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Beyond Panel-Based Testing: Exome Analysis Increases Sensitivity for Diagnosis of Genetic Kidney Disease

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Abstract

Background Next-generation sequencing (NGS) is a useful tool for evaluating patients with suspected genetic kidney disease. Clinical practice relies on the use of targeted gene panels that are ordered based on patient presentation. We compare the diagnostic yield of clinical panel-based testing to exome analysis.

Methods In total, 324 consecutive patients underwent physician-ordered, panel-based NGS testing between December 2014 and October 2018. Gene panels were available for four clinical phenotypes, including atypical hemolytic uremic syndrome (n=224), nephrotic syndrome (n=56), cystic kidney disease (n=26), and Alport syndrome (n=13). Variants were analyzed and clinical reports were signed out by a pathologist or clinical geneticist at the time of testing. Subsequently, all patients underwent retrospective exome analysis to detect additional clinically significant variants in kidney disease genes that were not analyzed as part of the initial clinical gene panel. Resulting variants were classified according to the American College of Medical Genetics and Genomics 2015 guidelines.

Results In the initial physician-ordered gene panels, we identified clinically significant pathogenic or likely pathogenic variants in 13% of patients (n=42/324). *CFHR3-CFHR1* homozygous deletion was detected in an additional 13 patients with aHUS without a pathogenic or likely pathogenic variant. Diagnostic yield of the initial physician-ordered gene panel was 20% and varied between groups. Retrospective exome analysis identified 18 patients with a previously unknown pathogenic or likely pathogenic variant in a kidney disease gene and eight patients with a high-risk *APOL1* genotype. Overall, retrospective exome analysis increased the diagnostic yield of panel-based testing from 20% to 30%.

Conclusions These results highlight the importance of a broad and collaborative approach between the clinical laboratory and their physician clients that employs additional analysis when a targeted panel of kidney disease–causing genes does not return a clinically meaningful result.

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Introduction

Next-generation sequencing (NGS) is as an important diagnostic tool for the characterization of kidney disease (1). Targeted gene panels aid in the diagnosis of steroid-resistant nephrotic syndrome (NS) (2), nephronophthisis (3), autosomal dominant polycystic kidney disease (4), atypical hemolytic uremic syndrome (aHUS) (5,6), and congenital anomalies of the kidney and urinary tract (7). Renal biopsy is useful for evaluating patients with suspected genetic kidney disease, but it may reveal nonspecific findings. A genetic diagnosis is critical for assessing risk in family members and may provide therapeutic options for otherwise untreatable diseases (8–13).

Recent efforts have explored the utility of exome sequencing in large populations of adults with CKD, however, these studies were done retrospectively on selected populations (8,14). This is an important distinction because this approach significantly differs from physician-ordered testing performed in real time in an accredited clinical laboratory. With these caveats in mind, Groopman *et al.* (8) reported that 9% of adults with CKD have a monogenic cause, encompassing 66 different disorders. Similarly, Lata *et al.* (14) identified a genetic cause for CKD in 24% of patients after selecting for individuals with CKD of unknown cause or family history of kidney disease. Interestingly, the majority of monogenic disorders were detected as singletons, which underscores the importance of a broad approach to genetic evaluation of kidney disease.

The selection of a genetic test is based on clinical assessment and family history. However, many kidney diseases have overlapping clinical and pathologic

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phenotypes, complicating selection of the appropriate panel-based test. Fortunately, many NGS-based targeted gene sets are built on an exome backbone. This approach allows the laboratory to use a single process for library preparation and sequencing followed by a customized informatics workflow that extracts a subset of genes for clinical reporting. In the event an initial gene set returns no pathogenic or likely pathogenic variants, additional clinically relevant gene sets can be "unmasked" and interrogated (5). Although there is an abundance of archived genetic data that may or may not have implications for the patients' health, there are currently no guidelines on which patients should undergo additional review, which genes should be reviewed, or how frequently this process should be repeated. Furthermore, new genes are implicated in renal disease on a regular basis (15–18).

