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/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
| Disorder                                                                 | Gene(s)                                      |
|------------------------------------------------------------------------|----------------------------------------------|
| 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, CEPI290, MKS5, PTHB1, TRIM32, TCT8 |
| COACH syndrome                                                        | CC2D2A, RPGRIPI1L, TMEM67                   |
| HANAC syndrome                                                        | COL4A1                                       |
| Jeune syndrome                                                        | IFIT80, IFIT40, DYNC2H1, NEK1, TCT21B        |
| Joubert syndrome                                                      | AHI1, ARL13B, ATXN10, CC2D2A, CEPI290, CEPI41, CPSPI1, INNPSE, KIF7, NPH1, OFDI1, RPGRIPI1L, TMEM216, TCTN1, TMEM138, TMEM237, TMEM67, TCT21B |
| Juvenile nephronophthisis (IN)                                         | AH1, ATXN10, IQCB1, CEPI290, GLIS2, INVS, NEK8, NPH1, NPH3, NPH4, RPGRIPI1L, TMEM67, TCT21B, WDR19, XPNPPEP3 |
| Juvenile nephronophthisis (IN)                                         |                                              |
| Meckel syndrome (MKS)/Meckel–Gruber syndrome                           | B9D1, B9D2, CC2DA, CEPI290, MKS1, NPH3, RPGRIPI1L, TCT2, TMEM216, TMEM67 |
| Medullary cystic kidney disease 2                                      |                                              |
| Oro-facial-digital syndrome 1                                          |                                              |
| Renal cysts and diabetes syndrome                                      |                                              |
| Serpentine fibula with polycystic kidney disease (SFPKS)/Hajdu–Cheney syndrome (HJCYS) |                                              |
| Sensenbrenner syndrome/(CED)                                           |                                              |
| Senior–Loken syndrome (IN with retinitis pigmentosa)                  |                                              |
| Disorders of tubular ion transport                                     |                                              |
| Apparent mineralocorticoid excess, syndrome of                        |                                              |
| APRT deficiency                                                       |                                              |
| Autosomal dominant hypocalcemia, ± Bartter syndrome                    |                                              |
| Bartter syndrome                                                       |                                              |
| Cystinosis                                                            |                                              |
| Cystinuria                                                            |                                              |
| Dent disease                                                          |                                              |
| Distal renal tubular acidosis                                         |                                              |
| Familial hypertension with hyperkalemia (Gordon syndrome),            |                                              |
| Pseudohypoaldosteronism II                                            |                                              |
| Gitelman syndrome                                                     |                                              |
| Hypophosphatemic rickets                                              |                                              |
| Isolated proximal renal tubular acidosis—generalized proximal defect  |                                              |
| (Fanconi syndrome)                                                    |                                              |
| Liddle syndrome (pseudo hyperaldosteronism)                           |                                              |
| Nephrogenic diabetes insipidus (NDI)                                   |                                              |
| Nephrogenic syndrome of inappopriate antiureasis (NSIAD)               |                                              |
| Primary hyperoxaluraria                                               |                                              |
| Pseudohypoaldosteronism I (PHA I)                                      |                                              |
| Renal glucosuria                                                      |                                              |
| Renal hypomagnesemia                                                  |                                              |
| Renal tubular acidosis, proximal, with ocular abnormalities           |                                              |
| Glomerular diseases                                                   |                                              |
| Alport syndrome                                                       |                                              |
| Alstrom syndrome                                                      |                                              |
| Congenital nephrotic syndrome (Finnish type)                           |                                              |
| DDS, Frasier syndrome                                                 |                                              |
| Diffuse mesangial sclerosis                                           |                                              |
| Epstein–Fechtner syndrome (renal disease with macrothrombocytopenia) |                                              |
| Fabry disease                                                         |                                              |
| FSGS–AD/XL                                                            |                                              |
| FSGS–AR                                                               |                                              |
| FSGS/steroid-resistant nephrotic syndrome (SRNS)–AR                   |                                              |
| Galloway–Mowat syndrome                                               |                                              |
| Glomerulopathy with fibronectin deposits                               |                                              |
| Hereditary systemic or renal amyloidiosis                              |                                              |

Continued
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

| Diseases                                      | Genes                      |
|-----------------------------------------------|----------------------------|
| 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             |
| CANKUT                                        | EYA1, SIX1, SIX5            |
| Branchio-oto-renal syndrome                   | TRAP1                      |
| CANKUT with VACTERL                           | NPHP1                      |
| Cogan oculomotor apraxia                      | AGTR1, AGTR2, CHD1L, DSYTYK, EYA1, GATA3, HNF1B, PAX2, RET, ROBO2, SALL1, SIX2, SIX5, TRAP1 |
| Common CANKUT                                 | GATA3                      |
| Fraser syndrome                               | BMP4, DSYTYK, FGF20, HNF1B, PAX2, RET, SALL1, SIX2, PAX2 |
| Hypoparathyroidism, sensorineural deafness and renal dysplasia | RET, UPK3               |
| Isolated renal hypo-dysplasia                 | ANOS1                      |
| Isolated renal hypoplasia and renal-coloboma syndrome (papillorenal syndrome) | WNT4                       |
| Isolated renal hypoplasia                     | CHD1L, HNF1B, ROBO2, SALL1 |
| Kallmann syndrome                             | CHD1L, HNF1B, ROBO2, SALL1, SIX2 |
| Mayer–Rokitansky–Küster–Hauser syndrome       | HNF1B                      |
| Multicystic dysplastic kidney                 | ACE, AGT, AGTR1, REN       |
| Posterior urethral valves                     | SALL1                      |
| Renal cysts and diabetes syndrome             | DSYTYK, HNF1B, RET, SALL1  |
| Renal tubular dysgenesis                      | DSYTYK, EYA1, HNF1B, RET, ROBO2, SALL1 |
| Townes–Brock syndrome                         | CHD1L, PAX2, SIX5          |
| Unilateral renal agenesis                     | DSYTYK, EYA1, GATA3, HNF1B, RET, ROBO2, SALL1, SOX17, TXNB, UPK3A |
| UPJ obstruction                               |                                 |
| UVJ obstruction                               |                                 |
| Vesicoureteral reflux                         |                                 |
| Other                                          | SALL4                      |
| Acrrenooculoor syndrome (Okihiro syndrome)    | COQ2                       |
| Mitochondrial cytopathy                       | GLI3                       |
| Pallister–Hall syndrome                       | CREBBP                     |
| Rubinstein–Taybi syndrome                     | SMARCAL1                   |
| Schimke immuno-osseous dysplasia              | WNT4                       |
| SERRAD syndrome (46XX sex reversal with dysgenesis of kidneys, adrenal and lungs) | GPC3                       |
| Simpson–Golabi–Behmel syndrome                | DHCRI7                     |
| Smith–Lemli–Opitz syndrome                    | TSC2                       |
| Tuberous sclerosis                            | TSC2                       |
| Williams syndrome                             | 7q11.23                    |

