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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 1 $\beta$ ; 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, Atails 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

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

Table 1: Continued

Accession         Locus/         Accession         alternative         Exon         HCGA1         NML 33413         DHDPSL         7           Gene         number         name         0.001         HCGA1         NML 0133413         DHDPSL         7           ACTH4         NML 003824         21         INF2         NML 0138174         FSGS5         22           ACTH4         NML 003882         SLCA1         20         INFPSE         NML 01425         NMLP12         17           ACTH4         NML 003884         SLCA1         20         INVS         NML 014257         NPHP12         17           ACTH         NML 00137146         FSGS4         6         KLH1.3         NML 001267734         18           APOLI         NML 00137465         SLAMS2         SCNU         NML 00174746         8         8           APOLI         NML 00174746         JBTS3         10         MKKS         NML 00174746         BS56         6           APIL3B         NML 00174746         JBTS3         10         MKKS         NML 0017474         BE56         6           APIL3E         NML 00174746         JBTS3         NML NT 70748         BE56         6         6           AP	screened by targeted genomic enrichment and massively parallel sequencing					Locus/		
Locusy         Locusy <thlocusy< th=""> <thlocusy< th=""> <thlocusy< th="" tr<=""><th></th><th></th><th></th><th></th><th>Cana</th><th>Accession</th><th>alternative</th><th>Exon</th></thlocusy<></thlocusy<></thlocusy<>					Cana	Accession	alternative	Exon
Gene         Inumber         Data         HOGA1         NML_01130241         CPUE         7           ACTMA         NML_0013924         21         INF2         NML_00131714         FSGS55         22           ACTMA         NML_000342         SLC4A1         20         INFPSE         NML_0191322         JBTS1         10           ACTMA         NML_000366         3         INVS         NML_011425         NPHPP2         17           ACTM         NML_00136743         JBTS3         27         KAL1         NML_0012757         NPHP5         15           ALMS1         NML_0013664         FSGS4         6         KLH3         NML_001271144         IS           APCL         NML_0013664         FSGS4         6         KLH3         NML_001271144         BS         ACP2           APRT         NML_00174150         JBTS8         10         MKKS         NML_00174748         BSS6         6           ARL138         NML_00178738         BTS3         8         MKS1         NML_001769         11           APPVAA         NML_0016697         JBTS4         20         20         23         24           APPVA         NML_002064         3         NEK8         NM		Accession	Locus/	Evon	Gene	number	name	count
Construct         Construct         Final         NML_001100241         FSGS         21           ACTIM4         NML_000342         SLCA1         20         INPPE         NML_0110807114         FSGSS         22           ACTIM4         NML_0003042         SLCA1         20         INPPE         NML_011425         NPHPE         17           AGXT         NML_00113666         3         INV         NML_001123570         NPHP5         15           ALMIN         NML_001136460         FSGS4         6         KLHL         NML_002157194         15           APRT         NML_0004860         5         LAMB2         NML_002257194         15           APRL         NML_001774160         BTSS         10         MKKS         NML_0027744         BSS6         6           APRL5         NML_0026323         ATPRIVA         NML_002773         18         20         MYH9         NML_002773         11           BSS1         NML_001226320         ATPS         3         NEK8         NML_00174465         20         27           BSS1         NML_002632         ATPRVAA         NML_002770         NPHP5         15         27           BSS2         NML_012866         17         <	Gene	number	name	count	HOGA1	NM_138413	DHDPSL	7
ACTNA         NM_004924         SLCA1         21         INF2         NM_001041714         FISDS         22           AGTR2         NM_000686         SLCA1         20         INFPS         INM_D11425         JBTS1         10           AGTR2         NM_000866         11         IQCB1         NM_0012370         NPHP2         17           AGXT         NM_00013640         PSGS4         27         KAL1         NM_0002370         NPHP5         15           AHI         NM_00136500         PSGS4         6         KLH13         NM_00127144         BS6         3           APOL         NM_000466         4         LMX15         NM_001174146         8         8           ARLI38         NM_001278233         BSS3         8         MKS1         NM_001165927         41           AVPPAD4         NM_0027823         ATPSN16         22         MYHP         NM_00107981         11         11           BSS1         NM_0027823         ATPSN16         24         MYHP         NM_00107981         11         11           BSS2         NM_00128678         17         NPHP1         NM_00007981         27         10           BSS1         NM_001286778         12					IFT80	NM_001190241	50005	21
AE1         NM_000842         SLCAA1         20         INP-Pote         NM_01019882         JB131         10           AGKT         NM_000030         11         ICCB1         NM_010123370         NPHP5         15           AGKT         NM_0011425         NPHP5         15         NM_011425         NPHP5         15           ALM1         NM_001124500         JBTS3         27         KCAL1         NM_0012370         NPHP5         14           ALM51         NM_001126640         FSGS4         6         KLM11         NM_002292         32           APR1         NM_000465         5         LAMB2         NM_00171446         8         8           APR1         NM_0002532         ATPSVDA4         NM_002773         18         11         10           APP2         NM_001250622         ATPSVDA4         NM_002773         JBTS         27         18           APP2         NM_001250678         17         NLRP3         NM_001278         27           BS5         NM_0128062         17         NLP13         NM_0012963         27           BS5         NM_0128062         17         NLP33         NM_00129193         27           BS5         NM_0012806	ACTN4	NM_004924	010111	21	INF2	NM_001031714	FSGS5	22
ALIR2         NML000280         13         INVS         NML01428         INPTP2         17           ARXT         NML000230         11         IQCB1         NML0012370         NPTP2         14           ALMS1         NML0012370         NPTP2         14           ALMS1         NML00125764         ROMK1         3           APQL         NML001257194         BS766         ROMK1         3           APQT         NML0001265         5         LAMB2         NML001724146         8           APRT         NML00174160         JBTS8         10         MKKS1         NML0174744         BBS6         6           APPL2         NML020054         3         NEK8         NML77774         BBS7         41           AVPP2         NML02062078         15         NML02070821         11         11           BS51         NML0262678         15         NPHP1         NML001079821         11           BS52         NML018190         18         NPH51         NML0101202         11           BS53         NML018190         18         NML0191705         10         10           CC2D2A         NML018190         18         NML0191705         10 <td< td=""><td>AE1</td><td>NM_000342</td><td>SLC4A1</td><td>20</td><td>INPPSE</td><td>NIVI_019892</td><td>JRISI</td><td>10</td></td<>	AE1	NM_000342	SLC4A1	20	INPPSE	NIVI_019892	JRISI	10
