OPEN

Genomic Testing in Patients with Kidney Failure of an Unknown Cause

A National Australian Study

Amali C. Mallawaarachchi,[1,2,3] *Lindsay Fowles*,[4] *Louise Wardrop*,[5] *Alasdair Wood*,[5] *Rosie O'Shea*,[3] *Erik Biros*,[3,6,7] *Trudie Harris*,[3,7] *Stephen I. Alexander*,[8,9,10] *Simon Bodek*,[11] *Neil Boudville*,[12] *Jo Burke*,[13,14] *Leslie Burnett*,[2,15,16] *Sarah Casauria*,[17] *Steve Chadban*,[18] *Aron Chakera*,[19,20] *Sam Crafter*,[21] *Pei Dai*,[22,23] *Paul De Fazio*,[24] *Randall Faull*,[25,26] *Andrew Honda*,[27] *Vanessa Huntley*,[28] *Sadia Jahan*,[29] *Kushani Jayasinghe*,[30,31,32] *Matthew Jose*,[33] *Anna Leaver*,[11] *Mandi MacShane*,[34,35] *Evanthia Olympia Madelli*,[17] *Kathy Nicholls*,[36,37] *Rhonda Pawlowski*,[38] *Gopi Rangan*,[39,40] *Paul Snelling*,[18] *Jacqueline Soraru*,[41,42] *Madhivanan Sundaram*,[43] *Michel Tchan*,[44,45] *Giulia Valente*,[11] *Mathew Wallis*,[13,14,46] *Laura Wedd*,[47] *Matthew Welland*,[47,48] *John Whitlam*,[49] *Ella J. Wilkins*,[24] *Hugh McCarthy*,[8,10,50] *Cas Simons*,[47,48,51] *Catherine Quinlan*,[51,52,53] *Chirag Patel*,[4] *Zornitza Stark*,[17,24,53] and *Andrew J. Mallett*[3,7,17,54]

Key Points

- Twenty-five percent of those with unexplained kidney failure have a monogenic cause.
- Whole genome sequencing with broad gene panel analysis is a feasible diagnostic approach in nephrology.

Abstract

Background The cause of kidney failure is unknown in approximately 10% of patients with stage 5 chronic kidney disease (CKD). For those who first present to nephrology care with kidney failure, standard investigations of serology, imaging, urinalysis, and kidney biopsy are limited differentiators of etiology. We aimed to determine the diagnostic utility of whole genome sequencing (WGS) with analysis of a broad kidney gene panel in patients with kidney failure of unknown cause.

Methods We prospectively recruited 100 participants who reached CKD stage 5 at the age of ≤50 years and had an unknown cause of kidney failure after standard investigation. Clinically accredited WGS was performed in this national cohort after genetic counseling. The primary analysis was targeted to 388 kidney-related genes with second-tier, genome-wide, and mitochondrial analysis.

Results The cohort was 61% male and the average age of participants at stage 5 CKD was 32 years (9 months to 50 years). A genetic diagnosis was made in 25% of participants. Disease-causing variants were identified across autosomal dominant tubulointerstitial kidney disease (6), glomerular disorders (4), ciliopathies (3), tubular disorders (2), Alport syndrome (4), and mitochondrial disease (1). Most diagnoses (80%) were in autosomal dominant, X-linked, or mitochondrial conditions (*UMOD*; *COL4A5*; *INF2*; *CLCN5*; *TRPC6*; *COL4A4*; *EYA1*; *HNF1B*; *WT1*; *NBEA*; *m.3243A>G*). Participants with a family history of CKD were more likely to have a positive result (odds ratio, 3.29; 95% confidence interval, 1.10 to 11.29). Thirteen percent of participants without a CKD family history had a positive result. In those who first presented in stage 5 CKD, WGS with broad analysis of a curated kidney disease gene panel was diagnostically more informative than kidney biopsy, with biopsy being inconclusive in 24 of the 25 participants.

Conclusions In this prospectively ascertained Australian cohort, we identified a genetic diagnosis in 25% of patients with kidney failure of unknown cause.

CJASN 19: 887–897, 2024. doi: https://doi.org/10.2215/CJN.0000000000000464

Due to the number of contributing authors, the affiliations are listed at the end of this article.

Correspondence: Prof. Andrew J. Mallett, email: Andrew.mallett@health.qld.gov.au

Received: October 30, 2023 **Accepted:** April 25, 2024
Published Online Ahead of Print: May 3, 2024

Introduction

Kidney failure carries high mortality, morbidity, and economic burden.[1] Approximately 10% of kidney failure cases lack a clear cause despite available diagnostic tools.[2–5] Uncertainty regarding disease etiology affects management—disease-appropriate therapies may not be employed, prognostication for the patient and their family is limited, post-transplant disease recurrence risk predictions are challenging, and uncertainty is added to the safe selection of living-related kidney donors.[2,6,7] Prolonged diagnostic odysseys have adverse quality of life and health-economic impacts.[8]

Approximately 30% of patients reach kidney failure without prior nephrology care.[4] In those who first present to medical care in kidney failure, diagnostic tools such as serology, kidney biopsy, imaging, and urinalysis are often inconclusive, given the difficulties in differentiating secondary changes of end-organ damage from the primary disease process.[7,9,10] An unknown cause of kidney failure is more likely to be the lower eGFR at the initial presentation.[4,11]

Genetic kidney disease is increasingly recognized as a significant cause of CKD, accounting for at least 10% of adult and up to 50% of pediatric cohorts.[12] In recent years, broad genomic testing using exome sequencing-based studies has been applied in varied CKD cohorts.[6,7,13–17] Overwhelmingly, currently available evidence is derived from retrospective, research genetic studies on patients with specific clinical features, suggestive of genetic disease or broad CKD cohorts that included patients already diagnosed with currently available nongenomic investigative tools.[7,13,18,19] Limited evidence exists on utility of genomic testing in those without a clinical diagnosis after standard diagnostic evaluation has been exhausted. This information is particularly useful for nephrologists considering the value of incorporating genomic diagnostics into their current practice. Therefore, we present the wHole genome Investigation to iDentify unDEtected Nephropathies (HIDDEN) study, which reports on the effectiveness of clinical genome sequencing in a prospective, national cohort of patients with kidney failure of unknown cause.

Methods

Recruitment

Participants were recruited from nephrology units and kidney genetics clinics across Australia between August 2018 and July 2022, with recruitment sites in every Australian capital city, with links to regional services. Recruitment faced intermittent delays primarily due to the coronavirus disease 2019 pandemic (2020–2022) and associated community, travel, and health system disruptions in Australia. The inclusion criteria required participants to have reached stage 5 CKD (eGFR $<$15 ml/min per 1.73 m^2 using CKD Epidemiology Collaboration equation) at the age of $\leq$50 years, with no identifiable cause for their CKD.[20] Exclusion criteria included participants with an existing kidney clinical or phenotypic diagnosis, including likely or proven diabetic nephropathy, renovascular disease, renal sarcoidosis, primary nephrotic-range proteinuric disorder, tuberculosis, paraproteinemia (except when excluded on kidney biopsy), exposure to nephrotoxin causing kidney dysfunction, obstructive uropathy, nephromegaly ($>$14 cm for adults; normagram for pediatric patients), and a family history of cystic kidneys or identified glomerular disorder on kidney biopsy that clarifies a diagnosis (Figure 1). The exclusion criteria also included isolated congenital anomaly of the kidney and urinary tract, which were excluded given the known relatively low yield of monogenic etiology (Figure 1).[21] Participants were identified through nephrology services and proposed to a central study team who assessed eligibility against the selection criteria. Participants who met the selection criteria were recruited by their local site after obtaining informed consent, including genetic counseling. Ethical approval was obtained through the Melbourne Health Human Research Ethics Committee (HREC/16/MH/251).