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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 2. Indications for testing

| CAKUT                                      | 2 |
|--------------------------------------------|---|
| Branchio-oto-renal syndrome                | 1 |
| HNF1-β                                     |   |
| Multicystic dysplastic kidney              |   |
| Papillorenal syndrome                      |   |
| Renal hypo/dysplasia                       |   |
| 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 |
| Hypercalmia                                | 3 |
| Hypokalemia                                | 2 |
| Hypomagnesemia                             | 3 |
| Hypophosphatemia                           | 3 |
| Kidney stones                              | 2 |
| Liddle syndrome                            | 2 |
| NDI                                        | 3 |
| Pseudohyypoaldosteronism                   | 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.
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 categorya,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 |
| 14   | Medullary cystic kidney disease/ nephronophthisis | Unknown | 2 | M | 31 | Caucasian |
| 15   | Hypercalcemia, hyocalciuria | 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 | Not provided |
| 24   | Bartter syndrome or other | Unknown | 3 | M | 1 | African/African-American |
| 25   | ESRD, tubulointerstitial disease | Y | 2 | M | 51 | Caucasian, Hispanic or Latino |
| 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. Nephrotic range 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
| Case | Indication for testing                                                                 | Family history | Disease category | Sex | Age (years) | Ethnicity                          |
|------|---------------------------------------------------------------------------------------|----------------|-----------------|-----|-------------|------------------------------------|
| 36   | Bilateral multicystic dysplastic kidneys, perinatal death                              | Unknown        | 1               | M   | 0b          | 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   | 0b          | Hispanic or Latino                |
| 39   | Multicystic bilateral kidneys                                                         | Unknown        | 1               | M   | 0b          | Caucasian, non-Hispanic           |
| 40   | Apparent mineral corticoid excess                                                    | Unknown        | 3               | M   | 2           | Not provided                      |
| 41   | Bartter syndrome. Polyuria, metabolic alkalosis                                      | Unknown        | 3               | F   | 3           | Taiwanese, 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, vescoureteral 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   | Nephronphthisis 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   | 0b          | Hispanic or Latino                |
| 66   | FSGS                                                                                  | Unknown        | 4               | F   | 10          | African/African-American, non-Hispanic |

*Continued*
| Case | Indication for testing                                                                 | Family history | Disease category<sup>a,b</sup> | Sex | Age (years) | Ethnicity                        |
|------|----------------------------------------------------------------------------------------|----------------|-------------------------------|-----|-------------|-----------------------------------|
| 67   | Horseshoe kidney, dysmorphic features, VSD                                            | Y              | 1                             | F   | <1          | Egyptian                         |
| 68   | Kidney stones, paresthesias, hypercalcuria, 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                                                                       |               |                               |     |             | 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<sup>b</sup> | 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 nephronphthisis 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<sup>b</sup> | 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           | African/African-American         |
| 91   | CKD, looking for APOL1 risk variants                                                   | N              | 4                             | F   | 18          | Caucasian, non-Hispanic          |
| 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<sup>b</sup> | 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<sup>b</sup> | Brazilian/Mexican, Hispanic or Latino |
| 103  | Nephrotic-range proteinuria                                                            | N              | 4                             | M   | <1          | Caucasian                        |

Continued
patients), Alport or Alport-like syndrome (10 patients), Bartter/Gitelman syndrome (7 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),

| Case | Indication for testing | Family history | Disease categorya,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*          | 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 nephronphthisis | 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  |

*Variant identification and diagnostic rates in renal patients.

