Genomics Integration Into Nephrology Practice

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Rationale & Objective: The etiology of kidney disease remains unknown in many individuals with chronic kidney disease (CKD). We created the Mayo Clinic Nephrology Genomics Clinic to improve our ability to integrate genomic and clinical data to identify the etiology of unexplained CKD.

Study Design: Retrospective study.

Setting & Participants: An essential component of our program is the Nephrology Genomics Board which consists of nephrologists, geneticists, pathologists, translational omics scientists, and trainees who interpret the patient's clinical and genetic data. Since September 2016, the Board has reviewed 163 cases (15 cystic, 100 glomerular, 6 congenital anomalies of kidney and urinary tract (CAKUT), 20 stones, 15 tubulointerstitial, and 13 other).

Analytical Approach: Testing was performed with targeted panels, single gene analysis, or analysis of kidney-related genes from exome sequencing. Variant classification was obtained based on the 2015 American College of Medical Genetics and Genomics and the Association for Molecular Pathology guidelines.

Results: A definitive genetic diagnosis was achieved for 50 families (30.7%). The highest diagnostic yield was obtained in individuals with tubulointerstitial diseases (53.3%), followed by congenital anomalies of the kidney and urological tract (33.3%), glomerular (31%), cysts (26.7%), stones (25%), and others (15.4%). A further 20 (12.3%) patients had variants of interest, and variant segregation, and research activities (exome, genome, or transcriptome sequencing) are ongoing for 44 (40%) unresolved families.

Limitations: Possible overestimation of diagnostic rate due to inclusion of individuals with variants with evidence of pathogenicity but classified as of uncertain significance by the clinical laboratory.

Conclusions: Integration of genomic and research testing and multidisciplinary evaluation in a nephrology cohort with CKD of unknown etiology or suspected monogenic disease provided a diagnosis in a third of families. These diagnoses had prognostic implications, and often changes in management were implemented.

Despite employing conventional biochemical, imaging, and biopsy data, a significant proportion of kidney disease patients do not obtain a firm diagnosis. Recent studies suggest that monogenic causes of chronic kidney disease (CKD) are more common than expected, accounting for 15% to 25% of kidney failure patients.[1-3] Although many monogenic kidney diseases manifest in childhood, leading to early kidney failure, adult presentations of these same genetic disorders are increasingly being recognized. Recent research has demonstrated the tremendous power of genomic testing via nephrology-focused gene panels or exome sequencing to detect variants that cause monogenic CKD.[4,5] As well as providing a diagnosis, these data can be of prognostic value, enabling disease-specific therapies or clinical trials for monogenic kidney diseases (such as for Fabry disease, Alport syndrome, tuberous sclerosis, and polycystic kidney disease[6-8]), and ultimately allowing for the development of new therapies. A precise diagnosis may also lead to lifestyle changes, influence family planning, inform selection of living donors, and improve knowledge about the disease.[8]

However, several factors continue to impede widespread integration of genomic testing into the clinical practice, including the complexity of interpretation of test results, lack of appropriate expertise; lack of recognition of the potential implications for patient care, cost, concerns about patient privacy, patient misunderstanding and confusion, and fear of employer or insurance discrimination for the patient and their family members.[9,10] Therefore, despite the increasing recognition of the value of genomic screening,[11,12] a close relationship between the testing facility with genomic expertise and nephrologists and access to expert genetic counseling are still needed to provide specific and precise information, assuage adverse psychosocial consequences from a diagnosis, and establish realistic pretest expectations through careful education.[4,13]

To improve the care of patients with monogenic kidney disease, we created an Inherited Renal Disease Clinic that combines clinical and genomic services. Importantly, we developed an expert internal adjudication committee known as the Nephrology Genomics Board, consisting of nephrologists, geneticists, pathologists, translational omics scientists, and trainees who would review genomic data in the context of patient and family clinical data. In addition, we embedded a process for pretest and posttest counseling that includes insurance approval and cost investigation.

Here we describe results from this integrated genomic testing and research program into the clinical practice.

METHODS

Ethics Statement

The Mayo Clinic institutional review board granted a waiver of consent for this study. All individuals provided written informed consent to perform genetic testing. Patients authorized the genetic testing laboratory to directly bill their health insurance plan on their behalf and to share health information that justified the testing. All individuals participating in research activities provided written informed consent to a study approved by the Mayo Clinic institutional review board (IRB #17-005255).

Cohort

The cohort was composed of individuals from different families with unexplained CKD per NKF KDOQI (National Kidney Foundation Kidney Disease Outcomes Quality Initiative) guidelines or stones/nephrocalcinosis with unknown or suspected genetic etiology who were evaluated at the Mayo Clinic Nephrology Division in Rochester, MN, and Scottsdale, AZ, from September 2016 to September 2020. Clinical and demographic data were obtained from review of electronic medical records.

Age at onset of symptoms was determined as the age at which the first sign or symptom of kidney disease was noted. This included first identification of abnormal laboratory results in blood or urine studies. Age at diagnosis of CKD was defined as age of first presentation to a nephrology service with CKD. Age at diagnosis of kidney failure was defined as the age of commencement of kidney replacement therapy (ie, date of receipt of first kidney transplant or date of commencement of dialysis). Individuals with suspected complement disorders were excluded because they were evaluated in a separate hematology cohort.

Genomic Testing and Variant Interpretation

Genomic DNA was isolated from whole blood or buccal swab samples. Targeted gene panels associated with the specific kidney phenotype and a customized, comprehensive nephrology panel retrieved from exome sequencing data including 346 genes associated with kidney-related diseases (and/or highly expressed in the kidney based on literature search) from the same Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory were used. Copy number variant analysis was performed based on the sequencing data for all cases. For a subset of individuals with tubulointerstitial kidney disease, targeted analysis of the pathogenic cytosine insertion in the variable-number tandem-repeat (VNTR) of *MUC1* (mucin 1) was obtained as a standalone test. For a subset of African/African American individuals with glomerulopathy, targeted analysis of the known *APOL1* (apolipoprotein L1)

risk alleles were evaluated. Some individuals for whom clinical testing was not possible or feasible had exome sequencing done on a research basis at the Mayo Clinic Medical Genome Facility in Rochester, MN, which was subsequently analyzed by a clinical geneticist member of the Board.

