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Screening of Living Kidney Donors for Genetic Diseases Using a Comprehensive Genetic Testing Strategy

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Related living kidney donors (LKDs) are at higher risk of end-stage renal disease (ESRD) compared with unrelated LKDs. A genetic panel was developed to screen 115 genes associated with renal diseases. We used this panel to screen six negative controls, four transplant candidates with presumed genetic renal disease and six related LKDs. After removing common variants, pathogenicity was predicted using six algorithms to score genetic variants based on conservation and function. All variants were evaluated in the context of patient phenotype and clinical data. We identified causal variants in three of the four transplant candidates. Two patients with a family history of autosomal dominant polycystic kidney disease segregated variants in *PKD1*. These findings excluded genetic risk in three of four relatives accepted as potential LKDs. A third patient with an atypical history for Alport syndrome had a splice site mutation in *COL4A5*. This pathogenic variant was excluded in a sibling accepted as an LKD. In another patient with a strong family history of ESRD, a negative genetic screen combined with negative comparative genomic hybridization in the recipient facilitated counseling of the related donor. This genetic renal disease panel will allow rapid, efficient and cost-effective evaluation of related LKDs.

Abbreviations: ADPKD, autosomal dominant polycystic kidney disease; CAKUT, congenital anomaly of the kidney and urinary tract; CKD, chronic kidney disease; ESRD, end-stage renal disease; FSGS, focal segmental glomerulosclerosis; HNF1B, hepatocyte nuclear factor 1β; LKD, living kidney donor; MAF, minor allele frequency; MPS, massively parallel sequencing; MRI, magnetic resonance imaging; NGS, next-generation sequencing; PCR, polymerase chain reaction; VUS, variant of unknown significance; WES, whole-exome sequencing

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Introduction

Kidney transplantation is superior to long-term dialysis for the management of end-stage renal disease (ESRD) because it provides greater long-term survival and better quality of life. Nevertheless, there is an ever-increasing gap between the need for transplantation and the availability of donor kidneys, with >120 000 patients currently on the deceased donor waitlist in the United States alone. This has resulted in an increasing push to encourage living donation, and today there are almost as many living donors as deceased donors annually in the United States (1). Living kidney donor (LKD) transplants, for those fortunate to receive one, bypass the long waiting time, reduce the likelihood of death while waiting and provide better long-term allograft and recipient survival compared with deceased donor kidneys (2,3). In some parts of the world, LKDs are the principal or only source of transplanted organs, and where long-term dialysis is

prohibitively expensive or unavailable, LKD transplants provide the only available therapy for ESRD.

Living donor nephrectomy is generally considered acceptable medical practice, even though there are real risks for the donor, including death, serious injury and failure of the remaining kidney. Recent retrospective studies examining long-term outcomes of living donation compared with matched nondonor cohorts reported an increased 15-year and lifetime risk of ESRD for LKDs (4,5). Although the absolute risk is arguably small, the relative risk is 30 per 10 000 over 15 years and 90 per 10 000 over a lifetime compared with four per 10 000 and 14 per 10 000 in matched controls. Within subpopulations, black men have a 15-year risk of 90 per 10 000 compared with just nine per 10 000 for white women (4). Although not statistically significant, there is a twofold increased risk of ESRD among biologically related LKDs compared with unrelated LKDs (4). The increased risk may reflect shared inheritance of genetic variants that are deleterious or a common environmental exposure that increases susceptibility to kidney disease.

In the United States, 40% of all LKDs are biologically related to their recipients (1). Many are siblings or adult children of patients with ESRD and are in their third and fourth decades of life, making it difficult to predict future risk of kidney disease. In addition, to guide focused genetic testing of related family members for a specific inherited disease, the transplant recipient's cause of ESRD must be known. Together, diabetes and hypertension are the two most important reported causes of ESRD and account for 60% of the waitlist (1,6). Most patients with diabetes and/or hypertension and chronic kidney disease (CKD) do not receive a kidney biopsy to verify the diagnosis, and recent studies estimated that as many as 35% of patients with presumed diabetic or hypertensive nephropathy may actually have an alternative diagnosis (7–9).

Traditionally, establishing and/or confirming the diagnosis of a presumed genetic disease has required Sanger sequencing of the suspected gene for pathogenic variants (10). When candidate genes are large, like *COL4A5*, sequencing is costly and time consuming. When the disease is heterogeneous, like focal segmental glomerulosclerosis (FSGS), serial gene-by-gene screening approaches are inefficient and impractical. These constraints can be largely overcome by using high-throughput approaches to DNA sequencing (i.e. next-generation sequencing [NGS] or massively parallel sequencing [MPS]) to sequence a large number of genes simultaneously. Targeted NGS panels have been developed to evaluate patients with a single phenotype, such as steroid-resistant nephrotic syndrome, FSGS and some ciliopathies (11–14).