**Table 3. Continued**

Unbiased testing advances the diagnosis of renal diseases
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 type | Sex | Age (years) | Race/ethnicity | Gene | Variant | Zygosity | ACMG classification | MAF gnomAD | Genetic diagnosis (AD/AR/XLR) | Disease category change | First reported |
|------|------------------------|----------------|--------------|-----|-------------|----------------|------|---------|----------|----------------------|-------------|--------------------------------|----------------------|--------------|
| 1    | Bilateral multicystic dysplastic kidneys | Y              | 1            | F   | <1          | H              | PKD1 | NM_000296: c.11575delG, p.Ala3859Pro*, 5'UTR NM_000297: c.2T>A, het p.Met1Lys | het       | Pathogenic (PVS1, PM1, PM2) | Not reported | ADPKD | 2 | This manuscript |
| 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) | Alport syndrome (XLD) | [18] |
| 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 | NFE | Nephronophthisis 1 (AR) | This manuscript |
| 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       | Likely pathogenic (PVS1, PM1, PM2, PP4) | Not reported | NFE | This manuscript |
| 8    | Nephronophthisis | Y              | 2            | F   | 10          | 2              | RPGRP1 | NM_001127897: c.1329_1330insA, p.Arg444Thrfs*10 | het       | Pathogenic known (PVS1, PM2, PP3) | Not reported | COACH syndrome (AR) | This manuscript |
| 11   | Autosomal dominant polycystic kidney disease | Unknown | 2            | M   | 27          | 1              | NPHP1 | NM_000091: c.1408+2T>C, p.Leu2866Pro | het       | Pathogenic known (PVS1, PM2, PP3) | Not reported | NFE | Nephronophthisis 1 (AR) | [20] |
| 20   | Alport syndrome | Unknown | 4            | M   | 5           | 4              | COL4A5 | NM_000495: c.1843G>A, p.Gly615Arg, Deletion of NPHP1 gene region on chr2 | hemi      | Pathogenic known (PVS1, PS1, PM2, PM3) | Not reported | Alport syndrome (XLD) | This manuscript |
| 23   | U/S prenatal echo-genic kidneys, postnatal bilateral cysts, HNF1B disease | Unknown | 1            | M   | <1          | 1              | PKD1 | NM_000296: c.8597T>C, p.Leu2866Pro | het       | Pathogenic known (PS1, PM2, PP3, PP5) | Not reported | ADPKD | 2 | This manuscript |
| 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) | This manuscript |
| 29   | Alport or thin basement membrane disease | Unknown | 4            | M   | 18          | 1              | COL4A3 | NM_000091: c.1408+2T>C | het       | Pathogenic known (PVS1, PM2, PP3) | Not reported | NFE | Alport syndrome (AD)/thin basement membrane disease (AD) | This manuscript |
| 33   | Unknown | 4            | M   | 12          | 1              | COL4A4 | NM_000296: c.11575delG, p.Ala3859Pro*, 5'UTR NM_000297: c.2T>A, het p.Met1Lys | het       | Pathogenic (PVS1, PM1, PM2) | 0.00089% NFE | NFE | | |
| Gene | Mutation Details | Genomic Location | Pathogenicity | Comment |
|------|-----------------|------------------|---------------|---------|
| **Alport syndrome** | NM_000092: c.4522G>A, p.Gly1508Ser; NM_000092: c.227892566_227974060 del | chr2: 227892566-227974060 | Likely pathogenic (PS1, PM2, PP3) | Alport syndrome (AR) |
| **Bartter syndrome, NDI or Dent disease; polyuria, polydipsia, hypercalcuria, medullary nephrocalcinosis** | Unknown 3 M 16 1 SLC12A1 | NM_000338: c.1652C>T, p.Thr551Ile; NM_000338: c.2807G>A, p.Trp936* | Likely pathogenic (PM1, PM2, PM3, PP3) | Not reported [26] |
| **Pseudohypoaldosteronism; hyperkalemia, polyuria** | Unknown 3 M <1 H SCNN1B | NM_000336: c.682delG, p.Ala228Hisfs*8; chr16: 23313555-23315510 del | Likely pathogenic (PM1, PM2, PM3) | Pseudohypoaldosteronism I (AR) |
| **Liddle syndrome; early onset hypertension and hypokalemia** | Y 3 F 19 1H HSD11B2 | NM_000196: c.623G>A, p.Arg208His; NM_000196: c.667G>A, p.Asp223Asn | Likely pathogenic (PS1, PM2, PP3) | Syndrome of apparent mineralocorticoid excess (AR) |
| **Cystinuria** | Y 3 F 19 1 SLC7A9 | NM_001126335: c.775G>A, p.Gly259Arg; NM_001126335: c.854C>A, p.Ala285Glu | Likely pathogenic (PS1, PM2, PP3) | Cystinuria (AR) |
| **PKD1, PKD2, HNF1B** | Unknown 2 M 6 H PKD1 | NM_000296: c.9395C>T, p.Ser3132Leu; NM_000296: c.10102G>A, p.Asp3368Asn | Likely pathogenic (PM1, PM2, PM3, PP3) | ADPKD |
| **Renal cysts** | Y 2 F 49 4 PKD1 | NM_000296: c.10102G>A, p.Asp3368Asn; NM_001008389: c.854C>A, p.Ala285Glu | Likely pathogenic (PS1, PM2, PP3) | ADPKD |
| **Bartter/Gitelman syndrome; hypokalemia, hypomagnesemia and metabolic alkalosis** | Unknown 3 M 12 Unknown SLC12A3 | NM_000092: c.1836G>T, p.Trp612Cys; NM_000338: c.4522G>A, p.Gly1508Ser; NM_000092: c.227892566_227974060 del; NM_001008389: c.854C>A, p.Ala285Glu | Likely pathogenic (PM1, PM2, PM3, PP3) | Not reported Gitleman syndrome (AR) |
| Case | Indication for testing | Family history | Disease categorya | Sex | Age (years) | Race/ethnicity | Gene | Variant | Zygosity | ACMG classification [17] | MAF gnomADb | Genetic diagnosis (AD/AR/XLR) | Disease category changea | First reported |
|------|------------------------|----------------|-------------------|-----|-------------|----------------|------|---------|----------|--------------------------------|----------------|---------------------------------|-------------------|---------------|