Whole Genome Sequencing

All participants underwent clinically accredited whole genome sequencing (WGS) (National Association of Testing Authorities, Australian Clinical Laboratory Improvement Amendments-equivalent) on DNA extracted from peripheral blood. Illumina 150-bp paired-end sequencing was performed with alignment (GRCh37) and variant calling performed according to the previously published methods[22–24] (Supplemental Methods). Analysis performed for single-nucleotide and copy number variants was first targeted to a precurated virtual gene panel of 388 genes that had a monogenic kidney disease association (KidneyOme V1) (https://panelapp.agha.umccr.org/panels/275/). Variant classification was performed according to the American College of Medical Genetics and Genomics and the Association for Molecular Pathology guidelines.[25] Participants without disease-causing (pathogenic or likely pathogenic [LP]) variants identified during this initial targeted analysis underwent an analysis of the coding region of all genes with a reported human disease association (this panel of genes is termed the "Mendeliome") (https://panelapp.agha.umccr.org/panels/137/). Variants were curated using population frequencies, annotations in ClinVar (pathogenic and LP), and *in silico* pathogenicity prediction tools. Mitochondrial-specific variant calling was performed using a reimplementation of the gnomAD mitochondrial pipeline[26] (https://github.com/populationgenomics/production-pipelines/blob/main/cpg_workflows/stages/mito.py) and analyzed using MitoReport curation tool (https://github.com/bioinfomethods/mitoreport) (Supplemental Methods).

Only variants classified as "Pathogenic" or "LP" were considered a diagnostic or "positive" result. All sequencing performed in this study was funded through a competitively awarded research grant. For local health care context, at the time of this study, National Federal Government (Medicare) funding was not available for investigation of suspected genetic kidney disease in Australia. Diagnostic investigation was funded by individual state or territory hospitals, according to varying local guidelines, with limited or no cost to the participant. Commercially available capture-based panels and exome-based sequencing were widely used diagnostic tests in this context.[6,18,27]

Clinical Data Collection

Age at stage 5 CKD, sex, and self-declared race-ancestry were collected from all participants, along with detailed phenotype information (Study Protocol[15]; Supplemental Methods). This information was sourced from the medical

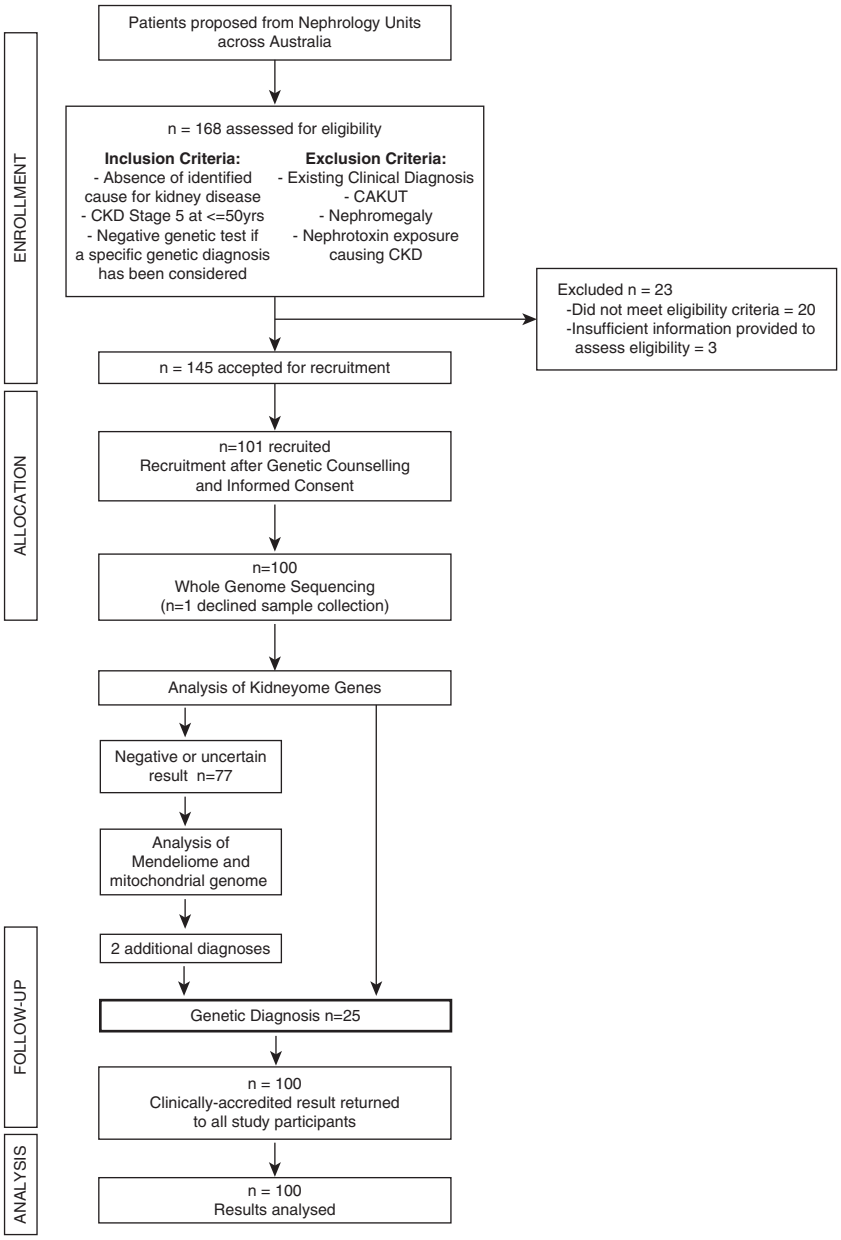

Figure 1. Study flow chart. Flow chart of study methodology and recruitment process. CAKUT, congenital anomalies of the kidney and urinary tract.

record or from the participant. Data were collected into a secure Research Electronic Data Capture database (Supplemental Methods).[28] Self-declared race-ancestry was collected for interpretation of individual genomic results.

Results Return

A diagnostic laboratory clinical report was generated for all participants and returned to the local referring clinician. All participants then had their genomic results (whether informative or uninformative) returned to them through the KidGen multidisciplinary kidney genetics network at their local study sites and received genetic counseling. Segregation analysis was performed by local sites as clinically indicated.

Statistical Analysis

The chi-squared test was used to compare categorical values. The Wilcoxon rank-sum parametric test was used to compare age distributions. Analysis was performed using RStudio, version 2022.07.1.

Results

Overall, 168 patients were assessed for eligibility for this study, with 23 excluded for not meeting the eligibility criteria ($N = 20$) or insufficient information was provided to assess eligibility ($N = 3$) (Figure 1). One hundred and forty-five patients were accepted for recruitment, with 101 participants consenting to participate (Figure 1). One

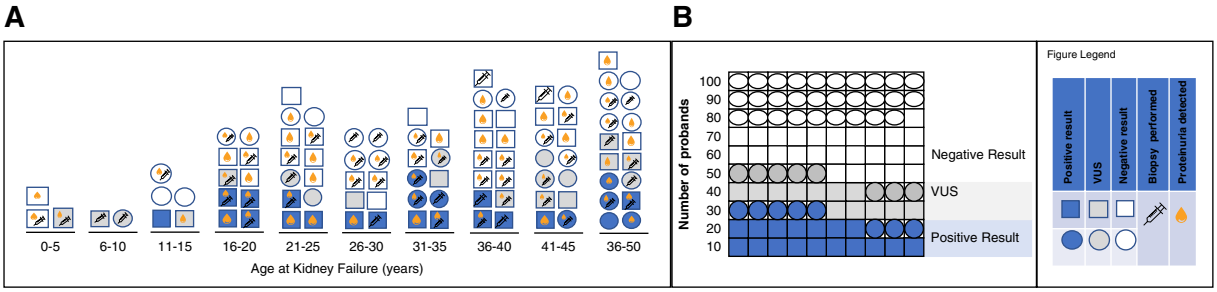

Figure 2. Cohort characteristics. (A) Age of kidney failure in all participants, with positive and negative results highlighted. (B) Overview of diagnostic yield within the cohort. The figure legend is applicable to both (A) and (B). Circles=female probands; squares=male probands; solid blue represents a disease-causing or positive result; gray represents a VUS; white represents a negative result; positive=pathogenic or LP result. LP, likely pathogenic; VUS, variant of uncertain significance.

hundred participants underwent WGS. One participant did not provide DNA and was omitted from further analysis.