We developed a targeted renal panel that includes 115 genes implicated in a variety of kidney diseases to facilitate a diagnosis in patients with suspected genetic renal disease. We validated this panel for the evaluation of selected LKDs in whom the related transplant recipient's phenotype raised suspicion of or clearly indicated an inherited renal disease. We reported our findings from a pilot study of six controls, four transplant candidates and their six related donors.

Methods

Patient selection

Renal transplant candidates referred to the Organ Transplant Center at the University of Iowa were recruited to the study if they had a known or suspected genetic renal disease and had an asymptomatic younger biological relative who volunteered to be an LKD. Clinical and laboratory data were obtained from the medical record or from patient interviews. Control samples were unrelated persons with no medical or familial history of renal disease. The study was approved by the institutional review board (IRB no. 201301818) for human subject research.

Targeted gene panel

A set of 115 genes implicated in a variety of genetic renal diseases was assembled by enumerating renal phenotypes (e.g. ciliopathy, FSGS, and congenital anomaly of the kidney and urinary tract [CAKUT]) and then assembling a list of known causal genes by literature review. Genes that are implicated in the development of atypical hemolytic-uremic syndrome and other complement-mediated glomerular diseases were excluded from this panel. Targeted genomic enrichment and MPS of these 115 genes (hereafter referred to as KidneySeq) was completed as described (genes included in this panel are shown in Tables 1 and S3). Genomic DNA was assessed for quality by gel electrophoresis and spectrophotometry (260/280 ratio of 1.8–2.2; Nanodrop 1000; Thermo Fisher Scientific, Waltham, MA) and quantity by fluorometry (Qubit 2.0 fluorometer; Life Technologies, Carlsbad, CA). Libraries were prepared using a modification of the solution-based Agilent SureSelect target enrichment system (Agilent Technologies, Santa Clara, CA) using liquid-handling automation equipment (Perkin Elmer, Waltham, MA). In brief, 3 μg of genomic DNA was randomly fragmented to an average size of 250 bp (Covaris Acoustic Solubilizer; Covaris Inc., Woburn, MA), fragment ends were repaired, A-tails were added, and sequencing adaptors were ligated before the first amplification. Solid-phase reverse immobilization purifications were performed between each enzymatic reaction. Hybridization and capture with RNA baits were followed by a second amplification before pooling for sequencing. Minimal amplification was used, typically six cycles for the prehybridization polymerase chain reaction (PCR) and 14 cycles for the posthybridization PCR, using Agilent Herculase II Fusion DNA Polymerase (Agilent Technologies). All samples were bar coded and multiplexed before sequencing on an Illumina MiSeq in pools of five (Illumina Inc, San Diego, CA; performance metrics are shown in Table S1).

Bioinformatic analysis

Data storage and analysis were performed on dedicated computing resources maintained by the Iowa Institute of Human Genetics at the University of Iowa. Sequencing data were archived as fastq files on a secured storage server and then analyzed using locally implemented open source Galaxy software on a high-performance computing cluster (15). The workflow for variant calling integrated publicly available tools: Reads were mapped using Burrows–Wheeler alignment (BWA–MEM) against human reference genome GRCh37/hg19; duplicates were removed by Picard; realignment, calibration and variant calling were performed with the Genome Analysis Toolkit; and variant annotation was

Table 1: Genes implicated in genetic renal diseases and screened by targeted genomic enrichment and massively parallel sequencing