| 60   | Nephronophthisis or medullary cystic kidney disease | Y              | 2                 | M   | 58          | 1              | UMOD | c.278_289del | het     | Likely pathogenic known (PS1, PM, PM4) | Not reported | Tubulo-interstitial kidney disease (AD) | [36]               |               |
| 61   | Polycystic kidney disease | Unknown        | 2                 | F   | 51          | 2              | PKD1 | NM_000296: | c.6356delTA | het     | Pathogenic (PV1, PM2, PP3) | Not reported | ADPKD                           | This manuscript   |               |
| 63   | FSGS, multivesicular 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     | Likely pathogenic (PV1, PM2, PP3) | Not reported | Distal renal tubular acidosis (AR) | This manuscript   |               |
| 68   | Kidney stones, par-thesia, hypercalcuria, hyperparathyroidism, ESRD | Y              | 3                 | M   | 58          | 1              | CASR | NM_000296: | c.8311G>A, p.Glu2711Lys | het     | Likely pathogenic (PS1, PM1, PM2, PM3) | Not reported | Hypocalcemia (AD)                  | This manuscript   |               |
| 69   | Large cystic kidneys | N              | 2                 | M   | 27          | 1              | PKD1 | NM_000296: | c.811G>T, p.Val836Leu | het     | Likely pathogenic (PS1, PM2, PP3) | Not reported | ADPKD                           | [37]               |               |
| 70   | Renal cystic dysplasia, ectopic atrial tachycardia, CUA, seizures, LVH; dialysis from birth | Unknown        | 2                 | F   | <1          | 1              | WTI  | c.1249C>T, p.Arg417Cys | het     | Likely pathogenic (PS1, PM2, PP3) | Not reported | DDS (AD)                        | 3                 |               |
| 79   | Premature newborn with severely enlarged cystic kidneys noted mid-trimester, severe oligohydramnios, pulmonary hypoplasia | N              | 2                 | F   | <1          | 1H             | PKHD1 | NM_13694.3: | c.9689delTA, p.Asp3230Valfs*34 | het     | Pathogenic known (PV1, PM2, PP3, PP4) | 0.039% LAT | ARPKD                           | [39]               |               |
| 80   | Alport syndrome | Unknown        | 4                 | F   | 11          | 1              | COL4A5 | c.1117C>T, p.Arg373* | hom     | Whole gene deletion | Pathogenic known (PS1, PM1, PM2, PP3) | Not reported | Alport syndrome (XLD)               | [18]               |               |
| 84   | Not provided | Unknown        | 5                 | M   | 14          | Unknown NP1H  |       |            |         | Pathogenic known (PS1, PM1, PM2, PP3) | Not reported | Nephronophthisis 1 (AR)             | 2                 |               |
| 86   | Orofaciodigital syndrome l | Unknown        | 2                 | F   | 21          | 1              | OFD1 | NM_000611: | c.875_876delAT, p.Met293Glyfs*15 | het     | Pathogenic known (PV1, PM2, PP3) | Not reported | Orofaciodigital syndrome 1 (AD)   | [42]               |               |
| 87   | Bilateral cystic kidneys | Unknown        | 2                 | M   | <1          | 3H             | PKHD1 | c.6356delTA | het     | Pathogenic known (PV1, PM2, PP3) | Not reported | ARPKD                           | This manuscript   |               |
| # | Condition                                      | Gender (M/F/S) | Age | Race | Gene | NM | Location | Mutation | Phenotype and Notes |
|---|-----------------------------------------------|----------------|-----|------|------|-----|----------|----------|---------------------|
| 90 | Alport syndrome                               | N 4 F 6 1      | NPFS2 | Likely pathogenic known (PS1, PM1, PP3) | 0.08% NFE | Pathogenic known (PS1, PM1, PP3) | HNF1B-related nephropathy |
| 93 | Congenital bilateral echogenic kidneys with small cysts | N 2 F 5      | Unknown | Pathogenic known (PS1, PM1, PM2, PP3) | 0.029% EA | Pathogenic known (PS1, PM1, PM2, PP3, PP5) | Steroid-resistant nephrotic syndrome (AR) |
| 94 | Failure to thrive, presented with hypertension and CKD | N 5 F 6 1       | TTC21B | Pathogenic (PVS1, PM1, PP3) | 0.0009% NFE | Pathogenic known (PS3 PM2, PP3, PP5) | Juvenile nephropathesis (AR), Jeune syndrome (AR), or Joubert syndrome (AR) |
| 96 | Alport syndrome, branchio-oto-renal syndrome (BOR), ESRD, nephropathies | Unknown 4 M 16 1 | COL4A5 | Pathogenic known (PS1, PM1, PM2, PP3) | 6.98% FE | Pathogenic known (PS3 PM1, PP2, PP3, PP5) | Not reported |
| 97 | Bartter syndrome                               | Unknown 3 F 2 H | KCNJ1 | Pathogenic (PVS1, PM2, PP3, PP5) | 0.0018% NFE | Not reported | Bartter syndrome (AR) |
| 98 | Autosomal recessive polycystic kidney disease | Unknown 2 M <1 1 | PKHD1 | Pathogenic (PS1, PM1, PM2, PP3, PP4) | 0.0058% EA | Pathogenic known (PS3 PM2, PP3, PP4) | ARPKD |
| 99 | Polycystic kidney disease                      | Y 2 M 7 1      | PKD1 | Pathogenic (PS1, PM1, PM2, PP3) | Not reported | Not reported | ADPKD |
| 103 | Nephrotic range proteinuria                   | N 4 M <1 1     | CLCN5 | Pathogenic known (PS1, PS3, PM2, PP2, PP3, PP5) | Not reported | Not reported | Dent disease |
| 104 | Papillorenal syndrome (renal-coloboma syndrome) | N 1 M 2 1H    | PAX2 | Pathogenic known (PS1, PM2, PP3, PP4, PP5) | Not reported | Not reported | Not reported |
| 106 |                                      | N 2 M 12 1      | PKD1 | Pathogenic known (PS1, PM1, PM2, PP3, PP4, PP5) | 0.027% NFE | ADPKD | This manuscript |