Materials and Methods

Clinical Laboratory Workflow

This study was approved by the Washington University School of Medicine Institutional Review Board and adheres to the Declaration of Helsinki. All patients provided informed consent before clinical genetic testing. Clinical gene sets (Table 1) were ordered by licensed physicians and performed in a College of American Pathologists (CAP)–accredited and Clinical Laboratory Improvement Amendments–certified laboratory. The data represent consecutive samples submitted to Genomics and Pathology Services at Washington University in Saint Louis between December 1, 2014 and October 31, 2018. Library preparation for the NGS assay began by fragmenting DNA to approximately 200 bp by ultrasonication, followed by end repair, A-tailing, and ligation to sequencing adapters. Libraries were prepared using the Agilent SureSelect XT Clinical Research Exome reagent, and sequenced using an Illumina (San Diego, CA) HiSeq2500 in 2×101-bp paired-end configuration. Analytic sensitivity, specificity, and reproducibility were established per the CAP NGS-testing checklist (19). Alignment was performed with Novoalign (version 2.08.02) and duplicates were marked with Picard (version 1.53). Single nucleotide variants were called with samtools (version 0.1.19) and insertions and deletions up to 21 bp were called using Unified Genotyper (GATK version 1.2) and pindel (version 0.2.4). *In silico* functional prediction was performed with SIFT (version 5.0.2) and polyPhen-2 (version 2.2.2). Patients referred for aHUS genetic testing underwent additional multiplex ligation-dependent probe amplification to detect deletion of *CFHR1-CFHR3* at Cincinnati Children's Hospital as previously described (5).

Table 1. Clinical gene sets

Clinical Gene Sets
Atypical hemolytic uremic syndrome, thrombotic microangiopathy, and C3 glomerulopathy gene set (13 genes and *CFHR3-CFHR1* deletion status) *ADAMTS13*, *C3*, *CD46*, *CFB*, *CFH*, *CFHR1*, *CFHR2*, *CFHR3*, *CFHR4*, *CFHR5*, *CFI*, *DGKE*, and *THBD*; *CFHR3-CFHR1* deletion by multiplex ligation-dependent probe amplification
Alport syndrome gene set (3 genes) *COL4A3*, *COL4A4*, and *COL4A5*
Cystic disease and nephronophthisis gene set (23 genes) *AHI1*, *CEP290*, *GLIS2*, *INVS*, *IQCB1*, *NEK8*, *NPHP1*, *NPHP3*, *NPHP4*, *RPGRIP1L*, *TMEM67*, *TTC21B*, *XPNPEP3*, *BICC1*, *CRB2*, *EYA1*, *HNF1B*, *PAX2*, *PKD1*, *PKD2*, *PKHD1*, *SIX5*, and *UMOD*.
Nephrotic syndrome and FSGS gene set (34 genes) *ACTN4*, *ADCK4* (*COQ8B*), *ANLN*, *APOL1*, *ARHGAP24*, *ARHGDIA*, *CD2AP*, *COL4A3*, *COL4A4*, *COL4A5*, *COQ2*, *COQ6*, *CRB2*, *CUBN*, *EMP2*, *INF2*, *ITGA3*, *ITGB4*, *LAMB2*, *LMX1B*, *MEFV*, *MYH9*, *MYO1E*, *NEIL1*, *NPHS1*, *NPHS2*, *PDSS2*, *PLCE1*, *PTPRO*, *SCARB2*, *SMARCAL1*, *TRPC6*, *TTC21B*, and *WT1*.

Variant Interpretation and Reporting

Variant classifications were based on standards and guidelines published by the American College of Medical Genetics and Genomics (ACMG) and the Association of Molecular Pathology (20). Variant calls were reported using Human Genome Variation Society nomenclature (http://www.hgvs.org/mutnomen) and variant attributes were examined using population databases, including the Exome Aggregation Consortium (ExAC version 0.3.1), Genome Aggregation Database (gnomAD version 2.1.0), the National Center for Biotechnology Information's short genetic variations database (dbSNP version 135), the FH aHUS mutation database (http://www.fh-hus.org/v3.0), ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/), the Human Gene Mutation Database public resource (http://www.hgmd.cf.ac.uk/ac/index.php access 6/3/2018), and an internally curated clinical-grade database of variants and interpretations housed in the Clinical Genomics Workspace (PierianDx, St. Louis, MO) (20,21). All results were reviewed by a pathologist with subspecialty boards in molecular genetics from the American Board of Pathology, or a clinical laboratory geneticist certified in clinical molecular genetics and clinical cytogenetics from the American Board of Medical Genetics and Genomics before release to the patient's medical record. High-risk *APOL1* genotype was defined as the presence of a G1G1, G1G2, or G2G2 genotype (22). Diagnostic yield was defined as the presence of a pathogenic or likely pathogenic variant, *APOL1* high-risk genotype, or *CFHR3-CFHR1* homozygous deletion in patients referred for aHUS testing.