Gene	Accession number	Locus/ alternative name	Exon count
ACTN4	NM_004924		21
AE1	NM_000342	SLC4A1	20
AGTR2	NM_000686		3
AGXT	NM_000030		11
AHI1	NM_001134830	JBTS3	27
ALMS1	NM_015120		23
APOL1	NM_001136540	FSGS4	6
APRT	NM_000485		5
AQP2	NM_000486		4
ARL13B	NM_001174150	JBTS8	10
ARL6	NM_001278293	BBS3	8
ATP6V0A4	NM_020632	ATP6N1B	22
AVPR2	NM_000054		3
BBS1	NM_024649		17
BBS2	NM_031885		17
BBS4	NM_001252678		15
BBS5	NM_152384		12
BBS7	NM_018190		18
BMP4	NM_001202		4
BSND	NM_057176		4
CaSR	NM_000388		7
CC2D2A	NM_001080522	JBTS9	38
CD2AP	NM_012120		18
CEP290	NM_025114	JBTS5, MKS4, NPHP6	54
CLCN5	NM_000084	CLC5	12
CLCNKA	NM_004070		20
CLCNKB	NM_000085		20
CLDN16	NM_006580	HOMG3	5
CLDN19	NM_001123395	HOMG5	4
CNNM2	NM_017649	HOMG6	8
COL4A1	NM_001845		52
COL4A3	NM_000091		52
COL4A4	NM_000092		48
COL4A5	NM_000495		51
COQ2	NM_015697		7
CREBBP	NM_001079846		30
CTNS	NM_001031681		13
CUL3	NM_001257197		15
DHCR7	NM_001163817		9
EGF	NM_001178130	HOMG4	23
EYA1	NM_000503		18
FGF23	NM_020638		3
FN1	NM_002026		46
FRAS1	NM_001166133		42
FREM2	NM_207361		24
GATA3	NM_001002295		6
GLA	NM_000169		7
GLI3	NM_000168		15
GLIS2	NM_032575	NPHP7	6
GPC3	NM_001164617		9
GRHPR	NM_012203		9
HNF1B	NM_000458		9
HOGA1	NM_138413	DHDPSL	7
IFT80	NM_001190241		21
INF2	NM_001031714	FSGS5	22
INPP5E	NM_019892	JBTS1	10
INVS	NM_014425	NPHP2	17
IQCB1	NM_001023570	NPHP5	15
KAL1	NM_000216		14
KCNJ1	NM_153766	ROMK1	3
KLHL3	NM_001257194		15
LAMB2	NM_002292		32
LMX1B	NM_001174146		8
MKKS	NM_170784	BBS6	6
MKS1	NM_001165927		18
MYH9	NM_002473		41
NEK8	NM_178170	NPHP9	15
NLRP3	NM_001079821		11
NPHP1	NM_000272	JBTS4	20
NPHP3	NM_153240		27
NPHP4	NM_001291593		27
NPHS1	NM_004646		29
NPHS2	NM_001297575		7
NR3C2	NM_000901		9
OCRL1	NM_000276		24
OFD1	NM_003611	JBTS10	23
PAX2	NM_000278		10
PHEX	NM_000444		22
PKD1	NM_000296	ADPKD-1	46
PKD2	NM_000297	ADPKD-2	15
PKHD1	NM_138694		67
PLCE1	NM_001165979	NPHS3	32
REN	NM_00537		10
RET	NM_020630		19
RPGRIP1L	NM_001127897	JBTS7, NPHP8, MKS5	25
SALL1	NM_001127892		3
SALL4	NM_020436		4
SCNN1A	NM_001038		13
SCNN1B	NM_000336		13
SCNN1G	NM_001039		13
SIX1	NM_005982		2
SIX2	NM_016932		2
SIX5	NM_175875		3
SLC12A1	NM_000338	NKCC2	27
SLC12A3	NM_000339	NCCT	26
SLC26A4	NM_000441		21
SLC34A1	NM_001167579	NPT2a	9
SLC34A3	NM_001177316	NPT2C	13
SLC3A1	NM_000341		10
SLC4A4	NM_001098484		26
SLC7A9	NM_001126335		13
SMARCAL1	NM_001127207		18
TCTN1	NM_001082537	JBTS13	15
TMEM216	NM_001173990	JBTS2, MKS2	5
TMEM237	NM_001044385	JBTS14	12
TMEM67	NM_001142301	JBTS6, MKS3, NPHP11	29

(*continued*)

Table 1: Continued

Gene	Accession number	Locus/ alternative name	Exon count
TRPC6	NM_004621	FSGS2	13
TTC21B	NM_024753	JBTS11	29
TTC8	NM_144596	BBS8	15
UMOD	NM_001008389		11
UPK3A	NM_001167574		4
WNK1	NM_001184985		28
WNK4	NM_032387		19
WNT4	NM_030761		5
WT1	NM_000378		9

performed with a CLCG annotation and reporting tool developed by our bioinformatics team (16–18).

Variant prioritization and Sanger validation

The total number of reads per sample varied as a function of the number of samples per run and DNA input per sample. Low-quality variants (depth <10 or QD <5) were filtered out by quality control. Common variants with minor allele frequency (MAF) >1% in any population were excluded (based on the National Heart, Lung, and Blood Institute GO Exome Sequencing Project [http://evs.gs.washington.edu], the 1000 Genomes Project [http://www.1000genomes.org] and the Exome Aggregation Consortium [http://exac.broadinstitute.org]) unless the variant was a known risk allele. Variants also were filtered based on predicted effect, retaining nonsynonymous single-nucleotide variants, canonical splicing changes and indels, which were prioritized based on MAF, nucleotide conservation, reported functional and expressive impact, and phenotype correlation. Reference databases that were routinely queried included the Human Gene Mutation Database, ClinVar and our in-house renal variant database. GERP++ (19), PhyloP (20), MutationTaster (21), PolyPhen-2 (22), SIFT (23) and likelihood ratio tests (24) were used to calculate variant-specific pathogenicity scores based on the sum of tools predicting a given variant to be deleterious. All reported variants were Sanger validated, as were specific portions of the KidneySeq panel not amenable to targeted genomic enrichment (Table S2).