Cohort Characteristics

Sixty-one percent of the cohort was male. The average age of participants with stage 5 CKD was 32 years (range, 9 months to 50 years) (Figure 2A). CKD stage at diagnosis was not reported for five participants, who were not included in the statistical analysis. For 53% of the cohort, their first presentation to nephrology services was at stage 5 CKD. Proteinuria was reported in 68% (68 of 100), with 70% (31 of 47) of those who presented having proteinuria before stage 5 CKD (Table 1). A kidney biopsy was performed on 54% (54 of 100) of the participants. Nearly half (25 of 53) of the participants who presented with stage 5 CKD had undergone kidney biopsy, with all but one having diagnostically inconclusive features of interstitial fibrosis and tubular atrophy, FSGS, or glomerulosclerosis. Details of whether biopsy was performed were not reported for four participants.

Eighty-three percent (83 of 100) of the cohort had never had prior genetic testing, and 3% of the cohort (three of 100) did not report details of prior genetic testing. Of those with prior genetic investigation, most (eight of 14) had a chromosome microarray alone (noting that three participants had no data reported regarding previous genetic testing). Thirty-seven percent (37 of 100) of the cohort reported a first-degree family member with kidney disease.

Genomic Results

The overall diagnostic yield from the HIDDEN study with Kidneyome (388 kidney-related genes), Mendeliome (all human-disease associated genes), and mitochondrial genome analysis was 25% (25 of 100) (Figure 2B). Only "pathogenic" or "LP" was considered a diagnostic or "positive" result.

A definitive genetic diagnosis was established for 23% (23 out of 100) of participants through genome sequencing and Kidneyome analysis (Table 2). Disease-causing variants were identified in a range of genetic kidney disease groups, including autosomal dominant tubulointerstitial kidney disease (6), glomerular disorders (4), ciliopathies (3), tubular disorders (2), and Alport syndrome (4) (Figure 3A). The diagnoses identified were inherited in an autosomal dominant (14%), autosomal recessive (5%), X-linked recessive (5%), and mitochondrial inheritance (1%) pattern. An additional 20 participants had variants of uncertain significance identified in genes in the Kidneyome (Figure 2B and Supplemental Table 1).

Overall, 77 participants who did not have a genetic diagnosis made on analysis of the Kidneyome gene panel had secondary analysis of the Mendeliome and mitochondrial genome, resulting in two additional diagnoses. Participant A0056 had a disease-causing variant in the *NBEA* gene, with multisystem findings, including intellectual disability, that were consistent with the genetic finding. A well-established, pathogenic mitochondrial variant m.3243A>G was identified on mitochondrial genome analysis in participant A0051 at 29.5% heteroplasmy in blood. No incidental findings were identified on phenotype-driven Mendeliome analysis. The American College of Medical Genetics and Genomics reportable genes (a list of genes in which variants may be considered medically actionable and therefore considered for voluntary review and reporting as a secondary finding, even if unrelated to the initial disease under investigation) were not analyzed, in keeping with the standard practice in Australia.[29]

There was a 13% yield in participants without a family history of kidney disease (6 of 46) (Figure 3B). Twenty-three percent (12 of 53) of participants who presented in stage 5 CKD had a positive diagnosis made on genomic testing.

Unadjusted analysis identified a positive family history as the only predictor of a positive diagnosis (odds ratio, 3.29; 1.10 to 11.29; $P = 0.02$). There was no significant difference between age at reaching kidney failure in participants with and without a positive diagnosis (average age 33 years in positives versus 32 years in negatives $P = 0.99$).

Extended Phenotype of Known Conditions

Several of the diagnoses expand the clinical spectrum associated with monogenic kidney disease. Participant A0052 had compound heterozygous disease-causing variants in the autosomal recessive polycystic kidney disease gene, *PKHD1*. They reached kidney failure at 46 years, without a family history of CKD (Figure 4A). Ultrasound demonstrated multiple small medullary kidney cysts and normal liver imaging (Figure 4B). Participant A0012 had compound heterozygous variants in *NPHP4* associated with nephronophthisis. They presented with kidney failure at 33 years, with moderate proteinuria, microscopic hematuria, and no relevant family history (Figure 4C). Kidney biopsy at

Table 1. Cohort characteristics

Characteristics	Whole Cohort (%), n=100	Positive Results (%), n=25	Negative Results (%), n=75
Male	61 (61)	17 (68)	44 (59)
Female	39 (39)	8 (32)	31 (41)
Age at stage 5 CKD (range)	32 yr (9 mo to 50 yr)	33 yr (14–50 yr)	32 yr (9 mo to 50 yr)
CKD stage at presentation			
Stage 1 CKD	6 (6)	1 (4)	5 (7)
Stage 2 CKD	14 (14)	6 (24)	8 (11)
Stage 3 CKD	9 (9)	4 (16)	5 (7)
Stage 4 CKD	13 (13)	1 (4)	12 (16)
Stage 5 CKD	53 (53)	12 (48)	41 (55)
Not reported	5 (5)	1 (4)	4 (5)
Urinalysis			
Urinalysis whole cohort			
Any proteinuria	68 (68)	19 (76)	49 (65)
Mild (150–500 mg/d)	20 (20)	8 (32)	12 (16)
Moderate (500–1000 mg/d)	18 (18)	4 (16)	14 (19)
Nephrotic range (>3.5 mg/d)	17 (17)	3 (12)	14 (19)
Unspecified	13 (13)	4 (16)	9 (12)
Hematuria	15 (15)	4(16)	11 (15)
Hematuria and proteinuria	14 (14)	3 (12)	11 (15)
Urinalysis only in participants who presented before CKD 5 (n=47)			
Any proteinuria	31 (31)	8 (32)	23 (31)
Mild (150–500 mg/d)	11 (11)	5 (20)	6 (8)
Moderate (500–1000 mg/d)	7 (7)	1 (4)	6 (8)
Nephrotic range (>3.5 mg/d)	7 (7)	0 (0)	7 (9)
Unspecified	6 (6)	2 (8)	4 (5)
Kidney biopsy			
Kidney biopsy whole cohort (n=100)			
Biopsy done	54 (54)	15 (60)	39 (52)
Tubulointerstitial fibrosis	27 (27)	6 (24)	21 (28)
FSGS	13 (13)	2 (8)	11 (15)
Not performed	42 (42)	10 (40)	32 (43)
Not reported if biopsy was done	4 (4)	0 (0)	4 (5)
Kidney biopsy only in participants who presented in kidney failure (n=53)			
Biopsy done	25 (47)	7	18
Tubulointerstitial fibrosis	15 (28)	4	11
FSGS	5 (9)	1	4
Not performed	26 (49)	5	21
Not reported if biopsy was done	2 (4)	0	2
Genetic testing before HIDDEN study			
No prior genetic testing	83 (83)	24 (96)	59 (79)
Any prior genetic test	14 (14)	1 (4)	13 (17)
Chromosome microarray	8 (8)	1 (4)	7 (9)
Single gene	3 (3)	0 (0)	3 (4)
Panel	5 (5)	0 (0)	5 (7)
WES	1 (1)	0 (0)	1 (1)
WGS	0 (0)	0 (0)	0 (0)
Not reported	3 (3)	0 (0)	3 (4)
Family history of kidney disease	48 (48)	18 (78)	30 (39)
First degree family member with kidney disease	37 (37)	16 (70)	21 (28)

HIDDEN, wHole genome Investigation to iDentify unDEtected Nephropathies; IFTA, interstitial fibrosis and tubular atrophy; WES, whole exome sequencing; WGS, whole genome sequencing.

presentation reported extensive glomerulosclerosis and tubulointerstitial fibrosis with interstitial inflammatory cell infiltrate on light microscopy (Figure 4, D and E). Electron microscopy demonstrated marked reduplication of the tubular basement membrane (Figure 4F).