Retrospective Review of Extended Renal Gene Set

We performed retrospective exome reanalysis of the variant call files for all patients using a curated list of genes implicated in renal disease (extended gene set in Supplemental Table 1) (8,14). Additional filtering criteria based on minor allele frequency in the gnomAD exome database, location of the variant within the gene, annotation based on comparison with clinical databases, and predicted *in silico* effects were used to enrich the data set for variants more likely to be causative of disease (Supplemental Figure 1). Selected variants outside of the clinically validated gene lists were confirmed by Sanger sequencing before returning results to the patient record.

Results

Patient Characteristics and Diagnostic Yield of Initial Panel-Based NGS Testing for Suspected Genetic Kidney Disease

In total, 324 patients received clinical, panel-based NGS testing for suspected genetic kidney disease between December 1, 2014 and October 31, 2018 (Table 2). Among

patients with available clinical history, 78 had ESKD and 48 had undergone kidney transplant at the time of referral (additional clinical information is provided in Supplemental Table 2).

The initial panel-based tests returned a clinically significant pathogenic or likely pathogenic variant in 42 patients (13%). An additional 101 (31%) patients had a variant of uncertain significance (VUS) with no pathogenic or likely pathogenic variant. The proportion of patients with a pathogenic or likely pathogenic variant was lowest in the aHUS group (6%) and significantly higher in the NS/FSGS (14%), cystic disease and nephronophthisis (46%), and Alport syndrome (30%) groups (Figure 1).

Genetic Variants in Patients Referred for aHUS Testing

aHUS is characterized by thrombotic microangiopathy and mutations in complement pathway genes. In total, 224 patients were referred for aHUS panel-based testing. A total of 18 patients had a clinically significant pathogenic or likely pathogenic variant detected in the initial aHUS gene set (Table 3). The genes most commonly affected were *CFH* ($n=6$), *C3* ($n=4$), and *CD46* ($n=4$). A total of 24 patients had homozygous loss of *CFHR3-CFHR1* detected by multiplex ligation-dependent probe amplification. We identified two novel variants not previously reported in aHUS. *CFH* p.D1119E was identified in a 9-month-old boy with anemia, thrombocytopenia, bloody diarrhea, and an *Escherichia coli*–positive stool sample. Similar variants (p.D1119G and p.D1119N) have been reported in aHUS (23,24), and the p.D1119G variant impairs the ability of CFH to bind C3b and C3d (25,26). *ADAMTS13* p.Q725* was identified in a 30-year-old man with isolated thrombocytopenia. Heterozygous nonsense variants in *ADAMTS13* have been reported in parents of patients with congenital thrombocytopenic purpura and are associated with decreased ADAMTS13 activity (27).

Exome reanalysis identified a previously unknown pathogenic or likely pathogenic variant in 12 patients initially referred for panel-based aHUS testing (Table 4). These data resulted in a new genetic diagnosis in eight patients and confirmed the genetic cause of an existing clinical diagnosis of autosomal dominant polycystic kidney disease ($n=3$) or Laurence–Moon–Biedl syndrome ($n=1$) in four patients. Two patients that received a new genetic diagnosis were newborns under evaluation for potential neonatal aHUS. In the first patient, exome reanalysis identified a previously reported homozygous nonsense mutation in *REN* (p.43*), which leads to absent renin expression at the protein and RNA level (28). In the second patient, exome reanalysis identified previously reported compound heterozygous mutations in *SLC26A1* (p.S358L, p.T185M) associated with ARF and calcium oxalate nephrolithiasis (29).

Exome reanalysis detected an additional seven patients with a high-risk *APOL1* genotype in the absence of a pathogenic, likely pathogenic, or VUS. *APOL1* high-risk genotype confers increased risk for ESKD and FSGS, but has not been previously associated with aHUS (30). All seven patients were black and the three patients with available clinical history had ESKD.

Genetic Variants in Patients Referred for NS, FSGS, and Alport Syndrome Testing

Genetic mutations in glomerular basement membrane proteins can lead to NS, FSGS, and Alport syndrome. In total, 56 patients were referred for the NS/FSGS gene set and 13 patients were referred for the Alport gene set. Initial panel-based NGS testing for the NS/FSGS and Alport gene sets identified 12 patients with a pathogenic or likely pathogenic variant (Table 3). The genes most commonly affected were *NPHS1* ($n=3$), *COL4A5* ($n=3$), and *NPHS2* ($n=2$). We identified a previously unreported pathogenic *NPHS1* frameshift variant (p.L16Gfs*3) in a 1-month-old girl with NS that is expected to result in loss of function.