Variant interpretation

To provide a clinically relevant report, a multidisciplinary board (KidneySeq group meeting) reviewed all genetic data in the context of the available clinical data (Table 3) (case descriptions follow). Standards developed by the American College of Medical Genetics were used to assign variants to one of five categories: pathogenic, likely pathogenic, variant of uncertain significance (VUS), likely benign and benign (25). Variants with MAF >1% known to be unrelated to disease were classified as "benign." Variants with an allele frequency greater than expected for the disease and for which computational evidence suggested low likelihood of pathogenicity were classified as "likely benign." Ultrarare variants reported as pathogenic in the literature and with supporting functional evidence were classified as "pathogenic." Null variants, such as partial or whole gene deletions, frame-shift mutations, initiation codon mutations, splice-site mutations (+1 or −1 or −2) and truncation mutations (if the stop codon was not in the terminal exon) that segregated with disease were classified as "pathogenic" when loss of function was a known mechanism of disease. Novel or rare missense variants that have an unknown impact on protein function were classified as either "likely pathogenic" or "VUS," a distinction that reflected two considerations: likely pathogenic variants were also (i) missense variants with pathogenicity scores ≥5 (based on GERP++, PhyloP, MutationTaster, PolyPhen-2, SIFT and LRT), ultrarare (MAF <0.00001%) and found in disease-related functional domains or loci or (ii) novel and caused loss of function. Based on genotypic findings and the clinical phenotype, additional testing was occasionally recommended.

Results

Massively parallel sequencing

The targeted regions of 115 candidate genes on KidneySeq covered ≈0.58 Mb of the genome (Table 1). On average, 4.4 million sequence reads per sample were generated for a mean depth of coverage of 586× with >99% of targeted regions covered at ≥10× (Table S1). Approximately 500 variants were detected per sample. These variants were annotated and filtered to identify high-quality rare and novel variants (Table 2). For each sample, we also identified regions with <10× coverage if they were associated with the disease phenotype (Table S2).

Sanger sequencing

For confirmation purposes, exons carrying a variant determined to be pathogenic were Sanger sequenced (Table 3). Primers for PCR and for sequencing were designed using Primer 3 and are available upon request (26). In addition, the duplicated regions of the *PKD1* gene (exons 1–34) were Sanger sequenced using published primers in those patients with suspected polycystic kidney disease (27).

Patients and KidneySeq multidisciplinary group meetings

Four transplant candidates with their six related LKDs participated in this study. The cohort included two patients with autosomal dominant polycystic kidney disease (ADPKD), one patient with suspected Alport syndrome and one patient with presumed hypertensive nephropathy who had a sibling with ESRD, raising suspicion of a genetically undefined inherited kidney disease (Figure 1). All patients and donors were white; the patients ranged in age from 40 to 63 years, and the donor candidates ranged in age from 20 to 36 years.

Case 1: The first patient was diagnosed with ADPKD in her early 50s when workup for a urinary tract infection in the setting of family history of ADPKD revealed multiple cysts in bilaterally enlarged kidneys (Figure 1A). She presented for transplant evaluation at age 63 years, and a daughter aged 25 years wished to be evaluated as a living donor. Genetic testing of the transplant candidate revealed a heterozygous 6-bp insertion in exon 41 of *PKD1*, which resulted in the in-frame insertion of Ala-Thr. This insertion has not been reported in the ADPKD Mutation Database (http://pkdb.mayo.edu) or in population databases. Segregation analysis identified this insertion in the patient's affected brother and in two other affected daughters. Based on the change in protein length, absence of controls, cosegregation with disease and close proximity of this in-frame insertion to another in-frame insertion classified as pathogenic in the ADPKD Mutation Database, this variant was classified as

Table 2: Variant filtering for the samples and controls included in this study

	Case 1	Case 2	Case 3	Case 4	Control 1	Control 2	Control 3	Control 4	Control 5	Control 6
Total number of variants	421	546	471	515	561	566	509	523	523	466
Quality filter (Q_VAR >50, QD >5 and observed % >30)	385	522	433	489	527	532	490	499	500	445
Rarity filter MAF <1%	8	30	11	14	44	19	42	23	12	16
Functional filters (exonic, nonsynonymous, splice)	2	7	4	5	5	5	6	5	5	5

Q_VAR, quality of the variant (quality of the identification of the nucleotide generated by automated DNA sequencing); QD, Phred-like quality score divided by depth; MAF, minor allele frequency.

Table 3: Transplant candidates tested with KidneySeq

Case	Clinical diagnosis	Result	Genotype	Genetic diagnosis
1	ADPKD	Positive	*PKD1*—NM_000296:c.7866C>G, p.Tyr2622Stop	ADPKD
2	Alport syndrome/FSGS	Positive	*COL4A5*—NM_000495:c.3604+1G>A	Alport syndrome
3	ADPKD	Positive	*PKD1*—NM_000296:c.11488_11489insGCGACC	ADPKD
4	CKD	No finding		

This table shows clinical diagnosis and genotype findings for the four transplant candidates tested in this pilot study. ADPKD, autosomal dominant polycystic kidney disease; CKD, chronic kidney disease; FSGS, focal segmental glomerulosclerosis.