The nephrologist in conjunction with the patient/family decided which was the most suitable test for patient's phenotype. Custom panels became available during the study and were employed when the patient's phenotype fitted into a clear disease category. Reportable genetic variants found in research testing were confirmed in a CLIA-certified and College of American Pathologists (CAP)-accredited laboratory. Genetic variants were classified according to the 2015 American College of Medical Genetics and Genomics (ACMG)/Association for Molecular Pathology (AMP) recommendations.[14] Segregation criteria (PP1) were applied as supporting evidence when variant segregates with ≥3 meioses. Prevalence of the variant in affected individuals (PS4) criteria was applied as supporting evidence when present in ≥2 probands with consistent phenotype.

Nephrology Genomics Board and Return of Results

All individuals received pretest counseling by the ordering provider and posttest counseling by a licensed genetic counselor member of the Board. The Board reviewed each case with the referring nephrologist present, and a kidney pathologist reassessed the kidney biopsy when slides were available. Critical review of genetic, laboratory, and clinical phenotypic information determined whether a variant fitted the clinical findings and appropriate follow-up. When relevant variants associated with syndromes with extrarenal manifestations were found, the individual was referred to the Department of Clinical Genomics for further investigation and management. The Board also determined whether to proceed with research testing after research consent. Genetic counseling and targeted variant analysis were provided for specific cases involving a family study, variant of uncertain significance (VUS) resolution, and family member living donor evaluation. Each individual and their health care provider were given a comprehensive report with detailed test results. A genetic family letter was offered to the proband to share with at-risk family members to introduce the genetic disorder and recommendations on how to pursue familial testing.

Genetic Counseling Process

Pretest counseling was provided by the ordering nephrologist who received supplemental training to review basic counseling concepts and was provided with patient-learning materials created by experienced genetic counselors. Posttest genetic counseling provided by a genetic counselor, primarily delivered through a phone or video call, was offered to patients with abnormal results after review by the Board experts.

Statistical Analysis

Descriptive statistics were expressed using frequencies and proportions.

RESULTS

Cohort Description

A total of 183 probands without a confirmed genetic diagnosis prior to the nephrology evaluation were referred to the Inherited Renal Disease Clinic (Fig 1). The patients were categorized into six phenotypic groups: glomerulopathy, tubulointerstitial kidney disease, cysts/ciliopathies, stones, congenital anomalies of kidney and urinary tract (CAKUT), and other (eg, channelopathies, metabolic disturbances, polyuria, renal tubular acidosis, and CKD of unknown etiology). Demographic and additional clinical information for each of the categories can be found in Table S1.

Twenty individuals did not undergo genomic testing due to reimbursement issues or because the patient declined, so 163 individuals were included in the analysis. Eight individuals had single-gene analysis, 10 exome sequencing, 19 phenotype-focused gene panels, and 126 had the 346-gene comprehensive nephrology panel (Table S2). Most were white (81.6%) and male (56.4%). Positive family history, defined as presence of first-degree relatives with the same kidney disease or unknown cause that resulted in kidney failure was present in 47.2% of probands (Table 1). Over a third of probands had onset of symptoms between 18 and 30 years of age, but 78.6% did not have genomic testing until after 31 years of age.

Diagnostic Yield and Genomic Findings

A definitive genetic diagnosis was achieved for 50 families (30.7%) (Table 2). The highest diagnostic yield was obtained in individuals with tubulointerstitial kidney disease (53.3%, 8 of 15), followed by glomerular (31%, 31 of 100), CAKUT (33.3%, 2 of 6), cysts (26.7%, 4 of 15), stones (25%, 5 of 20), and others (15.4%, 2 of 13). The causative genes for each of the disease groups are shown in Figure 2. The solve rate for individuals with a positive family history was 39% (30 of 77) while 24.1% of individuals (20 of 83) with no family history of kidney diseases obtained a genetic diagnosis. A genetic diagnosis was determined in 41.4% (36 of 87) of individuals with symptoms <30 years and 18.5% (14 of 76) of probands with symptoms beginning after 31 years of age. Out of the unsolved cases, 17.7% (20 of 113) of the individuals were deemed to have strong candidate variants for their phenotype (Table 3). These individuals had rare VUS deemed deleterious in autosomal dominant genes (8 of 20), single variants (pathogenic and VUS) deemed damaging in autosomal recessive genes (6 of 20), VUS in genes with not enough evidence to be called causative for the phenotype (2 of 20), African American individuals with focal segmental glomerulosclerosis (FSGS) who were biallelic for the *APOL1* risk alleles (2 of 20), two VUS in an autosomal recessive gene related to the phenotype, without phase confirmation (1 of 20), or a single pathogenic variant in a possible digenic disease (1 of 20). All the clinically reported variants for unsolved cases that were deemed not candidates for the patients' phenotypes are listed in Table S3.
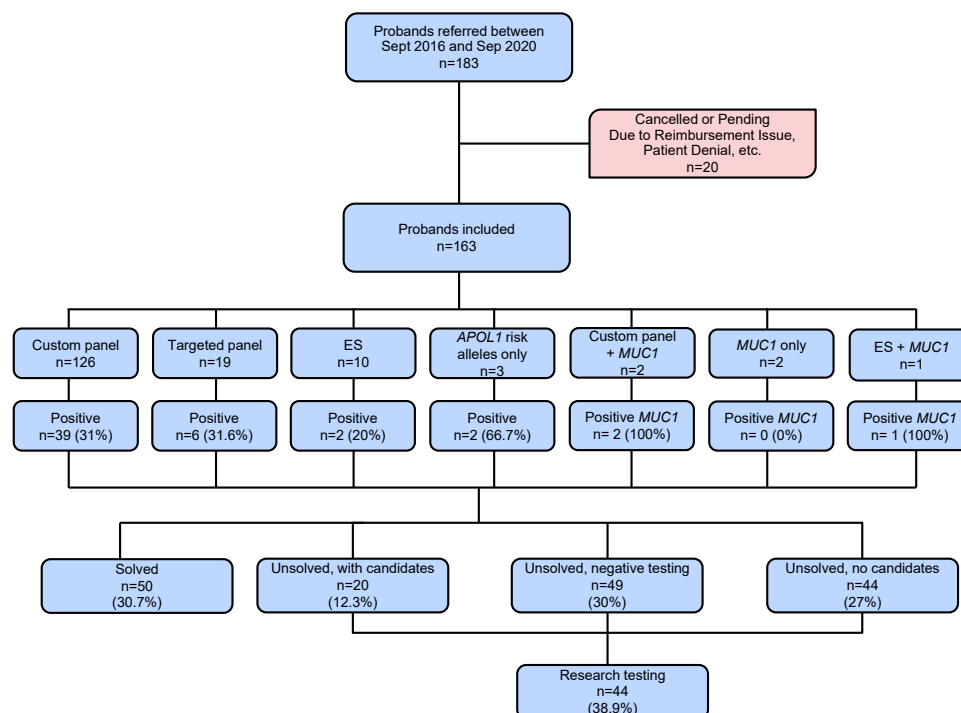