Exome reanalysis identified previously unknown pathogenic or likely pathogenic variants that resulted in a new genetic diagnosis in five patients (Table 4). Two of these patients were diagnosed with autosomal dominant conditions that have important implications for family members. The first patient had a strong family history of kidney disease and a previously reported variant in *UMOD* (p.H175_R185del) that causes autosomal dominant tubulointerstitial kidney disease. The second patient was a 37-year-old man with ESKD and biopsy specimen–proven FSGS that was presumed secondary to obesity. He had a strong family history of kidney disease and a novel heterozygous variant in *PAX2* (p.G76R) that is located at the same position as a variant previously described in the context of renal coloboma syndrome (31).

Genetic Variants in Patients Referred for Cystic Kidney Disease and Nephronophthisis Testing

Cystic kidney disease and nephronophthisis are a spectrum of disorders that can have phenotypic overlap with congenital anomalies of the kidney and urinary tract. In total, 26 patients were referred for the cystic kidney disease

Table 2. Patient demographics

Gene Set	*N*	Age in Years (SD)	Gender	Race and Ethnicity
aHUS	224	36.0 (19.1)	81 M, 143 F	W, 139; B, 34; AS, 7; HIS, 21; OTH, 23
NS/FSGS	56	15.9 (15.1)	31 M, 25 F	W, 24; B, 11; AS, 1; HIS, 5; OTH, 15
NPHP	26	20.4 (22.7)	13 M, 13 F	W, 11; B, 6; HIS, 1; OTH, 8
Alport	13	29.9 (24.0)	5 M, 8 F	W,8; B, 1; OTH, 4
Custom	5	40.2 (17.9)	3 M, 2 F	W, 3; B, 2
Total	324	31.1 (20.6)	133 M, 191 F	W, 185; B, 53; AS, 8; HIS, 27; OTH, 50

aHUS, atypical hemolytic uremic syndrome; M, male; F, female; W, white; B, black; AS, Asian; HIS, Hispanic; OTH, other; NS, nephrotic syndrome; NPHP, cystic renal disease and nephronophthisis.