"likely pathogenic." The donor candidate was negative for the insertion and was accepted to continue her donor evaluation. Unfortunately, the transplant candidate developed major complications from peripheral vascular disease, and that has precluded her transplant.

Case 2: The second patient was diagnosed with ADPKD in his late 30s when workup for severe hypertension in the setting of a positive family history of ADPKD revealed bilateral enlarged cystic kidneys (Figure 1B). He presented for a transplant evaluation at age 51 years, and his three children, aged 20, 22, and 25 years, wished to be evaluated as living donors. Genetic testing of the transplant candidate revealed a nonsense mutation in exon 21 of *PKD1* (p.Tyr2622X) that has been reported to be pathogenic (28). Pre- and posttest genetic counseling was provided to the candidate's three unaffected children. The mutation segregated in the family, and two of the three children were negative for the mutation. The 25-year-old son completed his evaluation and had normal urinalysis, normal kidney function, and no kidney cysts on computed tomography angiography. He underwent donor nephrectomy, and both recipient and donor are doing well.

Case 3: The third transplant candidate presented at age 40 years for an evaluation together with his sister, who wished to be considered as a donor (Figure 1C). The patient had had an earlier renal transplant that lasted 17 years. He first presented at age 18 years when hematuria and proteinuria were noted on an athletic physical examination. A renal biopsy at the time showed FSGS on light microscopy with segmental mesangial and glomerular capillary loop staining for IgM and C3 and glomerular basement membrane lamellations with segmental thickening and thinning on electron microscopy. Ophthalmology examination showed anterior lenticonus and mild retinal pigmentary epithelial clumping, but an audiogram showed no deafness. His mother has proteinuria and hematuria, and his maternal grandmother had "Bright's disease." The clinical picture with laboratory data was consistent with an X-linked or autosomal dominant hereditary nephritis suggestive of Alport syndrome, although hereditary FSGS was also a possibility. Genetic testing identified a splice site mutation in intron 38 of *COL4A5* (3657-9A>G). This variant has been reported as pathogenic, confirming X-linked Alport syndrome (29). The 35-year-old sister had negative urinalysis and a negative slit lamp examination and was negative for the splicing mutation. She was accepted as a donor but was blood type incompatible so is awaiting a match in the paired kidney donor program.

Case 4: The fourth case was a man aged 59 years who presented for a transplant evaluation with his 30-year-old son, who wished to be his living donor (Figure 1D). The patient had hypertension and advanced CKD with hematuria and proteinuria on dipstick testing. An ultrasound at first presentation several years earlier was noted to show a few small scattered cysts in both kidneys, consistent with hypertensive nephrosclerosis with acquired cysts, although other tubulointerstitial kidney diseases could not be ruled out. The patient's

