Monogenic causes of chronic kidney disease in adults

see commentary on page 743

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Approximately 500 monogenic causes of chronic kidney disease (CKD) have been identified, mainly in pediatric populations. The frequency of monogenic causes among adults with CKD has been less extensively studied. To determine the likelihood of detecting monogenic causes of CKD in adults presenting to nephrology services in Ireland, we conducted whole exome sequencing (WES) in a multi-centre cohort of 114 families including 138 affected individuals with CKD. Affected adults were recruited from 78 families with a positive family history, 16 families with extra-renal features, and 20 families with neither a family history nor extra-renal features. We detected a pathogenic mutation in a known CKD gene in 42 of 114 families (37%). A monogenic cause was identified in 36% of affected families with a positive family history of CKD, 69% of those with extra-renal features, and only 15% of those without a family history or extra-renal features. There was no difference in the rate of genetic diagnosis in individuals with childhood versus adult onset CKD. Among the 42 families in whom a monogenic cause was identified, WES confirmed the clinical diagnosis in 17 (40%), corrected the clinical diagnosis in 9 (22%), and established a diagnosis for the first time in 16 families referred with CKD of unknown etiology (38%). In this multi-centre study of adults with CKD, a molecular genetic diagnosis was established in over one-third of families. In the evolving era of precision medicine, WES may be an important tool to identify the cause of CKD in adults.

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The estimated global prevalence of chronic kidney disease[1] (CKD) is 11% to 13%. CKD is associated with high morbidity and resource utilization.[2] Mounting evidence highlights the urgency for early diagnosis and intervention, to stem the sequelae of elevated cardiovascular risk and delay progression to end-stage kidney disease (ESKD).[3] Monogenic causes of CKD in childhood are well established,[4] whereas only very limited data are available on monogenic causation of CKD in adults. In 34% of adults who have CKD, a positive family history is reported, suggesting a genetic cause.[5–7] However, genetic testing of adults is still not routinely performed in clinical practice. Panel sequencing of

CKD genes of specific diagnostic groups has revealed a genetic disorder in up to 43% of patients.[8] Using whole exome sequencing (WES), a single-center study demonstrated that a monogenic disease-causing gene can be identified in 24% of adults who have CKD.[9]

In this study, we aim to determine the contribution of monogenic CKD genes in an Irish adult cohort of CKD patients. We hypothesize that genetic causes of CKD in adults are under-recognized, particularly in patients who have a positive family history of CKD or the presence of extrarenal features. Employing WES in patients with familial nephropathy or extrarenal features may therefore reveal monogenic etiologic diagnoses in a high percentage of patients.

The estimated prevalence of CKD with unknown etiology (CKDU) is 10% to 36% in adults.[7,10] In this setting, patients often present late, with bilateral small kidneys that are not amenable to kidney biopsy. Even if a kidney biopsy is obtained, histological examination may be uninformative, as advanced CKD can result in histological findings that cannot be used to distinguish among multiple diseases.[11] We also hypothesize that WES may be especially useful in patients who have CKDU. Diagnosis of a molecular basis for disease in patients who have CKDU can therefore have implications for adequate clinical management, particularly in the era of "precision medicine."

RESULTS

A molecular genetic diagnosis was established in 37% of families, using WES

We performed WES in 114 families with members who had CKD (138 affected individuals). The median age at the time of recruitment was 48 years (range 18–85 years), and CKD was slightly more predominant among males (70 of 138, 51%; Table 1). We diagnosed a molecular genetic

Table 1 | Clinical characteristics of the 138 affected individuals (114 families) with chronic kidney disease that were submitted for whole exome sequencing analysis