Participant A0083 had a truncating variant in *EYA1*. They presented with kidney failure at 24 years, with bilateral kidney atrophy, nephrotic-range proteinuria, hematuria, and no relevant family history (Figure 4G). There was no branchial arch or auditory tract anomalies on head and neck computed tomography. Disease-causing variants in *EYA1* are associated with Branchiootorenal syndrome, which is characterized by otologic abnormalities, branchial malformations, and kidney tract malformations, all of varying severity.[30,31] The majority of cases previously reported have some form of otologic anomaly.[30,31]

Participant A0051 had a mitochondrial variant on mitochondrial genome analysis. She reached kidney failure at 33 years. She had mild proteinuria before kidney failure, with tubular atrophy on biopsy and nonspecific kidney imaging

Table 2. Disease-causing variants identified in wHole genome Investigation to iDentify unDEtected Nephropathies cohort

Publication ID	Age Range at Kidney Failure, yr	Sex	CKD Stage at dx	Urinalysis	Imaging	Kidney Biopsy	Family History	Gene	c.	p.	Variant Classification
A0079	16–20	M	5	Mild proteinuria	Hyperechogenic kidneys; normal renal length; cortical cysts	IFTA and glomerulosclerosis	Y	*NPHP4* (*variants in trans*)	c.834_841del c.1503+2dup	p.(Ala279Aspfs×28) p.?	LP
A0012	31–35	M	5	Moderate proteinuria and hematuria	Multiple renal cysts	Extensive glomerulosclerosis and tubulointerstitial fibrosis, marked reduplication of tubular basement membrane	N	*NPHP4* (*variants in trans*)	c.841G>T c.3004C>T	p.(Glu281×) p.(Gln1002×)	LP
A0052	46–50	F	2	Mild proteinuria	Multiple small medullary renal cysts; normal liver imaging	Not performed	N	*PKHD1*	c.6992T>A c.107C>T	p.(Ile2331Lys) p.(Thr36Met)	LP
A0046	31–35	M	2	Proteinuria without hematuria	Not available	Not available	Y	*NPHS2*	c.862G>A Homozygous variant	p.(Ala288Thr)	LP
A0088	16–20	M	5	Mild proteinuria and hematuria	Bilateral renal atrophy	IFTA and glomerulosclerosis, thin GBM	Y	*INF2* (*variants in cis*)	c.529C>G c.604A>C	p.(Arg177Gly) p.(Asn202His)	LP
A0077	21–25	M	5	Nephrotic range proteinuria	Hyperechogenic kidneys	Not reported	Y	*INF2*	c.490_498del	p.(Ala164_Asp166del)	LP
A0085	16–18	M	5	Nephrotic range proteinuria; no hematuria	Hyperechogenic kidneys	IFTA and glomerulosclerosis	U	*TRPC6*	c.2683C>T	p.(Arg895Cys)	P
A0062	31–35	F	1	Proteinuria and hematuria	Bilateral renal atrophy; renal cortical cysts	Thickening and thinning of GBM with foot process effacement	Y	*COL4A5*	c.2831G>A	p.(Gly944Glu)	LP
A0014	16–20	M	5	Proteinuria and hematuria	Bilateral renal atrophy	Extensive glomerulosclerosis with single fibrocellular crescent; IFTA	Y	*COL4A5*	c.359G>A	p.(Gly120Asp)	LP
A0076	36–40	M	3	Not reported	Not reported	FSGS	Y	*COL4A5*	c.142G>A	p.(Gly48Arg)	LP
A0044	41–45	M	5	Moderate proteinuria and hematuria	Two unilateral renal cysts	Not performed	Y	*COL4A4*	c.129C>G	p.(Tyr43×)	LP
A0024	46–50	F	5	Moderate proteinuria	Hyperechogenic kidneys	Not performed	Y	*UMOD*	c.278_289delins CCGCCTCCT	p.(Val93_Gly97delins AlaAlaSerCys)	P
A0074	31–35	F	2	Not reported	Not reported	Tubulointerstitial fibrosis	N	*UMOD*	c.502T>C	p.(Cys168Arg)	LP
A0030	21–25	M	5	Mild proteinuria	Bilateral normal kidney length	Tubular atrophy	Y	*UMOD*	c.328T>C	p.(Cys110Arg)	LP
A0034	46–50	F	5	Not reported	Not reported	Not performed	Y	*UMOD*	c.416G>A	p.(Cys139Tyr)	LP
A0045	41–45	F	5	Mild proteinuria	Hyperechogenic kidneys and bilateral renal atrophy	Tubular atrophy and tubulointerstitial fibrosis	Y	*UMOD*	c.263G>T	p.(Gly88Val)	LP
A0098	46–50	M	3	Mild proteinuria	Bilateral renal atrophy	Tubular atrophy	Y	*UMOD*	c.278_289delins CCGCCTCCT	p.(Val93_Gly97delins AlaAlaSerCys)	P
A0053	46–50	F	2	Mild proteinuria; no hematuria	Reduced renal corticomedullary differentiation; unilateral renal atrophy	GN	Y	*HNF1B*	c.865_870delinsGT	p.(Asn289Valfs×37)	LP
A0083	21–25	M	5	Proteinuria	Bilateral renal atrophy	Tubular atrophy; glomerulosclerosis; FSGS	N	*EYA1*	c.889C>T	p.(Arg297×)	LP
A0026	36–40	M	4	Renal salt wasting; renal potassium wasting	Bilateral renal atrophy and nephrocalcinosis	Tubulointerstitial fibrosis	Y	*CLCN5*	c.1231A>T	p.(Lys411×)	LP
A0061	26–30	M	2	Moderate proteinuria	Bilateral renal atrophy	Not performed	Y	*CLCN5*	c.1242del	p.(Glu414Aspfs×20)	LP
A0087	36–40	M	3	Moderate proteinuria	Bilateral renal atrophy	Markedly enlarged and bizarre tubular epithelial cells; moderate IFTA	Y	*FAN1*	c.2080_2081dup c.2928dup	p.(Leu694Phefs×27) p.(Lys977×)	LP
A0005	26–30	M	5	Not reported	Not reported	FSGS	N	*WT1*	c.1387C>T	p.(Arg463×)	P
A0051	31–35	F	2	Mild proteinuria; no hematuria	Hyperechogenic kidneys, bilateral renal atrophy, solitary renal cyst	Tubular atrophy	N	*MT-TL1*	m.3243A>G Heteroplasmy 29.7%		P
A0056	11–15	M	3	No proteinuria or hematuria	Not reported	Not performed	Y	*NBEA*	c.4477_4478del	p.Arg1493Glyfs×3	LP

NPHS2 NM_014625.4; NPHP4 NM_015102.5; PKHD1 NM_138,694.3; TRPC6 NM_004621.6; COL4A5 NM_033380.1; COL4A4 NM_000092.5; UMOD NM_001278614.1; HNF1B NM_000458.4; EYA1 NM_000503.5; CLCN5 NM_000084.4; FAN1 NM_014967.4; INF2 NM_022489.3; WT1 NM_024426.6; MT-TL1 NC_012920; NBEA NM_001385012.1.

When two variants were identified in a gene, these are noted as "in trans" if confirmed to be on separate gene alleles, or "in cis" if confirmed to be on the same gene allele. GBM, glomerular basement membrane; IFTA, interstitial fibrosis and tubular atrophy; LP, likely pathogenic; P, pathogenic; U, unknown.