Unbiased testing advances the diagnosis of renal diseases.
| Case | Indication for testing | Family history | Disease categorya | Sex | Age (years) | Race/ethnicity | Gene | Variant | Zygosity | ACMG classification [17] | MAFgnomAd | Genetic diagnosis (AD/AR/XLR) | Disease category changea | First reported |
|------|-----------------------|----------------|-------------------|-----|------------|----------------|-------|----------|----------|--------------------------|-----------|--------------------------------|------------------------|---------------|
| 113  | ESRD of unknown etiology | Y 5 M 20 H | NPHP1 | Whole gene deletion | hom | Likely pathogenic known (PS1, PM2, PP3, PP5) | Pathogenic known (PS1, PM2, PP3) | MAF | 0.0066% AFR | Nephronophthisis 1 (AR) | Not reported | Alport syndrome (AD) | [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) | Pathogenic known (PS1, PM2, PP3) | MAF | Not reported | DDS (AD) | [51] |
| 115  | FSGS or diffuse mesangial sclerosis | Unknown 4 M 4 1 | WT1 | NM_000095: c.1226G>A, p.Gly409Asp | het | Likely pathogenic known (PM1, PM2, PP2, PP3, PP5) | Not reported | MAF | 0.0068% AFR | Lowe syndrome (XLR) | [54] |
| 116  | Alport syndrome | Y 4 M 13 1 | COL4A5 | NM_000092: c.1024A>G, p.Ser342Gly | hom | Risk allele | Risk allele | MAF | 23% AFR | Dent disease (XLR) and APOL1 G1/G1 | [53] |
| 118  | Nephrotic syndrome | Unknown 4 M 8 2 | APOL1 | NM_001136540: c.1024A>G, p.Ser342Gly | hom | Risk allele | Risk allele | MAF | 22.9% AFR | [53] |
| 120  | ESRD due to FSGS | Unknown 4 F 20 | Unknown | NM_000078: c.1152T>G, p.Ile384Met | het | Pathogenic known (PS1, PM2, PP3, PP5) | Pathogenic known (PS1, PM2, PM1, PP3, PP5) | MAF | 0.0068% AFR | FSGS (AD); APOL1 G2/G2 | [55] |
| 122  | Zellweger syndrome, Galloway–Mowat syndrome, podocytopathy | Unknown 4 M 1 1 | OCRL | NM_000278: c.1484C>T, p.Pro495Leu | het | Pathogenic known (PS1, PM2, PM1, PM2, PP2, PP3, PP5) | Pathogenic known (PS3, PM1, PM2, PP2, PP3, PP5) | MAF | Not reported | Lowe syndrome (XLR) | [56] |
| 124  | Bartter/Gitelman syndromes, pseudohypoaldosteronism type 1 | Unknown 3 M <1 2 | NR3C2 | NM_000009: c.1002_1003insGT, p.Ser335Valfs*4 | het | Pathogenic (PS1, PM2, PM3, PP3) | Pathogenic (PS1, PM2, PM3, PP3) | MAF | Not reported | Pseudohypoaldosteronism I (AD) | [56] |
| 125  | Nephronophthisis | Unknown 2 M 15 1 | NPHP1 | Whole gene deletion | hom | Pathogenic known (PS1, PM2, PM3) | Pathogenic known (PS1, PM2, PM3) | MAF | Nephronophthisis 1 (AR) | [41] |
| 126  | Nephronophthisis | N 2 F 12 5 | NPHP1 | Whole gene deletion | hom | Pathogenic known (PS1, PM2, PM3) | Pathogenic known (PS1, PM2, PM3) | MAF | Nephronophthisis 1 (AR) | [41] |