NML_000134800         JBTS3         217         ICLS1         NML_00112320         NPFPS         14           ALMI         NML_001134800         JBTS3         27         KAL1         NML_0001257194         14           ALMIS         NML_00136640         FSGS4         6         KLH1         NML_001257194         15           APRT         NML_000485         4         LAMIS         NML_0012767194         88           APRT         NML_000486         4         LAMIS         NML_00176786         88           APRT         NML_000178293         BBS3         8         MKS         NML_010165927         18           ATP6V0A4         NML_002632         ATP6N1B         22         MYH9         NML_00165927         18           ATP6V0A4         NML_001252678         15         NPHP3         NML_0101291633         27           BS5         NML_0120120         18         NPH51         NML_001291503         27           BS44         NML_001202         4         NPH52         NM_001291503         27           BS5         NML_001202         4         NPH52         NM_001291503         27           BS50         NML_001202         4         NPH52         NM_00129151	AGTR2	NM_000686		3		NIVI_014425		17
AHIL         NML_DOI 134830         JB 15.3         27         KELL         NML_DOI25120         18           ARDLI         NML_001136540         FSGS4         6         KLHL3         NML_001257194         15           APCI         NML_001136540         FSGS4         6         KLHL3         NML_001257194         BS         32           APRT         NML_001174150         JBTS3         10         MKKS         NML_001174166         8           ARL13B         NML_0011774253         JBTS3         10         MKKS         NML_00116927         18           ARL5         NML_001774293         BBS3         8         MKS1         NML_001079821         11           AVPP2         NML_00054         3         NEKR         NML_178170         NPHP9         15           BS1         NML_01256278         15         NPHP3         NML_01291593         27           BS5         NML_012864         12         NPHP4         NML_00276         7           BS5         NML_0129102         18         NPHS1         NML_00276         7           BS4         NM_00018652         JBTS9         38         OFD1         NM_000276         74           CC22A         NM_0001804	AGXI	NIVI_000030				NIVI_001023570	INPHP5	15
ALMAIN         NM_013120         23         KLIKJ         NM_0123704         35           APRT         NM_000486         FSGS4         6         KLIKL3         NM_0127114         35           APRT         NM_000486         4         LAMB2         NM_002292         32           ARL13         NM_00174150         JBTS8         10         MKKS         NM_0170784         8           ARL14         NM_0016927         18         NM_0016927         18         18           ATP6V044         NM_002632         ATP6N18         22         MYH9         NM_0016927         18           ATP6V044         NM_001252678         15         NPHP3         NM_00107923         JBTS4         20           BSS         NM_01252678         15         NPHP3         NM_01291503         27           BSS         NM_0120122         4         NH52         NM_001291503         27           BSN         NM_0000852         JBTS9         38         OFD1         NM_000297         29           C20204         NM_001291         37         OCR1         NM_000297         20         23           C21204         NM_0000852         JBTS9         38         OFD1         NM_000297		NIVI_001134830	JD123	27		NIM 152766	POMK1	2
APRT         NML_001         NML_001         NML_001         NML_001         NML_001           APRT         NML_001174160         JBTS6         1         LMX1B         NML_001171416         8           ARL13B         NML_001174160         JBTS6         10         MKKS         NML_00177824         BBS6         6           ARL6         NML_000178293         BBS3         8         MKS1         NML_00177824         BBS6         6           APPEV0A         NML_000054         3         NEK8         NML_00179821         11           AVPR2         NML_000054         3         NEK8         NML_151240         27           BBS1         NML_00128678         17         NPHP1         NML_001291503         27           BBS4         NML_001220         4         NPHS2         NML_001291503         27           BS57         NML_013180         18         NPHS1         NML_001291503         27           BS58         NML_001202         4         NPHS2         NML_001291503         7           BS50         NML_001202         4         NPHS2         NML_001291575         7           BS50         NML_001080522         JBTS9         38         OFD1         NM		NIVI_015120	ESCSA	23	KUHI 3	NIM_001257194	NOMINI	15
ATH         INM_000486         JBTS         Denote         INM_00117416         S           ARL28         NM_001174150         JBTS8         10         MKKS         NM_1001174146         S           ARL158         NM_0017745293         BBS3         8         MKS1         NM_001169927         18           ATPEVDA4         NM_000264         3         NEKS         NM_1717170         NPHP9         15           BBS1         NM_000264         3         NEKS         NM_1717170         NPHP9         15           BBS1         NM_0012526778         15         NPHP3         NM_000272         JBTS4         20           BBS4         NM_012526778         15         NPHP3         NM_000275         7           BBS5         NM_152364         12         NPHP3         NM_001291593         27           BS50         NM_001202         4         NPHS2         NM_001291593         27           SBND         NM_001202         4         NPHS2         NM_001291593         27           C2D2A         NM_001205022         JBTS5         54         PHES2         NM_000276         29           C2D2A         NM_001205022         JBTS5         54         PHEX	APULI	NIVI_001130340	F3G34	5	LAMR2	NIM 002292		32
KLI 2         INV_00174150         JBTS8         10         MKS         NM_1070784         BBS6         6           ARLISB         NM_001278293         BBS3         8         MKS1         NM_00168927         18           ARLF         NM_000054         2         MYH9         NM_0024733         141           AVPR2         NM_000054         3         NEK8         NM_178170         NPHP9         15           BBS1         NM_0252678         15         NPHP1         NM_001291593         27           BBS5         NM_0122678         15         NPHP3         NM_001291593         27           BS5         NM_015120         18         NPHP4         NM_001291593         27           BS5         NM_015120         4         NPH52         NM_001291593         27           BS5         NM_001202         4         NPH52         NM_001291593         27           BS5         NM_001202         4         NPH52         NM_001291593         27           BS5         NM_001288         7         CFCL1         NM_000276         24           CC2D2A         NM_00198522         JBTS9         38         OFD1         NM_000278         10		NNA 000485		5	LAWD2	NM 001174146		8