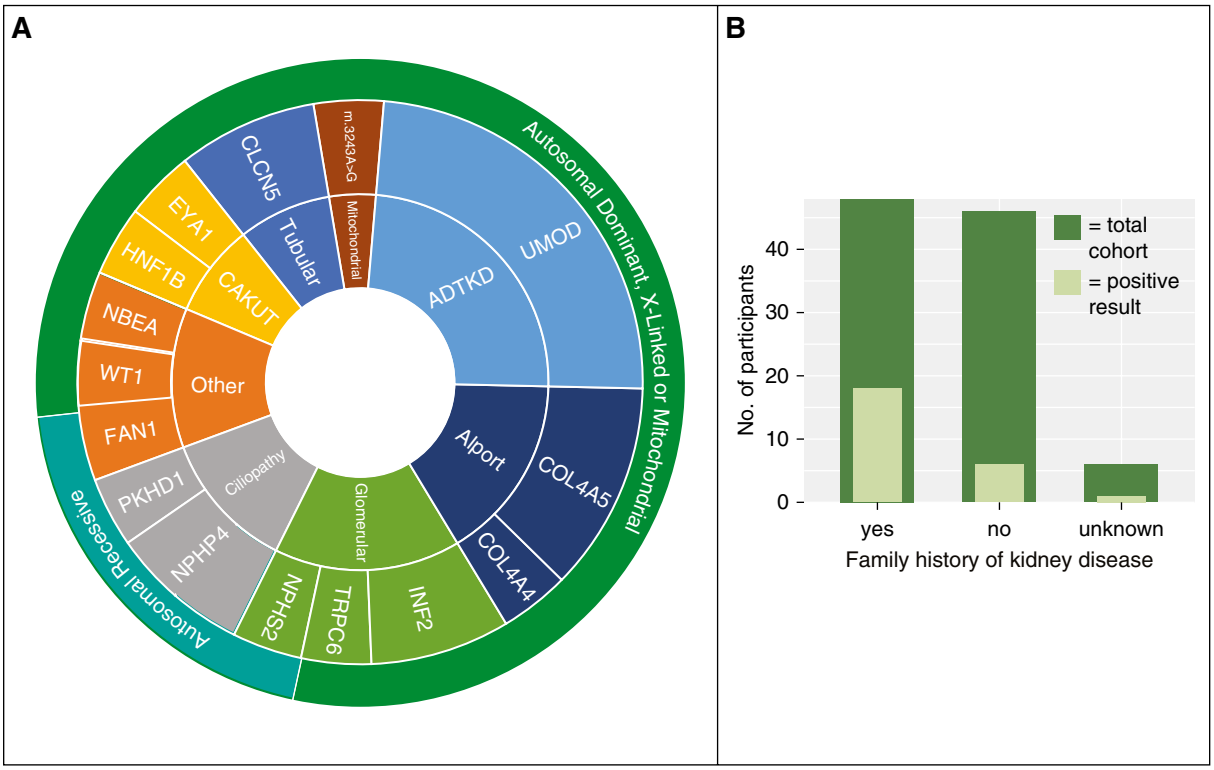

Figure 3. Diagnoses identified in the HIDDEN study. (A) Summary of overall study findings: the inner circle represents disease-groups identified, middle circle represents genes with disease-causing variant identified through the study, and outer circle represents the inheritance pattern of identified diagnoses. (B) Family history of kidney disease and positive and negative results. ADTKD, autosomal dominant tubulointerstitial kidney disease. HIDDEN, wHole genome Investigation to iDentify unDEtected Nephropathies.

findings. She developed diabetes mellitus after transplant. Her mother and a maternal uncle had diabetes mellitus, and her mother and two maternal uncles had nonspecific hearing loss (of eight siblings). There was no family history of kidney disease or stroke-like episodes.

Discussion

We report a 25% diagnostic rate using genome sequencing in patients younger than 50 years with kidney failure of unknown cause despite standard diagnostic evaluation. Our study provides evidence that genomics has a role in the diagnostic workup of patients presenting with kidney failure of unknown cause. Although some aspects of kidney failure management are agnostic to the cause of disease, there are clear benefits to understanding the underlying basis of disease. This allows for tailored treatment, more accurate prognostic information, improved estimation of recurrence risk post-transplant, and screening for extrarenal effects. In our study, 80% of diagnoses were made in conditions with autosomal dominant, X-linked, or mitochondrial inheritance; these results have clear implications for both the affected person and multiple generations of their family.

With improvements in genomic technologies, there has been increasing interest in genomic diagnostics in academic nephrology. However, there remains a significant lag in implementing these findings into mainstream nephrology practice.[32] There is increasing evidence of the yield of genomic testing in various areas of nephrology, from specific disease groups, such as cystic disease or nephrotic syndrome, to patient groups, such as pediatrics or transplant recipients. Studies are often retrospective analyses, single-center analyses of laboratory data, or subgroup analyses of datasets collected for alternate primary aims.[7,13,18,27] The HIDDEN study recruited patients to answer the primary question of genetic testing yield in kidney failure following standard diagnostic evaluation, with strict prepublished selection criteria. This reduces the likelihood of reporting bias and provides robust evidence for future clinical decision making. In contrast to many broad CKD cohort studies, we excluded patients with clear clinical features of specific conditions (such as autosomal dominant polycystic kidney disease) as these more common genetic conditions, with typically recognizable clinical features, can otherwise predominate diagnostic yield in genetic studies.[19] Crucially, our study undertook clinical-grade genome sequencing after genetic counseling, with results returned as clinically actionable, accredited results, reflecting a model of best practice application of clinical genetic testing. Our prospective methodology required stringent selection criteria reflected in the smaller participant numbers compared with retrospective analyses. This approach, however, allows collection of detailed clinical information and generation of clinically meaningful evidence.

It can be particularly challenging to confirm the underlying cause of CKD, especially for patients whose first presentation to nephrology care is when they are in kidney

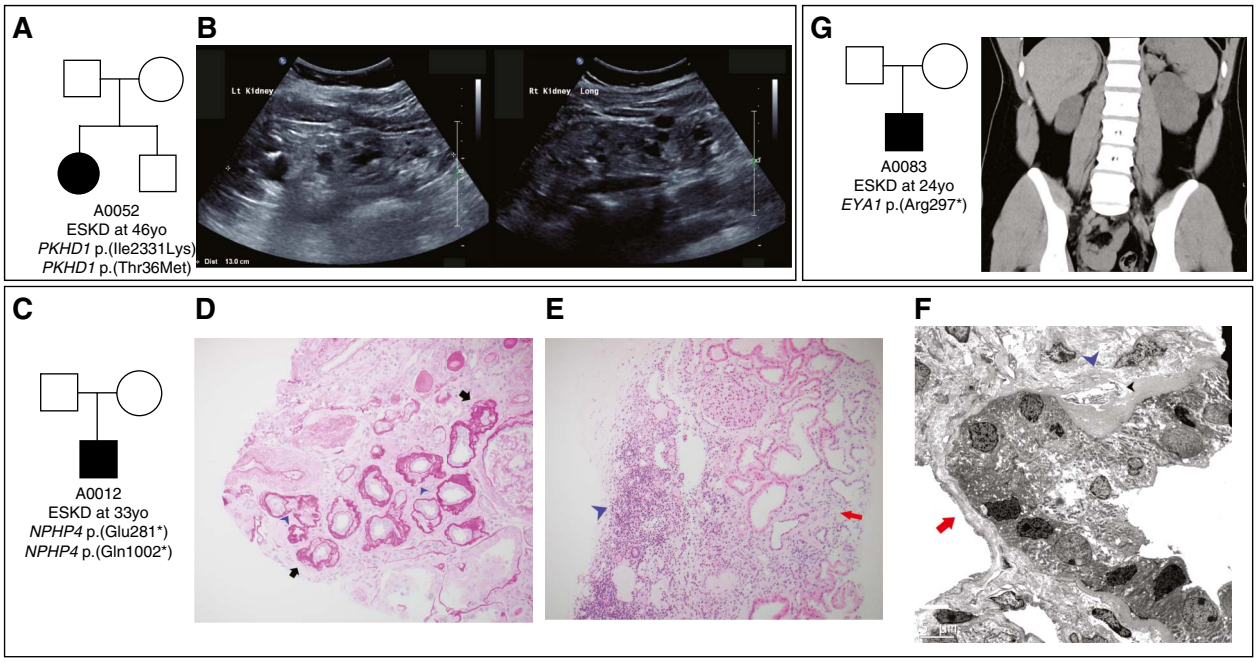

Figure 4. Expanding the phenotypic spectrum of known genetic kidney diseases. (A) Pedigree for participant A0052. (B) Abdominal ultrasound images of left and right kidneys in longitudinal view, demonstrating bilateral renal cysts. (C) Pedigree for participant A0012. (D) Periodic acid–Schiff stain 200× magnification demonstrating complete loss of tubular basement membrane (blue arrow head) and membrane reduplication (black arrow) seen within the same tubule. (E) Hematoxylin and eosin–stained slide 200× magnification with red arrow showing interstitial fibrosis and blue arrowheads showing interstitial inflammatory cell infiltrate. (F) Electron microscopy of tubule 3000× magnification with red arrow showing atrophic tubular basement membrane and blue arrowhead showing reduplicated, thickened tubular basement membrane. The black arrowhead shows split in the tubular basement membrane.