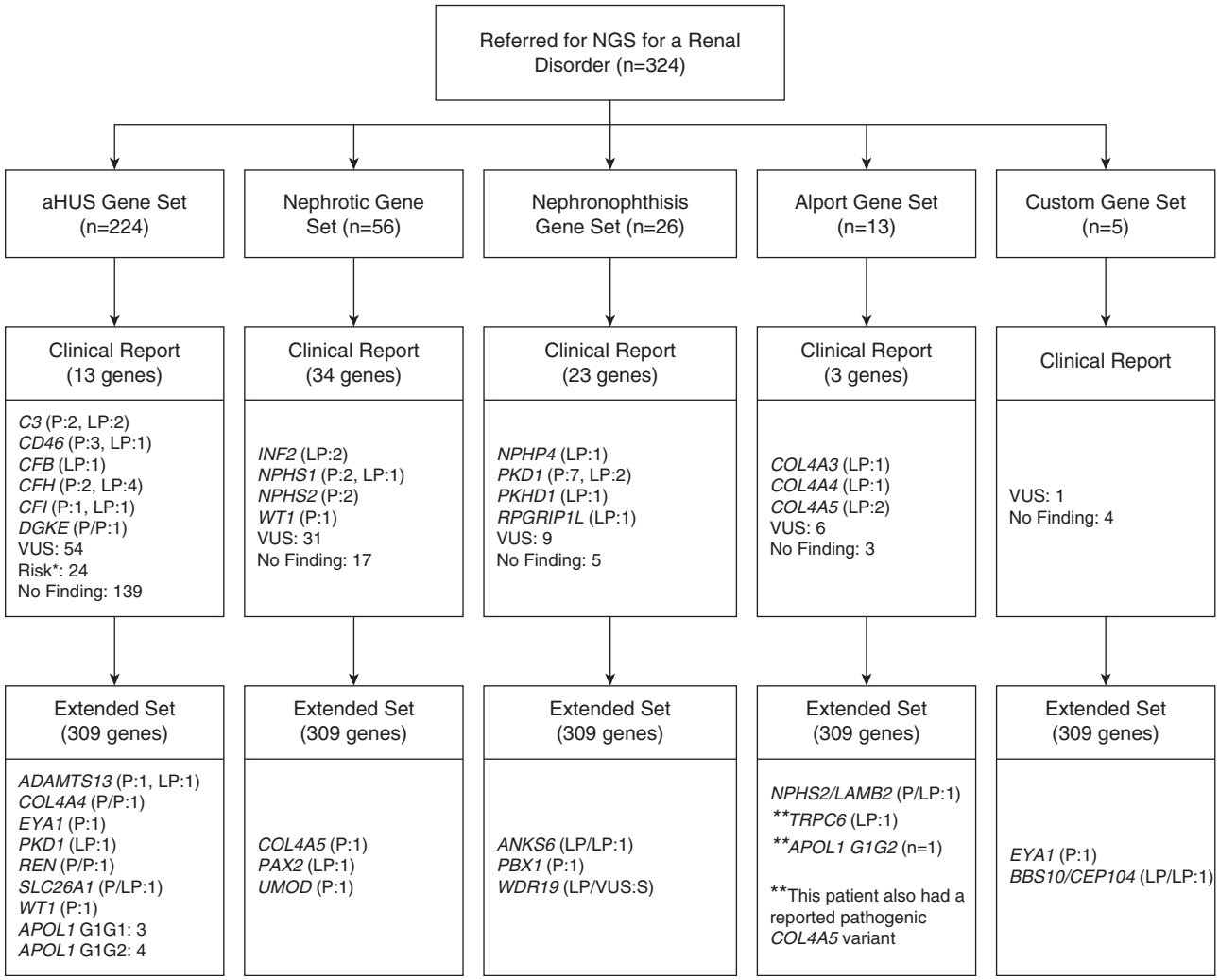

Figure 1. | **Expanding the gene panel increases sensitivity for detection of a clinically-significant variant.** Clinical next-generation sequencing (NGS) for renal disorders: physician-ordered, panel-based clinical testing for suspected genetic kidney disease was performed on 324 consecutive patients for the following indications: (*1*) atypical hemolytic uremic syndrome (aHUS), thrombotic microangiopathy, and C3 glomerulopathy; (*2*) nephrotic syndrome and FSGS; (*3*) nephronophthisis and cystic kidney disease; (*4*) Alport syndrome; and (*5*) other. Clinical reports were generated for the initial gene panel and submitted to the patient medical record. Retrospective exome analysis identified additional clinically significant pathogenic and likely pathogenic variants defined by American College of Medical Genetics and Genomics criteria. P, pathogenic; LP, likely pathogenic; VUS, variants of uncertain significance. *Risk, homozygous deletion of *CFHR3-CFHR1* are displayed as gene (assignment: number of patients) with "/" representing the presence of multiple variants (20).

and nephronophthisis gene set. Initial panel-based testing identified 13 patients with a pathogenic or likely pathogenic variant (Table 3). The majority of these patients had pathogenic or likely pathogenic variants in *PKD1* (n=9).

Exome reanalysis identified three patients with previously unknown pathogenic or likely pathogenic variants resulting in a new genetic diagnosis. The first patient was a 1-month-old girl with congenital echogenic kidneys and compound heterozygosity for a novel frameshift (p.L434fs) and previously reported splicing variant (c.1973-3C>G) in *ANKS6* (32). The second patient was a 5-month-old boy with bilateral renal dysplasia and a heterozygous nonsense variant in *PBX1* (p.Q48*). The third patient was a 3-year-old boy with congenital absence of the left kidney and compound heterozygosity for a novel frameshift variant (p.L92*) and previously reported missense variant (p.N273D) in *WDR19* (33).

Discussion

In this study, we report our 4-year experience with NGS panel-based testing for suspected genetic kidney disease in a clinically validated laboratory. The initial physician-ordered clinical gene sets resulted in a genetic diagnosis in 64 of 324 patients. These results include pathogenic (n=23) and likely pathogenic (n=19) variants in addition to *CFHR3-CFHR1* homozygous deletion in patients with aHUS (n=22). Retrospective exome reanalysis of kidney disease–related genes resulted in a clinically meaningful genetic diagnosis in an additional 26 patients. The retrospective results included additional pathogenic or likely pathogenic variants in kidney disease genes (n=18) and eight patients with a high-risk *APOL1* genotype. Our exome reanalysis indicates that affected patients are likely to have pathogenic variants in genes that rarely cause kidney

Table 3. Pathogenic and likely pathogenic variants identified in physician-ordered gene set

