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Targeted broad-based genetic testing by next-generation sequencing informs diagnosis and facilitates management in patients with kidney diseases

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ABSTRACT

Background. The clinical diagnosis of genetic renal diseases may be limited by the overlapping spectrum of manifestations between diseases or by the advancement of disease where clues to the original process are absent. The objective of this study was to determine whether genetic testing informs diagnosis and facilitates management of kidney disease patients.

Methods. We developed a comprehensive genetic testing panel (KidneySeq) to evaluate patients with various phenotypes including cystic diseases, congenital anomalies of the kidney and urinary tract (CAKUT), tubulointerstitial diseases, transport disorders and glomerular diseases. We evaluated this panel in 127 consecutive patients ranging in age from newborns to 81 years who had samples sent in for genetic testing.

Results. The performance of the sequencing pipeline for single-nucleotide variants was validated using CEPH (Centre de'Etude du Polymorphism) controls and for indels using Genome-in-a-Bottle. To test the reliability of the copy number variant (CNV) analysis, positive samples were re-sequenced and analyzed. For patient samples, a multidisciplinary review board interpreted genetic results in the context of clinical data. A genetic diagnosis was made in 54 (43%) patients and ranged from 54% for CAKUT, 53% for ciliopathies/tubulointerstitial diseases, 45% for transport disorders to 33% for glomerulopathies. Pathogenic and likely pathogenic variants included 46% missense, 11% nonsense, 6% splice site variants, 23% insertion–deletions and 14% CNVs. In 13 cases, the genetic result changed the clinical diagnosis.

Conclusion. Broad genetic testing should be considered in the evaluation of renal patients as it complements other tests and provides insight into the underlying disease and its management.

Keywords: copy number variants, genetic kidney disease, massively parallel sequencing, targeted gene panel

ADDITIONAL CONTENT

An author video to accompany this article is available at: https://academic.oup.com/ndt/pages/author_videos.

INTRODUCTION

The kidney is a complex organ that maintains physiological homeostasis through a myriad of complex processes that include the excretion of excess water, ingested drugs, toxins and metabolic waste products, the regulation of acid–base balance, the reclamation or elimination of various salts, and the synthesis of a variety of endocrine hormones to control blood pressure, erythropoiesis and bone mineralization. Disrupting this function leads to a broad spectrum of disease phenotypes. At one extreme are diseases that manifest as well-recognized Mendelian disorders such as Liddle syndrome, which is characterized by hypertension with hypokalemia from unregulated hyperactivity of the epithelial sodium channel in the connecting tubule and collecting duct [1]. At the other extreme are diseases in which a more global decline in renal function leads to chronic kidney disease (CKD) with a reduction in glomerular filtration rate, retention of urea, phosphorus and potassium, and the development of anemia and bone disease. The development of CKD may blur clues to the inciting insult even with extensive laboratory testing, renal imaging and renal histological examination [2].

Over the past decade, a number of discoveries relevant to renal diseases have improved our understanding of the ciliopathies [3], focal segmental glomerulosclerosis (FSGS) [4], steroid-resistant nephrotic syndrome [5, 6] and congenital anomalies of the kidney and urinary tract (CAKUT) [7, 8]. In recent years, the advancement of next-generation sequencing has facilitated the simultaneous interrogation of multiple genes for molecular diagnosis within many disease categories including those that cause a variety of renal diseases [9, 10]. In addition, exome sequencing (ES) has been used to diagnose monogenic renal diseases [11, 12]. The diagnostic success of disease-focused panels may be limited by difficulty in phenotyping renal diseases into specific categories. Similarly, ES may not be sensitive enough to detect variants in duplicated regions, such as the proximal portion of *PKD1*. We sought to test the clinical relevance of broad-based genetic testing that targets genes across a wide variety of renal disease phenotypes to inform diagnosis and facilitate management of the renal patient. Using a panel of 177 genes, we tested 127 consecutive renal patients whose samples we received and in this diverse cohort made a genetic diagnosis in 54 patients. Remarkably, in 13 patients, the genetic findings changed the clinical diagnosis.

MATERIALS AND METHODS

Study design

This was a retrospective study of the diagnostic accuracy of comprehensive genetic testing panel used a cohort of 127 consecutive patients where samples were sent to the University of Iowa Institute of Human Genetics for gene screening. There were no exclusion criteria. Patients were classified, based on clinical history provided, into the following broad disease subtypes: ciliopathies/tubulointerstitial diseases, CAKUT, tubular transport disorders and glomerulopathies. American College of Medical Genetics (ACMG) criteria were used to classify genetic variants as pathogenic, likely pathogenic, variant of unknown significance (VUS), likely benign and benign [13].

Gene selection, platform design and validation, and patient recruitment

Genes implicated in a large number of renal diseases were selected for inclusion in the kidney disease panel (KidneySeq v1) and grouped by renal phenotype (e.g. ciliopathy, glomerular diseases and CAKUT). Targeted capture of coding exons and splice sites was optimized using RNA baits selected with Agilent's SureDesign online software, incorporating 4-fold probe density and 25-base pairs of flanking intronic sequence. Performance metrics were assessed by studying 31 genomic DNA samples from the CEPH consortium (Centre de'Etude du Polymorphism) using results to improve depth-of-coverage (Supplementary data, Table S1). Additional genes were also added to increase the genetically relevant search space. The updated panel (KidneySeq v2) was used in the diagnostic evaluation of sequentially accrued samples from patients with renal disease (Table 1 and Supplementary data, Table S2). There were no exclusionary criteria.

Library preparation, targeted genomic enrichment and massively parallel sequencing

After preparing libraries from patient-derived gDNA, library preparation, targeted genomic enrichment and massively parallel sequencing (MPS) were completed as we have described [14]. In brief, libraries were prepared using a modification of the solution-based Agilent SureSelect target enrichment system (Agilent Technologies, Santa Clara, CA, USA) with liquid-handling automation. Hybridization and capture with RNA baits were followed by a second amplification. Before pooling for sequencing, all samples were bar coded and multiplexed. Sequencing was done using Illumina HiSeq (pool of 48 samples) or MiSeq (pools of five samples) instrumentation (Illumina Inc., San Diego, CA, USA). Sanger sequencing was used to amplify and resolve exons 1–34 of *PKD1* [15, 16].

Bioinformatics analysis

Data analysis was performed on dedicated computing resources maintained by the Iowa Institute of Human Genetics using a standardized workflow for sequence analysis and variant calling [14]. The freebayes variant caller was used to identify variants in *PKD1*. Variant annotation was performed with a custom-built reporting tool.

Variant filtering

Library quality was based on the total number of reads per sample and coverage at 30× or greater, excluding low-quality variants [depth <10 or quality by depth (QD) <5] and

Table 1. KidneySeq v2 gene list by disease category

Ciliopathies/tubulointerstitial diseases	
Alagille syndrome	*NOTCH2*
Autosomal recessive polycystic kidney disease	*PKHD1*
Autosomal dominant polycystic kidney disease	*PKD1*, *PKD2*
Autosomal dominant tubulointerstitial kidney disease	*HNF1B*, *REN*, *UMOD*
Bardet–Biedl syndrome (BBS)	*ARL6*, *BBS1*, *BBS2*, *BBS4*, *BBS5*, *BBS7*, *BBS10*, *BBS12*, *CEP290*, *MKKS*, *PTHB1*, *TRIM32*, *TTC8*
COACH syndrome	*CC2D2A*, *RPGRIP1L*, *TMEM67*
HANAC syndrome	*COL4A1*
Jeune syndrome	*IFT80*, *IFT140*, *DYNC2H1*, *NEK1*, *TTC21B*
Joubert syndrome	*AHI1*, *ARL13B*, *ATXN10*, *CC2D2A*, *CEP290*, *CEP41*, *CSPP1*, *INPP5E*, *KIF7*, *NPHP1*, *OFD1*, *RPGRIP1L*, *TMEM216*, *TCTN1*, *TMEM138*, *TMEM237*, *TMEM67*, *TTC21B*
Juvenile nephronophthisis (JN)	*AHI1*, *ATXN10*, *IQCB1*, *CEP290*, *GLIS2*, *INVS*, *NEK8*, *NPHP1*, *NPHP3*, *NPHP4*, *RPGRIP1L*, *TMEM67*, *TTC21B*, *WDR19*, *XPNPEP3*
Meckel syndrome (MKS)/Meckel–Gruber syndrome	*B9D1*, *B9D2*, *CC2DA*, *CEP290*, *MKS1*, *NPHP3*, *RPGRIP1L*, *TCTN2*, *TMEM216*, *TMEM67*
Medullary cystic kidney disease 2	*UMOD*
Oro-facial-digital syndrome 1	*OFD1*
Renal cysts and diabetes syndrome	*HNF1B*
Serpentine fibula with polycystic kidney disease (SFPKS)/Hajdu–Cheney syndrome (HJCYS)	*NOTCH2*
Sensenbrenner syndrome/(CED)	*IFT122*, *IFT43*, *WDR19*, *WDR35*
Senior–Loken syndrome (JN with retinitis pigmentosa)	*CEP290*, *IQCB1*, *NPHP1*, *NPHP3*, *NPHP4*, *WDR19*
Disorders of tubular ion transport	
Apparent mineralocorticoid excess, syndrome of	*HSD11B2*
APRT deficiency	*APRT*
Autosomal dominant hypocalcemia, ± Bartter syndrome	*CASR*
Bartter syndrome	*BSND*, *CLCNKA*, *CLCNKB*, *KCNJ1*, *SLC12A1*
Cystinosis	*CTNS*
Cystinuria	*SLC3A1*, *SLC7A9*
Dent disease	*CLCN5*, *OCRL*
Distal renal tubular acidosis	*ATP6V0A4*, *ATP6V1B1*, *SLC4A1*
Familial hypertension with hyperkalemia (Gordon syndrome), Pseudohypoaldosteronism II	*CUL3*, *KLHL3*, *WNK1*, *WNK4*
Gitelman syndrome	*CLCNKB*, *SLC12A3*
Hypophosphatemic rickets	*DMP1*, *CLCN5*, *ENPP1*, *FGF23*, *PHEX*, *SLC34A3*
Isolated proximal renal tubular acidosis—generalized proximal defect (Fanconi syndrome)	*ATP7B*, *CLCN5*, *CTNS*, *EHHADH*, *FAH*, *HNF4A*, *SLC34A1*
Liddle syndrome (pseudo hyperaldosteronism)	*SCNN1B*, *SCNN1G*
Nephrogenic diabetes insipidus (NDI)	*AQP2*, *AVPR2*
Nephrogenic syndrome of inappropriate antidiuresis (NSIAD)	*AVPR2*
Primary hyperoxaluria	*AGXT*, *GRHPR*, *HOGA1*
Pseudohypoaldosteronism I (PHA I)	*NR3C2*, *SCNN1A*, *SCNN1B*, *SCNN1G*
Renal glucosuria	*SLC5A2*
Renal hypomagnesemia	*CLDN16*, *CLDN19*, *CNNM2*, *EGF*, *HNF1B*, *TRPM6*
Renal tubular acidosis, proximal, with ocular abnormalities	*SLC4A4*
Glomerular diseases	
Alport syndrome	*COL4A3*, *COL4A4*, *COL4A5*
Alstrom syndrome	*ALMS1*
Congenital nephrotic syndrome (Finnish type)	*NPHS1*
DDS; Frasier syndrome	*WT1*
Diffuse mesangial sclerosis	*ARHGDIA*, *PLCE1*, *WT1*
Epstein–Fechtner syndrome (renal disease with macrothrombocytopenia)	*MYH9*
Fabry disease	*GLA*
FSGS–AD/XL	*ACTN4*, *ANLN*, *ARHGAP24*, *CD2AP*, *COL4A3*, *COL4A4*, *COL4A5*, *INF2*, *LMX1B*, *PAX2*, *TRPC6*, *WT1*
FSGS–AR	*APOL1*, *CRB2*, *MYO1E*, *NPHP4*, *TTC21B*
FSGS/steroid-resistant nephrotic syndrome (SRNS)–AR	*ADCK4*, *ALG1*, *ARHGDIA*, *CUBN*, *LAMB2*, *NPHS1*, *NPHS2*, *PLCE1*, *PDSS2*, *PMM2*, *PTPRO*, *SCARB2*, *ZMPSTE24*
Galloway–Mowat syndrome	*WDR73*
Glomerulopathy with fibronectin deposits	*FN1*
Hereditary systemic or renal amyloidosis	*APOA1*, *B2M*, *FGA*, *LYZ*, *NLRP3*