failure. Young adults are least likely to access prekidney failure care and also disproportionately affected by genetic kidney disease.[4,33] Our cohort reflects the challenges of making a diagnosis in patients who first presented in stage 5 CKD using the traditional clinical tools of serology, urinalysis, urinary tract imaging, and kidney biopsy. Half of the participants who presented in kidney failure had a kidney biopsy performed, and all but one had FSGS, interstitial fibrosis, tubular atrophy, or glomerulosclerosis as the primary finding. These patterns of injury can be seen in some primary kidney diseases but more commonly represent sequalae of cumulative injury in kidney failure and therefore have limited diagnostic utility in stage 5 CKD.[10] In comparison, 24% of the participants in our study who presented in stage 5 CKD had a positive diagnosis made on genomic testing. The data suggest that in patients at the age of ≤50 years who present with kidney failure and features of chronic disease, a genomic test is likely to have more diagnostic utility than a kidney biopsy. This test offers a valuable diagnostic advantage, while also avoiding the bleeding risks associated with native kidney biopsies in patients with advanced kidney disease.[34]

Consistent with other studies, a genetic diagnosis was more likely in participants with a family history of kidney disease.[6] However, even for those with no known family history of kidney disease, the diagnostic rate was 13%. Thirty-seven percent of the cohort had a family history of kidney disease. In comparison, studies in the United States and Ireland reported that 20%–36% of dialysis patients had a family history of kidney disease, suggesting that our cohort was not enriched for participants with a family history of kidney disease.[35,36]

We show that our approach of genome sequencing with initial analysis of kidney disease-associated genes (the Kidneyome) yields a high diagnostic rate, without high burden of uncertain or incidental findings.[29] Secondary extended analysis identified two additional findings *via* the Mendeliome and mitochondrial genome analysis. Compared with exome-based sequencing, WGS offers improved ability to sequence homologous regions and detect structural variants, and the opportunity to analyze noncoding regions and the mitochondrial genome.[37,38] Our identification of a participant with an established mitochondrial variant with nonspecific phenotype and family history highlights the value of mitochondrial genome analysis being part of a broad genomic test. Kidney disease associated with mitochondrial DNA variants is rarely reported and likely underreported.[39] As we were aiming to understand the yield of genetic investigation in the clinical setting, we did not perform noncoding analysis outside of immediate splice regions. This type of research analysis is outside the scope of clinical genetic testing. An additional benefit of a broad sequencing approach is that patients who received uninformative diagnostic results can have their genomic data transferred to research laboratories for ongoing reanalysis in light of new gene discoveries or bioinformatic improvements, without a requirement for resequencing.[40] We elected for WGS in this study to maximize clinical diagnostic yield and to best utilize opportunities for future reanalysis, which has been demonstrated in other disease systems to iteratively

increase diagnostic yield.[41] In jurisdictions where cost of WGS is a limitation, our results indicate that a broad kidney gene panel targeted to nuclear coding-region variants has significant yield, and this could also be achieved through whole exome or capture-based sequencing, though noting limitations in these techniques to identify mitochondrial, noncoding, or structural variants.

Many of the diagnoses were made in participants who had nonspecific clinical features that were not highly suggestive of genetic disease and challenged previous knowledge of the typical phenotypes and age of onset of particular conditions. This included adult participants who presented at or beyond the extremes of age reported for typically childhood-associated kidney failure conditions.[42–45] Broad sequencing in undifferentiated kidney cohorts will continue to expand the known disease spectrum of monogenic kidney disease and reduce ascertainment bias.

We report the findings of a dedicated study to investigate the yield of clinical genome sequencing in patients ≤50 years who have an unknown cause of kidney failure after standard diagnostic testing. We made a genetic diagnosis in 25% of our cohort, with most variants in X-linked, autosomal dominant, or mitochondrially inherited disorders that have direct familial implications. The study design, targeted to patients who are undiagnosed after diagnostic tests routinely performed in nephrology, and the use of clinically accredited sequencing, allows this finding to be directly applicable to current nephrology practice. Our findings suggest that genome sequencing should be considered the next diagnostic tool in patients with kidney failure of unknown cause and considered before kidney biopsy in patients who presented in stage 5 CKD with features of chronicity.

Disclosures

Disclosure forms, as provided by each author, are available with the online version of the article at http://links.lww.com/CJN/B889.

Funding

The HIDDEN study was funded through Australian Genomics. Australian Genomics is funded by the National Health and Medical Research Council (Grants GNT1113531 and GNT2000001). The study was also supported through funding from the Royal Prince Alfred Hospital Department of Renal Medicine, Royal Brisbane and Women's Hospital Foundation, and the Australian Government's Medical Research Future Fund Genomics Health Futures Mission (Grant MRF2008820 & MRF2008249). The study was further supported in kind by all participating clinical services. A.C. Mallawaarachchi was supported by the RACP Jacquot and Viertel Foundations. A. J. Mallett was supported by a Queensland Health Advancing Clinical Research Fellowship.

Author Contributions

Conceptualization: Lindsay Fowles, Amali C. Mallawaarachchi, Andrew J. Mallett, Chirag Patel, Catherine Quinlan, Zornitza Stark, Louise Wardrop.
Data curation: Laura Wedd.
Formal analysis: Erik Biros, Laura Wedd.
Methodology: Laura Wedd.
Supervision: Andrew J. Mallett, Chirag Patel, Catherine Quinlan, Zornitza Stark.
Writing – original draft: Amali C. Mallawaarachchi.
Writing – review & editing: Stephen Alexander, Erik Biros, Simon Bodek, Neil Boudville, Jo Burke, Leslie Burnett, Sarah Casauria, Steve Chadban, Aron Chakera, Sam Crafter, Pei Dai, Paul De Fazio, Randall Faull, Lindsay Fowles, Trudie Harris, Andrew Honda, Vanessa Huntley, Sadia Jahan, Kushani Jayasinghe, Matthew Jose, Anna Leaver, Mandi MacShane, Evanthia Olympia Madelli, Andrew J. Mallett, Hugh McCarthy, Kathy Nicholls, Rosie O'Shea, Chirag Patel, Rhonda Pawlowski, Catherine Quinlan, Gopi Rangan, Cas Simons, Paul Snelling, Jacqueline Soraru, Zornitza Stark, Madhivanan Sundaram, Michel Tchan, Giulia Valente, Mathew Wallis, Louise Wardrop, Laura Wedd, Matthew Welland, John Whitlam, Ella J. Wilkins, Alasdair Wood.

Data Sharing Statement

Data cannot be shared. Clinical genome sequencing data cannot be shared in a persistent repository due to privacy concerns. However, individual participant data (IPD) collected in this study can be made available upon request, in a de-identified format to ensure privacy. The authors will provide a comprehensive data-sharing plan outlining the data format, access procedures, and criteria for obtaining the data.

Supplemental Material

This article contains the following supplemental material online at http://links.lww.com/CJN/B890, http://links.lww.com/CJN/B891.

Supplemental Table 1. Phenotype information and variants identified in the HIDDEN cohort.