NLLIDD         INV_00178293         BBS3         8         MKS1         NM_0016927         18           ATPSVD44         NM_0002632         ATPSND4         3         NEKS1         NM_0016927         18           ATPSVD44         NM_0002632         ATPSND4         3         NEKS1         NM_0016927         18           BBS1         NM_0002652         ATPSND4         3         NEKS1         NM_000722         JBT54         20           BBS2         NM_01252678         15         NPHP3         NM_000272         JBT54         20           BBS5         NM_012192         4         NPHS1         NM_001297575         7           BBS1         NM_001202         4         NPHS1         NM_0012976         24           SEND         NM_001080522         JBT59         38         OFD1         NM_000276         24           C2D2A         NM_001080522         JBT55         54         PHX2         NM_000278         10           C2E2920         NM_00108052         JBT55         54         PHK2         NM_000286         ADPKD-1         46           C1CMS         NM_000084         CLC5         12         PKD1         NM_0018999         NPHS3         32 <td>ADFZ</td> <td>NM 001174150</td> <td>IBTS8</td> <td>10</td> <td>MKKS</td> <td>NM 170784</td> <td>BBS6</td> <td>6</td>	ADFZ	NM 001174150	IBTS8	10	MKKS	NM 170784	BBS6	6
ALTERVIDA         INL 020632         ATPENTB         22         MVH9         NL002473         41           AVPR2         INM_000064         3         NEK8         NM_17170         NPH99         15           BBS1         INM_02449         17         NLRP3         NN_001079821         11           BBS2         NM_031885         17         NPHP1         NM_0012722         JBTS4         20           BBS5         NM_152384         12         NPHP4         NM_001297575         7           BBS7         NM_018190         18         NPHS1         NM_001297575         7           BSNP4         NM_0010022         4         NPHS2         NM_000276         24           CC2D2A         NM_00160522         JBTS9         38         OFD1         NM_000278         10           CC2D2A         NM_00160522         JBTS9         38         OFD1         NM_000278         10           CC2D2A         NM_0016052         JBTS9         38         OFD1         NM_000278         10           CLCAK         NM_00070         20         PLC1         NM_000279         ADPKD-2         15           CLCNKB         NM_00065         20         REN         NM_000279<	ARLE	NIM_001278293	BBS3	8	MKS1	NM_001165927	2200	18
NML 000064         NML 000064         NML 0178170         NPHP9         15           BBS1         NML 024849         17         NLRP3         NML 0017921         11           BBS2         NML 01856         17         NPHP1         NML 000272         JBTS4         20           BBS4         NML 01252678         15         NPHP3         NML 01231593         27           BBS5         NML 0123264         12         NPHP3         NML 001291593         27           BBS5         NML 0118190         18         NPHS1         NML 00129765         7           BSND         NML 001202         4         NPHS2         NML 0012976         24           CC2D2A         NML 000388         7         OCRL1         NML 000311         JBTS1         23           CC2D2A         NML 001202         18         PAX2         NML 000444         22           CC2D2A         NML 012120         18         PAX2         NML 000444         22           CLCNKA         NML 000084         CLC5         12         PKHD1         NML 000297         ADPKD-1         46           CLCNKA         NML 000085         20         PEKN         NML 000037         10           CLCNKA<		NM_020632	ATP6N1R	22	MYH9	NM 002473		41
BBS1         NM_024649         17         NLRP3         NM_0002792,1         11           BBS2         NM_001252678         15         NPHP1         NM_001272,2         JBTS4         20           BBS5         NM_0153240         27         BBS5         NM_0153240         27           BBS5         NM_0153284         12         NPHP4         NM_004646         29           BBS7         NM_001702         4         NPHS1         NM_000001         9           BSND         NM_0018022         JBTS9         38         OFD1         NM_000001         9           GCSR         NM_010100522         JBTS9         38         OFD1         NM_000276         24           CC2D2A         NM_001006522         JBTS9         38         OFD1         NM_0002778         10           CE22A0         NM_0025114         JBTS5,         54         PHEX         NM_000277         ADPKD-2         15           CLCNA         NM_000084         CLC5         12         PKD1         NM_000297         ADPKD-2         16           CLCNKB         NM_000085         HOMG3         5         RET         NM_0002630         19           CLCNHS         NM_000085         HOMG6 </td <td>AVPR2</td> <td>NM_000054</td> <td></td> <td>.3</td> <td>NEK8</td> <td>NM 178170</td> <td>NPHP9</td> <td>15</td>	AVPR2	NM_000054		.3	NEK8	NM 178170	NPHP9	15
BBS2         NM_031885         17         NPHP1         NM_00272         JBTS4         20           BBS4         NM_01252678         15         NPHP3         NM_153240         27           BBS5         NM_018190         18         NPHP3         NM_00121993         27           BBS7         NM_018190         18         NPHS1         NM_001291593         27           BBS7         NM_001202         4         NPHS2         NM_001297575         7           BSND         NM_000361         JBTS1         NM_000276         24           C2D2AP         NM_001080522         JBTS9         38         OFD1         NM_000276         23           C2D2AP         NM_001080522         JBTS5         54         PHEX         NM_000276         20           C2C2D2A         NM_001084         CLC5         12         PKD1         NM_000276         20           CLCNKA         NM_00084         CLC5         12         PKD1         NM_000276         30           CLCNKA         NM_00085         20         REN         NM_00115897         NPH53         32           CLCNKA         NM_00112395         HOMG5         4         RPGRIPIL         NM_001127892	BBS1	NM 024649		17	NLRP3	NM 001079821		11
BB3A         NM_00125678         15         NPHP3         NM_153240         27           BBS5         NM_152384         12         NPHP4         NM_001291593         27           BBS7         NM_018190         18         NPHS1         NM_004646         29           BMP4         NM_001202         4         NPHS2         NM_0029755         7           BSND         NM_005022         JBTS9         38         OPD1         NM_000276         24           CC2D2A         NM_00108522         JBTS9         38         OPD1         NM_000276         10           CC2D2A         NM_00108522         JBTS5         54         PHEX         NM_00026         ADPKD-1         46           C222P         NM_000084         CLC5         12         PKHD1         NM_000297         ADPKD-1         46           CLCNK5         NM_000085         20         PEC1         NM_001185979         NPH53         32           CLCNK8         NM_000085         20         RET         NM_00037         19           CLCNK8         NM_000686         4         RPGRIP1L         NMS5         32           CLCNK8         NM_00035         5         RET         NM_00138	BBS2	NM 031885		17	NPHP1	NM 000272	JBTS4	20
BBSS         NM_152384         12         NPHP4         NM_01291593         27           BBS7         NM_018190         18         NPHS1         NM_0014646         29           BBS7         NM_0057176         4         NR3C2         NM_001297575         7           BSND         NM_001805522         JBTS9         38         OFD1         NM_000276         24           C2D2AP         NM_012120         18         PAX2         NM_000276         23           C2D2AP         NM_012120         18         PAX2         NM_000278         10           CEP290         NM_025114         JBTS5,         54         PHEX         NM_000278         20           CLCNKA         NM_00084         CLC5         12         PKD1         NM_000297         ADPKD-2         15           CLCNKA         NM_00085         20         REN         NM_00537         10           CLDN16         NM_0006560         HOMG5         52         SALL1         NM_001127892         3           COLA41         NM_001794         HOMG6         8         MK55         13         SCNN1A         NM_00136         13           COLA41         NM_000179846         30         SIX1	BBS4	NM 001252678		15	NPHP3	NM 153240		27