Figure 1. Flowchart of probands recruitment and genomic testing outcomes.

Table 1. Clinical Characteristics of the Cohort

Characteristic	No. of Patients (N = 163)
Sex	
Female	71 (43.6%)
Male	92 (56.4%)
Race or ethnic group	
White	133 (81.6%)
African American/African	10 (6.1%)
Asian	10 (6.1%)
Other or unspecified	10 (6.1%)
Age at onset of symptoms	
0-17 y	37 (22.7%)
18-30 y	50 (30.7%)
31-50 y	40 (24.5%)
51-70 y	32 (19.6%)
>70 y	4 (2.5%)
Age at time of testing	
0-17 y	10 (6.1%)
18-30 y	25 (15.3%)
31-50 y	56 (34.4%)
51-70 y	58 (35.6%)
>70 y	14 (8.6%)
Kidney transplant	
Yes	21 (12.9%)
No	142 (87.1%)
Family history	
Yes	77 (47.2%)
No	83 (50.9%)
Not available	3 (1.9%)
CKD stage	
1	33 (20.2%)
2	22 (13.5%)
3	48 (29.4%)
4	34 (20.9%)
5	26 (16.0%)
Phenotype[a]	
Glomerulopathy	100 (61.3%)
Tubulointerstitial	15 (9.2%)
Stones	20 (12.3%)
CAKUT	6 (3.7%)
Cysts/ciliopathy	15 (9.2%)
Other	13 (8.0%)

Values are number (percentage).
Abbreviations: CAKUT, congenital anomalies of the kidney and urological tract; CKD, chronic kidney disease.
[a]There are cases with multiple of phenotypes.

Board Recommendations and Research Activities

Based on the Board experts' recommendations and research testing, 14 probands had variants initially classified as VUS by the clinical laboratory but deemed likely causative for patients' phenotypes by the Board (Table 2). For 38.9% of the remaining unsolved cases (44 of 113), research testing such as trio exome sequencing, genome, and transcriptome sequencing was recommended.

Interesting Cases

Case 1: Variable Expressivity of Known Syndrome

A 67-year-old woman (NGB#24) with end-stage kidney disease and history of hypertension was referred with kidney failure with FSGS lesion and distal tubular microcysts on a kidney biopsy, kidney cysts on ultrasound, but no known extrarenal manifestations (Fig S1). Genetic testing identified a heterozygous likely pathogenic variant, *JAG1*: p.(Trp803Cysfs*17), consistent with a diagnosis of Alagille syndrome.[15]

Alagille syndrome is a multisystem genetic disorder and major features include bile duct abnormalities, cholestasis, congenital cardiac defects (typically pulmonary arteries), butterfly vertebrae, eye abnormalities (posterior embryotoxon), and kidney abnormalities.[16-18] Variable expressivity and incomplete penetrance have been reported in families with Alagille syndrome, and this individual and her family were counseled accordingly.[17]

After subspecialty clinical genetics referral, it was recommended she discuss this testing with her children. It emerged that one of her children underwent surgery at a young age due to congenital pulmonary valve defect that could be related.

Case 2: Variant Missed by Clinical Genetic Testing

A 45-year-old man (NGB#42) had had recurrent calcium oxalate kidney stones since he was a teenager (Fig S2). His sister was affected and died while on dialysis. Clinical panel based exome sequencing detected a heterozygous pathogenic variant, *GRPHR*: p.(Val289fs20*). Because the history was very suggestive of type 2 primary hyperoxaluria, a research panel with higher coverage for this gene was performed and also detected a 3-kb deletion encompassing exons 3 to 5. This case highlights the limitations of exome sequencing in detecting some types of genetic variants such as small deletions and duplications.

Case 3: Synonymous Variant Affecting Splicing

A 72-year-old man (NGB#30) had FSGS on kidney biopsy (Fig S3A) and a positive family history of hematuria. Clinical genetic testing detected a synonymous VUS in *COL4A3* [c.765G>A, p.(Thr255=)]. Because the variant lies at the exon-intron boundary, blood RNA studies were performed on a research basis and confirmed that the variant affects splicing (Fig S3B and S3C), which resulted in reclassification of the variant to pathogenic.