Supplemental Methods

References

1. Thurlow JS, Joshi M, Yan G, et al. Global epidemiology of end-stage kidney disease and disparities in kidney replacement Therapy. *Am J Nephrol.* 2021;52(2):98–107. doi:10.1159/000514550
2. Lim WH, Wong G, McDonald SP, et al. Long-term outcomes of kidney transplant recipients with end-stage kidney disease attributed to presumed/advanced glomerulonephritis or unknown cause. *Sci Rep.* 2018;8(1):9021. doi:10.1038/s41598-018-27151-4
3. ANZDATA Registry. *44th Report, Chapter 2: Prevalence of Kidney Failure with Replacement Therapy*; 2021.
4. USRD. *2021 USRDS Annual Data Report. Epidemiology of Kidney Disease in the United States.* National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases. 2021.
5. UK Renal Registry (2022). *UK Renal Registry 24th Annual Report.* UK Renal Registry; 2022.
6. Jayasinghe K, Stark Z, Kerr PG, et al. Clinical impact of genomic testing in patients with suspected monogenic kidney disease. *Genet Med.* 2021;23(1):183–191. doi:10.1038/s41436-020-00963-4
7. Groopman EE, Marasa M, Cameron-Christie S, et al. Diagnostic utility of exome sequencing for kidney disease. *New Engl J Med.* 2019;380(2):142–151. doi:10.1056/NEJMoa1806891
8. McConkie-Rosell A, Hooper SR, Pena LDM, et al.; Undiagnosed Diseases Network. Psychosocial profiles of parents of children with undiagnosed diseases: managing well or just managing? *J Genet Couns.* 2018;27(4):935–946. doi:10.1007/s10897-017-0193-5
9. Groopman EE, Rasouly HM, Gharavi AG. Genomic medicine for kidney disease. *Nat Rev Nephrol.* 2018;14(2):83–104. doi:10.1038/nrneph.2017.167
10. Liapis H, Gaut JP. The renal biopsy in the genomic era. *Pediatr Nephrol.* 2013;28(8):1207–1219. doi:10.1007/s00467-012-2356-9
11. Bragg-Gresham J, Morgenstern H, Shahinian V, Robinson B, Abbott K, Saran R. An analysis of hot spots of ESRD in the United

States: potential presence of CKD of unknown origin in the USA? *Clin Nephrol.* 2020;93(1):113–119. doi:10.5414/CNP92S120
12. Mallett A, Patel C, Salisbury A, Wang Z, Healy H, Hoy W. The prevalence and epidemiology of genetic renal disease amongst adults with chronic kidney disease in Australia. *Orphanet J Rare Dis.* 2014;9:98. doi:10.1186/1750-1172-9-98
13. Ottlewski I, Münch J, Wagner T, et al. Value of renal gene panel diagnostics in adults waiting for kidney transplantation due to undetermined end-stage renal disease. *Kidney Int.* 2019;96(1):222–230. doi:10.1016/j.kint.2019.01.038
14. Lata S, Marasa M, Li Y, et al. Whole-exome sequencing in adults with chronic kidney disease: a pilot study. *Ann Intern Med.* 2018;168(2):100–109. doi:10.7326/M17-1319
15. Mann N, Braun DA, Amann K, et al. Whole-exome sequencing enables a precision medicine approach for kidney transplant recipients. *J Am Soc Nephrol.* 2019;30(2):201–215. doi:10.1681/ASN.2018060575
16. Schrezenmeier E, Kremerskothen E, Halleck F, et al. The underestimated burden of monogenic kidney disease in adults waitlisted for kidney transplantation. *Genet Med.* 2021;23(7):1219–1224. doi:10.1038/s41436-021-01127-8
17. Robert T, Raymond L, Dancer M, et al. Beyond the kidney biopsy: genomic approach to undetermined kidney diseases. *Clin Kidney J.* 2024;17(1):sfad099. doi:10.1093/ckj/sfad099
18. Mallett AJ, McCarthy HJ, Ho G, et al. Massively parallel sequencing and targeted exomes in familial kidney disease can diagnose underlying genetic disorders. *Kidney Int.* 2017;92(6):1493–1506. doi:10.1016/j.kint.2017.06.013
19. Dahl N, Bloom MS, Chebib FT, et al. The clinical utility of genetic testing in the diagnosis and management of adults with chronic kidney disease. *J Am Soc Nephrol.* 2023;34(12):2039–2050. doi:10.1681/ASN.0000000000000249
20. Soraru J, Jahan S, Quinlan C, et al. The HIDDEN Protocol: an Australian prospective cohort study to determine the utility of whole genome sequencing in kidney failure of unknown aetiology. *Front Med (Lausanne).* 2022;9:891223. doi:10.3389/fmed.2022.891223
21. Khan K, Ahram DF, Liu YP, et al. Multidisciplinary approaches for elucidating genetics and molecular pathogenesis of urinary tract malformations. *Kidney Int.* 2022;101(3):473–484. doi:10.1016/j.kint.2021.09.034
22. Sadedin SP, Dashnow H, James PA, et al.; Melbourne Genomics Health Alliance. Cpipe: a shared variant detection pipeline designed for diagnostic settings. *Genome Med.* 2015;7(1):68. doi:10.1186/s13073-015-0191-x
23. Minoche AE, Lundie B, Peters GB, et al. ClinSV: clinical grade structural and copy number variant detection from whole genome sequencing data. *Genome Med.* 2021;13(1):32. doi:10.1186/s13073-021-00841-x
24. Mallawaarachchi A, Lundie B, Hort Y, et al. Genomic diagnostics in polycystic kidney disease: an assessment of real-world use of whole-genome sequencing. *Eur J Hum Genet.* 2021;29(5):760–770. doi:10.1038/s41431-020-00796-4
25. Richards S, Aziz N, Bale S, et al.; ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and genomics and the Association for Molecular Pathology. *Genet Med.* 2015;17(5):405–424. doi:10.1038/gim.2015.30
26. Laricchia KM, Lake NJ, Watts NA, et al.; Genome Aggregation Database Consortium. Mitochondrial DNA variation across 56,434 individuals in gnomAD. *Genome Res.* 2022;32(3):569–582. doi:10.1101/gr.276013.121
27. Tanudisastro H, Holman K, Ho G, et al. Australia and New Zealand renal gene panel testing in routine clinical practice of 542 families. *npj Genomic Med.* 2021;6(1):20. doi:10.1038/s41525-021-00184-x
28. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)–a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform.* 2009;42(2):377–381. doi:10.1016/j.jbi.2008.08.010
29. Kalia SS, Adelman K, Bale SJ, et al. Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. *Genet Med.* 2017;19(2):249–255. doi:10.1038/gim.2016.190
30. Orten DJ, Fischer SM, Sorensen JL, et al. Branchio-oto-renal syndrome (BOR): novel mutations in the EYA1 gene, and a review of the mutational genetics of BOR. *Hum Mutat.* 2008;29(4):537–544. doi:10.1002/humu.20691
31. Smith RJH. Branchiootorenal spectrum disorder. *GeneReviews® [Internet].* In: Adam MP, Feldman J, Mirzaa GM, et al., ed. University of Washington; 1999. Accessed September 6, 2018.
32. Jayasinghe K, Quinlan C, Mallett AJ, et al. Attitudes and practices of Australian nephrologists toward implementation of clinical genomics. *Kidney Int Rep.* 2021;6(2):272–283. doi:10.1016/j.ekir.2020.10.030
33. Vivante A, Hildebrandt F. Exploring the genetic basis of early-onset chronic kidney disease. *Nat Rev Nephrol.* 2016;12(3):133–146. doi:10.1038/nrneph.2015.205
34. Dhaun N, Bellamy CO, Cattran DC, Kluth DC. Utility of renal biopsy in the clinical management of renal disease. *Kidney Int.* 2014;85(5):1039–1048. doi:10.1038/ki.2013.512
35. Freedman BI, Soucie JM, McClellan WM. Family history of end-stage renal disease among incident dialysis patients. *J Am Soc Nephrol.* 1997;8(12):1942–1945. doi:10.1681/ASN.V8121942
36. Connaughton DM, Bukhari S, Conlon P, et al. The Irish kidney gene project - prevalence of family history in patients with kidney disease in Ireland. *Nephron.* 2015;130(4):293–301. doi:10.1159/000436983
37. Davis RL, Kumar KR, Puttick C, et al. Use of whole-genome sequencing for mitochondrial disease diagnosis. *Neurology.* 2022;99(7):e730–e742. doi:10.1212/WNL.0000000000200745
38. Mallawaarachchi AC, Hort Y, Cowley MJ, et al. Whole-genome sequencing overcomes pseudogene homology to diagnose autosomal dominant polycystic kidney disease. *Eur J Hum Genet.* 2016;24(11):1584–1590. doi:10.1038/ejhg.2016.48
39. Parasyri M, Brandstrom P, Uusimaa J, et al. Renal phenotype in mitochondrial diseases: a multicenter study. *Kidney Dis (Basel).* 2022;8(2):148–159. doi:10.1159/000521148
40. Hort Y, Sullivan P, Wedd L, et al. Atypical splicing variants in PKD1 explain most undiagnosed typical familial ADPKD. *NPJ Genomic Med.* 2023;8(1):16. doi:10.1038/s41525-023-00362-z
41. Ewans LJ, Minoche AE, Schofield D, et al. Whole exome and genome sequencing in mendelian disorders: a diagnostic and health economic analysis. *Eur J Hum Genet.* 2022;30(10):1121–1131. doi:10.1038/s41431-022-01162-2
42. Bouchireb K, Boyer O, Gribouval O, et al. NPHS2 mutations in steroid-resistant nephrotic syndrome: a mutation update and the associated phenotypic spectrum. *Hum Mutat.* 2014;35(2):178–186. doi:10.1002/humu.22485
43. Hwang D-Y, Dworschak GC, Kohl S, et al. Mutations in 12 known dominant disease-causing genes clarify many congenital anomalies of the kidney and urinary tract. *Kidney Int.* 2014;85(6):1429–1433. doi:10.1038/ki.2013.508
44. Feng H, Xu H, Chen B, et al. Genetic and phenotypic variability in Chinese patients with branchio-oto-renal or branchio-oto syndrome. *Front Genet.* 2021;12:765433. doi:10.3389/fgene.2021.765433
45. Lipska BS, Ranchin B, Iatropoulos P, et al.; PodoNet Consortium. Genotype-phenotype associations in WT1 glomerulopathy. *Kidney Int.* 2014;85(6):1169–1178. doi:10.1038/ki.2013.519

