age/ethnicity	Donor relationship	Familial variant	Donor genetic screen	Impact of genetic screen
1-2	M/33/EUR	Child	HNF1B	Negative for familial deletion	Counseled in favor of donation
2-2	F/22/EUR	Child	PKD1	Negative for familial variant	Counseled in favor of donation
2-3	M/20/EUR	Child	PKD1	Positive for familial variant	Counseled against donation
2-4	M/26/EUR	Child	PKD1	Negative for familial variant	Counseled in favor of donation
3-2	F/25/EUR	Child	PKD1	Negative for familial PKD	Allowed to donate
4-2	F/36/EUR	Sibling	COL4A5	Negative for familial Alport	Allowed to donate
5-2	M/29/EUR	Child	nd	Not performed	Donor given choice
6-2	M/25/EUR	Child	ARHGAP24	Positive for familial variant	Counseled against donation
6-3	M/35/EUR	Child	ARHGAP24	Positive for familial variant	Counseled against donation
6-4	F/33/EUR	Child	ARHGAP24	Negative for familial variant	Counseled in favor of donation
7-2	F/22/EUR	Child	nd	Not performed	Donor given choice
8-2	M/41/EUR	Sibling	MUC1	Negative for familial variant	Counseled in favor of donation
8-3	F/45/EUR	Sibling	MUC1	Negative for familial variant	Counseled in favor of donation
9-2	F/27/EUR	Sibling	PKD1	Negative for familial variant	Counseled in favor of donation
10-2	F/47/EUR	Aunt	CFH	Negative for familial variant	Counseled in favor of donation
11-2	M/23/EUR	Child	nd	Not performed	Donor given choice
12-2	M/35/LAT	Sibling	nd	Not performed	Donor given choice
13-2	F/41/EUR	Child	nd	Not performed	Donor given choice
14-2	M/36/EUR	Parent	nd	Not performed	Donor given choice
15-2	M/51/EUR	Sibling	COL4A3	Positive for familial variant	Counseled against donation
15-3	M/54/EUR	Sibling	COL4A3	Positive for familial variant	Counseled against donation
15-4	F/66/EUR	Sibling	COL4A3	Positive for familial variant	Counseled against donation
16-2	F/41/EUR	Child	COL4A3	Positive for familial variant	Counseled against donation
2-6	F/33/EUR	Child	PKD1	Negative for familial PKD	Counseled in favor of donation
17-2	F/33/EUR	Child	nd	Not performed	Donor given choice
17-3	M/27/EUR	Child	nd	Not performed	Donor given choice
18-2	F/27/EUR	Child	nd	Not performed	Donor given choice
18-3	F/29/EUR	Child	nd	Not performed	Donor given choice
18-4	M/22/EUR	Child	nd	Not performed	Donor given choice
19-2	F/34/EA	Sibling	nd	Not performed	Donor given choice
20-2	M/34/EUR	Grandchild	PKD1	Negative for familial variant	Counseled in favor of donation
21-2	F/46/EUR	Child	nd	Not performed	Donor given choice
22-2	F/32/EUR	Sibling	PKD1	Not performed	Patient transplanted prior to donor screening
23-2	F/36/EUR	Sibling	SDCCAG8	Heterozygous carrier of recessive variant	Counseled in favor of donation

Ethnicity: EA, East Asian; EUR, European; LAT, Latino.

nd, not detected.