Continued

Table 1. Continued

Muckle–Wells syndrome	*NLRP3*
Nail patella syndrome	*LMX1B*
Nephrotic syndrome, steroid sensitive	*PLCG2*
Pierson syndrome (nephrotic syndrome with microcoria)	*LAMB2*
Thin basement membrane disease (benign familial hematuria)	*COL4A3*, *COL4A4*
CAKUT	
Branchio-oto-renal syndrome	*EYA1*, *SIX1*, *SIX5*
CAKUT with VACTERL	*TRAP1*
Cogan oculomotor apraxia	*NPHP1*
Common CAKUT	*AGTR1*, *AGTR2*, *CHD1L*, *DSTYK*, *EYA1*, *GATA3*, *HNF1B*, *PAX2*, *RET*, *ROBO2*, *SALL1*, *SIX2*, *SIX5*, *TRAP1*
Fraser syndrome	*FRAS1*, *FREM2*, *GRIP1*
Hypoparathyroidism, sensorineural deafness and renal dysplasia	*GATA3*
Isolated renal hypo-dysplasia	*BMP4*, *DSTYK*, *FGF20*, *HNF1B*, *PAX2*, *RET*, *SALL1*, *SIX2*
Isolated renal hypoplasia and renal-coloboma syndrome (papillorenal syndrome)	*PAX2*
Isolated renal hypoplasia	*RET*, *UPK3*
Kallmann syndrome	*ANOS1*
Mayer–Rokitansky–Küster–Hauser syndrome	*WNT4*
Multicystic dysplastic kidney	*CHD1L*, *HNF1B*, *ROBO2*, *SALL1*
Posterior urethral valves	*CHD1L*, *HNF1B*, *ROBO2*, *SALL1*, *SIX2*
Renal cysts and diabetes syndrome	*HNF1B*
Renal tubular dysgenesis	*ACE*, *AGT*, *AGTR1*, *REN*
Townes–Brocks syndrome	*SALL1*
Unilateral renal agenesis	*DSTYK*, *HNF1B*, *RET*, *SALL1*
UPJ obstruction	*DSTYK*, *EYA1*, *HNF1B*, *RET*, *ROBO2*, *SALL1*
UVJ obstruction	*CHD1L*, *PAX2*, *SIX5*
Vesicoureteral reflux	*DSTYK*, *EYA1*, *GATA3*, *HNF1B*, *RET*, *ROBO2*, *SALL1*, *SOX17*, *TNXB*, *UPK3A*
Other	
Acrorenoocular syndrome (Okihiro syndrome)	*SALL4*
Mitochondrial cytopathy	*COQ2*
Pallister–Hall syndrome	*GLI3*
Rubinstein–Taybi syndrome	*CREBBP*
Schimke immuno-osseous dysplasia	*SMARCAL1*
SERKAL syndrome (46XX sex reversal with dysgenesis of kidneys, adrenal and lungs)	*WNT4*
Simpson–Golabi–Behmel syndrome	*GPC3*
Smith–Lemli–Opitz syndrome	*DHCR7*
Tuberous sclerosis	*TSC2*
Williams syndrome	7q 11.23

common variants with a minor allele frequency (MAF) >1% in any population (except for known risk alleles). Nonsynonymous single-nucleotide variants (SNVs), canonical splicing changes and insertion–deletions (indels) were retained. Reference databases routinely queried included the Human Gene Mutation Database, ClinVar, the autosomal dominant polycystic kidney disease (ADPKD) mutation database, the ARUP (*COL4A5*) database and our in-house Renal Variant Database (RVD). GERP++, PhyloP, MutationTaster, PolyPhen2, SIFT and LRT were used to calculate variant-specific pathogenicity scores as described [14].

Copy number variant analysis

Copy number variant (CNV) analysis was performed using ExomeCopy and ExomeDepth [17]. CNV calls from both programs were manually curated and validated if breakpoints were identified.

Sanger sequencing

Sanger sequencing was performed for platform validation, for *PKD1* testing and to confirm pathogenic variants, designing primers using Primer 3 (http://bioinfo.ut.ee/primer3-0.4.0/primer3/) [14].

Variant interpretation

A multidisciplinary board was held semimonthly to discuss all genetic results on a patient-by-patient basis in the context of the available clinical data. Variants were classified following ACMG guidelines. Variants with a MAF >1% were classified as 'benign' with a few notable exceptions (*APOL1* G1 and G2 alleles). Variants reported as 'pathogenic' in the literature with

Table 2. Indications for testing

CAKUT	
Branchio-oto-renal syndrome	2
HNF1-β	1
Multicystic dysplastic kidney	3
Papillorenal syndrome	1
Renal hypo/dysplasia	6
Unspecified	5
Total	18
Ciliopathy/tubulointerstitial	
ADPKD	7
ARPKD	3
Medullary cystic kidney disease/nephronophthisis	14
Orofacial digital syndrome	1
Renal cysts	5
Total	32
Tubular ion transport	
Apparent mineralocorticoid excess	1
Bartter/Gitelman	9
Cystinuria	1
Dent	5
Fanconi	2
Hypercalcemia	3
Hypokalemia	2
Hypomagnesemia	3
Hypophosphatemia	3
Kidney stones	2
Liddle syndrome	2
NDI	3
Pseudohypoaldosteronism I	2
Renal tubular acidosis	2
Total	40
Glomerulopathy	
Alport/Alport like	10
FSGS	17
Nephrotic proteinuria/nephrotic syndrome	9
Other glomerular	2
Total	40
Other	
Nephrogenic rests	1
Nonrenal	1
No information	5
Unclassified kidney disease	10
Total	17

Some patients had multiple laboratory abnormalities or clinical diagnosis that is listed individually, resulting in larger totals. ARPKD, autosomal recessive polycystic kidney disease.

supporting functional evidence were classified as 'pathogenic'. The 'likely pathogenic' classification was assigned to missense variants with pathogenicity scores ≥4 (based on GERP++, PhyloP, MutationTaster, PolyPhen2, SIFT and LRT) if they were also ultra-rare and in a disease-related functional domain. Novel or rare variants that changed protein sequence but had an unknown impact on protein function were classified as VUSs. Based on the clinical phenotype and the genotypic findings, clinical correlation and segregation analysis were recommended.

Institutional Review Board

The study was approved by the Institutional Review Board (IRB No. 201805825) for human subject research and informed consent was waived. The study adheres to the Principles of Medical Research as stated in the Declaration of Helsinki.

RESULTS

Performance metrics

Performance and validation of KidneySeq v1 using 31 CEPH samples showed that >70% of sequence reads overlapped target regions with a mean coverage of ≥400×; >99% of bases were covered by at least 30 reads (30×). This threshold was achievable with at least 5 million reads per sample (Supplementary data, Figure S1). Targeted regions covered at less than 30× were Sanger sequenced; no additional variants were identified (Supplementary data, Table S3). These performance metrics were used to refine the panel by changing probe density.

Variant analysis

Call accuracy in the 31 CEPH controls was determined by Sanger sequencing 29 variants with MAF >1% and 32 variants with QD <10 in all samples (Supplementary data, Table S4); 256 variants that were either heterozygous or homozygous alternate were identified (Supplementary data, Figure S2). All validated variants with a QD <5 were false positives. Between QD >5 and QD <10, there were false-positive calls for both SNVs and indels. Of the 1643 sites, there were 252 true positives, 4 false positives, 1387 true negatives and no false negatives. Specificity (99.71), sensitivity (100), and positive (98.44) and negative (100) predicted values were very high (Supplementary data, Tables S4 and S5).

Validation of sequencing and analysis pipeline

A high-density SNP array was used to interrogate the CEPH sample, NA12287 (1421-14). A comparison of genotype calls from the SNP array and KidneySeq v1 identified only one discordant variant from the 3008 identified (Supplementary data, Figure S3a). Through Sanger confirmation, we verified that the KidneySeq v1 variant call was correct and the SNP array was incorrect. To validate indels, we used Genome-in-a-Bottle (GIAB), which predicts 314 indels in the KidneySeq v1 targeted regions. All predicted indels were identified by KidneySeq v1 in addition to two other indels at QD >5 not reported in the GIAB reference sequence but confirmed by Sanger sequencing (Supplementary data, Figure S3b). To test the reliability and sensitivity of the CNV analysis workflow, positive samples were re-sequenced and re-analyzed. All known CNVs were detected successfully on the repeat samples (Supplementary data, Table S6).

PKD1 gene proximal region

The duplicated region of *PKD1* (exons 1–34) was Sanger sequenced to verify variant detection. The panel detected 36 variants in the homologous region of the *PKD1* gene in seven patients selected for this validation. Overall, 94.4% (34 of 36) of these variants were verified by Sanger sequence. The variant detected only by MPS (the same variant was detected in two patients) was a false positive in exon 15. No false negatives were detected by Sanger sequencing.