AFFILIATIONS

[1]Clinical Genetics Service, Institute of Precision Medicine and Bioinformatics, Royal Prince Alfred Hospital, Sydney, New South Wales, Australia
[2]Genomic and Inherited Diseases Program, Garvan Institute of Medical Research, Sydney, New South Wales, Australia

[3]KidGen Collaborative, Australian Genomics Health Alliance, Murdoch Children's Research Institute, Melbourne, Victoria, Australia
[4]Genetic Health Queensland, Royal Brisbane and Women's Hospital, Herston, Queensland, Australia
[5]KidGen Collaborative, Kidney Regeneration, Murdoch Children's Research Institute, Melbourne, Victoria, Australia
[6]College of Medicine and Dentistry, James Cook University, Townsville, Queensland, Australia
[7]Townsville University Hospital, Townsville, Queensland, Australia
[8]Centre for Kidney Research at the Children's Hospital at Westmead, Sydney, New South Wales, Australia
[9]Department of Nephrology, Children's Hospital at Westmead, Sydney, New South Wales, Australia
[10]Discipline of Child and Adolescent Health, Faculty of Medicine and Health, University of Sydney, Sydney, New South Wales, Australia
[11]Clinical Genetics Service, Austin Health, Melbourne, Victoria, Australia
[12]Medical School, University of Western Australia, Crawley, Western Australia, Australia
[13]School of Medicine and Menzies Institute for Medical Research, University of Tasmania, Hobart, Tasmania, Australia
[14]Tasmanian Clinical Genetics Service, Royal Hobart Hospital, Hobart, Tasmania, Australia
[15]Faculty of Medicine and Health, Northern Clinical School, University of Sydney, Sydney, New South Wales, Australia
[16]St Vincent's Healthcare Clinical Campus, UNSW Sydney, Sydney, New South Wales, Australia
[17]Australian Genomics, Murdoch Children's Research Institute, Melbourne, Victoria, Australia
[18]Renal Medicine, Royal Prince Alfred Hospital, Camperdown, New South Wales, Australia
[19]Harry Perkins Institute for Medical Research, University of Western Australia, Crawley, Western Australia, Australia
[20]Renal Unit, Sir Charles Gairdner Hospital, Nedlands, Western Australia, Australia
[21]The Women's and Children's Hospital, North Adelaide, South Australia, Australia
[22]Precision Immunology Program, Garvan Institute of Medical Research, Sydney, New South Wales, Australia
[23]Faculty of Medicine, St Vincent's Clinical School, UNSW Sydney, Sydney, New South Wales, Australia
[24]Victorian Clinical Genetics Services, Murdoch Children's Research Institute, Melbourne, Victoria, Australia
[25]Renal Unit, Royal Adelaide Hospital, Adelaide, South Australia, Australia
[26]University of Adelaide, Adelaide, South Australia, Australia
[27]The Kinghorn Centre for Clinical Genomics, Garvan Institute of Medical Research, Sydney, New South Wales, Australia
[28]Adult Genetics Service, Royal Adelaide Hospital, Adelaide, South Australia, Australia
[29]The Central and Northern Renal and Transplantation Service, Royal Adelaide Hospital, Adelaide, South Australia, Australia
[30]Department of Medicine, Monash University, Melbourne, Victoria, Australia
[31]Department of Nephrology, Monash Health, Melbourne, Victoria, Australia
[32]Melbourne Health, Melbourne, Victoria, Australia
[33]Royal Hobart Hospital, Hobart, Tasmania, Australia
[34]Genetic Services of WA, KEMH, Subiaco, Western Australia, Australia
[35]Harry Perkins Institute of Medical Research, Nedlands, Western Australia, Australia
[36]Nephrology Unit, Royal Melbourne Hospital, Parkville, Victoria, Australia
[37]The University of Melbourne, Parkville, Victoria, Australia
[38]Anatomical Pathology, Monash Health, Melbourne, Victoria, Australia
[39]Department of Renal Medicine, Westmead Hospital, Western Sydney Local Health District, Sydney, New South Wales, Australia
[40]Michael Stern Laboratory for Polycystic Kidney Disease, Westmead Institute for Medical Research, Westmead, New South Wales, Australia
[41]Department of Nephrology and Hypertension, Perth Children's Hospital, Nedlands, Western Australia, Australia
[42]Department of Nephrology and Renal Transplantation, Fiona Stanley Hospital, Murdoch, Western Australia, Australia
[43]Royal Darwin Hospital, Tiwi, Northern Territory, Australia
[44]Genetic Medicine, Westmead Hospital, Sydney, New South Wales, Australia
[45]Sydney Medical School, University of Sydney, Sydney, New South Wales, Australia
[46]Menzies Institute for Medical Research, University of Tasmania, Hobart, Tasmania, Australia
[47]Centre for Population Genomics, Garvan Institute of Medical Research and UNSW Sydney, Sydney, New South Wales, Australia
[48]Centre for Population Genomics, Murdoch Children's Research Institute, Melbourne, Victoria, Australia
[49]Department of Nephrology, Austin Health, Melbourne, Victoria, Australia
[50]Department of Nephrology, Sydney Children's Hospitals Network, Sydney, New South Wales, Australia
[51]Department of Kidney Regeneration, Murdoch Children's Research Institute, Melbourne, Victoria, Australia
[52]Department of Nephrology, Royal Children's Hospital, Melbourne, Victoria, Australia
[53]Department of Paediatrics, University of Melbourne, Melbourne, Victoria, Australia
[54]Institute for Molecular Bioscience and Faculty of Medicine, The University of Queensland, Brisbane, Queensland, Australia