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

*Disease category: 1 = CAKUT; 2 = ciliopathies or tubulointerstitial disease; 3 = disorders of tubular ion transport; 4 = glomerulopathies; 5 = undiagnosed 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, hemizygous.

*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.
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 \(RPGRIP1L\), 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.

![figure1](image.png)

**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.
| Case | Indication for testing   | Family history | Disease category | Sex | Age (years) | Ethnicity | Gene | Variant | Zygocity | ACMG classification/rules [17] | MAF Gnomad | First reported by | Possibly causal |
|------|--------------------------|----------------|------------------|-----|-------------|-----------|------|---------|----------|---------------------------------|------------|------------------|-----------------|
| 5    | Proteinuria, FSGS        | Y              | 4                | M   | 54          | 2         | FN1  | NM_0003206: c.5779C>T, p.Arg1927Cys | het       | PM1, PM2, PP3 | 0.007% NFE | Glomerulopathy with fibronectin deposits (AD) | N          |                  |
| 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 | SLCA1   | 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_000342: c.2680G>A, p.Gly894Ser | het       | PP3       | 0.18% NFE | This manuscript | Y          |                  |
| 19   | Severe CAKUT             | Unknown        | 1                | M   | <1          | Caucasian, Hispanic | DSTYK  | NM_013753: c.2216G>A, p.Arg739Gln | het       | PM2, PP3 | 0.25% LAT | This manuscript | Y          |                  |
| 22   | Interstitial nephritis   | Unknown        | 2                | F   | 10          | 2         | NPHP4 | NM_015102: c.2542G>A, p.Arg848Trp | het       | PM2, PP3, BP6 | 2.56% EF | [58]                  | Y          |                  |
| 25   | ESRD, tubulointerstitial disease | Y              | 2                | M   | 51          | African/African-American | CC2D2A | NM_01080522: c.3157A>G, p.Ile1053Val | het       | PM2, PP3 | 0.047% AFR | This manuscript | Y          |                  |
| 30   | FSGS, SRNS, hypoalbuminemia | Unknown        | 4                | M   | 17          | Caucasian non-Hispanic | NPHP3  | NM_01080522: c.3503G>A, p.Arg1168His | het       | PM1, PM2, PP3 | 0.035% AFR | This manuscript | Y          |                  |
| 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          |                  |
| 35   | Gitelman/Bartter syndrome; metabolic alkalosis, hypomagnesemia, hypokalemic nephropathy | Unknown        | 3                | F   | 17          | Caucasian | KLHL3  | NM_001257194: c.1357G>A, p.Val453Leu | 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 | ANOSI  | NM_000216: c.1759G>T, p.Val587Leu | het       | PM1, PM2, PP3 | Not reported | [60]                  | N          |                  |
| 46   | FSGS or minimal change disease. Persistent proteinuria | Unknown        | 4                | M   | 5           | Caucasian, non-Hispanic | ANOSI  | 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          | Hispanic  | FAH    | NM_000337: 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          |                  |
| #  | Condition                                      | Gender | Age | Ethnicity       | Gene      | variant | consequence  | Mutation Type | Other Comments | Johns Hopkins Hospital | Johns Hopkins University | Johns Hopkins Medicine |
|----|------------------------------------------------|--------|-----|----------------|-----------|---------|--------------|---------------|------------------|-------------------------|------------------------|----------------------|
| 53 | Renal cysts. Family history of hereditary nephritis | 2      | F   | 49             | LAMB2     | NM_002292: c.5234C>A, p.Ala1745Asp | het   | PM2, PP3      | Not reported     | This manuscript       | Y                     |                     |
| 54 | Polycystic kidney disease, undescended testes, HTN | 2      | M   | <1             | UMOD      | NM_001008389: c.854C>A, p.Ala285Glu     | het   | PM2, PP2, PP3 | Not reported     | This manuscript       | N                     |                     |
| 55 | Y 2 F 49 Asian                                  |        |     |                | NPHS1     | NM_004646: c.563A>T, p.Asn188Le        | het   | LR* (PM1, PP5, BP4, BP6) |                |                      | N                     |                     |
| 56 | TRAP1                                          |        |     |                | NM_001272049: c.598A>G, p.Ile200Val   | het   | PP3          | 2.05% EF         | ClinVar          |                |                      |                      |
| 57 | Moderate CKD                                    | Unknown|     |                | ACE       | NM_000789: c.907C>T, p.Arg265*         | het   | Pathogenic known | (PVS1, PM2, PM4, PP3) |                |                      | Y                     |
| 58 | Not provided                                   | Unknown|     |                | ACE       | NM_000789: c.3136G>A, p.Glu1046Ser     | het   | PM2           | Not reported     | This manuscript       | N                     |                     |
| 59 | GLI3                                           |        |     |                | NM_000168: c.1616G>A, p.Arg539Lys      | het   | PM2           | Not reported     | This manuscript       | U                     |                     |
| 60 | Nephronophthisis or medullary cystic kidney disease | 2      | M   | 58             | TRAP1     | NM_001272049: c.598A>G, p.Ile200Val   | het   | PM2, BS1      | 2.05% EF         | ClinVar          | N                     |                     |
| 61 | FSGS/multicystic dysplastic kidney              | 1, 4   | M   | 15             | PKD1      | NM_000296: c.971G>T, p.Arg324Leu      | het   | PM1, PP5      | 0.59% EF         | Uniprot          | N                     |                     |
| 62 | Hyperplastic nephrogenic rests, features seen with underlying syndromes such as Beckwith-Wiedemann | Unknown|     |                | CHD1L     | NM_001256336: c.2179A>G, p.Glu581Gln  | het   | 0.47% NFE     | This manuscript   |                      | N                     |                     |
| 63 | Horseshoe kidney, dysmorphic features, VSD     | 1      | F   | <1             | IQCB1     | NM_001023570: c.1441G>A, p.Glu481Lys  | het   | PM1, PP3, BP1 | 0.19% NFE       | ClinVar          | N                     |                     |
| 64 | Steroid-resistant nephrotic syndrome           | 4      | M   | 8              | ANOS1     | NM_000216: c.1759G>T, p.Arg539Lys   | het   | PM1, PM2, PP5 | Not reported     | [60]            | N                     |                     |
| 65 | Glomerulocystic kidneys and hepatoblastoma     | 2      | M   | 3              | ANLN      | NM_0018685: c.1741G>C, p.Glu581Gln     | het   | 0.023% EA     | This manuscript   | Y                 |                      |                     |
| 66 | PKD2                                           |        |     |                | CUBN      | NM_001081: c.6095G>A, p.Cys2032Tyr    | het   | PM2, PP3, BP1 | 0.019% NFE       | This manuscript   | N                     |                     |
| 67 | TMEM67                                         |        |     |                | CUBN      | NM_001142301: c.272G>A, p.Arg91Gln     | het   | PM2, PP, BP3, PP5 | 0.012% LAT        | ClinVar          | N                     |                     |
| 68 | ANLN                                           |        |     |                | TMEM67    | NM_001142301: c.272G>A, p.Arg91Gln     | het   | PM2, PP, BP3, PP5 | 0.012% LAT        | ClinVar          | N                     |                     |