Patients

Genetic testing was completed on 127 patients (77 males). The most common indication was FSGS (17 patients), followed by medullary cystic kidney disease/nephronophthisis (14

Table 3. Clinical renal samples: all patients with indication for testing, family history, disease type and demographics; family history, when known, are shown as positive (Y) or negative (N)

Case	Indication for testing	Family history	Disease category[a,b]	Sex	Age (years)	Ethnicity
1	Bilateral multicystic dysplastic kidneys	Y	1	F	6	Hispanic
2	Renal dysplasia	Unknown	1	M	1	Caucasian, non-Hispanic
3	Stage 5 (CKD), hearing loss	Unknown	4	M	37	Asian
4	FSGS at age 40 years	N	4	M	66	Caucasian, non-Hispanic
5	Proteinuria, FSGS	Y	4	M	54	African/African-American, non-Hispanic
6	Alport syndrome	Y	4	M	34	White
7	Dent disease (NDI, failure to thrive)	Unknown	3	M	1	Caucasian, Hispanic or Latino
8	Nephronophthisis	Y	2	F	10	African/African-American
9	FSGS	Unknown	4	M	54	African/African-American
10	Nephrotic syndrome	Unknown	4	M	3	Hispanic or Latino
11	Medullary cystic kidney disease	Unknown	2	M	27	Caucasian, non-Hispanic
12	Hypomagnesemia	Unknown	3	F	11	Not provided
13	FSGS	Unknown	4	M	58	Caucasian, non-Hispanic
14	Medullary cystic kidney disease/ nephronophthisis	Unknown	2	M	31	Caucasian
15	Hypercalcemia, hypocalciuria	N	3	F	81	Caucasian
16	Dilated cardiomyopathy and hypomagnesemia	N	3	M	3	Caucasian
17	Fanconi syndrome, hypophosphatemic rickets	Unknown	3	M	2	Caucasian, aboriginal
18	ESRD, primary FSGS	Unknown	4	M	55	Caucasian
19	Severe CAKUT	Unknown	1	M	<1	Caucasian, Hispanic or Latino
20	Alport syndrome	Unknown	4	M	5	Asian (India), non-Hispanic
21	Hypercalcemia, hypercalciuria, short stature	Unknown	3	M	2	Caucasian, non-Hispanic
22	Interstitial nephritis	Unknown	2	F	10	Caucasian
23	U/S prenatal echogenic kidneys, postnatal bilateral cysts, HNF1B disease	Unknown	1	M	<1	Caucasian, non-Hispanic
24	Bartter syndrome or other	Unknown	3	M	1	Not provided
25	ESRD, tubulointerstitial disease	Y	2	M	51	African/African-American
26	Bilateral hypoplastic dysplastic kidneys	Unknown	1	M	<1	Caucasian, Hispanic or Latino
27	Microhematuria, Alport or TBM disease	Unknown	4	M	2	Caucasian, Hispanic or Latino
28	FSGS or MCKD	Y	2, 4	M	60	African/African-American, non-Hispanic
29	Alport or TBM disease	Unknown	4	M	18	Caucasian, non-Hispanic
30	FSGS, SRNS, hypoalbuminemia	Unknown	4	M	17	Caucasian, non-Hispanic
31	FSGS or Dent disease. Nephroticrange proteinuria, global glomerulosclerosis	Unknown	3, 4	M	18	African/African-American
32	ADTKD, tubular proteinuria, no signs of Fanconi	Y	2	M	18	Unknown
33	Alport syndrome. Hearing loss, microscopic hematuria, CKD	Unknown	4	M	12	Caucasian
34	Renal agenesis/hypoplasia or nephronophthisis	Y	1, 2	F	16	Hispanic or Latino
35	Gitelman/Bartter syndrome	Unknown	3	F	17	Caucasian

Continued

Table 3. Continued

Case	Indication for testing	Family history	Disease category[a,b]	Sex	Age (years)	Ethnicity
36	Bilateral multicystic dysplastic kidneys, perinatal death	Unknown	1	M	0[b]	Unknown
37	Bartter syndrome, NDI or Dent disease. Polyuria, polydipsia, hypercalciuria, medullary nephrocalcinosis	Unknown	3	M	16	Caucasian, non-Hispanic
38	Pseudohypoaldosteronism. Hyperkalemia, polyuria	Unknown	3	M	0[b]	Hispanic or Latino
39	Multicystic bilateral kidneys	Unknown	1	M	0[b]	Caucasian, non-Hispanic
40	Apparent mineral corticoid excess	Unknown	3	M	2	Not provided
41	Bartter syndrome. Polyuria, metabolic alkalosis	Unknown	3	F	3	Caucasian, non-Hispanic
42	Liddle syndrome. Early onset hypertension and hypokalemia	Y	3	F	19	Caucasian, Hispanic or Latino
43	PKD (bilateral renal cysts and hypertension)	Unknown	2	M	15	Hispanic or Latino
44	NDI, medullary nephrocalcinosis, vesicoureteral reflux, hypophosphatemia	Unknown	3	F	3	Caucasian, non-Hispanic
45	Cystinuria	Y	3	F	19	Caucasian
46	FSGS or minimal change disease. Persistent proteinuria	Unknown	4	M	5	Caucasian, non-Hispanic
47	Hypokalemia, hypomagnesemia, high urinary Na and K, prior diagnosis of NDI	Unknown	3	F	59	Caucasian, non-Hispanic
48	Hypotonia, dysmorphic features, developmental delay, obesity	Unknown	5	F	2	Caucasian, non-Hispanic
49	Horseshoe kidney asymptomatic; daughter, son perinatal/fetal demise with CAKUT	Y	1	F	33	Caucasian, Native American, non-Hispanic
50	Proximal tubulopathy or Dent or hypophosphatemic rickets, nephrocalcinosis, small stature	Unknown	3	F	13	Asian, non-Hispanic
51	FSGS, ESRD, post-kidney transplant	Unknown	4	M	15	Hispanic or Latino
52	*PKD1*, *PKD2*, *HNF1B*	Unknown	2	M	6	Hispanic or Latino
53	Renal cysts, family history of hereditary nephritis	N	2	F	49	Asian, non-Hispanic
54	Polycystic kidney disease, undescended testes, HTN	N	2	M	<1	Caucasian, non-Hispanic
55	ESRD, FSGS	Y	4	M	64	African/African-American
56	HTN, AKI, LVH, congenital nephrotic syndrome or ARPKD	Unknown	2, 4	F	<1	Not provided
57	Moderate CKD	Unknown	5	M	1	Not provided
58	Not provided	Unknown	5	F	16	Not provided
59	Bartter/Gitelman syndrome, hypokalemia, hypomagnesemia and metabolic alkalosis	Unknown	3	M	12	Not provided
60	Nephronophthisis or MCKD	Y	2	M	58	Caucasian, non-Hispanic
61	Polycystic kidney disease	Unknown	2	F	51	African/African-American
62	FSGS or MCKD	Y	2, 4	M	56	African/African-American
63	FSGS/multicystic dysplastic kidney	Y	1, 4	M	15	Caucasian, non-Hispanic
64	Hyperplastic nephrogenic rests, features seen with underlying syndromes such as Beckwith–Wiedemann	Unknown	5	F	<1	Not provided
65	Hypophosphatemic rickets; distal renal tubular acidosis; isolated proximal renal tubular acidosis, generalized proximal defect	N	3	F	0[b]	Hispanic or Latino
66	FSGS	Unknown	4	F	10	African/African-American, non-Hispanic

Continued

Table 3. Continued

Case	Indication for testing	Family history	Disease category[a,b]	Sex	Age (years)	Ethnicity
67	Horseshoe kidney, dysmorphic features, VSD	Y	1	F	<1	Egyptian
68	Kidney stones, paresthesias, hypercalciuria, hypoparathyroidism, ESRD	Y	3	M	58	Caucasian
69	Large cystic kidneys	N	2	M	27	Caucasian, non-Hispanic
70	Renal cystic dysplasia, ectopic atrial tachycardia, CUA, seizures, LVH; dialysis from birth	Unknown	2	F	<1	Caucasian
71	Steroid-resistant nephrotic syndrome	N	4	F	8	Asian, multiracial
72	MCD, unresponsive to steroids	N	2	F	3	African/African-American
73	Glomerulocystic kidneys and hepatoblastoma	N	2	F	3	Hispanic or Latino
74	Alport syndrome		4	M	13	Caucasian
75	Steroid-resistant nephrotic syndrome	Y	4	M	4	Dominican Republic
76	Gitelman syndrome	N	3	F	23	Not provided
77	Not provided	Y	5	M	57	Not provided
78	Nephronophthisis	Y	2	F	38	Caucasian
79	Premature newborn with severely enlarged cystic kidneys noted mid-trimester, severe oligohydramnios, pulmonary hypoplasia	N	2	F	0[b]	Caucasian, Hispanic or Latino
80	Alport syndrome	Unknown	4	F	11	Caucasian
81	Hyponatremia, hypokalemia, nephrotic-range proteinuria, glucosuria	N	3	M	1	Caucasian, non-Hispanic
82	Global glomerulosclerosis	Y	4	F	65	African/African-American, non-Hispanic
83	Juvenile nephronophthisis and MCKD	Unknown	2	F	29	Not provided
84	Not provided	Unknown	5	M	14	Not provided
85	X-linked hypophosphatemic rickets	Unknown	3	F	1	Caucasian, non-Hispanic
86	Orofaciodigital syndrome I	Unknown	2	F	21	Caucasian, non-Hispanic
87	Bilateral cystic kidneys	Unknown	2	M	0[b]	Native American, Hispanic or Latino
88	Renal tubular acidosis	Unknown	1	F	9	Caucasian, Hispanic
89	Childhood nephrotic syndrome, possibly collapsing FSGS	Unknown	4	F	9	African/African-American, non-Hispanic
90	Alport syndrome	N	4	F	6	Caucasian
91	CKD, looking for APOL1 risk variants	N	4	F	18	African/African-American
92	Bilateral cystic kidney disease	Unknown	2	F	14	Caucasian, non-Hispanic
93	Congenital bilateral echogenic kidneys with small cysts	N	2	F	5	Not provided
94	Failure to thrive, presented with HTN and chronic renal failure	N	5	F	6	Caucasian
95	FSGS and hypertension	Unknown	4	M	54	Not provided
96	Alport syndrome, branchio-oto-renal syndrome (BOR), ESRD, nephronophthisis	Unknown	2, 4	M	16	Caucasian
97	Bartter syndrome	Unknown	3	F	2	Multiracial, Hispanic or Latino
98	Autosomal recessive polycystic kidney disease	Unknown	2	M	0[b]	Caucasian
99	Polycystic kidney disease	Y	2	M	7	Caucasian
100	Nephrotic syndrome	N	4	M	2	Caucasian
101	Chronic kidney stones and alkaline urine	Unknown	2	M	18	Not provided
102	Autosomal recessive polycystic kidney disease	Unknown	2	M	0[b]	Brazilian/Mexican, Hispanic or Latino
103	Nephrotic-range proteinuria	N	4	M	<1	Caucasian

Continued

Table 3. Continued

Case	Indication for testing	Family history	Disease category[a,b]	Sex	Age (years)	Ethnicity
104	Papillorenal syndrome (renal-coloboma syndrome)	N	1	M	2	Caucasian, Hispanic or Latino
105	Not provided	N	5	M	14	Caucasian
106	ADPKD	N	2	M	12	Caucasian
107	Congenital nephrotic syndrome	Unknown	4	F	0[b]	Hispanic or Latino
108	Not provided	Unknown	5	F	6	Not provided
109	Isolated multicystic dysplastic kidney disease and polycystic kidney disease	Unknown	2	M	7	Not provided
110	NDI	N	3	M	1	Caucasian, non-Hispanic
111	BOR or isolated CAKUT	Unknown	1	F	2	Not provided
112	Dent disease, Bartter or Gitelman syndromes	Unknown	3	M	23	Caucasian, non-Hispanic
113	ESRD of unknown etiology	Y	5	M	20	Hispanic or Latino
114	IgA nephropathy or FSGS	N	4	M	11	African/African-American
115	FSGS or diffuse mesangial sclerosis	Unknown	4	M	4	Caucasian
116	Alport syndrome	Y	4	M	13	Caucasian, non-Hispanic
117	Liddle syndrome	Unknown	3	F	4	Not provided
118	Nephrotic syndrome	Unknown	4	M	8	African/African-American
119	CDK Stage 2, FSGS	Unknown	4	F	16	African/African-American, non-Hispanic
120	ESRD due to FSGS	Unknown	4	F	20	Not provided
121	Juvenile nephronophthisis	Unknown	2	M	<1	Not provided
122	Zellweger syndrome, Galloway–Mowat syndrome, podocytopathy	Unknown	4	M	1	Caucasian, non-Hispanic
123	Steroid-resistant nephrotic syndrome	Unknown	4	M	<1	Caucasian, non-Hispanic
124	Bartter/Gitelman syndromes, pseudohypoaldosteronism Type 1	Unknown	3	M	<1	African/African-American
125	Nephronophthisis	Unknown	2	M	15	Caucasian
126	Nephronophthisis	N	2	F	12	Native Hawaiian or other Pacific Islander, non-Hispanic
127	Bartter syndrome, Gitelman syndrome or NDI	Y	3	M	2	Caucasian, non-Hispanic

[a]Disease category is associated with indication for testing.
[b]Disease categories: 1 = CAKUT; 2 = ciliopathies or tubulointerstitial disease; 3 = disorders of tubular ion transport; 4 = glomerulopathies; 5 = unclassified or other.
ADTKD, autosomal dominant tubulointerstitial disease; ARPKD, autosomal recessive polycystic kidney disease; CUA, calcific uremic arteriolopathy; F, female; HTN, hypertension; LVH, left ventricular hypertrophy; M, male; MCD, minimal change disease; MCKD, medullary cystic kidney disease; TBM, thin basement membrane disease; U/S, ultrasound VSD, ventricular septal defect; Y/N, yes/no.

patients), Alport or Alport-like syndrome (10 patients), Bartter/Gitelman syndrome (9 patients) and ADPKD (7 patients) (Table 2). Age ranged from newborn to 81 years (0–6 years, 56 patients; 7–14 years, 22 patients; 15–30 years, 26 patients; >30 years, 23 patients) (Table 3).