Patient	Gene	Variant	Genomic Coordinates (hg37)	ACMG	Novel	Gene Set	Family History[a]
4	*C3*	p.R161W	chr19:g.6718128G>A	LP	Yes	aHUS	No
66	*C3*	p.R1042L	chr19:g.6694471C>A	LP	Yes	aHUS	No
123	*C3*	p.K65Q	chr19:g.6719296T>G	P	Yes	aHUS	No
295	*C3*	p.K65Q	chr19:g.6719296T>G	P	Yes	aHUS	No
2	*CD46*	p.C35Y	chr1:g.207930365G>A	LP	Yes	aHUS	No
9	*CD46*	c.287-2A>G	chr1:g.207930883A>G	P	Yes	aHUS	No
23	*CD46*	c.1127+2T>G	chr1:g.207959029T>G	P	Yes	aHUS	No
35	*CD46*	c.287-2A>G	chr1:g.207930883A>G	P	Yes	aHUS	No
313	*CFB*	p.K323E	chr6:g.31916220A>G	LP	Yes	aHUS	No
113	*CFH*	p.D1119E	chr1:g.196714993C>G	LP	No	aHUS	No
166	*CFH*	p.R1210C	chr1:g.196716375C>T	LP	Yes	aHUS	No
234	*CFH*	p.R53H	chr1:g.196642207G>A	LP	Yes	aHUS	No
298	*CFH*	p.T956M	chr1:g.196709833C>T	LP	Yes	aHUS	No
267	*CFH*	c.3493+1G>A	chr1:g.196715130G>A	P	Yes	aHUS	No
18	*CFH*	c.619+1G>A	chr1:g.196646798G>A	P	Yes	aHUS	No
71	*CFI*	p.I370N	chr4:g.110670413A>T	LP	Yes	aHUS	No
304	*CFI*	p.R474*	chr4:g.110667387G>A	P	Yes	aHUS	No
85	*DGKE*	p.W322*,p.W322*	chr17:g.54926134G>A	P	Yes	aHUS	No
102	*COL4A3*	p.G695R	chr2:g.228142227G>A	LP	Yes	AS	Yes
280	*COL4A4*	p.G478E	chr2:g.227954610C>T	LP	Yes	AS	Yes
271	*COL4A5*	c.231+1G>A	chrX:g.107802384G>A	P	Yes	AS	Yes
264	*COL4A5*	c.4315+1G>A	chrX:g.107929360G>A	P	Yes	NPHP	No
188	*NPHP4*	p.K424Rfs*7	chr1:g.5993238delT	LP	Yes	NPHP	Yes
244	*PKD1*	p.G3326D	chr16:g.2149718C>T	LP	Yes	NPHP	Yes
252	*PKD1*	p.G1433R	chr16:g.2160661C>T	LP	Yes	NPHP	Yes
250	*PKD1*	c.9712+1G>A	chr16:g.2150166C>T	P	Yes	NPHP	Yes
273	*PKD1*	p.Q1203*	chr16:g.2161561G>A	P	Yes	NPHP	No
176	*PKD1*	p.V2569Rfs*43	chr16:g.2143887_2143888insG	P	Yes	NPHP	No
190	*PKD1*	p.A3082Cfs*96	chr16:g.2152218_2152219delAT	P	Yes	NPHP	Yes
225	*PKD1*	p.L3834Cfs*111	chr16:g.2141819delG	P	No	NPHP	Yes
245	*PKD1*	p.Q2900*	chr16:g.2153360G>A	P	Yes	NPHP	No
248	*PKD1*	p.L3999Wfs*40	chr16:g.2140892delA	P	No	NPHP	No
207	*PKHD1*	p.V1741M	chr6:g.51889387C>T	LP	Yes	NPHP	No
233	*RPGRIP1L*	p.D596Efs*5	chr16: g.53686809_53686823delinsAC	LP	No	NPHP	No
102	*COL4A3*	p.G695R	chr2:g.228142227G>A	LP	Yes	NS/ FSGS	Yes
98	*INF2*	p.R177C	chr14:g.105169653C>T	LP	Yes	NS/ FSGS	Yes
161	*INF2*	p.R177C	chr14:g.105169653C>T	LP	Yes	NS/ FSGS	No
277	*NPHS1*	p.R743C	chr19:g.36334481G>A	LP	Yes	NS/ FSGS	No
24	*NPHS1*	p.T847Rfs*57	chr19: g.36333146_36333149delTTAG	P	Yes	NS/ FSGS	No
213	*NPHS1*	p.L16Gfs*3	chr19:g.36342694_36342695insCC	P	No	NS/ FSGS	No
193	*NPHS2*	p.R138Q	chr1:g.179530462C>T	P	Yes	NS/ FSGS	Yes
238	*NPHS2*	p.R291W	chr1:g.179521740G>A	P	Yes	NS/ FSGS	No
216	*WT1*	p.H445R	chr11:g.32414217T>C	P	Yes	NS/ FSGS	No

Variant pathogenicity assignments based upon American College of Medical Genetics and Genomics guidelines (20). ACMG, American College of Medical Genetics and Genomics; chr, chromosome; LP, likely pathogenic; aHUS, atypical hemolytic uremic syndrome; P, pathogenic; AS, Alport syndrome; NPHP, cystic renal disease and nephronophthisis; del, deletion; ins, insertion; NS, nephrotic syndrome.
[a]First or second-degree relative with CKD or kidney transplant.

disease, because no two patients had a variant in the same gene. These data highlight the difficulty in designing a disease-focused gene set for renal disorders that often have overlapping clinical and pathologic phenotypes.

