

Genetic Etiologies for Chronic Kidney Disease Revealed through Next-Generation Renal Gene Panel

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Keywords

Genetic testing · Chronic kidney disease · Next-generation sequencing · Nephrology

Abstract

Introduction: Chronic kidney disease (CKD) is a major public health issue in the USA. Identification of monogenic causes of CKD, which are present in ~10% of adult cases, can impact prognosis and patient management. Broad gene panels can provide unbiased testing approaches, which are advantageous in phenotypically heterogeneous diseases. However, the use and yield of broad genetic panels by nephrologists in clinical practice is not yet well characterized. **Methods:** Renal genetic testing, ordered exclusively for clinical purposes, predominantly by general and transplant nephrologists within the USA, was performed on 1,007 consecutive unique patient samples. Testing was performed using a commercially available next-generation sequencing-based 382 gene kidney disease panel. Pathogenic (P) and likely pathogenic

(LP) variants were reported. Positive findings included a monoallelic P/LP variant in an autosomal dominant or X-linked gene and biallelic P/LP variants in autosomal recessive genes. **Results:** Positive genetic findings were identified in 21.1% (212/1,007) of cases. A total of 220 positive results were identified across 48 genes. Positive results occurred most frequently in the *PKD1* (34.1%), *COL4A5* (10.9%), *PKD2* (10.0%), *COL4A4* (6.4%), *COL4A3* (5.9%), and *TTR* (4.1%) genes. Variants identified in the remaining 42 genes comprised 28.6% of the total positive findings, including single positive results in 26 genes. Positive results in >1 gene were identified in 7.5% (16/212) of cases. **Conclusions:** Use of broad panel genetic testing by clinical nephrologists had a high success rate, similar to results obtained by academic centers specializing in genetics.

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Introduction

Chronic kidney disease (CKD) affects 37 million adults in the USA [1]. Recent studies suggest that disease-causing genetic variants are identifiable in ~10% of adults and ~20% of children with CKD [2, 3], most of whom are unaware of the genetic etiology for their kidney dysfunction. Identification of monogenic causes of CKD can inform prognosis, personalize treatments, inform counseling and testing of at-risk relatives, influence reproductive decision-making, and enable referrals for evaluation of extrarenal manifestations. For the ~800,000 individuals in the USA with end-stage kidney disease (ESKD), genetic diagnosis may inform the selection of potential-related kidney donors, assess the risk of disease recurrence, and guide clinical management following transplant.

Broad gene panels offer several advantages over mutational analysis of individual genes or targeted panels. The phenotypic variability of rare and multisystem disorders, including the unpredictable interaction of causative variants, complicates the selection of appropriate targets [3, 4]. Screening for single-gene disorders in a stepwise manner can preclude identification of the causative variants and can be expensive and time consuming. Broad gene panels provide an economical, comprehensive analysis that can reduce barriers to testing by streamlining testing procedures, reimbursement, report structure, and genetic counseling capabilities.

To date, most studies utilizing genetic testing for kidney disease focus on selected cohorts with a high suspicion for monogenic disorders in an academic setting or have tested an unselected population of individuals with CKD as part of a systematic approach to determine the prevalence of genetic disorders causing kidney disease [3, 5–10]. Additionally, most of these studies perform genetic testing using whole-exome sequencing (WES) or with a panel of genes selected based on clinical presentation. One recent study that tested 127 patients with kidney diseases with a broad genetic panel, comprised of 177 genes identified positive findings in 43% of patients [11]. In addition to these seminal studies, characterizing the results of genetic testing performed exclusively for clinical purposes and ordered by nephrologists in clinical practice will provide an understanding of the real-world value of these tests. Understanding the testing patterns and the yield and scope of test findings will provide better insight into the clinical utility of genetic testing and how to improve its application in nephrology.

Recently, Natera, Inc. developed a next-generation sequencing (NGS)-based broad panel test for the identifica-

tion of monogenic causes of CKD. This panel encompasses genes associated with disorders spanning multiple types of kidney diseases, including cystic, tubulointerstitial, glomerular, tubular, and structural disorders. Additionally, this panel covers a broad range of diseases from those which primarily affect the kidney to multisystem diseases with known renal components. The panel, which included 382 genes, was designed to capture both well-established and rare genetic kidney diseases, as well as multi-organ syndromes that may be missed through targeted tests. The panel is available to clinicians in the USA, and the ordering of this test is solely at the discretion of the clinical nephrologist and the patient. Here, we present the findings from the first 1,007 tests performed with this broad panel for kidney diseases.

Materials and Methods

Study Subjects

This study was a retrospective analysis of 1,007 consecutive tests performed on patients with a 382 renal gene NGS panel (the Renasight™ test, Natera, San Carlos, CA, USA). These tests were ordered by transplant and general nephrologists at 204 clinics across the USA between May and September 2020. Demographic information of the patients tested, including age, ethnicity, sex, transplant status, and testing indications (ICD-10 codes) specifying CKD stage and a limited set of CKD diagnoses was provided on the requisition form by the patient or physician (Table 1, online suppl. Table S1; for all online suppl. material, see www.karger.com/doi/10.1159/000522226). Patients were determined to be affected either by the ICD-10 code or the clinical information provided by the clinician on the requisition form. Thus, there is a discrepancy between the number of cases analyzed based on ICD-10 codes and with affected status (Table 1, online suppl. Table S1). All patients or legal guardians (in the cases of minors) provided informed consent for the performance of genetic testing and the data were de-identified prior to analysis. The study was performed in adherence with the Declaration of Helsinki.