BBS7         NM_018190         18         NPHS1         NM_004646         29           BMP4         NM_001202         4         NPHS2         NM_001297575         7           BSND         NM_000388         7         OCRL1         NM_000276         24           CC2D2A         NM_001080522         JBTS9         38         OFD1         NM_000276         23           CC2D2A         NM_025114         JBTS5,         54         PHEX         NM_000296         ADPKD-1         46           CC2NS         NM_0000862         LC5         12         PKD1         NM_000297         ADPKD-2         15           CLCNKB         NM_000085         20         REN         NM_000537         10           CLCNKB         NM_000085         20         REN         NM_00537         10           CLDN16         NM_00012395         HOMG5         4         RPGRIP1L         NM_001127897         JBTS7, NPHP8,         25           CNM2         NM_0011345         52         SALL4         NM_001127892         3         3           CLCNKB         NM_0001845         52         SALL4         NM_001127892         3         3           COLAA1         NM_000495         51	BBS5	NM 152384		12	NPHP4	NM_001291593		27
BMP4         NM_001202         4         NPH22         NM_001297675         7           BSND         NM_007776         4         NR3C2         NM_000361         9           CSR         NM_00180522         JBTS9         38         OFD1         NM_0003611         JBTS10         23           CD2AP         NM_012120         18         PAX2         NM_000276         10           CEP290         NM_025114         JBTS5,         54         PHEX         NM_000276         22           CL2P30         NM_00084         CLC5         12         PKD1         NM_000297         ADPKD-2         15           CLCNKA         NM_000085         20         PLCE1         NM_0015979         NPHS3         32           CLCNKA         NM_0006580         HOMG3         5         RET         NM_00112797         JBTS7, NPHP8,         25           CLN16         NM_00163395         HOMG3         5         RET         NM_0012863         10           CLAN18         NM_00091         52         SALL1         NM_001286         13         33           COL4A1         NM_000092         48         SCNN1A         NM_000386         13         33           COL4A5	BBS7	NM 018190		18	NPHS1	NM_004646		29
BSND         NM_0057176         4         NR3C2         NM_000801         9           CaSR         NM_0008652         JBTS9         38         OFD1         NM_000276         24           CC2D2A         NM_012120         18         PAX2         NM_0003611         JBTS10         23           CD2AP         NM_025114         JBTS5,         54         PHEX         NM_000276         ADPKD-1         46           CEP290         NM_025114         JBTS5,         54         PHEX         NM_000296         ADPKD-2         15           CLCNS         NM_000084         CLC5         12         PKD1         NM_000297         ADPKD-2         16           CLCNKB         NM_000085         200         REN         NM_00537         10           CLDN19         NM_000850         HOMG3         5         RET         NM_020600         19           CLDN19         NM_001728395         HOMG6         8         MKS5         MKS5         MKS5         10           COL4A1         NM_000127849         HOMG6         8         SCNN1A         NM_00127892         22         2           COL4A4         NM_00163161         13         SCN1A         NM_0010386         133	BMP4	NM 001202		4	NPHS2	NM_001297575		7
CASR         NM_000288         7         OCRL1         NM_000276         24           CC2D2A         NM_001060522         JBTS9         38         OFD1         NM_003611         JBTS10         23           CD2AP         NM_025114         JBTS5,         54         PHEX         NM_000276         10           CEP30         NM_025114         JBTS5,         54         PHEX         NM_000276         ADPKD-1         46           NPHP6         PKD2         NM_000297         ADPKD-2         15           CLCNK5         NM_000084         CLC5         12         PKHD1         NM_0165979         NPHS3         32           CLCNK6         NM_00065         20         REN         NM_000165979         NPHS3         32           CLCNK6         NM_00065         4         RPGRIP1L         NM_001127897         JBTS7, NPHP8,         25           CNMM2         NM_017649         HOMG6         8         MKS5         MKS5         33           COL4A1         NM_00091         52         SALL4         NM_001127892         33           COL4A4         NM_00017846         30         SIX1         NM_001038         13           COL4A5         NM_00107846         <	BSND	NM_057176		4	NR3C2	NM_000901		9
CC2D2A         NM_0101080522         JBTS9         38         OFD1         NM_003611         JBTS10         23           CD2AP         NM_012120         18         PAX2         NM_000278         10           CEP290         NM_025114         JBTS5,         54         PHEX         NM_000296         ADPKD-1         46           CEP290         NM_000086         CLC5         12         PKD1         NM_000297         ADPKD-2         15           CLCNKA         NM_000084         CLC5         12         PKHD1         NM_00537         10           CLDN16         NM_000685         20         REN         NM_001127897         JBTS7, NPHP8,         25           CNMM2         NM_01123395         HOMG5         4         RPGRIP1L         NM_001127897         JBTS7, NPHP8,         25           COL4A1         NM_001845         52         SALL1         NM_001038         4         4           COL4A3         NM_00091         52         SALL4         NM_001038         13         33           COL4A4         NM_001031681         13         SIX2         NM_001039         13         33           COL4A4         NM_001031681         13         SIX2         NM_001038 <td>CaSR</td> <td>NM_000388</td> <td></td> <td>7</td> <td>OCRL1</td> <td>NM_000276</td> <td></td> <td>24</td>	CaSR	NM_000388		7	OCRL1	NM_000276		24
CD2AP         NM_012120         18         PAX2         NM_000278         10           CEP290         NM_025114         JBTS5,         54         PHEX         NM_000296         ADPKD-1         46           NKS4,         PKD1         NM_000297         ADPKD-2         15           CLCN5         NM_00084         CLC5         12         PKHD1         NM_001165979         NPHS3         32           CLCNK8         NM_00085         20         PLC1         NM_001165979         NPHS3         32           CLCNK8         NM_00123395         HOMG3         5         RET         NM_001127897         JBTS7, NPHP8,         25           CNMM2         NM_0017649         HOMG6         8         MKS5         4         COL4A1         NM_001036         13           COL4A1         NM_00091         52         SALL1         NM_001038         13         3           COL4A5         NM_000092         48         SCNN1A         NM_001038         13         3           COL4A5         NM_00103681         13         SIX2         NM_00139         13         3           COL4A5         NM_0010361681         13         SIX2         NM_001389         NCCT         26	CC2D2A	NM_001080522	JBTS9	38	OFD1	NM_003611	JBTS10	23