Variant identification and diagnostic rates in renal patients

A genetic diagnosis was made in 54 patients (43%) (Table 4; 46% solve rate between 0–14 years; 46% from 15–30 years and 22% in those >30 years). By disease group, the solve rate was 54% for CAKUT (7 of 13 patients), 53% for ciliopathies/tubulointerstitial diseases (17 of 32 patients), 45% for disorders of tubular transport (13 of 29 patients) and 33% for glomerulopathies (15 of 43 patients) (Figure 1 and Table 4). A number of identified variants were classified as VUSs as they did not meet ACMG criteria for pathogenicity or likely pathogenicity (Tables 5–7).

DISCUSSION

We identified a genetic basis for disease in 54 of 127 (44%) patients, demonstrating that broad-based genetic testing can augment current clinical algorithms used to evaluate the renal patient. The solve rate for cases decreased with age from 46% for patients between 0 and 14 years to 22% for patients >30 years old. Among solved cases, 9 were X-linked, 22 were autosomal dominant and 22 were autosomal recessive (6 homozygous and 16 compound heterozygous variants). Family history was positive in six autosomal dominant disorders (13 unknown), four autosomal recessive disorders (14 unknown) and in one X-linked disorder (7 unknown). Pathogenic and likely pathogenic variants included missense (32 of 75),

Table 4. Patients with a positive genetic diagnosis, showing indication(s) for testing, disease type, genetic variant(s), zygosity, ACMG classification, mean allele frequency and genetic diagnosis

Case	Indication for testing	Family history	Disease category[a]	Sex	Age (years)	Race/ ethnicity	Gene	Variant	Zygosity	ACMG classification [17]	MAF gnomAD[b]	Genetic diagnosis (AD/AR/XLR)	Disease category change[a]	First reported
1	**Bilateral multicystic dysplastic kidneys**	**Y**	**1**	**F**	**<1**	**H**	***PKD1***	**NM_000296: c.11575delG, p.Ala3859Profs*85**	**het**	**Pathogenic (PVS1, PM1, PM2)**	**Not reported**	**ADPKD**	**2**	**This manuscript**
							PKD2	**NM_000297: c.2T>A, p.Met1Lys**	**het**	**Likely pathogenic (PVS1, PM2, PP3)**	**0.02% LAT**			http://pkdb.mayo.edu/
2	Renal dysplasia	Unknown	1	M	2	1	*HNF1B*	NM_000458: c.516C>G, p.Tyr172*	het	Likely pathogenic (PVS1, PM2, PP3)	Not reported	*HNF1B*-related nephropathy (AD)		This manuscript
3	Stage V (CKD), hearing loss	Unknown	4	M	37	4	*COL4A5*	NM_000495: c.529G>C, p.Gly177Arg	hemi	Pathogenic known (PS1, PM1, PM2, PP3)	Not reported	Alport syndrome (XLD)		[18]
7	Dent disease (NDI, failure to thrive, anion gap metabolic acidosis)	Unknown	3	M	2	1	*AQP2*	NM_000486: c.502G>A, p.Val168Met	het	Pathogenic known PS1, PM2, PP3	0.041% LAT	NDI (AR)		[19]
								NM_000486: c.656A>G, p.Tyr219Cys	het	Likely pathogenic PM1, PM2, PP3, PP4	Not reported			This manuscript
8	**Nephronophthisis**	**Y**	**2**	**F**	**10**	**2**	***RPGRIP1L***	**NM_001127897: c.3118 + 1G>A**	**het**	**Pathogenic (PVS1, PM2, PP3)**	**0.011% AFR**	**COACH syndrome (AR) or Joubert syndrome (AR)**		**This manuscript**
								NM_001127897: c.1329_1330insA, p.Arg444Thrfs*10	**het**	**Pathogenic (PVS1, PM2, PP3)**	**Not reported**			**This manuscript**
11	**Autosomal dominant polycystic kidney disease**	Unknown	**2**	**M**	**27**	**1**	***NPHP1***	**NM_000272: c.1756C>T, p.Arg586***	**het**	**Pathogenic known (PVS1, PS1, PM2, PP3)**	**0.0009% NFE**	**Nephronophthisis 1 (AR)**		[20]
								Deletion of *NPHP1* gene region on chr2	**het**	**Pathogenic (PVS1, PS1, PM3, PP3)**				[21]
20	Alport syndrome	Unknown	4	M	5	4	*COL4A5*	NM_000495: c.1843G>A, p.Gly615Arg	hemi	Likely pathogenic known (PM1, PM2, PM5, PP2, PP3, PP5)	Not reported	Alport syndrome (XLD)		[22]
23	**U/S prenatal echogenic kidneys, postnatal bilateral cysts, HNF1B disease**	**Unknown**	**1**	**M**	**<1**	**1**	***PKD1***	**NM_000296: c.8597T>C, p.Leu2866Pro**	**het**	**Likely pathogenic known (PS1, PM2, PP3, PP5)**	**Not reported**	**ADPKD**	**2**	[23]
24	Bartter syndrome or other	Unknown	3	M	1	Unknown	*KCNJ1*	NM_000220: c.123G>C, p.Arg41Ser	hom	Likely pathogenic (PM1, PM2, PM3, PP2 PP3)	Not reported	Bartter syndrome (AR)		This manuscript
26	**Bilateral hypoplastic dysplastic kidneys**	**Unknown**	**1**	**M**	**<1**	**1H**	***EYA1***	**NM_000503: c.922C>T, p.Arg308***	**het**	**Pathogenic known (PVS1, PS3, PM2, PP3)**	**Not reported**	**Branchio-oto-renal syndrome (AD)**		[24]
29	Alport or thin basement membrane disease	Unknown	4	M	18	1	*COL4A3*	NM_000091: c.1408 + 2T>C	het	Pathogenic (PVS1, PM2, PP3)	Not reported	Alport syndrome (AD)/thin basement membrane disease (AD)		This manuscript
33		Unknown	4	M	12	1	*COL4A4*		het		0.00089% NFE			[25]

	Alport syndrome; hearing loss, microscopic hematuria, CKD							NM_000092: c.4522G>A, p.Gly1508Ser		Likely pathogenic (PS1, PM2, PP3)		Alport syndrome (AR)	
								chr2: 227892566 227974060 del	het	Pathogenic (PVS1, PM2, PM4, PP3)			This manuscript
37	Bartter syndrome, NDI or Dent disease; polyuria, polydipsia, hypercalciuria, medullary nephrocalcinosis	Unknown	3	M	16	1	*SLC12A1*	NM_000338: c.1652C>T, p.Thr551Ile	het	Likely pathogenic (PM1, PM2, PM3, PP3)	0.0009% NFE	Bartter syndrome (AR)	This manuscript
								NM_000338: c.2807G>A, p.Trp936*	het	Pathogenic (PVS1, PM2, PM4)	Not reported		[26]
38	Pseudohypoaldosteronism; hyperkalemia, polyuria	Unknown	3	M	<1	H	*SCNN1B*	NM_000336: c.682delG, p.Ala228Hisfs*8	het	Pathogenic (PVS1, PM2, PM4, PP3)	Not reported	Pseudohypoaldosteronism I (AR)	This manuscript
								chr16: 23313555-23315510 del	het	Pathogenic (PVS1, PM2, PP3)			This manuscript
39	Multicystic bilateral kidneys	Unknown	1	M	<1	1	*HNF1B*	Full gene deletion chr17: 36047234-36104883 del	het	Pathogenic known (PVS1, PM2, PP3)		*HNF1B*-related nephropathy (AD)	[27]
41	Bartter syndrome; polyuria, metabolic alkalosis	Unknown	3	F	3	1	*SLC12A1*	NM_000338: c.2873 + 2_2873 + 3insT	het	Pathogenic (PVS1, PM2, PP3)	0.0017 % NFE	Bartter syndrome (AR)	This manuscript
								NM_000338: c.3164 + 1G>A	het	Pathogenic known (PVS1, PS1 PM2, PP3)	0.0012% NFE		[28]
42	**Liddle syndrome; early onset hypertension and hypokalemia**	**Y**	**3**	**F**	**19**	**1H**	***HSD11B2***	**NM_000196: c.623G>A, p.Arg208His**	het	**Pathogenic (PS1, PS3, PM2, PP3)**	**0.006% LAT**	**Syndrome of apparent mineralocorticoid excess (AR)**	[29]
								NM_000196: c.667G>A, p.Asp223Asn	**het**	**Pathogenic (PS1, PS3, PM2, PP3)**	**0.033% LAT**		[30]
45	Cystinuria	Y	3	F	19	1	*SLC7A9*	NM_001126335: c.775G>A, p.Gly259Arg	hom	Pathogenic known (PS1, PM2, PM3, PP2, PP3	0.0018% NFE	Cystinuria (AR)	[31]
52	*PKD1, PKD2, HNF1B*	Unknown	2	M	6	H	*PKD1*	NM_000296: c.9395C>T, p.Ser3132Leu	het	Likely pathogenic known (PM1, PM2, PP3, PP5)	Not reported	ADPKD	[32]
53	Renal cysts	Y	2	F	49	4	*PKD1*	NM_000296: c.10102G>A, p.Asp3368Asn	het	Likely pathogenic known (PS1, PM2, PP3)	0.3% EA	ADPKD	[33]
							UMOD	NM_001008389: c.854C>A, p.Ala285Glu	het	VUS (PM2, PP2, PP3)	Not reported		
59	Bartter/Gitelman syndrome; hypokalemia, hypomagnesemia and metabolic alkalosis.	Unknown	3	M	12	Unknown	*SLC12A3*	NM_000339: c.1836G>T, p.Trp612Cys	hom	Likely pathogenic known (PM1, PM2, PM3, PP3, PP5)	Not reported	Gitelman syndrome (AR)	[34]
							CLCNKB	Full gene deletion	het	Likely pathogenic known (PS1, PM2, PM4)			[35]