Panel Design

The broad renal genetic panel included 382 genes associated with cystic and tubulointerstitial disorders, glomerular disorders, complement-related kidney disorders, congenital anomalies of the kidney and urinary tract (CAKUT) and structural disorders, tubulopathy and tubular disorders, diabetic nephropathies, hypertension-related disorders, nephrolithiasis, and electrolyte abnormalities (online suppl. Table S2) [3].

Renasight NGS Panel Sequencing and Data Analysis

Genomic DNA isolated from the accessioned samples (blood or buccal saliva) was prepared into libraries using a customized hybrid capture enrichment protocol targeting key coding exons and splicing junctions based on IDT xGen Lockdown probe chemistry (Integrated DNA Technologies, Inc., Coralville, IA, USA). Paired-end sequencing was then performed on DNA libraries on

Table 1. Demographics of patients

Age range	All patients		Positive cases	
	N = 1,007	(%)	250	(%)
≤18	21	(2.1)	1	(0.4)
19–29	144	(14.3)	41	(16.4)
30–39	234	(23.2)	68	(27.2)
40–49	170	(16.9)	60	(24.0)
50–59	171	(17.0)	41	(16.4)
60–69	164	(16.3)	26	(10.4)
70–79	84	(8.3)	11	(4.4)
>80	19	(1.9)	2	(0.8)
Ethnicity	All patients		Positive cases	
	N = 737	(%)	N = 204	(%)
African American	171	(23.3)	59	(28.9)
Ashkenazi Jewish	9	(1.2)	0	(0.0)
Asian	1	(0.1)	0	(0.0)
Caucasian	381	(51.7)	101	(49.5)
East Asian	6	(0.8)	3	(1.5)
Hispanic	120	(16.3)	27	(13.2)
Mediterranean	4	(0.5)	3	(1.5)
Pacific Islander	2	(0.3)	0	(0.0)
Southeast Asian	14	(1.9)	2	(1.0)
Sephardic Jewish	2	(0.3)	1	(0.5)
South Asian	9	(1.2)	4	(2.0)
Mixed/Other	18	(2.4)	4	(2.0)
Affected status	All patients		Positive cases	
	N = 973	(%)	N = 220	(%)
Affected	924	(95.0)	210	(95.5)
Unaffected	49	(5.0)	10	(4.5)

the Illumina platform 2,500 HiSeq or NovaSeq 6,000, using 300bp reads. The average coverage across the panel was >150× with 99.6% of the targeted regions covered at ≥20×. Copy number was calculated from NGS coverage data using a customized algorithm [12], which involved comparing normalized exonic coverage to controls.

Variant Interpretation

All variants detected in the reportable region (i.e., coding exons and ±20bp flanking introns) were assessed based on the American College of Medical Genetics and Genomics guideline for sequence variant interpretation [13]. Variants were classified into five-tier categories: pathogenic (P), likely pathogenic (LP), variants of uncertain significance (VUS), likely benign and benign. P and LP variants were reported and VUS findings were reported if requested by the provider but were not considered positive results. A monoallelic P/LP variant in an autosomal dominant (AD) or X-linked gene, and biallelic P/LP variants in an autosomal recessive (AR) gene were considered as positive findings. One P/LP variant in an AR gene imparted carrier status. For P/LP variants identified in genes associated with both

AD and AR diseases, clinical relevance was interpreted based on variant type, frequency, molecular mechanism of disease, and previously reported clinical cases in literature. Heterozygous P/LP variants within the *COL4A3* and *COL4A4* were considered positive, as were heterozygous P/LP variants in *COL4A5* in female patients [14].

Confirmatory Analysis

Confirmatory testing was performed for all P/LP cases, except for copy number events spanning ≥12 exons not overlapping regions of genomic complexity. When needed, confirmatory testing of the NGS-detected variants was performed on the original DNA sample. Sequence variants detected by NGS were confirmed by Sanger sequencing. Sequence variants detected in the regions with pseudogenes or homologous sequences were confirmed by long-range PCR followed by Sanger sequencing. Deletions or duplications were confirmed as indicated by quality score by an orthogonal method (qPCR or MLPA). Deletions or duplications in the *PKD1* and *PKD2* genes were confirmed by SALSA® MLPA® Probemix (P351-C1 for *PKD1*, P352-D1 for *PKD1-PKD2*).