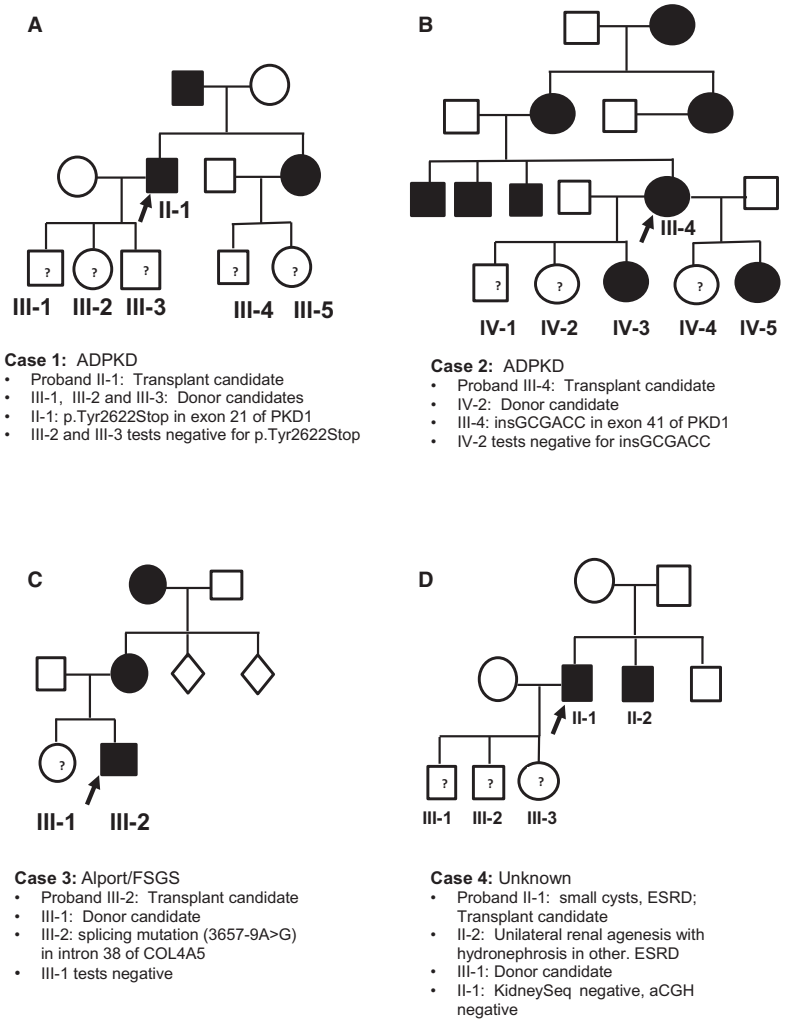

Figure 1: Pedigree chart of candidates and donors tested. Transplant candidates are shown as the probands. ADPKD, autosomal dominant polycystic kidney disease; ESRD, end-stage renal disease; FSGS, focal segmental glomerulosclerosis.

younger sibling had presented at age 37 years with advanced CKD, an absent left kidney and right-sided hydronephrosis on ultrasound. On retrograde pyelography, this sibling had moderate right-sided caliectasis with a possible filling defect in the ureter and narrowing consistent with obstructive right-sided urolithiasis or congenital ureteropelvic junction obstruction or unilateral vesicoureteric reflux. The left ureteric orifice was cannulated and appeared to have a blind end within 1 cm, consistent with an involuted multicystic dysplastic kidney or left-sided renal agenesis.

In these two siblings, we considered disease associated with hepatocyte nuclear factor 1β (*HNF1B*) presenting as interstitial kidney disease in one and as a CAKUT in the other. Comprehensive renal gene panel testing in the transplant candidate did not identify any likely pathogenic variants in any of the genes on KidneySeq, including *HNF1B*. Of note, copy number variant analysis of *HNF1B* was normal, a relevant finding because about half of *HNF1B*-associated disease arises from gene or chromosomal microdeletions on 17q12 (30,31). We confirmed this finding using array chromosomal gene hybridization as an orthogonal technology. Having found no likely pathogenic variants, the son was counseled and completed his donor evaluation with no detectable abnormalities on functional testing and proceeded to donor nephrectomy. Both recipient and donor are doing well.

Discussion

LKDs have a greater lifetime risk of ESRD than otherwise matched controls (4,5). Whether this increase reflects unrecognized risk factors that are not affected by the donation process or whether the loss of one kidney increases the risk of kidney disease in a subset of donors is not known. In either case, genetic susceptibility may

contribute to the risk, with nephrectomy either promoting progressive CKD or simply shortening the time to reach ESRD once CKD begins.

Because ≈40% of LKDs are close biological relatives of the transplant recipient, it is imperative, if appropriate, to exclude presymptomatic genetic disease prior to accepting a donor candidate for nephrectomy. There are published instances in which this precaution was not taken and the genetic risk to a sibling LKD was unrecognized, only to have the donor develop the same kidney disease years later (32,33). Assessing this risk is difficult because recipient candidates who progress to ESRD are often not appropriately phenotyped with a renal biopsy and are seldom genotyped for possible genetic causes of disease.

We designed, developed and validated a targeted gene panel to provide comprehensive genetic testing for 115 genes implicated in a wide variety of renal diseases (Table S3). Although this gene panel was developed to facilitate the genetic diagnosis in patients with hereditary kidney diseases, in this publication, we described its utility for the evaluation of asymptomatic LKDs without evident kidney disease who nevertheless have a family history of kidney disease.

There are many reasons to consider comprehensive gene panel testing in this setting. First, although >60% of transplant-eligible patients have diabetes or hypertension as the stated cause of their renal disease, this diagnosis is often based on association rather than probable causality. If biopsy correlation is available, up to one-third of patients with diabetes or hypertension may have an alternative diagnosis to explain their ESRD (7–9). In another 20% of transplant candidates, the cause of ESRD is unknown, preventing a focused genetic evaluation of related family members (1,6).

Second, some diseases such as *HNF1B*-associated kidney disease (also known as renal cysts and diabetes) have limited penetrance and variable expression, which makes clinical diagnosis challenging. Although heterozygosity for pathogenic variants in *HNF1B* represents the most common monogenic cause of developmental kidney disease (30,34), the disease is a multisystem disorder. Renal cysts are the most frequently presenting feature, but the spectrum of possible renal structural abnormalities includes renal hypodysplasia, pelvic–ureteric junction obstruction, horseshoe kidney, unilateral renal agenesis, single kidneys and renal hypoplasia (35). Extrarenal phenotypes also occur, and other affected family members might present with early onset diabetes (maturity onset diabetes of the young type 5) or genital abnormalities (36,37). This complexity and the often apparently limited number of affected relatives can reduce suspicion of a genetic disease.

