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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 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 two-fold 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 36% 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 \textit{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 Hercule 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        | JBTS3                  | 11         |
| AH1        | NM_00113483      |                        | 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         |
| AVPR1      | 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        |                        | 12         |
| CLCNKA     | NM_004070        |                        | 20         |
| CLCNKB     | NM_000085        |                        | 20         |
| CLDN16     | NM_006580        | HOMG3                  | 5          |
| CLDN19     | NM_00123395      | HOMG5                  | 4          |
| CNNM2      | NM_017649        | HOMG6                  | 8          |
| COLA1      | NM_001845        |                        | 52         |
| COLA3      | NM_000091        |                        | 52         |
| COLA4      | NM_000092        |                        | 48         |
| COLA5      | 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         |
| FGFR2      | NM_020636        |                        | 3          |
| FN1        | NM_002026        |                        | 46         |
| FRAS1      | NM_001166133     |                        | 42         |
| FREM2      | NM_027361        |                        | 24         |
| GATA3      | NM_001002295     |                        | 6          |
| GLA        | NM_000169        |                        | 7          |
| GLI3       | NM_000168        |                        | 15         |
| GLIS2      | NM_032575        | NPHP7                  | 6          |
| GPY3       | NM_001164617     |                        | 9          |
| GRHPR      | NM_012203        |                        | 9          |
| HNF1B      | NM_000458        |                        | 9          |

(continued)

Table 1: Continued

| Gene       | Accession number | Locus/alternative name | Exon count |
|------------|------------------|------------------------|------------|
| HOQA1      | 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        |                        | 22         |
| 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          |
| NRS2C2     | NM_000901        |                        | 9          |
| OCR1L      | 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         |
| RPGIP1L    | NM_001127897     | JBTS7, NPHP8, MKS5    | 25         |
| SALL1      | NM_001127892     |                        | 3          |
| SALL4      | NM_020436        |                        | 4          |
| SCN1A      | NM_001038        |                        | 13         |
| SCN1B      | NM_000336        |                        | 13         |
| SCN1G      | NM_001039        |                        | 13         |
| SIX1       | NM_005982        |                        | 2          |
| SIX2       | NM_016932        |                        | 2          |
| SIX5       | NM_175875        |                        | 3          |
| SLC12A1    | NM_000338        | NKCC2                  | 27         |
| SLC12A3    | NM_000339        | NCKT                   | 26         |
| SLC26A1    | NM_000441        |                        | 21         |
| SLC3A1     | NM_001167579     | NPT2a                  | 9          |
| SLC3A3     | NM_001177316     | NPT2C                  | 13         |
| SLC3A1     | NM_000341        |                        | 10         |
| SLC4A4     | NM_001089484     |                        | 26         |
| SLC7A9     | NM_001126335     |                        | 13         |
| SMARCA1    | NM_001172207     |                        | 18         |
| TCTN1      | NM_001082537     | JBTS13                 | 15         |
| TMEM216    | NM_001173990     | JBTS2, MKS2            | 5          |
| TMEM237    | NM_001040385     | JBTS14                 | 12         |
| TMEM67     | NM_001142301     | JBTS6, MKS3, NPHP11    | 29         |

(continued)
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 alterations as “pathogenic.” Other variants with a low likelihood of pathogenicity were classified as “likely pathogenic” or “VUS,” a distinction that reflected two considerations: likely pathogenic variants were those where the genetic change segregated with disease and was associated with the disease phenotype (Table S2).

**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...
“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 \( \text{PKD1} \) (\( \text{p.Tyr2622X} \)) 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 \( \text{COL4A5} \) (\( 3657-9A > G \)). 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

### 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| \( \text{PKD1-NM_000296:c.7866C>G, p.Tyr2622Stop} \) | ADPKD            |
| 2    | Alport syndrome/FSGS| Positive| \( \text{COL4A5-NM_000495:c.3604+1G>A} \) | Alport syndrome  |
| 3    | ADPKD              | Positive| \( \text{PKD1-NM_000296:c.11488_11489insGCGACC} \) | 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.
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 1B (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
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 hypoplasia, 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 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 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 inheri-
tance; 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 ultra-
structural examination of a renal biopsy, there were
some inconsistencies; for example, there was no hearing
deficit, and the light microscopy and immunofluores-
cence 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. Sec-
ond, 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, phenot-
types 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 consid-
ered 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 biolog-
ically 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 dis-
ease 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 set-
ting 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 bioin-
formatic 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 Lab-
oratory Improvement Amendments program). In addition,
we strongly advocate that sequencing and bioinformatic
data be reviewed by a multidisciplinary group in the con-
text 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 addi-
tion 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 sec-
ondary 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 exo-
nic regions. Third, a negative screen may falsely reduce per-
ceived 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 dissua-
donation from donation. Finally, significant variants unrelated to
the phenotype (unsolicited but nevertheless medically sig-
nificant 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.