Table 2. Positive genes

Gene	Associated conditions	Positive results, n	Inheritance pattern	Kidney disease category*
<i>ABCC8</i>	Familial hyperinsulinemia hypoglycemia, diabetes mellitus	1	AD/AR	T, G, D, H
<i>ADCY10</i>	Absorptive hypercalciuria	1	AD	T
<i>ALPL</i>	Hypophosphatasia	1	AD/AR	T
<i>APOL1</i>	Susceptibility to end-stage renal disease; focal segmental glomerulosclerosis 4	57	Complex	G
<i>ATP6V0A4</i>	Renal tubular acidosis, distal	1	AR	T
<i>ATP6V1B1</i>	Renal tubular acidosis with deafness	1	AR	T
<i>AVPR2</i>	Diabetes insipidus, nephrogenic	1	XL	T
<i>BBS1</i>	Bardet-Biedl syndrome	2	AR	CS, CTI
<i>CASR</i>	Hypocalcemia; familial hypocalciuric hypercalcemia with transient neonatal hyperparathyroidism	1	AD/AR	T
<i>CD2AP</i>	Focal segmental glomerulosclerosis	2	AD/AR	G
<i>CFI</i>	Hemolytic uremic syndrome, atypical; complement factor I deficiency	3	AD/AR	CR
<i>COL11A1</i>	Stickler syndrome	2	AD	CS?
<i>COL4A1</i>	HANAC	2	AD	CTI
<i>COL4A3</i>	Alport syndrome, COL4A3-related	13	AD/AR	G
<i>COL4A4</i>	Alport syndrome, COL4A4-related	14	AD/AR	G
<i>COL4A5</i>	Alport syndrome, X-linked	24	XL	G
<i>CUBN</i>	Megaloblastic anemia 1, Finnish type	1	AR	G
<i>CYP24A1</i>	Familial hypercalcemia	2	AR	T
<i>GANAB</i>	Polycystic kidney and/or polycystic liver disease 3	1	AD	CTI
<i>HBB</i>	Beta-hemoglobinopathies (HbSC disease)	1	AD/AR	G, T
<i>HNF1A</i>	Diabetes mellitus; maturity-onset diabetes of the young, type 3	1	AD/AR	CTI
<i>HNF1B</i>	Renal cysts and diabetes syndrome	1	AD	CTI, CS, D
<i>HNF4A</i>	Fanconi renotubular syndrome 4, with maturity-onset diabetes of the young, type 1	2	AD	G, D
<i>INF2</i>	Focal segmental glomerulosclerosis 5; Charcot-Marie-tooth disease E	3	AD	G
<i>KCNJ11</i>	Congenital hyperinsulinism; permanent neonatal diabetes mellitus	2	AD/AR	G, D
<i>MC4R</i>	Obesity risk	1	AD/AR	D, H
<i>MEFV</i>	Familial Mediterranean fever	1	AR	G
<i>NPHS2</i>	Nephrotic syndrome, type 2	2	AR	G
<i>NR3C2</i>	Pseudohypoaldosteronism type I, autosomal dominant hypertension, early-onset	1	AD	T, H
<i>OFD1</i>	Joubert syndrome, type 10; orofacioidigital syndrome I; Golabi-Behmel syndrome, type 2	1	XL	CTI
<i>PAX2</i>	Isolated renal hypoplasia; papillorenal syndrome; focal segmental glomerulosclerosis 7	1	AD	CS, G
<i>PBX1</i>	CAKUTHEd	1	AD	CS
<i>PKD1</i>	Polycystic kidney disease 1	75	AD	CTI
<i>PKD1/TSC2</i> gene deletion	Polycystic kidney disease 1/tuberous sclerosis contiguous gene deletion	1	AD	CTI
<i>PKD2</i>	Polycystic kidney disease 2	22	AD	CTI
<i>PKHD1</i>	Autosomal recessive polycystic kidney disease	2	AR	CTI
<i>PRKCSH</i>	Polycystic liver disease 1	1	AD	CTI
<i>PTPN11</i>	Noonan syndrome 1	1	AD	CS
<i>SLC12A3</i>	Gitelman syndrome	3	AR	T
<i>SLC34A1</i>	Fanconi renotubular syndrome 2; hypercalcemia, infantile, 2; nephrolithiasis/osteoporosis, hypophosphatemic, 1	1	AD/AR	T
<i>SLC3A1</i>	Cystinuria	4	AD/AR	T
<i>SLC4A1</i>	Renal tubular acidosis, distal	1	AD/AR	T
<i>SLC7A9</i>	Cystinuria	1	AD/AR	T
<i>SMAD9</i>	Pulmonary hypertension, primary 2	1	AD	T, H
<i>TSC2</i>	Tuberous sclerosis 2	1	AD	CTI
<i>TTR</i>	Amyloidosis, hereditary, transthyretin-related	9	AD	G
<i>UMOD</i>	Medullary cystic kidney disease 2; hyperuricemic nephropathy; glomerulocystic kidney disease	2	AD	CTI
<i>VHL</i>	Von Hippel-Lindau syndrome	1	AD	CTI
<i>WNK4</i>	Pseudohypoaldosteronism, type 2B	2	AD	T
<i>WT1</i>	Denys-Drash syndrome; Frasier syndrome; nephrotic syndrome, type 4	1	AD	CS, G

HANAC, hereditary angiopathy with nephropathy, aneurysms, and muscle cramps; CAKUTHEd, congenital anomalies of the kidney and urinary tract syndrome with or without hearing loss, abnormal ears, or developmental delay. * Kidney disease categories: CTI, cystic and tubulointerstitial disorders; G, glomerular disorders; CR, complement-related kidney disorders; CS, congenital anomalies of the kidney and urinary tract (CAKUT) and structural disorders; T, tubulopathy and tubular disorders (tubular ion transport, nephrolithiasis, cystinuria, nephrogenic diabetes); D, diabetes-related; H, hypertension-related.