Continued

Table 4. Continued

Case	Indication for testing	Family history	Disease category[a]	Sex	Age (years)	Race/ ethnicity	Gene	Variant	Zygosity	ACMG classification [17]	MAF gnomAD[b]	Genetic diagnosis (AD/AR/XLR)	Disease category change[a]	First reported
60	Nephronophthisis or medullary cystic kidney disease	Y	2	M	58	1	*UMOD*	UMOD (c.278_289del TCTGCCCCGAAGinsCCGCCTCCT; p.V93_G97del/ins AASC	het	Likely pathogenic known (PS1, PM, PM4)	Not reported	Tubulointerstitial kidney disease (AD)		[36]
61	Polycystic kidney disease	Unknown	2	F	51	2	*PKD1*	NM_000296: c.6356delA	het	Pathogenic (PVS1, PM2, PP3)	Not reported	ADPKD		This manuscript
63	FSGS, multicystic dysplastic kidney	Y	4/1	M	15	1	*PAX2*	NM_000278: c.419G>T, p.Arg140Leu	het	Likely pathogenic (PM1, PM2, PP1, PP3)	Not reported	FSGS (AD)/CAKUT		This manuscript
65	Hypophosphatemic rickets; distal renal tubular acidosis; isolated proximal renal tubular acidosis, generalized proximal defect	N	3	F	<1	H	*ATP6V0A4*	NM_020632: c.154_157 del GTGAp.Val 52 Metfs*25	het	Pathogenic (PVS1, PM2, PP3)	Not reported	Distal renal tubular acidosis (AR)		This manuscript
								NM_020632: c.1231G>T, p.Asp411Tyr	het	Likely pathogenic (PM2, PM3, PP3, PP5)	0.042% LAT			ClinVar (likely pathogenic)
68	Kidney stones, paresthesias, hypercalciuria, hypoparathyroidism, ESRD	Y	3	M	58	1	*CASR*	NM_000388: c.2506G>C, p.Val836Leu	het	Likely pathogenic (PM1, PM2, PP2, PP3)	Not reported	Hypocalcemia (AD)		This manuscript
69	Large cystic kidneys	N	2	M	27	1	*PKD1*	NM_000296: c.8311G>A, p.Glu2771Lys	het	Likely pathogenic known (PS1, PM1, PM2, PP3)	Not reported	ADPKD		[37]
70	Renal cystic dysplasia, ectopic atrial tachycardia, CUA, seizures, LVH; dialysis from birth	Unknown	2	F	<1	1	*WT1*	NM_000378: c.1249C>T, p.Arg417Cys	het	Likely pathogenic (PS1, PM2, PP3)	Not reported	DDS (AD)	3	[38]
79	Premature newborn with severely enlarged cystic kidneys noted mid-trimester, severe oligohydramnios, pulmonary hypoplasia	N	2	F	<1	1H	*PKHD1*	NM_138694.3: c.9689delA, p.Asp3230Valfs*34	het	Pathogenic known (PVS1, PM2, PP3, PP4)	0.039% LAT	ARPKD		[39]
								NM_138694.3: c.6297_6300delTG, p.Gln2100Glyfs*7	het	Pathogenic known (PVS1, PM2, PP3, PP4)	Not reported			[40]
80	Alport syndrome	Unknown	4	F	11	1	*COL4A5*	NM_000495: c.1117C>T, p.Arg373*	het	Pathogenic known (PVS1, PM1, PM2, PP3)	Not reported	Alport syndrome (XLD)		[18]
84	Not provided	Unknown	5	M	14	Unknown	*NPHP1*	Whole gene deletion	hom	Pathogenic known (PVS1, PS1, PM2)		Nephronophthisis 1 (AR)	2	[41]
86	Orofaciodigital syndrome I	Unknown	2	F	21	1	*OFD1*	NM_003611: c875_876delAT, p.Met293Glyfs*15	het	Pathogenic known (PVS1, PM2, PP3)	Not reported	Orofaciodigital syndrome I (AD)		[42]
87	Bilateral cystic kidneys	Unknown	2	M	<1	3H	*PKHD1*		het	Pathogenic (PVS1, PM2, PP3)	Not reported	ARPKD		This manuscript

								NM_138694: c.9559delT, p.Ser3187Leufs*33						
								NM_138694: c.107C>T, p.Thr36Met	het	Likely pathogenic known (PS1, PP3, PP5)	0.08% NFE			[43]
90	**Alport syndrome**	**N**	**4**	**F**	**6**	**1**	***NPHS2***	**NM_014625: c.871C>T, p.Arg291Trp**	**het**	**Likely pathogenic known (PS1, PM1, PM2, PP2, PP3, PP5)**	**0.029% EA**	**Steroid-resistant nephrotic syndrome (AR)**		[44]
								NM_014625: c.686G>A, p.Arg229Gln	**het**	**Likely pathogenic when inherited with a pathogenic known (PS3, PM1, PP2, PP3, PP5)**	**6.98% FE**			[44]
93	Congenital bilateral echogenic kidneys with small cysts	N	2	F	5	Unknown	*HNF1B*	Whole gene deletion	het	Pathogenic known (PVS1, PM2, PP3)		*HNF1B*-related nephropathy		[27]
94	**Failure to thrive, presented with hypertension and CKD**	**N**	**5**	**F**	**6**	**1**	***TTC21B***	**NM_024753: c.1516 + 2T>C**	**het**	**Pathogenic (PVS1, PM1, PP3)**	**0.0009% NFE**	**Juvenile nephronophthisis (AR), Jeune syndrome (AR), or Joubert syndrome (AR)**	**2**	**This manuscript**
								NM_024753: c.626C>T, p.Pro209Leu	**het**	**Likely pathogenic known (PS3 PM2, PP3, PP5)**	**0.03% LAT**			[45]
96	Alport syndrome, branchio-oto-renal syndrome (BOR), ESRD, nephronophthisis	Unknown	4	M	16	1	*COL4A5*	NM_000495: c.796C>T, p.Arg266*	hemi	Pathogenic known (PVS1, PM1, PM2, PM4, PP3, PP5)	Not reported	Alport syndrome (XLD)		[46]
97	Bartter syndrome	Unknown	3	F	2	H	*KCNJ1*	NM_000220: c.924C>A, p.Cys308*	het	Pathogenic (PVS1, PM2, PP3, PP5)	Not reported	Bartter syndrome (AR)		This manuscript
								NM_000220: c.683G>A, p.Gly228Glu	het	Likely pathogenic known (PS1, PM2, PP3, PP4)	0.0018% NFE			[47]
98	Autosomal recessive polycystic kidney disease	Unknown	2	M	<1	1	*PKHD1*	NM_138694: c.7717C>T, p.Arg2573Cys	het	Likely pathogenic known (PS1, PM2, PP3, PP4, PP5)	0.0058% EA	ARPKD		[48]
								NM_138694: c.3766delC, p.Gln1256Argfs*47	het	Pathogenic known (PVS1, PS1, PP3)	0.12% LAT			[48]
99	Polycystic kidney disease	Y	2	M	7	1	*PKD1*	NM_000296: c.12230_12231delAG, p.Glu4077Valfs*78	het	Pathogenic (PVS1, PM2, PP3)	Not reported	ADPKD		This manuscript
103	**Nephrotic range proteinuria**	**N**	**4**	**M**	**<1**	**1**	***CLCN5***	**NM_000084: c.1546C>T, p.Arg516Trp**	**hemi**	**Pathogenic known (PS1, PS3, PM2, PP2, PP3, PP5)**	**Not reported**	**Dent disease**	**3**	[49]
104	Papillorenal syndrome (renal-coloboma syndrome)	N	1	M	2	1H	*PAX2*	NM_000278: c.69delC, p.Val26CysfsX2	het	Pathogenic known (PVS1, PM2, PP3, PP4, PP5)	Not reported	Papillorenal syndrome (AD)		[50]
106		N	2	M	12	1	*PKD1*		het		0.027% NFE	ADPKD		[23]

Continued

Table 4. Continued

Case	Indication for testing	Family history	Disease category[a]	Sex	Age (years)	Race/ ethnicity	Gene	Variant	Zygosity	ACMG classification [17]	MAF gnomAD[b]	Genetic diagnosis (AD/AR/XLR)	Disease category change[a]	First reported
	Autosomal dominant polycystic kidney disease							NM_000296: c.776G>A, p.Cys259Tyr		Likely pathogenic known (PS1, PM2, PP3, PP5)				
113	**ESRD of unknown etiology**	**Y**	**5**	**M**	**20**	**H**	***NPHP1***	**Whole gene deletion**	**hom**	**Pathogenic known (PVS1, PS1, PM2)**		**Nephronophthisis 1 (AR)**	**2**	[41]
114	**IgA nephropathy or FSGS**	**N**	**4**	**M**	**11**	**2**	***COL4A4***	**NM_000092: c.1856G>A, p.Gly619Asp**	**het**	**Likely pathogenic known (PS1, PM2, PP3)**	**0.0066% AFR**	**Alport syndrome (AD)**		[51]
115	FSGS or diffuse mesangial sclerosis	Unknown	4	M	4	1	*WT1*	NM_000378: c.1333C>T, p.Arg445Trp	het	Pathogenic known (PS1, PS3, PM2, PP3)	Not reported	DDS (AD)		[52]
116	Alport syndrome	Y	4	M	13	1	*COL4A5*	NM_000495: c.1226G>A, p.Gly409Asp	het	Likely pathogenic known (PM1, PM2, PP2, PP3, PP5)	Not reported	Alport syndrome (XLD)		[18]
118	**Nephrotic syndrome**	**Unknown**	**4**	**M**	**8**	**2**	***APOL1***	**NM_001136540: c.1024A>G, p.Ser342Gly**	**hom**	**Risk allele**	**23% AFR**	**Dent disease (XLR) and *APOL1* G1/G1**	**3**	[53]
								NM_001136540: c.1152T>G, p.Ile384Met	**hom**	**Risk allele**	**22.9% AFR**			[53]
							CLCN5	**NM_000084: c.1909C>T, p.Arg637***	**hemi**	**Pathogenic known (PS1, PVS1, PM2, PP3, PP5)**	**Not reported**			[54]
120	ESRD due to FSGS	Unknown	4	F	20	Unknown	*PAX2*	NM_000278: c.70_71insG, p.Val26Glyfx*28	het	Pathogenic known (PS1, PVS1, PM1, PM2, PP3, PP5)	0.0068% AFR	FSGS (AD); *APOL1* G2/G2		[55]
							APOL1	NM_001136540: c.1160_1165 delATAATT	het	Risk allele	14.14% AFR			[53]
122	**Zellweger syndrome, Galloway–Mowat syndrome, podocytopathy**	**Unknown**	**4**	**M**	**1**	**1**	***OCRL***	**NM_000276: c.1484C>T, p.Pro495Leu**	**hemi**	**Pathogenic known (PS3, PM1, PM2, PP2, PP3, PP5)**	**Not reported**	**Lowe syndrome (XLR)**	**3**	[56]
124	Bartter/Gitelman syndromes, pseudohypoaldosteronism type 1	Unknown	3	M	<1	2	*NR3C2*	NM_000901: c.1002_1003insGT, p.Ser335Valfs*4	het	Pathogenic (PVS1, PM2, PP3)	Not reported	Pseudohypoaldosteronism I (AD)		This manuscript
125	Nephronophthisis	Unknown	2	M	15	1	*NPHP1*	Whole gene deletion	hom	Pathogenic known (PVS1, PS1, PM2)		Nephronophthisis 1 (AR)		[41]
126	Nephronophthisis	N	2	F	12	5	*NPHP1*	Whole gene deletion	hom	Pathogenic known (PVS1, PS1, PM2)		Nephronophthisis 1 (AR)		[41]

Patients in whom the genetic diagnosis changed the clinical diagnosis are shown in bold font.