Case 4: Variant Not Amenable to Conventional Next-Generation Sequencing Approach

A 24-year-old woman had CKD stage 4 (NGB#48), tubulointerstitial nephritis (Fig S4A), discrepant kidney size, but no proteinuria. Clinical genetic testing revealed a VUS in *SLC22A12* deemed not related to the phenotype. Due to the high suspicion of autosomal dominant tubulointerstitial kidney disease (ADTKD)-*MUC1*, specific

Table 2. Patients With a Confirmed Genetic Diagnosis After Genetic Testing

NGB ID	Disease Group	Gene[a]	Disease Associated With Gene	MOI	cDNA	Amino Acid	Zygosity	ACMG Classification[b]	Additional Information From NGB	Final Variant Classification	Reference	Consequences of Testing
1	CAKUT	WT1	NS, type 4	AD	c.1432+4C>T	p.?	Het	P				PD, PI, CT, ER
2[c]	CAKUT	GATA3	Hypoparathyroidism, deafness, and renal dysplasia	AD	c.551_572del	p.(Leu184Profs*4)	Het	P				PD, PI, ER
3	Cysts	HNF1B	Renal cysts and diabetes syndrome	AD	c.541C>T	p.(Arg181*)	Het	P				PD, PI
4[c]	Cysts	PKD1	PKD 1	AD	c.2820_2826del	p.(Ser940Argfs*9)	Mosaic	P				PD, PI
5	Cysts	PKD1	PKD 1	AD	c.5968_5969del	p.(Arg1990Glufs*59)	Het	P				PD, PI, CT
6	Cysts	PKD1	PKD 1	AD	c.6184C>T	p.(Gln2062*)	Mosaic	P				PD, PI
7[c]	GN	INF2	FSGS	AD	c.147C>A	p.(Asn49Lys)	Het	VUS	Damaging VUS in AD gene related to phenotype, segregates with disease in the family	LP		PD, PI, FT, CT
8[c]	GN	COL4A3	AS	AD/AR	c.689G>A	p.(Gly230Asp)+	Het	LP				PD, PI, CT, KT, ER
9	GN	INF2	FSGS	AD	c.217G>A	p.(Gly73Ser)	Het	LP				PD, PI, KT, ER
10	GN	SMARCAL1	NS, steroid resistant	AR	c.[2114C>T]; [666_667insA]	p.[(Thr705Ile)]; [(Gln223Thrfs*41)]	Het;Het	LP;LP				PD, PI, FT, ER
11	GN	COL4A4	AS	AD/AR	c.2734G>A	p.(Gly912Ser)	Het	P				PD, PI, KT, ER
12[c]	GN	INF2	FSGS	AD	c.470G>A	p.(Gly157Asp)	Het	LP				PD, PI, FT
13[c]	GN	COL4A4	AS	AD/AR	c.3982G>A	p.(Gly1328Arg)	Het	VUS	Damaging VUS (Gly-X-Y tripeptide) in AD gene related to phenotype	LP		PD, PI, ER
14	GN	NPHS2	NS type 2	AR	c.[983A>G]; [686G>A]	p.[(Gln328Arg)]; [(Arg229Gln)]	Het;Het	VUS;RA	Risk allele in trans with variant in exon 7 known to be associated with phenotype. Variants have been published in combination in individuals with the same phenotype.	LP;RA	41	PD, PI, ER
15[c]	GN	COL4A3	AS	AD/AR	c.2126-1G>C	p.?	Het	LP				PD, PI, FT, KT, ER
16	GN	COL4A5	AS	X-linked	c.1871G>A	p.(Gly624Asp)	Het	P				PD, PI, KT
17[c]	GN	COL4A5	AS	X-linked	c.796C>T	p.(Arg266*)	Het	P				PD, PI, FT, CT
18[c]	GN	UMOD	ADTKD-UMOD	AD	c.317G>T	p.(Cys106Phe)	Het	VUS	Damaging VUS in AD gene related to phenotype. Variant has been reported in other individuals with the same phenotype.	LP	42	PD, PI, FT, KT

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Table 2 (Cont'd). Patients With a Confirmed Genetic Diagnosis After Genetic Testing

NGB ID	Disease Group	Gene[a]	Disease Associated With Gene	MOI	cDNA	Amino Acid	Zygosity	ACMG Classification[b]	Additional Information From NGB	Final Variant Classification	Reference	Consequences of Testing
19[c]	GN	ARHGAP24	FSGS	AD	c.120G>A	p.(Trp40*)	Het	VUS	Damaging VUS in AD gene related to phenotype. Variant has been reported in other individuals with the same phenotype.	LP	43	PD, PI, KT, ER
20[c]	GN	COL4A5	AS	X-linked	c.919G>A	p.(Gly307Ser)	Het	P				PD, PI, FT, KT, ER
21[c]	GN	COL4A4	AS	AD/AR	c.2270G>A	p.(Gly757Glu)	Het	LP				PD, PI, FT, ER
22[c]	GN	COL4A3	AS	AD/AR	c.2083G>A	p.(Gly695Arg)	Het	LP				PD, PI, ER
23	GN	COL4A3	AS	AD/AR	c.2215G>A	p.(Gly739Arg)	Het	LP				PD, PI, KT, ER
24[c]	GN	JAG1	Alagille syndrome	AD	c.2409del	p.(Trp803Cysfs*17)	Het	LP				PD, PI, ER
25[c]	GN	TRPC6	FSGS	AD	c.2683C>T	p.(Arg895Cys)	Het	P				PD, PI, KT
26[c]	GN	TRPC6	FSGS	AD	c.2686T>A	p.(Tyr896Asn)	Het	VUS	Damaging VUS (several other pathogenic variants adjacent to the amino acid) in AD gene related to phenotype	LP		PD, PI, FT
27[c]	GN	COL4A5	AS	X-linked	c.2288G>A	p.(Gly763Glu)	Hemi	P				PD, PI, ER
28[c]	GN	COL4A3	AS	AD/AR	c. 1559G>A	p.(Gly520Asp)	Het	LP				PD, PI, FT, ER
29[c]	GN	COL4A5	AS	X-linked	c.340G>A	p.(Gly114Arg)	Hemi	P				PD, PI, KT, ER
30[c]	GN	COL4A3	AS	AD/AR	c.765G>A	p.(Thr255=)	Het	VUS	Research RNA studies showed alternative splicing.	LP		PD, PI, FT, ER
31[c]	GN	JAG1	Alagille syndrome	AD	c.2230C>T	p.(Arg744*)	Het	P				PD, PI, FT, ER
32[c]	GN	COL4A5	AS	X-linked	c.1871G>A	p.(Gly624Asp)	Het	P				PD, PI, FT, ER
33	GN	CLCN5	Dent disease	X-linked	c.82C>T	p.(Arg28*)	Hemi	P				PD, PI
34[c]	GN	COL4A4	AS	AD/AR	c.[81_86del];[595-7T>A]	p.[(Ile29_Leu30del)];[?]	Het;Het	VUS;VUS	Likely solved, damaging VUS, predicted to eliminate a splice acceptor in AD gene related to phenotype. Variant has been reported in other individuals with the same phenotype.	LP;VUS	44-46	PD, PI, FT, ER
35	GN	COL4A5	AS	X-linked	c.1691G>A	p.(Gly564Asp)	Het	P				PD, PI, FT, ER