Continued
| Case | Indication for testing | Family history | Disease category | Sex | Age (years) | Ethnicity | Gene | Variant | Zygocityb | ACMG classification/rules [17] | MAF Gnomadb | First reported by | Possibly causalib |
|------|-----------------------|----------------|-----------------|-----|------------|-----------|------|---------|-----------|---------------------|-------------|-----------------|-------------------|
| 76   | Steroid-resistant nephrotic syndrome Gtelman syndrome | N | 3 | F | 23 | Dominican Republic | Not provided | EYA1 | NM_198428: c.1648A>G, p.Ile550Val | het | PP3 | 0.064% EA | ClinVar | N |
| 77   | Not provided | Y | 5 | M | 57 | Not provided | Not provided | TRIM32 | NM_01099679: c.1688G>A, p.Arg663His | het | PM2, PP3 | 0.013% NFE | ClinVar | N |
| 78   | Nephronophthisis | Y | 2 | F | 38 | Caucasian | Not provided | GLIS2 | NM_032575: c.278A>G, p.Asn93Ser | het | BP1, BP4 | 0.09% EF | This manuscript | N |
| 82   | Global glomerulosclerosis | Y | 4 | F | 65 | African/African-American | Not provided | COL4A4 | NM_000339: c.1967C>T, p.Pro656Leu | het | PP2, PP3 | 0.021% NFE | This manuscript | N |
| 83   | Juvenile nephronophthisis and medullary cystic kidney disease | Y | 2 | F | 29 | Not provided | Not provided | SLC12A3 | NM_002936: c.544G>A, p.Ala182Thr | het | PP3, PP5 | 0.208% NFE | ClinVar | N |
| 85   | X-linked hypophosphatemic rickets | Unknown | 3 | F | 1 | Caucasian, non-Hispanic | Not provided | HOX11 | NM_138413: c.700 + 5G>T | het | PP3, PP5 | 0.208% NFE | ClinVar | N |
| 88   | Renal tubular acidosis | Unknown | 3 | F | 9 | Caucasian, Hispanic | Not provided | 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 | Not provided | PKD1 | NM_000296: c.5866G>A, p.Val1956Met | – | – | 0.002% NFE | This manuscript | N |
| 90   | Alport syndrome | N | 4 | F | 6 | Caucasian | Not provided | SLC7A9 | NM_001126335: c.544G>A, p.Ala182Thr | het | PP2, PP3, PP5 | 0.43% NFE | ClinVar | N |
| 92   | Bilateral cystic kidneys | Unknown | 2 | F | 14 | 1 | Caucasian, non-Hispanic | Not provided | TMEM67 | NM_00142301: c.803T>C, p.Leu268Ser | het | PM2, PP2, PP3, PP5 | 0.004% NFE | [64] | N |
| 93   | Congenital bilateral echogenic kidneys with small cysts | 2 | F | 5 | Not provided | Not provided | SLC3A1 | NM_000396: c.8971T>G, p.Tyr2991Asp | het | PM1, PM2, PP3 | Not reported | This manuscript | Y |
| 102  | Autosomal recessive polycystic kidney disease | Unknown | 2 | M | 0 | Brazilian/Mexican Hispanic | Not provided | HNF4A | NM_000457: c.1133C>T, p.Ser378Phe | het | PM2, PP2, PP3, PP5 | 0.018% NFE | [65] | N |
| 107  | Congenital nephrotic syndrome | Unknown | 4 | F | 0 | Brazilian/Mexican Hispanic or Latino | Not provided | 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 | Not provided | IFT140 | NM_014714: c.886G>A, p.Gly296Arg | het | PM2, PP3 | 0.023% SA | This manuscript | N |
| 109  | Isolated multicystic dysplastic kidney disease and polycystic kidney disease | Unknown | 1, 2 | M | 7 | Not provided | Not provided | ANOS1 | NM_000296: c.2974A>G, p.Ile929Val | het | – | 0.413% SA | This manuscript | N |
| 110  | NDI | N | 3 | M | 1 | Caucasian, non-Hispanic | Not provided | AGTR2 | NM_000686: c.395delT, p.Glu132del | het | PP3, BP6 | 0.102% NFE | ClinVar | N |
| 111  | Branchio-oto-renal syndrome or isolated CAKUT | Unknown | 1 | F | 2 | Not provided | Not provided | CREBBP | NM_001079846: c.2458C>T, p.Pro820Ser | het | PP3, BP6 | 0.915% AFR | ClinVar | N |
| Case ID | Disease Category | Zygosity | Age | Gender | Ethnicity | Allele 1 | Allele 2 | Modality | Genes | Reference | Zygosity | Ethnicity | Modality | Genes | Reference | Zygosity | Ethnicity | Modality | Genes | Reference |
|---------|------------------|----------|-----|--------|-----------|----------|----------|----------|--------|----------|----------|-----------|----------|--------|----------|-----------|----------|--------|----------|--------|----------|-----------|----------|
| 112     | Dent disease, Bartter or Gitelman syndromes | Unknown | 3   | M      | Caucasian, non-Hispanic | NM_001178074: c.2633G>A, p.Arg878His | 0.052% AFR | This manuscript | N  |
| 114     | IgA nephropathy or FSGS | N        | 4   | M      | African/African-American | NM_001166133: c.4648C>T, p.Leu1550Phe | 0.22% EF | ClinVar | N  |
| 121     | Juvenile nephronophthisis | Unknown | 2   | M      | Not provided | NM_001079821: c.128G>A, p.Arg43Lys | 0.002% NFE | This manuscript | N  |
| 123     | Steroid-resistant nephrotic syndrome | Unknown | 4   | M      | Caucasian, non-Hispanic | NM_001126335: c.544G>A, p.Ala182Thr | 0.43% NFE | ClinVar | N  |
| 125     | Nephronophthisis | Unknown | 2   | M      | Caucasian | NM_001166133: c.4648C>T, p.Leu1550Phe | 0.22% EF | ClinVar | N  |
| 127     | Bartter syndrome, Gitelman syndrome or NDI | Y        | 3   | M      | Caucasian, non-Hispanic | NM_001166133: c.4648C>T, p.Leu1550Phe | 0.22% EF | ClinVar | N  |