[a]Disease category: 1 = CAKUT; 2 = ciliopathies or tubulointerstitial disease; 3 = disorders of tubular ion transport; 4 = glomerulopathies; 5 = unclassified or other. Ethnicity: 1 = Caucasian; 2 = African/African-American; 3 = American Indian or Alaska Native; 4 = Asian; 5 = Native Hawaiian or other Pacific Islander; H = Hispanic or Latino. Zygosity: het, heterozygous; hom, homozygous; hemi, hemizigous.

[b]gnomAD: highest MAF reported.

AFR, African; EA, East Asian; FE, European Finnish; NFE, European (non-Finnish); LAT, Latino; SA, South Asian; AD, autosomal dominant; AR, autosomal recessive; XLR, X-linked recessive; LVH, left ventricular hypertrophy; ARPKD, autosomal recessive polycystic kidney disease; M, male; F, female.

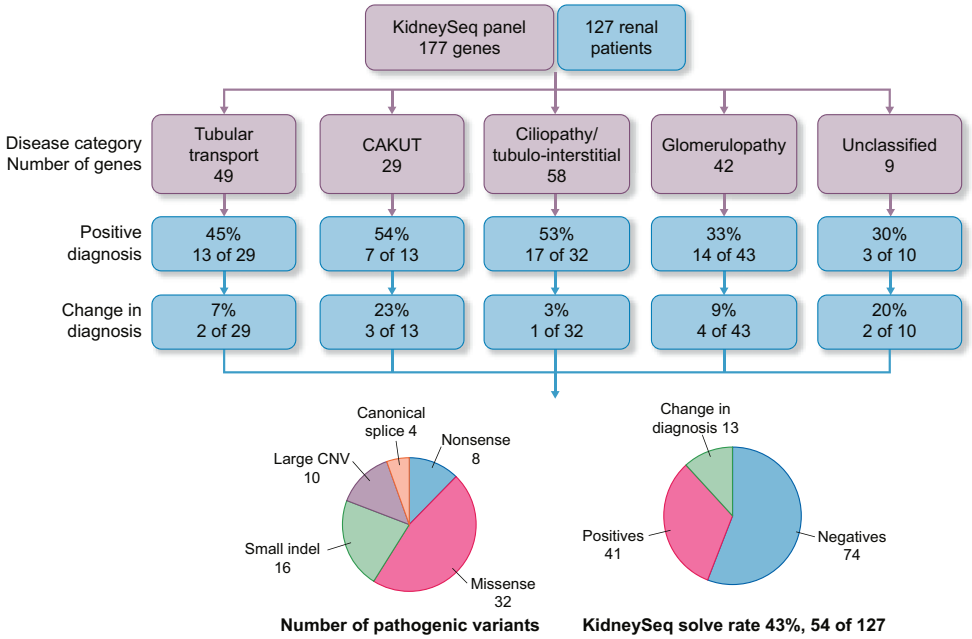

FIGURE 1: Outcome of KidneySeq panel testing in 127 renal patients. The positive diagnosis rate in each disease category is shown together with the percentage where diagnosis changed. A pie chart shows the number and types of pathogenic variants and the overall solve rate.

nonsense (9 of 75), canonical splice site variants (4 of 75), small indels (17 of 75) and large CNVs (10 of 75), demonstrating the power to detect all types of genetic variants (Figure 1).

In 41 of 54 patients with a genetic diagnosis, data confirmed the clinical impression (i.e. ADPKD as ADPKD, Bartter as Bartter, etc.) but also provided prognostic information, guided clinical management and/or enabled counseling (Figure 1 and Table 4). For example, the identification of a truncating variant in *PKD1* (NM_000296: c.12230_12231delAG) in a 7-year-old child with polycystic kidney disease (Case 99) mandates regular evaluation for increasing kidney volume, since truncating *PKD1* variants predict a median onset of end-stage renal disease (ESRD) at 55 years of age, substantially earlier than non-truncating *PKD1* variants or any *PKD2* variant [72]. In another example, the diagnosis of CKD at age 10 years (Case 8) in two fraternal twins born prematurely led to a clinical suspicion of juvenile nephronophthisis. We identified two null variants in *RPGRIP1L*, which is reported in the allelic disorders Joubert syndrome, COACH syndrome and Meckel syndrome. Patients with hypomorphic *RPGRIP1L* variants develop Joubert syndrome or COACH syndrome (Joubert features with congenital hepatic fibrosis), while those with null variants develop Meckel syndrome, which is considered to be at the more severe end of the clinical disease spectrum [73]. While the phenotype can be variable with Joubert and COACH syndrome, awareness of the type of genetic variants should prompt a careful and guided evaluation for extrarenal features, such as liver disease, that may require treatment.

In the remaining 13 cases (24%), genetic testing changed the clinical diagnosis, helped to direct future care, guided genetic counseling, and/or directed the evaluation process for living donor candidates. For example, the indication for screening in a 1-month-old (Case 26) was bilateral hypoplastic dysplastic kidneys. Upon testing, a null variant was identified in *EYA1*, consistent with the diagnosis of branchio-oto-renal syndrome 1 (BOR1). BOR1 exhibits variable penetrance and is characterized by hearing loss, branchial defects, preauricular pits and CAKUT [74]. On further evaluation, the child was found to have hearing loss and preauricular pits.

We also identified bilineal autosomal dominant diseases and digenic autosomal recessive disease. As an example of the former, in a 6-year-old female (Case 1) with bilateral multicystic dysplastic kidneys, pathogenic variants were identified in both *PKD1* (a single nucleotide deletion) and *PKD2* (a nucleotide substitution that converts the start codon to lysine). Each of these variants alone is sufficient to cause ADPKD, and the co-inheritance in this patient is consistent with her severe and atypical phenotype. Bilineal disease is rare in humans, although it has been noted in experimental mice [75–77].

In one case (Case 70), a medically actionable variant in *WT1* was incidentally identified in a 6-month-old infant with renal cystic dysplasia, ESRD, ectopic atrial tachycardia, left ventricular hypertrophy and seizures. The variant, p.Arg417Cys, is ultra-rare, predicted pathogenic and previously reported in two patients—one with Denys–Drash syndrome (DDS) and Wilms' tumor and one child with DDS who died shortly after birth [38, 78]. In light of these reports, the variant was reported to the clinician as likely pathogenic for DDS with the attendant risks of Wilms' tumor.

Table 5. VUSs

Case	Indication for testing	Family history	Disease category[a]	Sex	Age (years)	Ethnicity	Gene	Variant	Zygocity[b]	ACMG classification/ rules [17]	MAF Gnomad[c]	First reported by	Possibly causal[d]
5	Proteinuria, FSGS	Y	4	M	54	2	*FN1*	NM_002026: c.5779C>T, p.Arg1927Cys	het	PM1, PM2, PP3	0.007% NFE	Glomerulopathy with fibronectin deposits (AD)	Y
16	Dilated cardiomyopathy and associated hypomagnesemia	N	3	M	3	Caucasian	*ROBO2*	NM_002942: c.2834T>C, p.Ile945Thr	het	PS3, PM2, PP5	0.0027% NFE	[57]	N
17	Fanconi syndrome, hypophosphatemic rickets	Unknown	3	M	2	Caucasian, Aboriginal	*SLC4A1*	NM_000342: c.2396C>T, p.Ser799Leu	het	PM2, PP3	0.0045% NFE	This manuscript	N
18	ESRD, primary FSGS	Unknown	4	M	55	Caucasian	*ACTN4*	NM_004924: c.2680G>A, p.Gly894Ser	het	PP3	0.18% NFE	This manuscript	Y
19	Severe CAKUT	Unknown	1	M	<1	Caucasian, Hispanic	*DSTYK*	NM_015375: c.2216G>A, p.Arg739Gln	het	PM2, PP3	0.25% LAT	This manuscript	Y
22	Interstitial nephritis	Unknown	2	F	10	Caucasian	*NPHP4*	NM_015102: c.2849G>A, p.Arg950Gln	het	PM2, PP3	0.082% EA	This manuscript	Y
								NM_015102: c.2542G>A, p.Arg848Trp	het	PM2, PP3, BP6	2.56% EF	[58]	Y
25	ESRD, tubulointerstitial disease	Y	2	M	51	Africa/African-American	*CC2D2A*	NM_001080522: c.3157A>G, p.Ile1053Val	het	PM2, PP3	0.047% AFR	This manuscript	Y
								NM_001080522: c.3503G>A, p.Arg1168His	het	PM1, PM2, PP3	0.035% AFR	This manuscript	
30	FSGS, SRNS, hypoalbuminemia	Unknown	4	M	17	Caucasian non-Hispanic	*NPHP3*	NM_153240: c.2881C>G, p.Gln961Glu	het	PP3, PM2	0.055% NFE	This manuscript	N
34	Renal agenesis/hypoplasia or nephronophthisis	Y	1, 2	F	16	Hispanic	*SIX2*	NM_016932: c.126C>G, p.His42Gln	het	PM2, PP3	Not reported	This manuscript	Y
							NPHP4	NM_015102: c.3055G>A, p.Asp1019Asn	het	PM2, PP3	Not reported	This manuscript	N
35	Gitelman/Bartter syndrome; metabolic alkalosis, hypomagnesemia, hypokalemia	Unknown	3	F	17	Caucasian	*KLHL3*	NM_001257194: c.1357G>A, p.Val453Ile	het	PM2, PP2	0.002% NFE	This manuscript	N
42	Liddle syndrome. Early onset hypertension and hypokalemia	Y	3	F	19	Caucasian, Hispanic	*KLHL3*	NM_001257194: c.988C>T, p.Arg330Trp	het	PM2, PP2, PP3,	0.002% NFE	[59]	N
44	NDI, medullary nephrocalcinosis, vesicoureteral reflux, hypophosphatemia	Unknown	3	F	3	Caucasian, non-Hispanic	*ANOS1*	NM_000216: c.1759G>T, p.Val587Leu	het	PM1, PM2, PP3, PP5	Not reported	[60]	N
46	FSGS or minimal change disease. Persistent proteinuria	Unknown	4	M	5	Caucasian, non-Hispanic	*ANOS1*	NM_000216: c.2015A>G, p.His672Arg	het	PP5	0.044% NFE	[61]	N
50	Proximal tubulopathy or Dent or hypophosphatemic rickets. Nephrocalcinosis, small stature	Unknown	3	F	13	Asian	*FAH*	NM_000137: c.181G>T, p.Val61Phe	het	PP3	1.907% EA	This manuscript	Y
51	FSGS. Post deceased kidney transplant	Unknown	4	M	15	Hispanic	*LMX1B*	NM_001174146: c.875G>T, p.Arg292Leu	het	PP2, PP3	0.21% LAT	This manuscript	Y