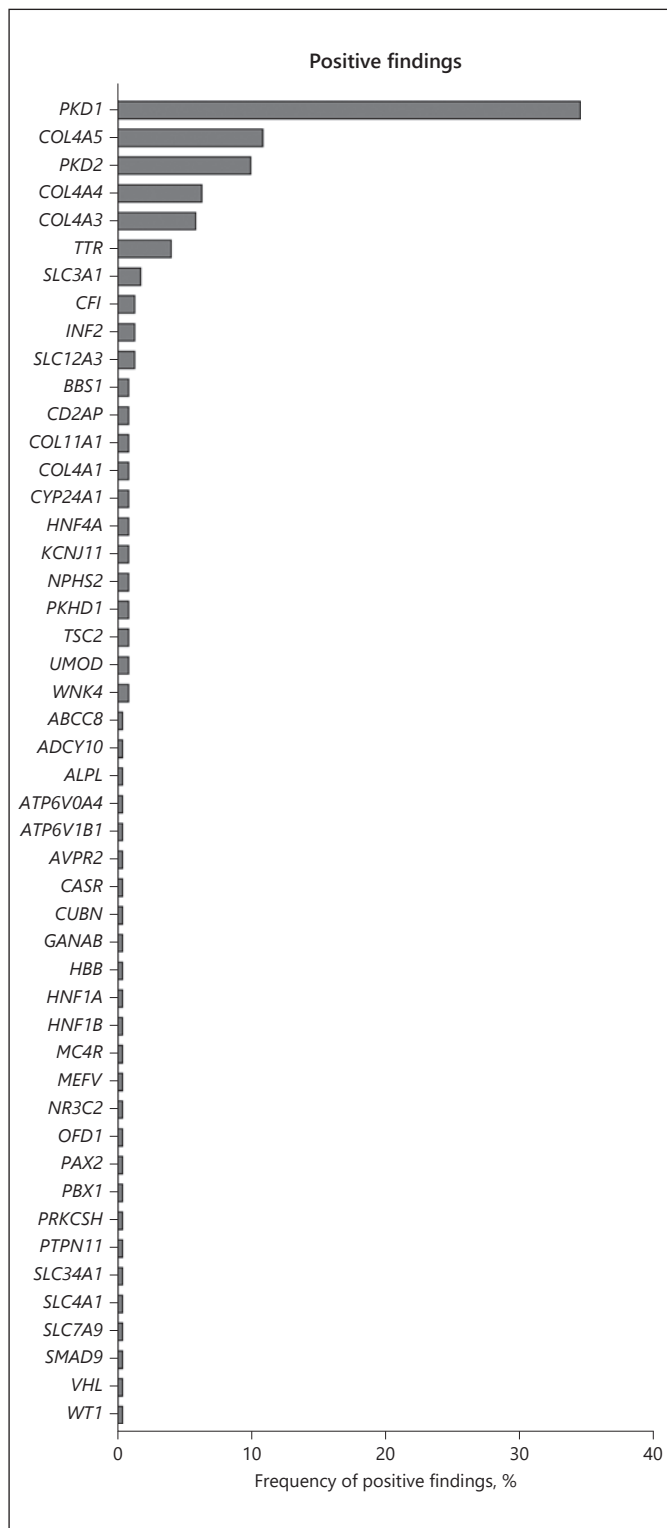


Fig. 1. Positive findings from testing with renal gene panel. Frequency of positive P/LP findings in each of 48 different genes (excluding *APOL1*) was determined out of 220 total positive results in 212 individuals. Frequency of positive findings in *PKD1* and *TSC2* include a *PKD1/TSC2* contiguous deletion identified in 1 case.

Results

Patient Characteristics

Renal genetic testing was performed on samples from 1,007 individuals with a median age of 46 years (range 5–91), of which, 52.7% (531/1,007) were female. Information about a patient's kidney disease status was available for 96.5% (973/1,007) of cases, of which 95.0% (924/973) were affected (Table 1). Testing indications, as designated by ICD-10 codes, were provided for 933 patients, of which, CKD (stages 1–5 or unspecified), or ESKD were submitted as the indication for 76.4% (713/933) of the tests ordered (online suppl. Table S1).

Ethnicity was reported for 73.2% (737/1,007) of cases, of which 51.7% (381/737) were Caucasian, 23.2% (171/737) were African American (AA), and 16.3% (120/737) were Hispanic. Among those with positive findings from genetic testing ($n = 260$; including *APOL1*), the median age was 44 years (range: 18–89), and the proportion of each ethnic group was similar to that of the full cohort (Table 1).