CEP290         NM_025114         JBTS5, MKS4, NPHP6         54         PHEX         NM_000444         22           CLCN5         NM_000084         CLC5         12         PKD2         NM_000297         ADPKD-2         15           CLCN5         NM_0004070         20         PLCE1         NM_0165979         NPHS3         32           CLCNKA         NM_00085         20         PLC1         NM_000537         10           CLCNKA         NM_000558         HOMG3         5         RET         NM_0001127897         JBTS7, NPHP8,         25           CLN16         NM_001123395         HOMG6         4         RCGU4A1         NM_001845         32           COL4A1         NM_00091         52         SALL4         NM_00138         13           COL4A3         NM_000495         51         SCNN1A         NM_000336         13           COL4A4         NM_0013681         13         SIX1         NM_00139         13           COL4A5         NM_001031681         13         SIX2         NM_016332         2           CTNS         NM_00103681         13         SIX2         NM_016332         2           CHS         NM_00116313         46         SLC12A1	CD2AP	NM_012120		18	PAX2	NM_000278		10
MKS4, NPHP6         PKD1         NM_000296         ADPKD-1         46           CLCN5         NM_000084         CLC5         12         PKHD1         NM_138694         67           CLCNKA         NM_00085         20         PLCE1         NM_001165979         NPHS3         32           CLCNKB         NM_000680         HOMG5         5         RET         NM_002637         19           CLDN16         NM_01123395         HOMG5         4         RPGRIP1L         NM_001127897         JBTS7, NPHP8,         25           CNM2         NM_017649         HOMG6         8         MKS5         3           COL4A1         NM_0001845         52         SALL4         NM_001127897         JBTS7, NPHP8,         25           COL4A3         NM_000091         51         SCNN1A         NM_00138         4           COL4A4         NM_0001695         51         SCNN1B         NM_001336         13           COL4A5         NM_0010257197         15         SIX5         NM_16932         2           CNS         NM_001265197         15         SIX5         NM_00338         NCC2         27           CH4A5         NM_00178130         HOMG4         23         SLC12A1 <td>CEP290</td> <td>NM_025114</td> <td>JBTS5,</td> <td>54</td> <td>PHEX</td> <td>NM_000444</td> <td></td> <td>22</td>	CEP290	NM_025114	JBTS5,	54	PHEX	NM_000444		22
NHP6         PKD2         NM_000297         ADPKD-2         15           CLCN5         NM_00084         CLC5         12         PKHD1         NM_138694         67           CLCNKA         NM_00085         20         PLCE1         NM_0115597         NPHS3         32           CLCNKB         NM_00085         20         REN         NM_00537         10           CLDN16         NM_006580         HOMG3         5         RET         NM_001127897         JBTS7, NPHP8,         25           CNMM2         NM_017649         HOMG6         8         MKS5         3           COL4A1         NM_00091         52         SALL4         NM_00127892         3           COL4A3         NM_00092         48         SCNN1A         NM_00138         13           COL4A4         NM_00095         51         SCNN1B         NM_001039         13           COL4A5         NM_00131681         13         SIX2         NM_01032         2         2           CTNS         NM_00131681         13         SIX2         NM_01632         2         2           CUL3         NM_00138161         13         SIX2         NM_00388         NCCT         26			MKS4,		PKD1	NM_000296	ADPKD-1	46
CLCN5         NM_000084         CLC5         12         PKHD1         NM_138694         67           CLCNKA         NM_004070         20         PLCE1         NM_00116579         NPHS3         32           CLCNKA         NM_00085         20         REN         NM_0016577         10           CLDN16         NM_00123395         HOMG5         4         RPGRIP1L         NM_00127397         JBTS7, NPHP8,         25           CNNM2         NM_017649         HOMG6         8         MKS5         3           COL4A1         NM_00092         48         SCN11A         NM_001038         13           COL4A3         NM_000495         51         SCN11B         NM_001038         13           COL4A4         NM_00179846         30         SIX1         NM_001039         13           COQ2         NM_01016811         13         SIX2         NM_016992         2           CUI3         NM_001318817         9         SLC12A1         NM_00038         NKCC2         27           EGF         NM_001178130         HOMG4         23         SLC12A3         NM_00038         NKC2         27           EGF         NM_0016133         42         SLC3A1         NM_0			NPHP6		PKD2	NM_000297	ADPKD-2	15
CLCNKA         NM_0004070         20         PLCE1         NM_001165979         NPHS3         32           CLCNKB         NM_00085         20         REN         NM_00057         10           CLDN16         NM_001123395         HOMG3         5         RET         NM_001127897         JBTS7, NPHP8,         25           CNMM2         NM_017649         HOMG6         8         MKS5         MKS5           COL4A1         NM_000091         52         SALL4         NM_00138         4           COL4A4         NM_000092         48         SCNN1A         NM_00036         4           COL4A5         NM_000495         51         SCNN1B         NM_00036         13           COQ2         NM_01079846         30         SIX1         NM_00039         13           COQ2         NM_00131681         13         SIX2         NM_0038         NKCC2         27           CHS         NM_0013817         9         SLC12A1         NM_000339         NCCT         26           EGF         NM_00163181         18         SLC26A4         NM_000339         NCC2         27           FGF23         NM_020638         3         SLC34A1         NM_001167579         NPT2a	CLCN5	NM_000084	CLC5	12	PKHD1	NM_138694		67
CLCNKB         NM_00085         20         REN         NM_00537         10           CLDN16         NM_006580         HOMG3         5         RET         NM_020630         19           CLDN19         NM_001123395         HOMG5         4         RPGRIP1L         NM_001127897         JBTS7, NPHP8, 25           CNNM2         NM_017649         HOMG6         8         MKS5         3           COL4A1         NM_000091         52         SALL4         NM_001127892         3           COL4A4         NM_000092         48         SCNN1A         NM_001038         13           COL4A5         NM_001079846         30         SIX1         NM_001039         13           COL2         NM_01013681         13         SIX2         NM_016932         2           CTINS         NM_001257197         15         SIX5         NM_175875         3           DHCR7         NM_00116817         9         SLC12A1         NM_000339         NCCT         26           EYA1         NM_001633         18         SLC26A4         NM_00116779         NPT2a         9           FNAS1         NM_001166133         42         SLC3A1         NM_00118357         13	CLCNKA	NM_004070		20	PLCE1	NM_001165979	NPHS3	32