	Total cohort		Positive family history		Negative family history with extrarenal features		Negative family history and no extrarenal features	
	138 (114)		102 (78)		16 (16)		20 (20)	
Clinical characteristics	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
***A priori* clinical diagnosis**								
Cystic kidney disease	16	12	9	9	6	38	1	5
CAKUT	53	38	38	37	3	19	12	60
Chronic GN	9	7	7	7	1	6	1	5
TIKD	10	7	10	10	0	0	0	0
SRNS	7	5	4	4	1	6	2	10
Renal tubulopathy	2	1	1	1	1	6	0	0
CKD etiology unknown	41	30	33	32	4	25	4	20
Total	138	100	102	100	16	100	20	100
ESKD								
Yes	90	66	64	65	11	69	15	70
No	48	34	38	35	5	31	5	30
Total	138	100	102	100	16	100	20	100
Age in years at onset of CKD[a]								
<18 (childhood onset)	50	36	27	26	9	56	14	70
≥18 (adult onset)	85	62	74	73	6	38	5	25
Missing data	3	2	1	1	1	6	1	5
Total	138	100	102	100	16	100	20	100
Age in years at onset of ESKD[b]								
<18 (childhood onset)	21	15	8	8	5	31	8	40
≥18 (adult onset)	69	50	56	55	6	38	7	35
CKD only in adulthood	48	35	38	37	5	31	5	25
Total	138	100	102	100	16	100	20	100
Sex								
Male	70	51	49	48	9	56	12	60
Female	68	49	53	52	7	44	8	40
Total	138	100	102	100	16	100	20	100
Self-reported ethnicity								
Irish	135	98	101	99	14	88	20	100
Other European	2	1	1	1	1	6	0	0
Asian	1	1	0	0	1	6	0	0
Total	138	100	102	100	16	100	20	100

A priori clinical diagnosis of CKD is defined as pre-WES clinical diagnosis per referral by primary nephrologist.
CAKUT, congenital anomalies of the kidney and urinary tract; CKD, chronic kidney disease; ESKD, end-stage kidney disease; GN, glomerulonephritis; SRNS, steroid-resistant nephrotic syndrome; TIKD, tubulointerstitial kidney disease; WES, whole exome sequencing.
[a]Age at first presentation to medical services with evidence of CKD.
[b]Age at start of renal replacement therapy, i.e., dialysis or kidney transplantation.

cause in 42 of the 114 families (37%; Figure 1a, navy-blue segment). The genetic diagnostic rate varied by recruitment group (Figure 2). We detected mutations in 29 different genes across a diverse spectrum of known monogenic CKD genes (Table 2; Figure 3). These categories included the following types of genes: cystic kidney disease (8 of 42 families; Figure 1b, red segment); syndromic congenital anomalies of the kidney and urinary tract (CAKUT) (8 of 42 families; Figure 1b, light-blue segment); isolated CAKUT (6 of 42 families; Figure 1b, dark-blue segment); chronic glomerulonephritis (GN) (5 of 42 families; Figure 1b, orange segment); tubulointerstitial kidney disease (TIKD) (4 of 42 families; Figure 1b, brown segment); renal tubulopathy (4 of 42; Figure 1b, purple segment); nephrolithiasis/ nephrocalcinosis (NLNC) (4 of 42 families; Figure 1b, pink segment); steroid-resistant nephrotic syndrome (SRNS) (2 of 42 families; Figure 1b, green segment); and Fabry disease (1 of 42 families; Figure 1b, cream segment).

Detection of a molecular genetic diagnosis in families with an *a priori* clinical diagnosis of cystic kidney disease

In families with an *a priori* clinical diagnosis of cystic kidney disease (12 of 114), we detected a pathogenic mutation in 10 of 12 (83%). In 6 families, the molecular genetic diagnosis confirmed the pre-WES clinical diagnosis, with detection of mutations in cystic kidney disease or nephronophthisis (NPHP) genes (Table 2, red segment: P13, *IFT140*; P80 and P389, *NPHP1*; P324, *BBS9*; P231 and P317, *PKHD1*). In 4 families, we detected mutations in CKD genes known to phenocopy cystic kidney disease. This finding pertained mostly to bilateral small kidneys that were thought to represent the phenotype of small cystic kidneys but in fact represented the CAKUT phenotype of renal hypodysplasia (Table 2, light-blue segment: B2328, *GLI3;* B2454, *TBX1*; P320, *MAP2K2*). In 1 family, WES identified a likely pathogenic mutation in the gene *GLA*, as previously reported in patients who have Fabry disease (Table 2, cream segment: B2327).[12]