In light of these findings, we are in the process of reviewing our testing paradigm that reflexes to an expanded panel in cases where pathogenic or likely pathogenic variants are not identified in the initial, clinically indicated gene set. Furthermore, as new genes are added to our clinical gene sets, all patients that were tested before the addition of the new gene will be interrogated for pathogenic variants. Although there are currently no consensus guidelines, we

Table 4. Pathogenic and likely pathogenic variants identified in exome reanalysis

Patient	Age (yr)	Gene	Variant	Genomic Coordinates (hg37)	Clinical Diagnosis/Indication	Initial Ordered Gene Set	Family History[a]
138	75	*ADAMTS13*	p.D187H[b]	chr9:g.136291338G>C	aHUS	aHUS	No
302	30	*ADAMTS13*	p.Q725*[b]	chr9:g.136307803C>T	Thrombocytopenia	aHUS	No
318	19	*COL4A4*	p.R989fs p.R989fs	chr2:g.227917021_227917022delCT chr2:g.227917021_227917022delCT	ESKD and thrombotic microangiopathy	aHUS	No
79	26	*EYA1*	p.Y226*	chr1:g.248813510A>G	ESKD due to membranoproliferative GN	aHUS	No
231	19	*PKD1*	p.R3277C	chr16:g.2149956G>A	ESKD due to C3 glomerulopathy and cystic kidneys.	aHUS	No
152	74	*PKD1*	p.A2375V[c]	chr16:g.2156891G>A	ADPKD rule out aHUS	aHUS	No
198	33	*PKD2*	p.R213*[c]	chr4:g.88940651C>T	ADPKD rule out aHUS	aHUS	Yes
219	58	*PKD2*	p.R213*[c]	chr4:g.88940651C>T	ADPKD rule out aHUS	aHUS	Yes
195	<1	*REN*	p.R43* p.R43*	chr1:g.204131263G>A chr1:g.204131263G>A	Congenital ARF	aHUS	No
69	<1	*SLC26A1*	p.S358L p.T185M	chr4:g.983654G>A chr4:g.984938G>A	Neonatal aHUS	aHUS	No
52	35	*USH2A*	p.R4935*[c]	chr1:g.215814065G>A	Laurence–Moon–Biedl syndrome	aHUS	No
206	9	*WT1*	p.R462W	chr11:g.32413566G>A	aHUS	aHUS	No
119	17	*LAMB2* *NPHS2*	p.C1058fs p.R138Q	chr3:g.49161980_49161981delCA chr1:g.179530462C>T	Gross hematuria	AS	Yes
264	15	*TRPC6*	p.N125S	chr11:g.101375326T>C	Collapsing FSGS and thin basement membranes	AS	No
212	<1	*ANKS6*	p.L434fs c.1973-3C>G	chr9:g.101542538delA chr9:g.101530535G>C	Congenital renal dysplasia, heart abnormalities, and hepatosplenomegaly	NPHP	No
268	<1	*PBX1*	p.Q48*	chr1:g.164529201C>T	Congenital renal dysplasia	NPHP	No
168	3	*WDR19*	p.L92* p.N273D	chr4:g.39191386T>G chr4:g.39207283A>G	Congenital absence left kidney and enlarged, dysplastic right kidney	NPHP	No
96	9	*COL4A5*	c.2042–2A>T	chrX:107845113A>T	Nephrotic syndrome	NS/FSGS	Yes
197	37	*PAX2*	p.G107R	chr10:102510464G>C	Adult-onset FSGS	NS/FSGS	Yes
281	53	*UMOD*	p.H177_R185del	chr16:g.20360068_20360094del	FSGS	NS/FSGS	Yes
67	64	*EYA1*	p.L513P	chr8:g.72127681A>G	Congenital renal dysplasia and hearing loss	*GRHPR*	No
134	10	*BBS10* CEP104	p.Y559* p.Q335*	chr12:g.76740088G>T chr1:g.3753972G>A	Cystic renal disease	NS/FSGS, NPHP	No

Variant pathogenicity assignments based upon American College of Medical Genetics and Genomics guidelines (20). chr, chromosome; aHUS, atypical hemolytic uremic syndrome; ADPKD, autosomal dominant polycystic kidney disease; AS, Alport syndrome; NPHP, cystic renal disease and nephronophthisis; NS, nephrotic syndrome.