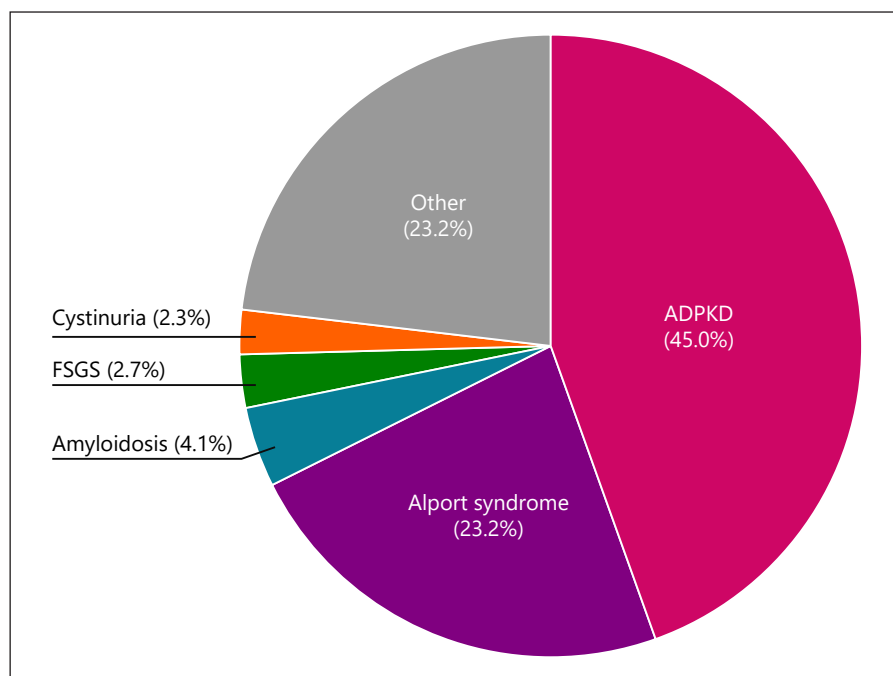
Genetic Findings

Of 1,007 individuals tested, 220 positive P/LP variants were identified across 48 genes (excluding *APOL1*) in 212 cases (Table 2, Fig. 1). Among the positive P/LP variants identified, 10 copy number variations were identified in nine genes, including a large deletion of *PKD1* and *TSC2* (online suppl. Table S3). Positive P/LP variants were identified most frequently in the *PKD1* (34.1%), *COL4A5* (10.9%), *PKD2* (10.0%), *COL4A4* (6.4%), *COL4A3* (5.9%), and *TTR* (4.1%) genes. Disease-causing P/LP variants identified in the remaining 42 genes comprised 28.6% of the total positive findings. A single positive P/LP result was identified in 26 genes, representing 11.8% of the 220 total positive results (shown in Fig. 1).

Among the 220 positive P/LP findings, the most frequent genetic diagnoses were AD polycystic kidney disease (ADPKD; 45.0%; $n = 99$), Alport syndrome (23.2%, $n = 51$), amyloidosis (4.1%; $n = 9$), focal segmental glomerulosclerosis (FSGS, 2.7%; $n = 6$), and cystinuria (2.3%; $n = 5$). Together, these five conditions comprised 77.2% of the total positive P/LP findings (shown in Fig. 2).

Biallelic G1 and G2 alleles in the *APOL1* gene confer an increased risk for the development of FSGS. Among the cohort, positivity for these *APOL1* high-risk genotypes (G1/G1, G1/G2, G2/G2) were identified in 57 individuals (Table 2). The G1 and G2 alleles are present at a high frequency in individuals of African descent. Among the high-risk genotype-positive cases in our cohort, 77.2%

Fig. 2. Top positive disorders identified by renal genetic testing. Disorders associated with positive P/LP variants identified in 48 genes were categorized to determine the most prevalent disorders among the cohort. ADPKD is associated with variants in the *PKD1*, *PKD2*, or *GANAB* genes; Alport syndrome is associated with variants in the *COL4A3*, *COL4A4*, or *COL4A5* genes, Amyloidosis is associated with variants in the *TTR* gene; FSGS is associated with variants in the *INF2*, *CD2AP*, or *PAX2* genes; and Cystinuria is associated with variants in the *SLC3A1* gene.



(44/57) of cases were AA, 7.0% (4/57) were Hispanic, 1.8% (1/57) were Caucasian, and ethnicity was not provided for 14.0% (8/57) of cases. Together, positive findings in disease-causing P/LP variants and *APOL1* high-risk genotypes were identified in 25.8% (260/1,007) of individuals. Positive findings in more than one gene (including both P/LP variants and *APOL1* high-risk genotypes) were identified in 16 cases, 9 of whom were positive for an *APOL1* high-risk genotype (online suppl. Table S6).

We assigned the 220 positive P/LP findings and their associated conditions to one or multiple broad kidney disease categories that we defined based on the framework developed by the ClinGen Kidney Disease Working Group [15]. Positive P/LP findings were most prevalent in genes associated with cystic and tubulointerstitial disorders (51.4%, 113/220), glomerular diseases (35.0%, 77/220), and tubulopathies (10.9%, 24/200) (Table 2). Additionally, 6.4% of the total positive findings were in genes and associated conditions that were assigned multiple kidney disease categories, highlighting the variable presentations of many kidney disorders (Table 2; online suppl. Fig. S1).