CLDN16         NM_006580         HOMG3         5         REI         NM_020630         19           CLDN19         NM_001123395         HOMG5         4         RPGRIP1L         NM_001127897         JBTS7, NPHP8, 25           CNNM2         NM_017649         HOMG6         8         MKS5         3           COL4A1         NM_00091         52         SALL4         NM_001386         4           COL4A3         NM_000092         48         SCNN1A         NM_000386         13           COL4A5         NM_000495         51         SCNN16         NM_001386         13           COQ2         NM_016697         7         SCNN16         NM_00139         13           CREBBP         NM_001031881         13         SIX2         NM_016932         2           CIL3         NM_001257197         15         SIX5         NM_175875         3           DHCR7         NM_001178130         HOMG4         23         SLC12A1         NM_000339         NCCT         26           EYA1         NM_002063         18         SLC26A4         NM_00116779         NPT2a         9           FRI         NM_001002295         6         SLC34A1         NM_00117835         13	CLCNKB	NM_000085		20	REN	NM_00537		10
CLDN19         NM_001123395         HOMG5         4         HPGRIP1L         NM_001127897         JBIS7, NPHP8, 25           CNNM2         NM_017649         HOMG6         8         MKS5           COL4A1         NM_001845         52         SALL1         NM_001127892         3           COL4A3         NM_000091         52         SALL4         NM_001038         4           COL4A4         NM_000092         48         SCNN1A         NM_001038         13           COL4A5         NM_0010994         51         SCNN1B         NM_000336         13           COL2         NM_0116697         7         SCNN1G         NM_001039         13           CREBBP         NM_001031681         13         SIX2         NM_016932         2           CUL3         NM_00115817         9         SLC12A1         NM_000338         NKCC2         27           EGF         NM_001178130         HOMG4         23         SLC12A1         NM_00039         NCCT         26           EYA1         NM_002063         18         SLC26A4         NM_001167579         NPT2a         9           FN1         NM_002063         24         SLC34A3         NM_001167579         NPT2a	CLDN16	NM_006580	HOMG3	5	REI	NM_020630		19
CNNM2         NM_017649         HOMG6         8         MKS5           COL4A1         NM_001845         52         SALL4         NM_020127892         3           COL4A3         NM_000091         52         SALL4         NM_02036         4           COL4A3         NM_000495         51         SCNN1A         NM_00036         13           COL4A5         NM_001079846         30         SIX1         NM_001039         2           CNL3         NM_00105861         13         SIX2         NM_016932         2           CUL3         NM_001257197         15         SIX5         NM_175875         3           DHCR7         NM_001163817         9         SLC12A1         NM_000338         NKCC2         27           EGF         NM_001163817         9         SLC12A1         NM_000338         NKCC2         27           EGF         NM_001163817         9         SLC12A3         NM_000338         NKCC2         27           EGF23         NM_020638         3         SLC34A1         NM_000167579         NPT2a         9           FN1         NM_00026         46         SLC34A1         NM_001167579         NPT2C         13           GATA3 <td>CLDN19</td> <td>NM_001123395</td> <td>HOMG5</td> <td>4</td> <td>RPGRIP1L</td> <td>NM_001127897</td> <td>JBIS7, NPHP8,</td> <td>25</td>	CLDN19	NM_001123395	HOMG5	4	RPGRIP1L	NM_001127897	JBIS7, NPHP8,	25
COL4A1         NM_001845         52         SALL1         NM_001127892         3           COL4A3         NM_000091         52         SALL4         NM_001038         4           COL4A4         NM_00092         48         SCNN1A         NM_001038         13           COL4A5         NM_001455         51         SCNN1B         NM_001039         13           COQ2         NM_0115697         7         SCNN1G         NM_001039         13           COQ2         NM_001257197         15         SIX5         NM_016932         2           CUL3         NM_001163817         9         SLC12A1         NM_000338         NKCC2         27           EGF         NM_001163817         9         SLC12A1         NM_000338         NKCC2         27           EGF         NM_00163817         9         SLC12A1         NM_000338         NKCC2         27           EGF         NM_002063         18         SLC26A4         NM_000441         21           FGF23         NM_020638         3         SLC34A3         NM_001177316         NPT2a         9           FN1         NM_00266         46         SLC34A3         NM_001177316         NPT2         13      <	CNNM2	NM_017649	HOMG6	8	0.4.1.4		MKS5	0
COLAA3         NM_000091         52         SALL4         NM_020436         4           COL4A4         NM_000092         48         SCNN1A         NM_001038         13           COL4A5         NM_001695         51         SCNN1B         NM_001039         13           COQ2         NM_015697         7         SCNN1G         NM_001039         13           CREBBP         NM_001079846         30         SIX1         NM_005982         2           CTNS         NM_001257197         15         SIX5         NM_175875         3           DHCR7         NM_001163817         9         SLC12A1         NM_000339         NCCT         26           EGF         NM_001163817         9         SLC12A3         NM_000339         NCCT         26           EYA1         NM_0020638         3         SLC12A3         NM_000441         21         21           FGF23         NM_020206         46         SLC3A41         NM_00117716         NPT2a         9           FN1         NM_001002295         6         SLC7A9         NM_001126335         13           GLA         NM_000168         15         TCTN1         NM_001082537         JBTS13         15      <	COL4A1	NM_001845		52	SALLI	NM_001127892		3
COLAAA         NM_000092         48         SCNNTA         NM_0001038         13           COLAA5         NM_000495         51         SCNNTB         NM_000336         13           COQ2         NM_015697         7         SCNNTG         NM_001039         13           CREBBP         NM_001031681         13         SIX1         NM_005982         2           CTNS         NM_001257197         15         SIX5         NM_175875         3           DHCR7         NM_001163817         9         SLC12A1         NM_000338         NKCC2         27           EGF         NM_000503         18         SLC34A1         NM_000441         21         FGF23         NM_020638         3         SLC34A1         NM_001167579         NPT2a         9           FRAS1         NM_001166133         42         SLC34A1         NM_001177316         NPT2C         13           FRAS1         NM_001002295         6         SLC34A1         NM_001098484         26           GATA3         NM_00169         7         SMARCAL1         NM_001098484         26           GATA3         NM_00168         15         TCTN1         NM_001082537         JBTS13         15           GLIS2 </td <td>COL4A3</td> <td>NM_000091</td> <td></td> <td>52</td> <td>SALL4</td> <td>NIVI_020436</td> <td></td> <td>4</td>	COL4A3	NM_000091		52	SALL4	NIVI_020436		4
COLAAS         NM_000495         51         SCINITB         NM_00036         13           COQ2         NM_015697         7         SCINITB         NM_001039         13           CREBBP         NM_001079846         30         SIX1         NM_005982         2           CUL3         NM_001257197         15         SIX5         NM_175875         3           DHCR7         NM_001178130         HOMG4         23         SLC12A1         NM_000339         NCCT         26           EYA1         NM_000503         18         SLC26A4         NM_000441         21         17           FGF23         NM_001166133         42         SLC34A1         NM_000441         21         10           FRAS1         NM_001166133         42         SLC34A3         NM_001177316         NPT2a         9           FRAS1         NM_001166133         42         SLC3A41         NM_001355         13           GATA3         NM_001002295         6         SLC7A9         NM_001127207         18           GLI3         NM_001068         15         TCTN1         NM_001127207         18           GLI3         NM_00168         15         TCTN1         NM_001014385         JBTS13 <td>COL4A4</td> <td>NM_000092</td> <td></td> <td>48</td> <td>SCINITA SCINITA</td> <td>NIVI_001038</td> <td></td> <td>13</td>	COL4A4	NM_000092		48	SCINITA SCINITA	NIVI_001038		13