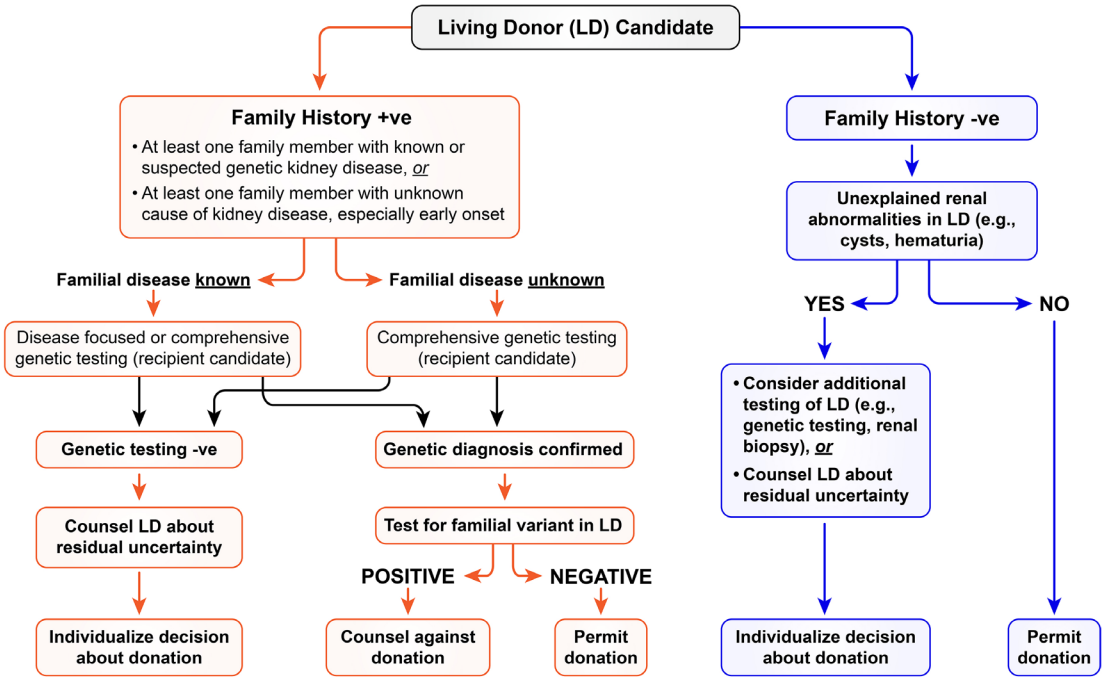

Figure 4 Flow chart depicting a systematic approach to genetic testing of living donor (LD) candidates. A positive family history (FH) is defined as kidney disease in a parent, grandparent, sibling, or child. Generally, when testing donor for genetic disease, the choice of test is driven by the knowledge of the familial renal disease. Genetic testing in asymptomatic or minimally symptomatic LD candidates should not be done without appropriate counseling about the implications of test results. This schematic does not include ancestry-based testing for kidney disease risk alleles such as APOL1 renal risk variants or the sickle cell trait.

renal disease in their related transplant candidate is suspected or known to be genetic in nature [31]. The transplant candidate is first seen and evaluated by a genetic counselor and then screened with a targeted comprehensive renal gene panel [33]. If a genetic etiology is identified that information is used to screen the related living donor. The genetic test results in the donor are then used for counseling and to determine whether the donor candidate can proceed to donation. A simplified flowchart outlines a systematic approach to genetic testing of living donor candidates (Fig. 4). In addition, in donors of appropriate ancestry with or without a family history of renal disease, we offer testing for sickle cell trait and APOL1 risk alleles [31,34–36]. The costs of genetic testing of recipient and donor candidates are covered by recipient insurance or recorded as part of the organ acquisition cost on the Medicare cost report similar to any other test required to determine a living donor's medical eligibility [37].

In an unselected series of patients with ESKD who underwent exome sequencing, as many as 10% had an identifiable cause of genetic disease, although this number may be an underestimate as the bioinformatic pipeline did not include an analysis for PKD1 variants or for CNVs in any relevant renal disease gene [38]. In another cohort specifically referred for genetic testing, we identified monogenic kidney disease in 43% of patients by comprehensive genetic testing of genes causally implicated in renal disease; 14% of diagnosed patients had pathogenic CNVs [15]. In another series of patients seen in a renal genetics clinic, exome sequencing confirmed a genetic diagnosis in 39% of patients [39]. Importantly, alternative or orthogonal technologies are sometimes necessary to confirm monogenic kidney disease, as in the case of MUC1-mediated disease or when chromosomal microdeletions are suspected to be the cause of disease. Physicians and genetic counselors need to be aware of the limitations of genetic testing by next-generation sequencing and to know when additional diagnostic techniques may be necessary.

There are several limitations to this study. First, this is a single-center retrospective series of a small number of patients, and therefore, these results may not be generalizable. Second, there were no formal criteria to identify waitlist candidates for genetic testing or a systematic sequence for testing, but most candidates were selected

after a related, typically younger, person volunteered to be a donor. Third, we did not formally determine whether donors found the information helpful in their decision-making process, although we know that without genetic testing many younger donors with a family history of certain genetic conditions like ADPKD would have otherwise been advised against donation by the transplant center even with a negative ultrasound because of the limitations of imaging at a younger age.

In conclusion, this series of renal transplant candidates and related living donors who have received genetic testing is the largest to date and appears to demonstrate the value of testing selected transplant candidates to exclude monogenic disease and allow donation to proceed safely. This series builds on our experience where we demonstrated the feasibility of a comprehensive testing strategy for evaluation of candidates and donors, and the integration of a renal genetics service with a genetics counselor into the renal transplant clinic for efficient deployment of genetic testing [14,33]. Current practice may err on the side of caution when uncertainty exists and deny a candidate the opportunity to undergo donor nephrectomy (as in the case of young donors with family history of ADPKD). In other instances, demonstrating genetic risk in asymptomatic, clinically normal individuals where no other test can provide such confirmation enhances donor safety by avoiding inadvertent nephrectomy in individuals destined to develop genetic kidney disease. Finally, the absence of genetic findings in recipients after unbiased comprehensive genetic screening substantially diminishes the likelihood of future genetic disease in related LKDs and provides prognostic information that refines risk estimates and facilitates donor counseling.

Authorship

CPT: conceived and designed the study. MID, MAM and BWD: contributed analytic tools. SG, DGH, BWD, MID, MAM and CPT: gathered and analyzed the data. MAM, RJS and CPT: interpreted genetic test results. SG and CPT: wrote the manuscript with contributions from MEF, KC, MID, DAK, MAM and RJS. Each author contributed content during manuscript drafting or revision, accepts personal accountability for the author's own contributions, and agrees to ensure that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved. All authors approved the final version of the manuscript.

Funding

No external funds supported this work.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

None.

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