(Continued)

NGB ID	Disease Group	Gene[a]	Disease Associated With Gene	MOI	cDNA	Amino Acid	Zygosity	ACMG Classification[b]	Additional Information From NGB	Final Variant Classification	Reference	Consequences of Testing
36	GN	*APOE*	Lipoprotein glomerulopathy	SD	c.127C>T	p.(Arg43Cys)	Het	VUS	*APOE* Kyoto variant seen in several individuals with the same phenotype	LP	47	PD, PI
37	Other	*SLC12A3*	Gitelman syndrome	AR	c.[1196_1202dup]; [434G>A]	p.[(Ser402*)]; [(Arg145His)]	Het;Het	P;VUS	Pathogenic variant predicted in trans with a VUS in AR gene related to phenotype. Biochemical findings consistent with the disease. Variant has been published in other individuals with the same phenotype.	P; LP	48	PD, PI
38	Other	*CFI*	Hemolytic uremic syndrome	AD/AR	c.[1149-2A>T]; [1570G>T]	p.[?];[(Asp524Tyr)]	Het;Het	LP;VUS	Variant lies on the same amino acid that has pathogenic variants reported in individuals with the same phenotype.	LP;LP		PD, PI, FT
39	Stones	*SLC34A3*	Hypophosphatemic rickets with hypercalciuria	AR	c.[734dup]; [575C>T]	p.[(Leu246Alafs*23)]; [(Ser192Leu)]	Het;Het	P;P				PD, PI, CT
40[c]	Stones	*CYP24A1*	Hypercalcemia	AR	c.[1186C>T]; [475C>T]	p.[(Ala396Thr)]; [(Ala159Thr)]	Het;Het	P;VUS				PD, PI
41	Stones	*CLDN16*	Hypomagnesemia	AR	c.[697G>T]; [310G>A]	p.[(Gly233Cys)]; [(Asp104Asn)]		LP;VUS	VUS predicted in trans with a LP variant in AR gene related to phenotype. Biochemical findings consistent with disease.	LP;LP	49	PD, PI, FT
42[c]	Stones	*GRHPR*	Primary hyperoxaluria type 2	AR	c.[864_865del]; [exon3-5 del]	p.[(Val289Aspfs* 22)];[?]	Het;Het	LP;P				PD, PI, FT
43[c]	TI	*MUC1*	ADTKD-MUC1	AD	VNTR C insertion	p.?	Het	P				PD, PI
44	TI	*NPHP1*	Nephronophthisis	AR	c.[1027G>A]; [gene deletion]	p.[(Gly343Arg)];[?]	Het;Het	P;P				PD, PI, KT, ER
45[c]	TI	*MUC1*	ADTKD-MUC1	AD	VNTR C insertion	p.?	Het	P				PD, PI, FT, KT
46[c]	TI	*COL4A3*	AS	AD/AR	c.1984G>A	p.(Gly662Arg)	Het	LP				PD, PI, FT, KT, ER
47[c]	TI	*COL4A4*	AS	AD/AR	c.4953G>A	p.(Trp1651*)	Het	LP				PD, PI, FT, KT, ER
48[c]	TI	*MUC1*	ADTKD-MUC1	AD	VNTR C insertion	p.?	Het	P				PD,PI
49[c]	TI and GN	*UMOD*	ADTKD-UMOD	AD	c.817G>T	p.(Val273Phe)	Het	LP				PD, PI, CT

(Continued)

Table 2 (Cont'd). Patients With a Confirmed Genetic Diagnosis After Genetic Testing

NGB ID	Disease Group	Gene[a]	MOI	cDNA	Amino Acid	Zygosity	ACMG Classification[b]	Additional Information From NGB	Final Variant Classification	Reference	Consequences of Testing
50	TI and stones	SLC34A3	AR	c.[1579_1581del]; [413C>T]	p.[(Leu527del)]; [(Ser138Phe)]	Het	VUS;VUS	VUS predicted in trans in AR gene related to phenotype. Variants have been reported in other individuals with the same phenotype.	LP	48,50-52	PD, PI

Abbreviations: ACMG, American College of Medical Genetics and Genomics; AD, autosomal dominant; ADTKD-MUC1, autosomal dominant tubulointerstitial kidney disease, MUC1-related; ADTKD-UMOD, autosomal dominant tubulointerstitial kidney disease; AR, autosomal recessive; AS, Alport syndrome; CAKUT, congenital anomalies of the kidney and urological tract; cDNA, complementary DNA; CT, change in treatment; ER, evaluation of extrarenal manifestations; FSGS, focal segmental glomerulosclerosis; FT, family testing; GN, glomerulopathy; Het, heterozygous; KT, clarified for kidney transplant; LP, likely pathogenic; MOI, mode of inheritance; NGB, Nephrology Genomic Board; NS, nephrotic syndrome; P, pathogenic; PD, provided diagnosis; PI, prognostic information; PKD, polycystic kidney disease; RA, risk allele; SD, semidominant; TI, tubulointerstitial; VNTR, variable-number tandem-repeat; VUS, variant of uncertain significance.
aGene transcripts and demographics for positive cases are described in Table S4.
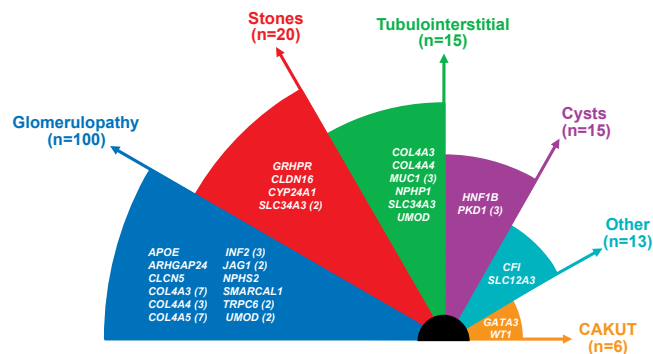bProvided by the clinical laboratory.
cCases with positive family history.