Notes:
- 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.
- Zygosity: het = heterozygous; hom = homozygous; hemi = hemizygous.
- gnomAD: highest minor allele frequency reported. AFR = African; EA = East Asian; FE = European Finnish; NFE = European (non-Finnish); LAT = Latino; SA = South Asian.
- Yes (Y), no (N) or unknown (U).
- Newborn.
- Jewish No gnomAD data.
- M, male; F, female. HTN, hypertension; VSD, ventricular septal defect; CUA, calcific uremic arteriolopathy.
- Newborn.
| Case | Indication for testing | Family history | Disease category | Sex | Age (year) | Ethnicity | Gene | Variant | Zygosity | ACMG classification/ rules | MAF gnomAD | Associated disease | First reported by |
|------|-----------------------|----------------|------------------|-----|------------|-----------|------|---------|----------|--------------------------|-------------|-------------------|------------------|
| 9    | FSGS                  | Unknown        | African/African-American | M   | 54         |           | APOL1 | NM_001136540: c.1024A>G, p.Ser342Gly | hom        | Risk allele | 23% AFR | FSGS, hypertensive nephrosclerosis and HIV associated nephropathy | [53]        |
| 15   | Hypercalcemia, hypocalciuria. Suspicion of CaSR inactivating mutation | N 3 F 81 | Caucasian | F   | 54         |           | CaSR  | NM_001136540: c.1152T>G, p.Ile384Met | het        | Risk allele | 22.9% AFR | Hypercalcemia | [67]        |
| 46   | FSGS or minimal change disease. Persistent proteinuria | Unknown        | African/African-American | M   | 55         |           | APOL1 | NM_001136540: c.1160_1165delATAATT, p.Asn388_Tyr389del | het        | Risk allele | 15% AFR | Steroid sensitive nephrotic syndrome | This manuscript |
| 101  | Chronic kidney stones and alkaline urine | Unknown        | African/African-American | M   | 18         |           | APOL1 | NM_001136540: c.1024A>G, p.Ser342Gly | hom        | Risk allele | 23% AFR | Kidney stones | This manuscript |
| 118  | Nephrotic syndrome    | Unknown        | African/African-American | M   | 80         |           | APOL1 | NM_001136540: c.1124A>G, p.Lys379Met | hom        | Risk allele | 23% AFR | FSGS, hypertensive nephrosclerosis and HIV associated nephropathy | [53]        |
| 119  | CDK Stage 2, FSGS     | Unknown        | African/African-American | F   | 16         |           | APOL1 | NM_001136540: c.1124A>G, p.Lys379Met | hom        | Risk allele | 23% AFR | FSGS, hypertensive nephrosclerosis and HIV associated nephropathy | [53]        |
| 120  | ESRD due to FSGS      | Unknown        | African/African-American | F   | 20         |           | APOL1 | NM_001136540: c.1124A>G, p.Lys379Met | hom        | Risk allele | 14.14% AFR | FSGS, hypertensive nephrosclerosis and HIV associated nephropathy | [53]        |

*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.

Zygosity: het, heterozygous; hom, homozygous; hemi, hemizygous.

*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 categorya | Sex | Age (years) | Ethnicity | Gene | Variant | Zygosityb | ACMG classification/rules [17] | MAF gnomADc | 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 nephromophthisis 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 | Jeune syndrome 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 | N          |
| 111  | Branchio-oto-renal syndrome or isolated CAKUT | Unknown        | 1                 | F   | 2           | Not provided       | FGF23  | NM_020638: c.59del5, p.Ser20Thrfs*20 | het        | Not reported | This manuscript |                  |
| 112  | Dent disease, Bartter or Gitelman syndromes | Unknown        | 3                 | M   | 23          | Caucasian, non-Hispanic | ATP7B  | NM_000053: c.2972C>T, pThr911Met | het        | Likely pathogenic (PS3, PM1, PP2, PP3, PP5) 0.24% NFE | [71]       | Wilson disease carrier |                   |

*aDisease 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.

*bZygosity: het, heterozygous; hom, homozygous; hemi, hemizygous.

cgnomAD: highest minor allele frequency reported. FE, European Finnish; NFE, European (non-Finnish); SA, South Asian.

Y, yes; M, male; F, female.
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 (Table 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 (Table 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 (Table 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 (Table 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 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 \( 227900000 \) to \( 227950000 \) on \( COL4A4 \), which are duplicated as \( 227900000 \) to \( 227950000 \) on \( COL4A4 \). ATP6V1B1, which increase the risk of hypercalcemia, steroid-sensitive nephrotic syndrome and kidney stones, respectively [81–83].

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, bilinear 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 nkd 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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