							LAMB2	NM_002292: c.5234C>A, p.Ala1745Asp	het	PM2, PP3	Not reported	This manuscript	N
53	Renal cysts. Family history of hereditary nephritis	Y	2	F	49	Asian	*UMOD*	NM_001008389: c.854C>A, p.Ala285Glu	het	PM2, PP2, PP3	Not reported	This manuscript	Y
54	Polycystic kidney disease, undescended testes, HTN	N	2	M	<1	Caucasian, non-Hispanic	*NPHS1*	NM_004646: c.563A>T, p.Asn188Ile	het	LB* (PM1, PP5, BP4, BP6)	0.93% NFE	[62]	N
							TRAP1	NM_001272049: c.598A>G, p.Ile200Val	het	PP3	2.05% EF	ClinVar	N
57	Moderate CKD	Unknown	5	M	1	Unknown	*ACE*	NM_000789: c.793C>T, p.Arg265*	het	Pathogenic known (PVS1, PM2, PM4, PP3)	0.0027% NFE	[63]	Y
								NM_000789: c.3136G>A, p.Glu1046Ser	het	PM2, PM3, PP3	Not reported	This manuscript	Y
58	Not provided	Unknown	5	F	16	Not provided	*ACE*	NM_000789: c.955G>T, p.Ala319Ser	het	PM2	Not reported	This manuscript	N
							GLI3	NM_000168: c.1616G>A, p.Arg539Lys	het	PM2	Not reported	This manuscript	U
							KLHL3	NM_001257194: c.203C>T, p.Thr68Met	het	PM2, PP2	0.012% NFE	This manuscript	U
							SLC3A1	NM_000341: c.788G>C, p.Ser263Thr	het	PP2, PP3	0.27% AFR	ClinVar	U
							SMARCAL1	NM_001127207: c.1271A>T, p.Asp424Val	het	PP3, BP6	0.35% NFE	ClinVar	N
60	Nephronophthisis or medullary cystic kidney disease	Y	2	M	58	Caucasian, non-Hispanic	*TRAP1*	NM_001272049: c.598A>G, p.Ile200Val	het	PP3, BS1	2.05% EF	ClinVar	N
63	FSGS/multicystic dysplastic kidney	Y	1, 4	M	15	Caucasian, non-Hispanic	*PKD1*	NM_000296: c.971G>T, p.Arg324Leu	het	PM1, PP5	0.59% EF	Uniprot	N
64	Hyperplastic nephrogenic rests, features seen with underlying syndromes such as Beckwith-Wiedemann	Unknown	5	F	<1	Not provided	*CHD1L*	NM_001256336: c.2179A>G, p.Ile727Val	het		0.47% NFE	This manuscript	N
							IQCB1	NM_001023570: c.1441G>A, p.Glu481Lys	het	PM1, PP3, BP1	0.19% NFE	ClinVar	N
							ANOS1	NM_000216: c.1759G>T, p.Val587Leu	het	PM1, PM2, PP5	Not reported	[60]	N
67	Horseshoe kidney, dysmorphic features, VSD	Y	1	F	<1	Egyptian	*PTPRO*	NM_002848: c.433G>A, p.Glu145Lys	het	PM2	0.001% NFE	This manuscript	N
							WT1	NM_000378: c.563C>T, p.Ala188Val	het		0.007% AFR	This manuscript	N
71	Steroid-resistant nephrotic syndrome	N	4	F	8	Asian, multiracial	*ANLN*	NM_018685: c.1741G>C, p.Glu581Gln	het		0.023% EA	This manuscript	Y
							CUBN	NM_001081: c.6095G>A, p.Cys2032Tyr	het	PM2, PP3, BP1	0.019% NFE	This manuscript	N
73	Glomerulocystic kidneys and hepatoblastoma	N	2	F	3	Hispanic	*CHD1L*	NM_001256336: c.1798G>A, p.Gly600Arg	het	PM2, BP4	Not reported	This manuscript	Y
							PKD2	NM_000297: c.2398A>C, p.Met800Leu	het	PM2	Not reported	Uniprot	N
							TMEM67	NM_001142301: c.272G>A, p.Arg91Gln	het	PM2, PP2, PP3, PP5	0.012% LAT	ClinVar	N
75		Y	4	M	4		*BBS9*		het	BP6	0.75% AFR	ClinVar	N

Continued

Table 5. Continued

Case	Indication for testing	Family history	Disease category[a]	Sex	Age (years)	Ethnicity	Gene	Variant	Zygocity[b]	ACMG classification/ rules [17]	MAF Gnomad[c]	First reported by	Possibly causal[d]
	Steroid-resistant nephrotic syndrome					Dominican Republic		NM_198428: c.1648A>G, p.Ile550Val					
76	Gitelman syndrome	N	3	F	23	Not provided	*EYA1*	NM_000503: c.403G>A, p.Gly135Ser	het	PP3	0.064% EA	ClinVar	N
77	Not provided	Y	5	M	57	Not provided	*TRIM32*	NM_001099679: c.1688G>A, p.Arg563His	het	PM2, PP3	0.013% NFE	ClinVar	N
78	Nephronophthisis	Y	2	F	38	Caucasian	*GLIS2*	NM_032575: c.278A>G, p.Asn93Ser	het	BP1, BP4	0.09% EF	This manuscript	N
							TRPC6	NM_004621: c.1030G>A, p.Ala344Thr	het	PM2	Not reported	This manuscript	N
82	Global glomerulosclerosis	Y	4	F	65	African/African-American	*COL4A4*	NM_000092: c.3143G>A, p.Gly1048Asp	het	PM2, PP3	Not reported	This manuscript	Y
83	Juvenile nephronophthisis and medullary cystic kidney disease	Y	2	F	29	Not provided	*SLC12A3*	NM_000339: c.1967C>T, p.Pro656Leu	het	PP2, PP3	0.021% NFE	This manuscript	N
85	X-linked hypophosphatemic rickets	Unknown	3	F	1	Caucasian, non-Hispanic	*HOGA1*	NM_138413: c.700 + 5G>T	het	PP3, PP5	0.208% NFE	ClinVar	N
88	Renal tubular acidosis	Unknown	3	F	9	Caucasian, Hispanic	*IFT140*	NM_014714: c.1541T>A, p.Leu514His	het	PP3, BP6	1.58% EF	ClinVar	N
89	Childhood nephrotic syndrome, possibly collapsing FSGS	Unknown	4	F	9	African/African-American	*PKD1*	NM_000296: c.5866G>A, p.Val1956Met	het	–	0.002% NFE	This manuscript	N
90	Alport syndrome	N	4	F	6	Caucasian	*SLC7A9*	NM_001126335: c.544G>A, p.Ala182Thr	het	PP2, PP3, PP5	0.43% NFE	ClinVar	N
							TMEM67	NM_001142301: c.803T>C, p.Leu268Ser	het	PM2, PP2, PP3, PP5	0.004% NFE	[64]	N
92	Bilateral cystic kidneys	Unknown	2	F	14	1	*PKD1*	NM_000296: c.8971T>G, p.Tyr2991Asp	het	PM1, PM2, PP3	Not reported	This manuscript	Y
93	Congenital bilateral echogenic kidneys with small cysts	N	2	F	5	Not provided	*SLC3A1*	NM_000341: c.647C>T, p.Thr216Met	het	PM2, PP2, PP3, PP5	0.018% NFE	[65]	N
102	Autosomal recessive polycystic kidney disease	Unknown	2	M	0[e]	Brazilian/Mexican Hispanic	*HNF4A*	NM_000457: c.1133C>T, p.Ser378Phe	het	PM2	Not reported	This manuscript	N
107	Congenital nephrotic syndrome	Unknown	4	F	0[e]	Hispanic or Latino	*COL4A1*	NM_001845: c.1366G>A, p.Glu456Lys	het	PM1, PP2, PP3	0.0058% EA	This manuscript	N
108	Not provided	Unknown	5	F	6	Not provided	*IFT140*	NM_014714: c.886G>A, p.Gly296Arg	het	PM2, PP3	0.023% SA	This manuscript	N
							LAMB2	NM_002292: c.2974A>G, p.Ile992Val	het	–	0.413% SA	This manuscript	N
109	Isolated multicystic dysplastic kidney disease and polycystic kidney disease	Unknown	1, 2	M	7	Not provided	*ANOS1*	NM_000216: c.98G>C, p.Arg33Pro	het	PP3	0.072% LAT	This manuscript	Y
110	NDI	N	3	M	1	Caucasian, non-Hispanic	*AGTR2*	NM_000686: c.395delT, p.Phe134Leufs*5	het	PP3, BP6	0.102% NFE	ClinVar	N
111	Branchio-oto-renal syndrome or isolated CAKUT	Unknown	1	F	2	Not provided	*CREBBP*	NM_001079846: c.2458C>T, p.Pro820Ser	het	PP3, BP6	0.915% AFR	ClinVar	N

							GRIP1	NM_001178074: c.2633G>A, p.Arg878His	het		0.052% AFR	This manuscript	N
112	Dent disease, Bartter or Gitelman syndromes	Unknown	3	M	23	Caucasian, non-Hispanic	*FRAS1*	NM_001166133: c.4648C>T, p.Leu1550Phe	het	BP1	0.22% EF	ClinVar	N
							NLRP3	NM_001079821: c.128G>A, p.Arg43Lys	het	PP2, BP4	0.002% NFE	This manuscript	N
							PKD1	NM_000296: c.7409C>A, p.Pro2470Gln	het	PP3	0.0022% NFE	This manuscript	N
114	IgA nephropathy or FSGS	N	4	M	11	African/African-American	*PAX2*	NM_000278: c.1178G>C, p.Arg393Pro	het	PM2, PP2	Not reported	This manuscript	Y
121	Juvenile nephronophthisis	Unknown	2	M	<1	Not provided	*NPHP3*	NM_153240: c.1181T>A, p.Ile394Asn	het	PM2, PP3	0.003% LAT	ClinVar	Y
								NM_153240: c.460G>C, p.Ala154Pro	het	PM2, PP3	Not reported	ClinVar	Y
123	Steroid-resistant nephrotic syndrome	Unknown	4	M	<1	Caucasian, non-Hispanic	*COQ2*	NM_015697: c.854C>G, p.Pro285Arg	het	PM2, PP3, PP5	0.001% NFE	ClinVar (likely pathogenic)	Y
125	Nephronophthisis	Unknown	2	M	15	Caucasian	*NR3C2*	NM_000901: c.731G>A, p.Arg244Gln	het		0.004% NFE	This manuscript	N
							SIX2	NM_016932: c.722C>T, p.Pro241Leu	het	BP6	0.44% FE	[66]	N
127	Bartter syndrome, Gitelman syndrome or NDI	Y	3	M	2	Caucasian, non-Hispanic	*TNXB*	Full gene deletion	het	LP* (PVS1, PM2)			N
							SLC7A9	NM_001126335: c.544G>A, p.Ala182Thr	het	PP2, PP3, PP5	0.43% NFE	ClinVar	N

[a]Disease category is associated with the indication for testing. 1 = CAKUT; 2 = Ciliopathies or tubulointerstitial disease; 3 = Disorders of tubular ion transport; 4 = Glomerulopathies; 5 = Unclassified or Other.
[b]Zygosity: het= heterozygous; hom=homozygous; hemi= hemizigous.
[c]gnomAD: highest minor allele frequency reported. AFR= African; EA= East Asian; FE= European Finnish; NFE= European (non-Finnish); LAT= Latino; SA= South Asian.
[d]Yes (Y), no (N) or unknown (U).
[e]Newborn.
Jewish# No gnomAD data.
M, male; F, female. HTN, hypertension; VSD, ventricular septal defect; CUA, calcific uremic arteriolopathy.
Newborn.