Identification of carriers for variants in AR genes has implications for reproductive and family planning. Carriers of one P/LP variant in one or more AR kidney disease genes or of one G1 or G2 allele in *APOL1* were identified in 45.3% (457/1,007) of cases; 23.0% (105/457) of

these cases also had positive findings in other genes. In total, 604 carrier variants were identified in 131 genes (online suppl. Table S1). VUS were reported in 100% (340/340) of cases where requested, with a mean number of 7 VUS identified (range 1–18).

Renal Genetic Test Findings among African Americans (AA)

In the USA, the incidence of developing ESKD is approximately 4-fold higher among AAs as compared to Caucasians [16]. This disparity is largely attributed to the high rate of positivity for *APOL1* high-risk genotypes. In our cohort, either positive P/LP variants or *APOL1* high-risk genotypes were identified in 34.5% (59/171) of AA patients, across 12 genes. Positivity for *APOL1* high-risk genotypes was identified in 74.6% (44/59) of the positive AA cases (Table 2). Among other findings in AA individuals, 5 cases were positive for variants in *PKD1* (6.7% of all *PKD1* positive cases), 4 in *TTR* (44.4% of all *TTR* cases), and 3 in *COL4A4* (21.4% of all *COL4A4* cases). Unique positive findings were identified in the *CASR*, *COL4A1*, *CUBN*, *HBB*, *PKD2*, *PTPN11*, and *SLC3A1* genes (online suppl. Table S5).

Reports have indicated that individuals with sickle cell trait (SCT) in the *HBB* gene may be at increased risk for the development of CKD [17]. Carriers of the *HBB* gene were identified in 15.2% (26/171) of AA patients, repre-

senting 66.7% (26/39) of all *HBB* carriers in our cohort. Five *HBB* carriers (4 AA; 1 unknown ethnicity) were also positive for *APOL1* high-risk genotypes, representing 7.0% of all *APOL1*-positive cases.

Unaffected Cases with Positive Findings

Among cases for which either positive P/LP variants or *APOL1* high-risk genotypes were identified, 3.8% (10/260) were clinically unaffected at the time of testing, and 1.5% (4/260) had an unknown disease status. Of the unaffected patients, five were prior or potential kidney donors, four of whom had *APOL1* high-risk genotypes, and two were positive for variants in either *PKD1* or *PKD2* and reported family histories of PKD. One patient with an LP *CD2AP* gene variant, which is associated with FSGS, for which no family history information was available, had preconception genetic testing performed to evaluate reproductive risk.

Positive Findings among CKD and ESKD Patients, and Kidney Transplant Recipients

Testing indications, based on ICD-10 codes were provided for 243 cases with positive findings (either P/LP variants or *APOL1* high-risk genotypes). Kidney transplant status information was provided by physicians for 202 of these positive cases. We investigated the positive findings among a sub-cohort of these cases for which severity of kidney disease progression was indicated (CKD stages 1–5 or unspecified, ESKD, or kidney transplant recipient [KTR]).

CKD or ESKD was indicated for 78.2% (190/243) of cases with positive results, of which 35.2% (67/190) had positive findings in the *PKD1* or *PKD2* genes, 23.2% (44/190) were positive for an *APOL1* high-risk genotype and 15.3% (29/190) had positive findings in *COL4A3*, *COL4A4*, or *COL4A5* (online suppl. Table S1, S7).

KTR comprised 8.1% (82/1,007) of patients in the cohort. Positive P/LP variants or *APOL1* high-risk genotypes were identified in 29.3% (24/82) of KTR in 13 genes, including 2 cases with positive findings in multiple genes. Findings included positivity for an *APOL1* high-risk genotype (4.2%; 10/24) and variants in the *COL4A3*, *COL4A4*, or *COL4A5* genes (16.7%; 4/24), which are associated with Alport syndrome (online suppl. Table S8). Among our cohort, 24 individuals were tested for potential organ donation. Positive findings (including P/LP variants and *APOL1* high-risk genotypes) were identified in 29.2% (7/24) of these cases, spanning multiple genes, including 4 patients with high-risk genotypes in *APOL1* (online suppl. Table S7).

Discussion/Conclusion

Here, we report the genetic findings observed during clinical use of a broad panel for monogenic kidney disorders by general and transplant nephrologists. This real-world application of this broad panel genetic test resulted in a positive genetic finding rate of 21.1%. In comparison, previously reported rates of positive findings have ranged from 9.3% in a cohort of unselected CKD/ESKD patients [3] to 51% in cohorts that were selected based upon family history, early onset of disease, or high suspicion of genetic kidney disease [6, 7, 10]. In our study, test ordering was determined solely at the discretion of the physician and patient, and criteria likely varied between physicians. As a result, the cohort tested likely included a combination of CKD/ESKD patients with low and high suspicions of genetic kidney disease, as well as asymptomatic individuals that may have been tested as a part of family testing or donor evaluation. Thus, the rate of positive findings in our cohort reflects selective screening and identification of at-risk patients by nephrologists.