CUC2         NM_015697         7         SCNNTG         NM_001039         13           CREBBP         NM_001079846         30         SIX1         NM_005982         2           CTNS         NM_001031681         13         SIX2         NM_016932         2           CUL3         NM_001157197         15         SIX5         NM_175875         3           DHCR7         NM_001163817         9         SLC12A1         NM_000338         NKCC2         27           EGF         NM_00503         HOMG4         23         SLC12A3         NM_000441         21           FGF23         NM_0206638         3         SLC34A1         NM_001167579         NPT2a         9           FN1         NM_001166133         42         SLC34A3         NM_001177316         NPT2C         13           FRAS1         NM_001166133         42         SLC444         NM_001126335         13           GLA         NM_001002295         6         SLC7A9         NM_001126335         13           GL3         NM_001168         15         TCTN1         NM_001082537         JBTS13         15           GL3         NM_00168         15         TCTN1         NM_0010173990         JBTS2, MKS2	COL4A5	NM_000495		51	SCININ I B	NIVI_000336		13
CREBBP         NM_001079846         30         SIX1         NM_000382         2           CTNS         NM_001031681         13         SIX2         NM_016932         2           CUL3         NM_001257197         15         SIX5         NM_75875         3           DHCR7         NM_001163817         9         SLC12A1         NM_000338         NKCC2         27           EGF         NM_001178130         HOMG4         23         SLC12A3         NM_000339         NCCT         26           EYA1         NM_000503         18         SLC26A4         NM_000441         21         21           FGF23         NM_0202638         3         SLC34A1         NM_001167579         NPT2a         9           FN1         NM_001166133         42         SLC3A1         NM_001167579         NPT2C         13           FRAS1         NM_001166133         42         SLC3A1         NM_001126335         13           GLA         NM_000169         7         SMARCAL1         NM_001127207         18           GLI3         NM_000168         15         TCTN1         NM_001082537         JBTS13         15           GLI3         NM_0011646177         9         TMEM216	COUZ	NIVI_015697		/	SUNNIG	NIVI_001039		13
CINS         NM_001031681         13         SIA2         NM_01032         2           CUL3         NM_001257197         15         SIX5         NM_175875         3           DHCR7         NM_001163817         9         SLC12A1         NM_000338         NKCC2         27           EGF         NM_001178130         HOMG4         23         SLC12A3         NM_000339         NCCT         26           EYA1         NM_000503         18         SLC26A4         NM_000441         21           FGF23         NM_020638         3         SLC34A1         NM_001167579         NPT2a         9           FN1         NM_00166133         42         SLC3A1         NM_001177316         NPT2C         13           FRAS1         NM_001166133         42         SLC3A1         NM_00198484         26           GATA3         NM_001002295         6         SLC7A9         NM_001126335         13           GLA         NM_000169         7         SMARCAL1         NM_001127207         18           GLI3         NM_001164617         9         TMEM216         NM_001173990         JBTS2, MKS2         5           GPC3         NM_001164617         9         TMEM237         N	CREBBP	NIVI_001079846		30	SIV1	NM 016022		2
COLOS       NM       NM       OD1257197       15       SIXS       NM       NM       SIXS       NM       SIXS       S		NIVI_001031081		13	SIXE	NM 175875		2
DHCH7         NM_001163817         9         SLC12A1         NM_000305         NRCC2         27           EGF         NM_001178130         HOMG4         23         SLC12A3         NM_000339         NCCT         26           EYA1         NM_000503         18         SLC26A4         NM_000441         21           FGF23         NM_020638         3         SLC34A1         NM_001167579         NPT2a         9           FN1         NM_002026         46         SLC34A3         NM_001177316         NPT2C         13           FRAS1         NM_001166133         42         SLC3A1         NM_000341         10           FREM2         NM_001002295         6         SLC7A9         NM_001126335         13           GLA         NM_000169         7         SMARCAL1         NM_001127207         18           GLI3         NM_00168         15         TCTN1         NM_001082537         JBTS13         15           GLI3         NM_001164617         9         TMEM216         NM_001173990         JBTS2, MKS2         5           GPC3         NM_001164617         9         TMEM237         NM_001044385         JBTS14         12           GRHPR         NM_0020458		NNI_001237197		15	SI C12A1	NIM 000338		27
EYA1       NM_00017/8130       NOVIG4       23       SLC12/30       NM_2000503       10011       21         FGF23       NM_020638       3       SLC26A4       NM_000411       21         FGF23       NM_020638       3       SLC34A1       NM_001167579       NPT2a       9         FN1       NM_001166133       42       SLC3A1       NM_001177316       NPT2C       13         FRAS1       NM_001166133       42       SLC3A1       NM_000341       10         FREM2       NM_001002295       6       SLC7A9       NM_001126335       13         GLA       NM_000169       7       SMARCAL1       NM_001127207       18         GLI3       NM_000168       15       TCTN1       NM_001082537       JBTS13       15         GLI3       NM_00116417       9       TMEM216       NM_001173990       JBTS2, MKS2       5         GPC3       NM_001164617       9       TMEM237       NM_001044385       JBTS14       12         GRHPR       NM_00203       9       TMEM67       NM_001142301       JBTS6, MKS3, 29       9         HNF1B       NM_000458       9        NM_001142301       JBTS6, MKS3, 29       9 <td></td> <td>NIVI_001103017</td> <td></td> <td>3</td> <td>SLC12A1</td> <td>NIM 000339</td> <td>NCCT</td> <td>26</td>		NIVI_001103017		3	SLC12A1	NIM 000339	NCCT	26
FGF23       NM_000003       10       GLC24A1       NM_0001167579       NPT2a       9         FGF23       NM_0020638       3       SLC34A1       NM_001167579       NPT2a       9         FN1       NM_002026       46       SLC34A3       NM_001177316       NPT2C       13         FRAS1       NM_001166133       42       SLC3A1       NM_0000341       10         FREM2       NM_001002295       6       SLC7A9       NM_001126335       13         GLA       NM_000169       7       SMARCAL1       NM_001127207       18         GLI3       NM_00168       15       TCTN1       NM_001082537       JBTS13       15         GLI32       NM_001164617       9       TMEM216       NM_001173990       JBTS2, MKS2       5         GPC3       NM_001164617       9       TMEM237       NM_001044385       JBTS14       12         GRHPR       NM_012203       9       TMEM67       NM_001142301       JBTS6, MKS3, 29       9         HNF1B       NM_000458       9        NPHP11		NM 000503	11010104	18	SL C2644	NM_000441	NOOT	20