[a]First or second-degree relative with CKD or kidney transplant.

[b]Variants were identified in patients that underwent testing before the addition of ADAMTS13.

[c]Variants are associated with an established clinical diagnosis.

believe that good laboratory practices should include retrospective review of negative cases on a semiannual to yearly basis

There is a wide spectrum of genetic testing options that range from single gene to panel-based and whole-exome sequencing for both somatic and constitutional disorders (34,35). Nephrology was a late adopter of clinical NGS testing and, as a result, relatively little is known about the optimal approach to diagnosis of genetic kidney disease. We can draw from the experience of other specialties to better understand how different modalities apply to diagnosis, prognosis, and precision medicine (34). To date, disease-focused panels have been the preferred approach for clinical applications; however, there are challenges to this approach that include development and validation of separate panels for each disease process, constant updating of existing panels, and adherence to changing medical guidelines. These challenges have raised the question of whether clinical testing should use a more comprehensive approach like whole-exome or whole-genome sequencing (11,34). However, the benefits of a comprehensive approach have to be weighed against their increased cost and turnaround time, in addition to the increased likelihood of discovering VUS that may or may not be related to the patient's disease process. Currently, the majority of clinical laboratories rely on panel-based approaches for the diagnosis of genetic kidney disease. An important caveat is that laboratories often use an "exome backbone" for their clinical panels. This approach simplifies laboratory workflow by employing a single library preparation, sequencing, and bioinformatics process for all patient samples, and is made possible by the decreasing cost of exome sequencing. The end result is that there is a large amount of unanalyzed genetic data that may contain clinically meaningful information. A simple compromise might be to establish a workflow where the remaining genetic data is unmasked and analyzed when the first disease-focused panel is negative.

Remaining challenges in the diagnosis of genetic kidney disease include difficulty in establishing the pathogenicity of VUS and the detection of structural variants, which represent a minority of inherited kidney disease (2). The difficulty with interpreting VUS will be partly resolved as clinical databases increase in size to include new patient populations and pathogenic variants.

However, there is a role for use of *in silico* computational prediction tools for assessing variant pathogenicity. In our study, we used multiple *in silico* tools to predict the functional consequence of variants encountered in exome reanalysis. By limiting our investigation to variants with a predicted damaging or deleterious effect, we were able to increase the likelihood that variants were assigned a likely pathogenic or pathogenic assertion under ACMG criteria (36). One of the drawbacks of our approach is that we likely missed clinically significant variants with a benign functional prediction. *In silico* analysis alone is insufficient to assign pathogenicity and is lacking in sensitivity and specificity (35,37). A potential solution could be to design clinically validated functional assays to provide evidence for pathogenicity.

Recently, at least one group has published a case series describing a comprehensive panel-based approach (approximately 200 genes) in a series of 127 patients (38). Diagnostic yield ranged from 30% to 54%, depending on disease category, and was higher in younger patients (0–14 years), which comprised the majority of their population. An important finding was that among the 54 patients with a genetic diagnosis, 13 patients had a change in clinical diagnosis due to genetic testing. These data raise the possibility that had these patients initially received a narrow, disease-focused panel; it is possible that their genetic diagnosis would have been missed. Together with our data, these findings argue for a comprehensive approach and highlight the benefits of clinical NGS for kidney disease. NGS-based testing will continue to be an important component of the diagnostic workup in patients with a suspected genetic component to their kidney disease and this study demonstrates the utility of variant reanalysis to improve diagnostic yield .

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Author Contributions

M. Corliss, J. Gaut, L. Love-Gregory, S. McNulty, and P. Wilson reviewed and edited the manuscript and were responsible for data curation, investigation, and methodology; J. Gaut, L. Love-Gregory, S. McNulty, and P. Wilson were responsible for formal analysis and software; J. Gaut, L. Love-Gregory, and P. Wilson conceptualized the study; J. Heusel, J. Gaut, L. Love-Gregory, and P. Wilson provided supervision; L. Love-Gregory and P. Wilson were responsible for visualization; P. Wilson wrote the original draft; and all authors were responsible for project administration, resources, and validation.

Supplemental Material

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Supplemental Table 1. Extended renal gene set.

Supplemental Table 2. Patient demographics and clinical history.

Supplemental Figure 1. Filtering strategy.

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