The genetic variants identified in this cohort encompassed a large range of genes and associated kidney diseases. Most of the positive findings were identified in six key genes; however, the remaining 28.6% of findings involved 42 genes in which variants were only observed in 1 to 4 patients. The high rate of overall findings in this long tail of genes highlights the value of a broad panel. The genes in which positive findings were identified as associated with conditions that span multiple disease types, including cystic, glomerular, tubulointerstitial, and electrolyte disorders. Additionally, many of these conditions can have phenotypes that could be classified into multiple kidney disease categories. The heterogeneity among the conditions for which positive findings were identified among this cohort suggests that genetic testing with a broad panel could assist in accurate diagnosis when clinical tools are insufficient.

The largest disease groups for which positive disease-causing genetic findings were identified were ADPKD and Alport syndrome (collagen 4A disorders), reflecting findings in other cohorts of CKD patients referred for genetic testing [3, 10]. Testing for monogenic causes of ADPKD can enable diagnosis when ultrasound criteria alone cannot exclude individuals without a family history, in individuals with atypical presentation, or in younger patients with fewer or smaller cysts [18]. As variants in additional genes, such as *GANAB* and *DNAJB11* have been implicated in atypical presentation of ADPKD or can have phenotypic overlap with non-ADPKD disorders, diagnoses based on ultrasound alone have become more

complicated [18]. Knowledge of the underlying genetic component can influence prognosis and treatment of ADPKD [19]. For instance, patients with mutations in *PKD1* are likely to have a more rapid decline in eGFR and benefit from emerging treatments such as tolvaptan [20]. Furthermore, presentation or treatment of the disease can be complicated by the presence of P/LP variants in additional genes, which was observed in 5.1% (5/99) of *PKD1*, *PKD2*, or *GANAB*-positive cases in our cohort.

Positive findings in variants associated with Alport syndrome were also highly prevalent in our cohort. Collagen disorders such as Alport syndrome may be difficult to diagnose clinically, as cardinal features such as hearing loss and hematuria present variably. Among CKD patients referred for genetic testing, 62% of those with *COL4A* mutations do not have a clinical diagnosis of Alport syndrome [3]. Additionally, Alport syndrome can often manifest as FSGS, for which genetic testing can result in reclassification of the clinical diagnosis [21]. As a result of variability in the clinical presentations of Alport syndrome, recent guidance recommended a classification system for Alport syndrome and other collagen 4 disorders that include the incorporation of genetic confirmation [22].

Testing has the potential to influence management for a multitude of less common genetic kidney diseases that were identified among our cohort. Variants in *INF2*, *CD2AP*, *PAX2*, and *WT1* can be associated with FSGS and nephrotic syndrome. As genetic FSGS and nephrotic syndrome are often steroid-resistant [23], testing can result in avoidance of unnecessary use of glucocorticoids, and its associated toxicity. Variants in *HNF1B* can be associated with hypomagnesemia, asymptomatic liver function test abnormalities, gout, and progressive kidney disease, which can be treated if detected. Identification of variants in *CUBN*, which causes Imerslund-Grasbeck syndrome, can inform disease prognosis, as these individuals often have normal renal function and do not need treatment, despite the presence of proteinuria [24]. Furthermore, as kidney biopsy is expensive and has serious risks, identification of variants in some genes, including *UMOD* and *APOL1* have the potential to obviate or supplement a kidney biopsy which may not provide a definitive diagnosis.

AA individuals comprised 23.3% of our cohort, of which, the most common genetic findings were *APOL1* high-risk genotypes. The G1 and G2 alleles are present in 11%–13% of people of African ancestry [25, 26], who have a 3.5-fold higher incidence rate of ESKD compared to Caucasians [27]. It is generally thought that a second genetic or environmental factor is needed for the development of disease in individuals with *APOL1* high-risk genotypes. In our

study, P/LP variants in a second gene were identified in 5.8% (9/57) of *APOL1*-positive cases. Additionally, 44.4% (4/9) of the positive variants in *TTR* were in AA patients, consistent with previous reports for increased risk of *TTR* mutations among this population [28, 29]. Evidence also supports an association between SCT, which is prevalent among AA individuals, and CKD and decline in eGFR [17, 30]. In our cohort, 15.2% of AA patients were carriers of *HBB*, of which 92.3% were affected, much higher than the 8%–9% of all AA with SCT [31]. AA individuals are under-screened for genetic kidney diseases compared to other races [32] but disproportionately comprise the CKD population. The high rates of non-*APOL1* findings among the AA in our cohort (30.5%; 18/59) highlight the importance of screening AA with a broad gene panel to identify coexisting causes of inherited kidney disease.

Limitations of this study include a lack of detailed information regarding clinical diagnoses, the purpose of testing, or clinical follow-up. Testing indications based on ICD-10 may not be reflective of an accurate clinical diagnosis, as these codes are required for billing, and physicians are under no obligation to be specific with their coding. Due to the lack of additional medical history, we are unable to determine if patients considered “unaffected” are healthy or have other underlying medical conditions that may not have been documented. This limits the evaluation of the utility of genetic diagnoses in this cohort. Second, this test was initially available only to adult patients, resulting in an underreporting of pediatric patients. Third, although this gene panel encompasses a broad range of kidney-related genes and phenotypes, test results are limited to the scope of the panel and may miss the identification of certain P/LP variants. Future clinical studies that include larger cohorts, follow-up information, and healthy controls will be able to further evaluate the utility of genetic testing with a broad kidney gene panel on the management of patients with CKD.