FN1       NM_02000       46       SLC34A3       NM_001177316       NPT2C       13         FRAS1       NM_001166133       42       SLC3A1       NM_001177316       NPT2C       13         FREM2       NM_001166133       42       SLC3A1       NM_001098484       26         GATA3       NM_001002295       6       SLC7A9       NM_001126335       13         GLA       NM_000169       7       SMARCAL1       NM_001082537       JBTS13       15         GLI3       NM_0011668       15       TCTN1       NM_001173990       JBTS2, MKS2       5         GPC3       NM_001164617       9       TMEM237       NM_001044385       JBTS14       12         GRHPR       NM_012203       9       TMEM67       NM_001142301       JBTS6, MKS3, 29       29         HNF1B       NM_000458       9        NM_001142301       JBTS6, MKS3, 29       NPHP11	EGE23	NM_020638		3	SI C34A1	NM_001167579	NPT2a	9
FRAS1       NM_001166133       42       SLC3A1       NM_000381       10         FREM2       NM_207361       24       SLC4A4       NM_001098484       26         GATA3       NM_001002295       6       SLC7A9       NM_001126335       13         GLA       NM_000169       7       SMARCAL1       NM_001127207       18         GLI3       NM_00168       15       TCTN1       NM_001082537       JBTS13       15         GLIS2       NM_001164617       9       TMEM216       NM_001173990       JBTS2, MKS2       5         GPC3       NM_0011203       9       TMEM237       NM_001044385       JBTS14       12         GRHPR       NM_012203       9       TMEM67       NM_001142301       JBTS6, MKS3, 29       9         HNF1B       NM_000458       9        NM_001142301       JBTS6, MKS3, 29       0	FN1	NM_002026		46	SI C34A3	NM 001177316	NPT2C	13
FREM2         NM_207361         24         SLC4A4         NM_001098484         26           GATA3         NM_001002295         6         SLC7A9         NM_001126335         13           GLA         NM_000169         7         SMARCAL1         NM_001127207         18           GLI3         NM_00168         15         TCTN1         NM_001082537         JBTS13         15           GLIS2         NM_001164617         9         TMEM216         NM_001173990         JBTS2, MKS2         5           GPC3         NM_001164617         9         TMEM237         NM_001044385         JBTS14         12           GRHPR         NM_0020358         9         TMEM67         NM_001142301         JBTS6, MKS3, 29           HNF1B         NM_000458         9         NMEM67         NM_001142301         JBTS6, MKS3, 29	FRAS1	NM_001166133		42	SLC3A1	NM 000341	111 120	10
GATA3         NM_001002295         6         SLC7A9         NM_001126335         13           GLA         NM_000169         7         SMARCAL1         NM_001127207         18           GLI3         NM_000168         15         TCTN1         NM_001082537         JBTS13         15           GLIS2         NM_032575         NPHP7         6         TMEM216         NM_001173990         JBTS2, MKS2         5           GPC3         NM_001164617         9         TMEM237         NM_001044385         JBTS14         12           GRHPR         NM_012203         9         TMEM67         NM_001142301         JBTS6, MKS3, 29         9           HNF1B         NM_000458         9          NPHP11	FRFM2	NM 207361		24	SLC4A4	NM 001098484		26
GLA         NM_000169         7         SMARCAL1         NM_001127207         18           GL3         NM_000168         15         TCTN1         NM_001082537         JBTS13         15           GLI3         NM_032575         NPHP7         6         TMEM216         NM_001173990         JBTS2, MKS2         5           GPC3         NM_001164617         9         TMEM237         NM_001044385         JBTS14         12           GRHPR         NM_012203         9         TMEM67         NM_001142301         JBTS6, MKS3, 29           HNF1B         NM_000458         9         NM_001142301         JBTS6, MKS3, 29	GATA3	NM_001002295		6	SLC7A9	NM 001126335		13
GLI3         NM_000168         15         TCTN1         NM_001082537         JBTS13         15           GLIS2         NM_032575         NPHP7         6         TMEM216         NM_001173990         JBTS2, MKS2         5           GPC3         NM_001164617         9         TMEM237         NM_001044385         JBTS14         12           GRHPR         NM_012203         9         TMEM67         NM_001142301         JBTS6, MKS3, 29           HNF1B         NM_000458         9         NM_001142301         JBTS6, MKS3, 29	GLA	NM 000169		7	SMARCAL1	NM_001127207		18
GLIS2         NM_032575         NPHP7         6         TMEM216         NM_001173990         JBTS2, MKS2         5           GPC3         NM_001164617         9         TMEM237         NM_001044385         JBTS14         12           GRHPR         NM_012203         9         TMEM67         NM_001142301         JBTS6, MKS3,         29           HNF1B         NM_000458         9          NM_001142301         NPHP11	GLI3	NM_000168		15	TCTN1	NM_001082537	JBTS13	15
GPC3         NM_001164617         9         TMEM237         NM_001044385         JBTS14         12           GRHPR         NM_012203         9         TMEM67         NM_001142301         JBTS6, MKS3,         29           HNF1B         NM_000458         9         MEM67         NM_001142301         NHP11	GLIS2	NM_032575	NPHP7	6	TMEM216	NM_001173990	JBTS2, MKS2	5
GRHPR         NM_012203         9         TMEM67         NM_001142301         JBTS6, MKS3,         29           HNF1B         NM_000458         9         NPHP11	GPC3	NM_001164617		9	TMEM237	NM_001044385	JBTS14	12
HNF1B NM_000458 9 NPHP11	GRHPR	NM_012203		9	TMEM67	NM_001142301	JBTS6, MKS3,	29
	HNF1B	NM_000458		9			NPHP11	

(continued)

(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
UРКЗА	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 gueried 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  $\geq 5$  (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  $\approx 0.58$  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  $\geq 10\times$  (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

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	Case 1	Case 2	Case 3	Case 4	Control 1	Control 2	Control 3	Control 4	Control 5	Control 6
Total number of variants Quality filter (Q_VAR >50,	421 385	546 522	471 433	515 489	561 527	566 532	509 490	523 499	523 500	466 445
QD >5 and observed % >30)										
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

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

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

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 vears, 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

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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  $1\beta$  (*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* 

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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

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

Because  $\approx$ 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-bygene 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 agedependent 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 areater 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. Ser342Glv 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 hypertensionassociated 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

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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 phenotypedefined 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 thetarget region covered.

Table S2: Target regions covered with <10×.

**Table S3:** Broad disease phenotypes, genes tested, andmodes of inheritance.