Figure 2. Disease groups and list of causative genes.

testing was obtained that detected a pathogenic cytosine insertion in the VNTR of MUC1.[19]

This case highlights that for individuals with tubulointerstitial kidney disease a gene panel should include UMOD, HNF1B, REN, and SEC61A1; if negative, targeted testing for MUC1 VNTR pathogenic variants should be performed.[20] These VNTR variants are not detected by conventional gene panels and exome sequencing.[19]

Case 5: Atypical Case of Cystic Disease

A 67-year-old man (NGB#6) had kidney cysts and CKD3A (iothalamate measured glomerular filtration rate of 50 mL/min/1.73 m^2) with a negative family history of cystic disease or CKD. Kidney ultrasound revealed normal cortical thickness and parenchymal echogenicity with no hydronephrosis and bilateral cysts (Fig S4B) with the largest measuring 6.5 × 7.0 × 7.0 cm. Clinical genetic testing using the cystic disease targeted panel detected a pathogenic p.(Gln2062*) variant in PKD1, but the variant was present in only 11% of the reads in blood DNA. This case highlights a milder disease course consistent with a truncating mosaic variant in PKD1.[21]

DISCUSSION

Our results demonstrate the feasibility of integrating genomic sequencing into the routine care at a large US academic clinical nephrology referral practice. The Nephrology Genomics Board reviews case details with the referring nephrologist and interprets the genetic results relevant to the patient's phenotype. In addition to verifying eligibility and approving research activities, and providing guidance to clinical care teams regarding possible ancillary testing, treatments, and clinical trials, the Board promotes genomic education and outreach within the practice group. Furthermore, there were many cases with overlapping phenotypes and complex genetic results where the Board experts were able to determine a diagnosis and provide advice regarding changes in management based on the genetic results.

Few US studies have reported the use of comprehensive genetic testing in a CKD population, and to date those that

Table 3. Patients With Candidate Variants

NGB ID	Disease Group	Gene[a]	Disease Associated With Gene	MOI	cDNA	Amino Acid	Zygosity	ACMG Classification[b]	NGB Conclusion	Reference	Follow-up
54	Cysts	NOTCH2	Alagille syndrome	AD	c.6139C>T	p.(Arg2047Trp)	Het	VUS	Unsolved with candidates, VUS predicted deleterious in AD gene related to phenotype		Clinical tests, segregation, and RNA sequencing
55	Cysts	NEK8	Renal-hepatic-pancreatic dysplasia 2	AR	c.67C>T	p.(Arg23*)	Het	LP	Unsolved with candidates, single hit in AR gene related to phenotype		RNA sequencing
56	GN	COL4A3	AS	AD/AR	c.4981C>T	p.(Arg1661Cys)	Het	VUS	Unsolved with candidates, VUS predicted deleterious in AD gene related to phenotype		Research WES and RNA sequencing
57	GN	KANK4	NS, steroid resistant	Unknown	c.487T>A	p.(Ser163Thr)	Het	VUS	Unsolved with candidates, VUS in a gene with lack of evidence	53	Research WES
58	GN	MYO1E	Steroid-resistant nephrotic syndrome	AR	c.1713C>G	p.(Asp571Glu)	Het	VUS	Unsolved with candidates, VUS in AR gene related to phenotype		RNA sequencing
59	GN	NPHS2	NS, type 2	AR	c.948del	p.(Ala317Leufs*31)	Het	P	Unsolved with candidates, single hit in AR gene related to phenotype		RNA sequencing
60	GN	NPHS2	NS, type 2	AR	c.138_142dup	p.(Ser48Trpfs*53)	Het	P	Unsolved with candidates, single hit in AR gene related to phenotype		RNA sequencing
61	GN	DLC1	NS	AD/AR	c.[1664T>C]; [117C>A]	p.[(Val555Ala)]; [(Ser39Arg)]	Het	VUS; VUS	Unsolved with candidates, VUSs in AR gene related to phenotype		RNA sequencing
62	GN	COL4A1	Angiopathy, nephropathy, aneurysms, and muscle cramps	AD	c.1085G>A	p.(Gly362Asp)	Het	VUS	Unsolved with candidates, VUS predicted deleterious in AD gene related to phenotype; patient with nephropathy but no other current symptoms		Clinical tests and follow-up
63	GN	INF2	FSGS	AD	c.1870A>T	p.(Arg624Trp)	Het	VUS	Unsolved with candidates, VUS predicted deleterious in AD gene related to phenotype		
64	GN	COL4A3	AS	AD/AR	c.1934G>C	p.(Arg645Thr)	Het	VUS	Unsolved with candidates, VUS in AD gene related to phenotype		Research WES
65	GN	TRPC6	FSGS	AD	c.432G>C	p.Glu144Asp	Het	VUS	Unsolved with candidates, VUS in AD gene related to phenotype		
66	GN	APOL1	FSGS	AR	c.[1024A>G]; [1164_1169del]	p.[(Ser342Gly)]; [(Asn388_ Tyr389del)]	Het;Het	RA;RA	Unsolved with candidates, risk alleles in trans in a gene possibly related to phenotype		Clinical management, transplant evaluation and donor genetic testing