Our study likely under-represents the true prevalence of genetic disorders in this population due to VUS and to unknown genetic causes of some disorders. Additionally, certain disease-causing variants, such as those in the *MUC1* gene, which account for 1% of ESKD cases [33], cannot be identified by multigene panels or WES. As variants identified through this genetic test are classified based on American College of Medical Genetics and Genomics criteria, many VUS are present. For instance, missense variants are the most common changes identified in the *COL4A* genes and as many of these variants are novel, they can be difficult to classify [14]. Thus, there may be many disease-causing variants that do not currently have enough evidence to

reach the level of P or LP. As information about genetic variants increases through additional functional studies, some VUS will likely be reclassified as disease-causing in the future. In addition, as more monogenic causes of kidney disease are identified in the future, expansion of genetic panels can enable higher positivity rates among patients. Thus, as with other studies in this area, the rate of positive findings in this study is likely an underestimate of the true prevalence of monogenic kidney disorders in this cohort.

Targeted phenotype-driven gene panels and WES both afford the ability to identify genetic disorders of the kidney but have drawbacks. Many commercial gene panels are restricted to groups of genes known to be causative of certain types of kidney disease (i.e., cystic or glomerulopathy). Thus, a negative result may lead to non-discovery of a genetic cause or could lead to subsequent testing with different disease panels. The comprehensive nature of WES enables the identification of variants in genes associated with both common and rare kidney disorders, as well as exploration of diseases for which the renal implications are not well defined. However, WES is costly and has longer turnaround time. A broad gene panel, such as the Renasight™ test, combines the benefits of both approaches and avoids the drawbacks.

The use of a broad panel for genetic diagnosis for kidney diseases has multiple advantages for clinicians, minimizing the need to identify the correct genetic panel or prioritize panels for individuals with an overlap of symptoms. Use of a single test for a wide range of patients allows physicians to become familiar with a single process for ordering, insurance ascertainment, cost, and results reporting, which have previously been identified as obstacles for use of genetic testing among nephrologists [4, 34]. In addition, unexpected findings for rare diseases that were not under consideration will improve diagnostic accuracy.

A recent study using a 177 gene panel spanning cilopathies/tubulointerstitial diseases, CAKUT, tubular transport disorders, and glomerulopathies to test a small cohort of individuals had a diagnostic yield of 43% [11]. This high yield is likely a result of clinicians selecting patients with a high suspicion of genetic kidney disease as well as the small cohort size. Yields are likely to be lower when genetic testing is used as part of routine clinical care of CKD patients.

In summary, genetic results from individuals tested with the Renasight test, a broad gene panel for evaluation for CKD, nephrolithiasis, and electrolyte abnormalities, revealed a high rate of positive findings representing a variety of both common, and rare genetic diagnoses. Our study revealed cases in which positive findings were identified in more than one

gene. These findings indicate that a broad kidney disease gene panel is highly effective in identifying monogenic variants underlying inherited kidney diseases and has utility for genetic diagnoses in the nephrology setting.

Statement of Ethics

No ethics approval was required for this study. All patients provided informed consent for genetic testing and the data were de-identified for analysis. The study was performed in adherence with the Declaration of Helsinki.

Conflict of Interest Statement

A.J.B. received speaker fees from Natera and has served on advisory boards for Horizon Therapeutics. J.J.E. received speaker fees from Natera, Otsuka, and Astra Zeneca. M.Z.M. received grant/research support from Viracor and CareDx and served as an advisor for Merck, CareDx, and AbbVie. W.K. is an employee and the owner of Florida Kidney Physicians; has ownership in/is a consultant for DaVita; received speaker fees and honoraria from Natera, Otsuka, Opko, and Astra-Zeneca; received research support from GSK, Tricida, Bayer, Sanifit, DiaMedica, Retrophin, and Astra-Zeneca; and is a member of the advisory boards for Retrophin and Astra-Zeneca. P.M. received support from Natera, Immunocor, CareDX, and Veloxis and is a consultant for CareDx and Natera. Y.X. and S.S. are employees of and own stock in Fulgent Genetics. M.W., J.X., M.S.B., K.B., S.P., Z.P.D., H.T., P.R.B., and T.M. are employees of Natera, Inc. with the option to own stock. F.J. and S.K. have no conflicts of interest to declare.

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Author Contributions

T.M. and J.X. conceived and designed the research; Y.X., S.S., J.J.E., M.Z.M., W.K., and P.M. performed data acquisition; M.W. and M.S.B. analyzed the data; A.B., J.X., S.P., M.S.B., S.K., and T.M. interpreted the results; A.B., M.W., K.B., J.X., M.S.B., Z.D., and T.M. drafted the manuscript; H.T. and P.R.B. oversaw the study design. All authors reviewed and approved the final version of the manuscript.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The clinical and demographic data are not publicly available due to privacy or ethical restrictions. All P/LP variants have been reported to and are accessible through ClinVar [35].

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