Third, some types of kidney diseases (e.g. FSGS) are genetically heterogeneous, with at least 15 known loci that cause dominant or recessive disease, and this list is growing, making traditional gene testing impractical (38,39). Furthermore, classically distinct genetic diseases can phenocopy other diseases, blurring the difference between phenotypes. Variants in, for example, other syndromic glomerular disease genes; the Alport genes, *COL4A3*/*COL4A4*; and the gene for nail–patella syndrome, *LMX1B*, can be identified in a number of patients without extrarenal features who have histological FSGS (12,40–42). Variants in ciliary disease genes *TTC21B* and *NPHP4* that typically cause juvenile nephronophthisis have been recently reported as causing inherited FSGS (43–45). Phenotypic similarities mean that often a large number of candidate genes are associated with a given renal disease, making gene-by-gene screening prohibitive in terms of cost and time.

Fourth, genetic diseases that present in adult life, with the exception of ADPKD, do not have accepted diagnostic tests—short of genetic testing—that have been validated for presymptomatic screening to exclude disease in a living donor at risk. Even with ADPKD, although age-dependent ultrasound and magnetic resonance imaging (MRI) criteria for the exclusion of disease have been developed, there are many scenarios in which diagnostic certainty is insufficient, making genetic screening requisite to establish or exclude a diagnosis (46,47).

Finally, comprehensive genetic testing takes on even greater importance for specific ethnic groups. A prime example is the contribution of West African ancestry to the risk of FSGS and CKD associated with two common alleles in the gene apolipoprotein L1 (*APOL1*), referred to as G1 and G2 (48,49). The G1 allele is composed of two missense variants in linkage disequilibrium, Ser342Gly and Ile384Met, and the G2 allele is an in-frame deletion of two amino acids, delN388/Y389. In the Yoruba people of Nigeria, the prevalence of these alleles is 40% and 8%, respectively, reflecting the heterozygous protection they afford to carriers from infection with *Trypanosoma brucei rhodesiense*. In African Americans, G1 is found in 52% of those with and 18–23% of those without FSGS; for the G2 allele, the percentages are 23% and 15%, respectively. Under a recessive model (i.e. carriers of two risk alleles: G1/G1, G1/G2 or G2/G2), there is a seven- to 10-fold increased risk of hypertension-associated renal disease and a 10- to 17-fold increased risk of FSGS. These two *APOL1* risk alleles also affect allograft outcomes of the donor kidney. Kidneys from deceased African American donors with two *APOL1* risk variants fail more rapidly after transplantation than kidneys from donors with no or one risk allele; however, the *APOL1* allele status of the transplant recipient does not affect outcome (50–52). Taken together, some have suggested that all African American kidney donors should be screened for these *APOL1* risk alleles (10,53,54).

In this pilot series, we tested four transplant candidates to determine the genetic basis of disease (Table 3). In

two candidates, the clinical diagnosis of ADPKD was easily made on the basis of strong family history of enlarged cystic kidneys and autosomal dominant inheritance; however, their children were all aged <30 years, limiting the utility of imaging-based screening. In a third candidate, although there was a high suspicion of Alport disease based on the clinical features of childhood-onset hematuria and proteinuria and glomerular-basement membrane lamellations with segmental thinning on ultrastructural examination of a renal biopsy, there were some inconsistencies; for example, there was no hearing deficit, and the light microscopy and immunofluorescence suggested FSGS. The fourth case was the most problematic because there was no unifying diagnosis for the two affected siblings in the pedigree. Nevertheless, negative screening in this case reduced concern about a common genetic disease and was valuable in providing counseling to the donor candidate.

The KidneySeq panel includes many genes not associated with ESRD or CKD but with other distinct renal phenotypes. The clinical utility of their inclusion is multifold. First, the added sequencing cost of additional genes is trivial. Second, by including all known causes of genetic renal disease, it becomes possible to restrict the bioinformatic analysis, if necessary, to the genes associated with the phenotype of interest. As more genes are discovered to be causes of renal diseases, updating a single targeted panel also becomes more practical than updating multiple phenotype-defined panels (e.g. a panel limited to FSGS). Third, phenotypes are often blurry with the absence of pathognomonic clinical, imaging or biopsy information, making it unclear whether the focus should be on a glomerular disease or a tubulointerstitial disease. Moreover, as stated earlier, even when the phenotype is clear, there is significant variability in the phenotypic expression of some genes.