(Continued)

Table 3 (Cont'd). Patients With Candidate Variants

NGB ID	Disease Group	Gene[a]	Disease Associated With Gene	MOI	cDNA	Amino Acid	Zygosity	ACMG Classification[b]	NGB Conclusion	Reference	Follow-up
67	GN	APOL1	FSGS	AR	c.[1024A>G]; [1164_1169del]	p.[(Ser342Gly)]; [(Asn388_ Tyr389del)]	Het;Het	RA;RA	Unsolved with candidates, risk alleles in trans in a gene possibly related to phenotype		Clinical management, transplant evaluation and donor genetic testing
68	GN	NPHS1	Nephronophthisis	AR	c.[2389C>A]; [2826C>A]	p.[(Pro797Thr)]; [(Asp942Glu)]	Het;Het	VUS;VUS	Unsolved with candidates, lacking phase of 2 VUS in AR gene related to phenotype		Variant segregation
69	Other	LAMA5	FSGS	AD/AR	c.[4105C>T]; [896G>T]	p.[(Arg1369Trp)]; [(Arg299Leu)]	Het	VUS	Unsolved with candidates, VUS in a gene with lack of evidence	54	RNA sequencing
70	Other	CACNA1H	Hyperaldosteronism	AD	c.5788G>A	p.(Asp1930Asn)	Het	VUS	Unsolved with candidates, VUS in AD gene related to phenotype		
71	Stones	SLC34A1	Nephrocalcinosis and hypercalcemia	AD/AR	c.1238C>A	p.(Thr413Asn)	Het	VUS	Unsolved with candidates, VUS predicted deleterious in AD gene related to phenotype		
72	Stones	CLCNKA	Bartter syndrome 4	Digenic	c.650_655+ 28del34	p.(Phe217_ Gly219delinsCys)	Het	VUS	Unsolved with candidates, single hit in gene related to phenotype		Research WGS
73	TI and stones	NPHP1	Nephronophthisis	AR	2q13 Deletion	p.?	Het	P	Unsolved with candidates, single hit in AR gene related to phenotype		RNA sequencing

Abbreviations: ACMG, American College of Medical Genetics and Genomics; AD, autosomal dominant; AR, autosomal recessive; AS, Alport syndrome; FSGS, focal segmental glomerulosclerosis; GN, glomerulopathy; Het, heterozygous; LP, likely pathogenic; MOI, mode of inheritance; NGB, Nephrology Genomic Board; NS, nephrotic syndrome; P, pathogenic; RA, risk allele; TI, tubulointerstitial; VUS, variant of uncertain significance; WES, exome sequencing; WGS, genome sequencing.

[a]Gene transcripts are described in the Table S2.

[b]Provided by the clinical laboratory.

have largely described results in research cohorts rather than embedded in a clinical practice.[4,22,23] KDIGO, a global organization developing evidence-based clinical practice guidelines in kidney disease, recognizes the promises of genomic medicine for kidney disease and the many technical, logistical, and ethical questions related to genetic testing in nephrology that must be addressed.[24]

A recent study used exome sequencing in a large research cohort that collectively included the major clinical CKD subtypes and achieved a solve rate of 9.3%.[4] Other studies using gene panels for focused phenotypes reported diagnostic yields ranging from 11% to 60% with higher solve rates for autosomal dominant polycystic kidney disease (ADPKD), pediatric patients, consanguineous families, patients with extrarenal manifestations, and familial cases.[11,22,25-27] Our solve rate was similar and, as expected, we had a higher solve rate in individuals with a positive family history and in the pediatric population (40.5%, 15 of 37). Interestingly, only 6.3% of the pediatric-onset cases had had genetic testing before adulthood, delaying the diagnosis for years. It is also important to note that a quarter of solved cases were individuals without a family history, highlighting that genetic testing should be offered to appropriate sporadic cases. Moreover, 22% of the affected individuals had onset of symptoms after 50 years of age, supporting a role for genetic testing in an adult population.

Smooth integration of research activities into a busy clinical practice is a unique feature of our program, and several of the cases highlighted above illustrate the value of this interaction. Case 1 illustrates an atypical cause of cystic disease, Alagille syndrome, and the variable expressivity of this disease, further highlighting the value of genetic testing for diagnostics. In Case 2, a multi-exon deletion missed by the clinical exome sequencing analysis was detected by employing a more targeted research gene panel with higher coverage of the genes tested. Exome sequencing and gene panels usually do not detect variants in regions outside the flanking regions of exons and might miss copy number variants. This case illustrates the value of the Board input that initiated follow-up research testing in a case where a second pathogenic variant was suspected. In Case 3, only a rare, synonymous variant at an exon-intron boundary of COL4A3 was detected. But in-house analysis and follow-up research RNA studies confirmed that this variant affected splicing, illustrating the value of genomic expertise and research capabilities. The impact of research might be even higher in our cohort because in almost 40% of the unsolved cases research testing is currently pending.

Noteworthy, variants in the VNTR region of MUC1—the cause of ADTKD-MUC1—are undetectable by short-read sequencing technology, owing to the repetitive nature and high guanosine and cytosine content of the VNTR region. In several cases (including case 4) we simultaneously ordered specialized clinical testing to detect the MUC1 pathogenic variant, solving three families' cases, and

limiting delays in obtaining a diagnosis. In case 5, a detected truncating PKD1 mutation did not match the mild cystic disease phenotype; however, the pathogenic allele was detected in just 11% of reads, suggesting mosaicism that is often associated with milder disease.[21,28] This screening of a mild ADPKD case resulted in actionable information important for the family.

PKD1 is a challenging gene to be analyzed by conventional sequencing due to segmentally duplicated regions. For instance, one individual in our cohort with FSGS and a low number of cysts had a likely pathogenic PKD1 variant detected in the duplicated region of the gene (exons 1-32). The region was poorly covered, so Sanger sequencing was performed and confirmed that the variant was indeed a sequencing artefact and that the very mild cystic phenotype was not related to ADPKD.