Table 6. Risk alleles

Case	Indication for testing	Family history	Disease category[a]	Sex	Age (year)	Ethnicity	Gene	Variant	Zygocity[b]	ACMG classification/ rules [17]	MAF gnomAD[c]	Associated disease	First reported by
9	FSGS	Unknown	4	M	54	African/African-American	*APOL1*	NM_001136540: c.1024A>G, p.Ser342Gly	hom	Risk allele	23% AFR	FSGS, hypertensive nephrosclerosis and	[53]
							G1/G1	NM_001136540: c.1152T>G, p.Ile384Met		Risk allele	22.9% AFR	HIV associated nephropathy	[53]
15	Hypercalcemia, hypocalciuria. Suspicion of CaSR inactivating mutation	N	3	F	81	Caucasian	*CaSR*	NM_000388: c.2956G>T, p.Ala986Ser	het	PM2, PP2, BP6	Not reported	Hypercalcemia	[67]
46	FSGS or minimal change disease. Persistent proteinuria	Unknown	4	M	5	Caucasian, non-Hispanic	*PLCG2*	NM_002661: c.3563C>T, p.Pro1188Leu	het		0.0067% NFE	Steroid sensitive nephrotic syndrome	This manuscript
72	MCD, unresponsive to steroids	N	2	F	3	African/African-American	*APOL1*	NM_001136540: c.1024A>G, p.Ser342Gly	het	Risk allele	23% AFR	FSGS, hypertensive nephrosclerosis and	[53]
							G1/G2	NM_001136540: c.1152T>G, p.Ile384Met	het	Risk allele	22.9% AFR	HIV associated nephropathy	[53]
								NM_001136540: c.1160_1165delATAATT, p.Asn388_Tyr389del	het	Risk allele	14.14% AFR		[53]
101	Chronic kidney stones and alkaline urine	Unknown	3	M	18	Not provided	*ATP6V1B1*	NM_001692: c.298G>A, p.Asp100Asn	het	PP2, PP3	0.16% EA	Kidney stones	This manuscript
118	Nephrotic syndrome	Unknown	4	M	8	African/African-American	*APOL1*	NM_001136540: c.1024A>G, p.Ser342Gly	hom	Risk allele	23% AFR	FSGS, hypertensive nephrosclerosis and	[53]
							G1/G1	NM_001136540: c.1152T>G, p.Ile384Met		Risk allele	22.9% AFR	HIV associated nephropathy	[53]
119	CDK Stage 2, FSGS	Unknown	4	F	16	African/African-American	*APOL1*	NM_001136540: c.1024A>G, p.Ser342Gly	hom	Risk allele	23% AFR	FSGS, hypertensive nephrosclerosis and	[53]
							G1/G1	NM_001136540: c.1152T>G, p.Ile384Met		Risk allele	22.9% AFR	HIV associated nephropathy	[53]
120	ESRD due to FSGS	Unknown	4	F	20	Not provided	*APOL1G2/G2*	NM_001136540: c.1160_1165delATAATT, p.Asn388_Tyr389del	hom	Risk allele	14.14% AFR	FSGS, hypertensive nephrosclerosis and HIV associated nephropathy	[53]

[a]Disease category is associated with the indication for testing. 1 = CAKUT; 2 = Ciliopathies or tubulointerstitial disease; 3 = Disorders of tubular ion transport; 4 = Glomerulopathies; 5 = Unclassified or Other.
[b]Zygosity: het, heterozygous; hom, homozygous; hemi, hemizigous.
[c]gnomAD: highest minor allele frequency reported. AFR, African; EA, East Asian; NFE, European (non-Finnish).
Jewish# No gnomAD data.
N, no; M, male; F, female.

Table 7. Pathogenic carriers

Case	Indication for testing	Family history	Disease category[a]	Sex	Age (years)	Ethnicity	Gene	Variant	Zygocity[b]	ACMG classification/rules [17	MAF gnomAD[c]	Reported in	Associated disease
75	Steroid-resistant nephrotic syndrome	Y	4	M	4	Dominican Republic	*BBS1*	Deletion chr11: 66278119-66301084	het			This manuscript	BBS carrier
83	Juvenile nephronophthisis and medullary cystic kidney disease	Y	2	F	29	Not provided	*SLC12A3*	NM_000339: c.1967C>T, p.Pro656Leu	het	PP2, PP3	0.021% NFE	[68]	Gitelman carrier
85	X-linked hypophosphatemic rickets	Unknown	3	F	1	Caucasian, non-Hispanic	*HOGA1*	NM_138413: c.700 + 5G>T	het	PP2, PP5	0.21% NFE	[69]	Primary hyperoxaluria III carrier
88	Renal tubular acidosis	Unknown	1	F	9	Caucasian, Hispanic	*IFT140*	NM_014714: c.1541T>A, p.Leu514His	het	PP3, BP6	1.58% FE	[70]	Jeune syndrome carrier
108	Not provided	Unknown	5	F	6	Not provided	*SLC12A1*	NM_000338: c.1872delC	het	Pathogenic (PVS1, PM2, PP3)	0.032% SA	This manuscript	Bartter syndrome 1 carrier
111	Branchio-oto-renal syndrome or isolated CAKUT	Unknown	1	F	2	Not provided	*FGF23*	NM_020638: c.59delG, p.Ser20Thrfs*20	het	LP* (PVS1, PM2)	Not reported	This manuscript	N
112	Dent disease, Bartter or Gitelman syndromes	Unknown	3	M	23	Caucasian, non-Hispanic	*ATP7B*	NM_000053: c.2972C>T, p.Thr991Met	het	Likely pathogenic PS3, PM1, PP2, PP3, PP5	0.24% NFE	[71]	Wilson disease carrier

[a]Disease category is associated with the indication for testing. 1 = CAKUT; 2 = Ciliopathies or tubulointerstitial disease; 3 = Disorders of tubular ion transport; 4 = Glomerulopathies; 5 = Unclassified or Other.
[b]Zygosity: het, heterozygous; hom, homozygous; hemi, hemizigous.
[c]gnomAD: highest minor allele frequency reported. FE, European Finnish; NFE, European (non-Finnish); SA, South Asian.
Jewish# No gnomAD data.
Y, yes; M, male; F, female.

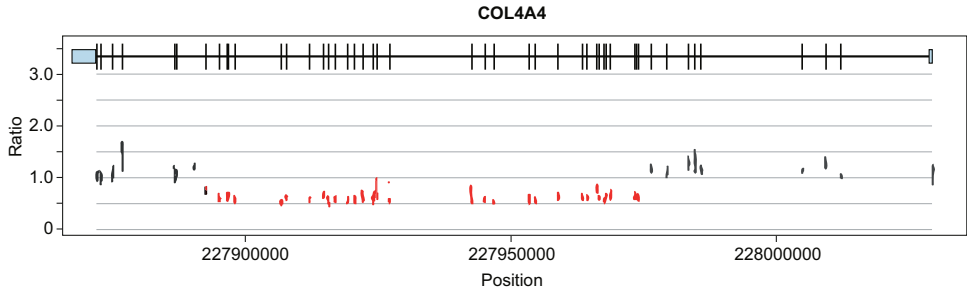

FIGURE 2: CNV identified in Case 33. The ratio of expected-to-observed sequence reads shows ∼50% reduction in signal, which is consistent with heterozygous deletion of exons 10–40 in *COL4A4*.

In some cases, identified variants had insufficient evidence to be labeled as likely pathogenic or pathogenic and were reported as VUSs (Tables 5–7). In two cases, the genetic variants did not meet strict ACMG criteria for likely pathogenicity and were labeled as VUSs, but in the clinical context, the multidisciplinary group considered these as probably causal (Tables 5–7, Cases 57 and 92). In two other cases, variants classified as likely pathogenic by ACMG criteria were reported as VUSs because the genetic disease appeared irrelevant to the clinical phenotype. One of these was a case with nephrogenic diabetes insipidus (NDI) and nephrocalcinosis with hypophosphatemia (Tables 5–7, Case 44), where an identified variant in *KAL1* was classified as likely pathogenic for Kallmann syndrome by ACMG criteria. In the other, a case with hypomagnesemia and dilated cardiomyopathy (Tables 5–7, Case 16), a likely pathogenic variant in ROBO2 for CAKUT was identified but reported as a VUS. In other instances, we identified alleles that increase risk for specific renal diseases (Tables 5–7). Five patients with FSGS, nephrotic syndrome or CKD were homozygous or compound heterozygous for variants in *APOL1* that substantially increase the risk for FSGS in Americans of sub-Saharan African descent [79, 80]. Other risk variants were identified in *CaSR*, *PLCG2* and *ATP6V1B1*, which increase the risk of hypercalcemia, steroid-sensitive nephrotic syndrome and kidney stones, respectively [81–83].

CNVs are significant contributors to genetic renal disease and their detection was an important component of our analysis [84]. We identified pathogenic CNVs in 18% of positive diagnoses, including four cases of autosomal recessive JN1 (*NPHP1*), two cases of autosomal dominant CAKUT (*HNF1B*), one case each of autosomal recessive Alport syndrome (*COL4A4*) and autosomal recessive pseudohypoaldosteronism (*SCNN1B*) and a possible tri-allelic form of Gitelman syndrome (*CLCNKB*; Figure 2).

Alternative methods to provide comprehensive unbiased screening for genetic renal disorders include genome sequencing (GS) and/or ES, both of which have been used to diagnose monogenic renal disorders in a research setting and have been used in the clinical setting when locus heterogeneity is extreme, the phenotype is very indistinct, or the renal features are only a minor part of a multisystem disease [85, 86]. Neither GS nor ES is optimized for the renal exome, which includes challenging regions like the first 32 exons of *PKD1*, which are duplicated as six pseudogenes on chromosome 16. Nevertheless, ES remains an alternative as described by Lata *et al.*, who report a 24% diagnostic rate in a selected population of adults with CKD, excluding ADPKD, where a genetic disease was suspected based on family history or there was early age-of-onset of disease [10]. In another study of a larger cohort of patients with CKD, ES identified diagnostic variants in 9.3% of patients [12].

There are some limitations and caveats to our testing strategy. First, as in ES, some types of genetic variants that occur within tandem oligonucleotide repeats, such as the cytosine insertion within a cytosine repeat sequence in *MUC1*, are difficult to identify [87]. Second, new genetic causes of kidney disease continue to be identified that may not have been present on the diagnostic gene panel at the time of testing. Included in this category are new and rare causes of kidney disease such as *DZIP1L*, *FAT1* and the *NUP* and *IFT* family genes. Third, we were not always able to verify the presence of variants *in trans* to confirm compound heterozygosity for autosomal recessive disorders due to lack of parent or offspring samples. In addition, we purposefully omitted complement genes on this panel because we have developed a discrete panel for ultra-rare complementopathies, including atypical hemolytic uremic syndrome and C3 glomerulopathy. Finally, it should be noted that our diagnostic yield is high and warrants confirmation with larger studies.

In summary, these data add to the body of literature suggesting that genetic renal diseases are underdiagnosed and underappreciated in both children and adults [10, 88–90]. In this cohort of patients, presumably selected by clinicians based on suspicion of monogenic kidney disease, the genetic diagnostic rate is very high and is likely to be lower if more indiscriminate patient testing becomes the norm. Nevertheless, panels facilitate identification of a broad range of Mendelian diseases, including cystic kidney disease, the CAKUTs, tubulointerstitial disease and glomerular disease, as well as non-Mendelian genetic disease, bilineal and digenic disease, atypical forms of disease and unsuspected disease. As such, comprehensive genetic testing has an important place in the evaluation and care of the renal patient [91].

SUPPLEMENTARY DATA

Supplementary data are available at ndt online.

AUTHORS' CONTRIBUTIONS

M.A.M., C.P.T. and R.J.S. conceived the study and wrote the manuscript; M.A.M. conducted genetic testing; R.R.S.

performed bioinformatic analysis; M.E.F., C.A.C., R.J.S. and C.P.T. interpreted genetic test results with contributions from C.J.N., A.E.K. and M.J.K. All authors approved the final version of the manuscript.

CONFLICT OF INTEREST STATEMENT

None declared.

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