Who are candidates for genetic screening? For living donors, we recommend genetic testing in all persons with a clear family history of CKD or ESRD or when two or more family members have kidney disease of unknown or uncertain etiology, unless an alternative screening test with a negative predictive value close to 100% is available. Genetic testing should also be considered for living donors with just one first-degree relative with CKD or ESRD unless that renal disease is clearly diabetic, immunologic (e.g. lupus nephritis), vascular, obstructive, or drug or toxin related. About 40% of the 5000 annual living donors in the United States are biologically related to their recipients; 8–10% of recipients have a known genetic diagnosis like polycystic kidney disease and 18–20% have an unknown cause of ESRD (1,6). At a conservative estimate, 5–10% of these unknown causes may have gene variants that confer a Mendelian risk of future disease. We suggest that 9–12% of LKDs may benefit from formal testing to exclude monogenic kidney disease. Such testing could include imaging studies with high negative predictive value (e.g. MRI for ADPKD), focused genetic testing for diseases like Alport (*COL3A3*, *COL3A4* and *COL3A5*) or comprehensive screening using targeted gene panels. Expanded genetic testing may also increase the living donor pool by excluding genetic disease in susceptible persons who are currently not being accepted because of clinical uncertainty.

Whole-exome sequencing (WES) is increasingly used for the diagnosis of monogenic disorders in a research setting and has been proposed by some as the preferred clinical genetic diagnostic test when locus heterogeneity is extreme or when the phenotype is indistinct (55,56). When applied to clinical diagnostics, however, the bioinformatic analysis of WES data must be restricted to genes known to be clinically implicated in the disease under consideration. Compared with targeted panels like KidneySeq, the aggregate sequencing and analysis costs of WES are far higher, the depth of sequencing is lower, the bioinformatic throughput is slower, and the type of analysis is more restricted—all points that favor the use of targeted panels in the clinical arena.

Diagnostic laboratories offering genetic panels must be certified (College of American Pathologists or Clinical Laboratory Improvement Amendments program). In addition, we strongly advocate that sequencing and bioinformatic data be reviewed by a multidisciplinary group in the context of the clinical data. This group should include, at a minimum, research scientists with expertise in targeted genomic enrichment and MPS, bioinformaticians, clinical geneticists and physicians with expertise in genetic renal diseases. We also recommend that biological relatives who are considering becoming LKDs be offered pre- and posttest genetic counseling. Genetic counselors can assist in the evaluation of an appropriate family history in addition to providing counseling and interpretation of test results. Last, both donor candidates and clinicians should understand the benefits and limitations of genetic testing.

There are several limitations to genetic testing for LKDs. First, the majority of kidney disease is polygenic or secondary to diabetes, hypertension or autoimmune conditions or from infections or toxins. Second, not all genetic variants are identified by targeted NGS panels (or WES), including variants in 5′ regulatory regions, introns or untranslated exonic regions. Third, a negative screen may falsely reduce perceived risk and thus provide misleading reassurance to the transplant center and the donor. Fourth, some identified VUSs may be exceedingly difficult to interpret, leading the transplant center and/or the donor to unwarranted dissuation from donation. Finally, significant variants unrelated to the phenotype (unsolicited but nevertheless medically significant discoveries) may be identified that are actionable and that need to be addressed.

In summary, the reasons to include comprehensive genetic testing in the evaluation of prospective renal transplant recipients and donors are compelling. We

showed that a targeted sequencing approach works well and detects single-nucleotide changes and more complex indels and copy number variants. Areas that are not adequately captured must be clearly defined so that complementary sequencing methods can be included in the analytical pipeline to ensure comprehensive coverage, and all likely pathogenic or pathogenic variants should be Sanger confirmed on a new DNA sample extracted from the originally received blood samples (Figures S1 and S2). Finally, to ensure a clinically meaningful report, a multidisciplinary review of all variants in the context of the phenotypic data is essential.

Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Figure S1: KidneySeq test workflow. The diagram in this figure shows the test workflow. Samples received in the laboratory were entered into a database. Quality of samples was assessed after several steps (DNA extraction, library preparation, and hybridization and capture). Successful samples were then pooled in batches of five samples and sequenced in the MiSeq. Sequencing data were analyzed through an in-house–developed pipeline (Figure S2), and an internal report was generated. Variants in this report were evaluated for interpretation at the multidisciplinary board meeting, those variants interpreted as etiologic were Sanger sequenced and a final results letter was generated.

Figure S2: Analysis pipeline for processing massively parallel sequencing data. The pipeline shows processing of raw sequencing reads to variant detection and report generation, which includes FastQC to monitor quality, Burrows–Wheeler alignment to map reads to thereference genome, Picard to remove read duplicates, the Genome Analysis Toolkit for variant detection across the KidneySeq target regions, Freebayes to call variants in the *PKD1* gene, and an in-house–developed tool to annotate and filter variants and generate a final complete report.

Table S1: Total sequence reads and percentage of the target region covered.

Table S2: Target regions covered with <10×.

Table S3: Broad disease phenotypes, genes tested, and modes of inheritance.