Despite progress with genetic diagnostics in nephrology and the demonstrated value of a firm diagnosis, clinical genetic testing is still often not part of a routine patient workup. A case in point is ADPKD, one of the commonest genetic causes of kidney failure. ADPKD displays genetic heterogeneity,[29,30] and knowing the gene and variant type can be of prognostic value. There is an expanding number of cystic/ciliopathy genes (allelic heterogeneity), somatic mosaicism is increasingly described,[31] and hypomorphic alleles and digenic inheritance are all capable of altering the prognosis.[28,32-34] Another example is primary hyperoxaluria, a monogenic cause of kidney stones and kidney failure that requires specific management strategies depending on the gene that is implicated (eg, intensive daily hemodialysis, monitoring for systemic oxalosis, combined liver kidney transplant), and for which novel small-interfering RNA (siRNA) treatment strategies are emerging.[35]

With evolving knowledge about gene-disease associations and the impact of genetic variants in known genes, individuals with inconclusive genetic results may still have monogenic disease or a genetic variant contributing to the phenotype.[24] In our cohort we found several individuals with a positive family history and/or suspected monogenic diseases for whom the reported variant(s) were not classified as pathogenic by the ACMG/AMP guidelines or a second variant in suspected recessive disease was not detected. This scenario triggered further genetic testing for affected individuals and family members, as illustrated here, resulting in a diagnosis.

Genetic counseling is an important component in the genomic evaluation of kidney disease.[36,37] This involves evaluating and understanding an individual's or family's risk of an inherited disorder and providing educational and psychosocial support. However, genetic counseling expertise embedded in a nephrology practice is rare.[38,39] At our center posttest genetic counseling was provided through a collaboration with the Mayo Clinic Center for Individualized Medicine, which also fostered the counselors' knowledge of kidney genetic diseases.[40] Meanwhile, pretest counseling concepts such as types of results,

informed consent, and testing logistics were often absorbed by the ordering nephrologist or other support staff, although additional training was required for these individuals to fulfill these roles.

A recent Columbia University study described return of clinically actionable results to a nephrology population. However, the testing was completed in a research setting, and ultimately only 62% of individuals could be recontacted from the original cohort of 108 individuals with medically actionable results.[23] In comparison, all individuals in our cohort had return of genetic testing results as part of their routine medical care and were counseled appropriately. Additionally, a letter explaining the results for other family members was provided to the proband.

The development of NGS methodologies and resulting expansion of clinical genetic testing has decreased the cost and improved availability. However, in the United States insurance coverage for genetic testing can limit its use for rare diseases. National guidelines provide eligibility criteria for genetic testing in other well-described monogenic disorders, such as hereditary cancer syndromes, and based on these guidelines most health insurance companies have created individual criteria for coverage of genetic testing (www.nccn.org). Greater awareness is needed regarding the value of genetic testing and the important legal, insurance, and emotional issues surrounding a genetic kidney diagnosis.

In addition to improving diagnostics and prognostics, genomic testing can facilitate clinical trials and highlight available specific therapies. This knowledge and a precise diagnosis can improve patient outcomes and quality of life, sometimes preempting more intensive treatments and resulting in cost savings. Our study suggests the reimbursement environment is already improving because >90% of probands had the test covered by insurance. Moreover, in most cases there was no out-of-pocket expense, or the expense was a few hundred dollars, like the cost of many common standard-of-care nephrology tests.

We acknowledge that a cost-effectiveness analysis of integrating genomic testing and a multidisciplinary team of experts into the nephrology practice is warranted. However, this type of analysis is difficult in the United States due to the way the health care system is established with variable costs of procedures and testing depending on the insurance payor. Moreover, a cost-effectiveness analysis is out of the scope of the present work.

In summary, genomic testing results evaluated by a multidisciplinary team coupled with state-of-art research techniques provided a definitive diagnosis for 30.7% of families with unexplained CKD or stones. The new diagnosis often resulted in change of management. Surprisingly, we detected a high prevalence of monogenic diseases in individuals without a family history and ones with an onset of symptoms at older ages. The incorporation of specialized genetic counselors into our nephrology practice improved access to genetic testing and provided a substantial impact in this patient population.

SUPPLEMENTARY MATERIAL

Supplementary File 1 (PDF)

Figure S1: 67-year-old with CKD stage 5, 3 g prot/24h with a pathogenic variant in *JAG1* associated with Allagille syndrome. Bilateral renal cysts (A and B). Kidney biopsy (periodic acid-Schiff stain) showing (C) focal glomerulus with segmental scarring and (D) focal distal tubular microcysts (black arrows).

Figure S2: 45-year-old man with recurrent calcium oxalate kidney stones and pathogenic variants in *GRPHR*. Ultrasound shows multiple nonobstructing renal stones bilaterally (black arrows).

Figure S3: 72-year-old man with FSGS lesion (A) and a *COL4A3* synonymous variant. (B) Agarose gel primer sets encompassing exons 10-16 which includes exon 13 where the variant c.765G>A lies. Product has two bands (highlighted in red). Lower band indicates a deletion. (C) Sequence alignment showing a 78-bp deletion consistent with exon 13 skipping (highlighted in red).

Figure S4: (A) 24-year-old woman with CKD stage 4 and kidney biopsy (H&E stain) showing tubulointerstitial nephropathy, interstitial fibrosis, and tubular atrophy with mild interstitial inflammation. (B) 67-year-old man with CKD stage 3 with a mosaic pathogenic variant in *PKD1*. Ultrasound showing bilateral kidney cysts, normal kidney size, and no liver cysts.

Supplementary File 2 (xlsx)

Table S1: Demographics and clinical characteristics by phenotype.

Supplementary File 3 (xlsx)

Table S2: Genes included in the multigene panels.

Supplementary File 4 (xlsx)

Table S3: Other genetic variants found in unsolved cases that were deemed not candidates.

Supplementary File 5 (xlsx)

Table S4: Clinical and genetic data for solved cases.

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