Detection of a molecular genetic diagnosis in families with an *a priori* clinical diagnosis of CAKUT

For families with CAKUT (45 of 114), we detected mutations in 10 of 45 (22%). Five families had mutations in isolated CAKUT genes (Table 2, dark-blue segment: P306, *HNF1B*; B2482, *UPK3A*; P69 and P307, *PAX2*; P162, *FREM2*), and 3 families had mutations in syndromic CAKUT genes (Table 2, light-blue segment: B2330, *PROKR2*; B2481, *TBX3*; B2463,

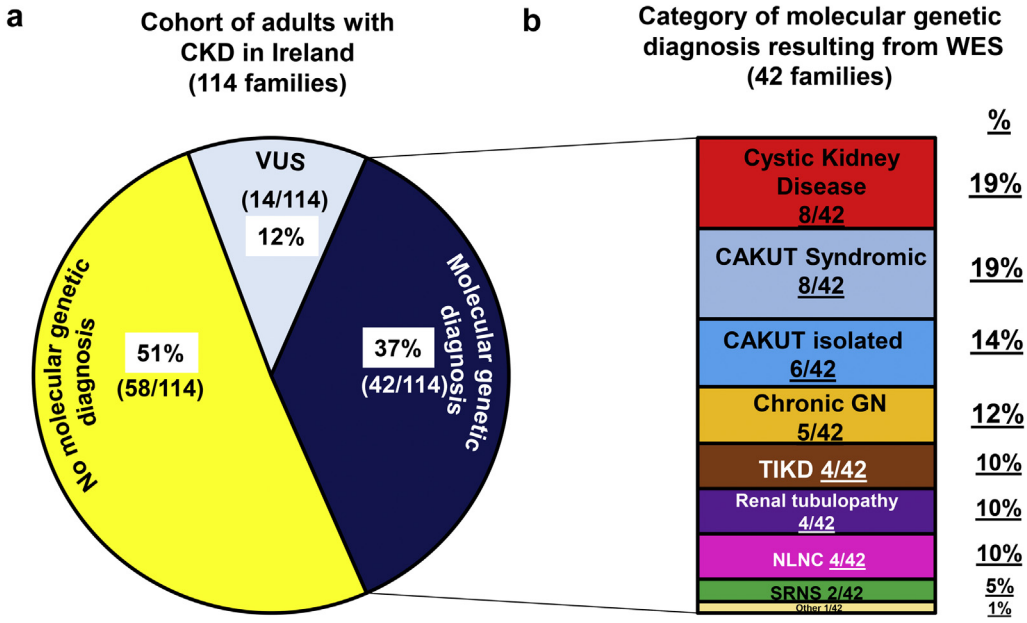

Figure 1 | Percentage of the 114 families in Ireland with chronic kidney disease (CKD) in whom whole exome sequencing (WES) established a molecular genetic diagnosis (i.e., a pathogenic or likely pathogenic monogenic mutation in a known CKD gene was detected post-WES). The 37% of families (42 of 114) in whom a pathogenic or likely pathogenic mutation in a known CKD disease gene was detected (i.e., molecular genetic diagnosis established post-WES) is indicated by navy blue. The 12% of families (14 of 114) in whom a variant of uncertain significance (VUS) in a known CKD gene was detected, is indicated by light blue. Yellow indicates that no meaningful genetic variant could be detected in a known CKD gene post-WES (i.e., no molecular genetic diagnosis established post-WES; **a**). The right-hand side shows the category and percentage of monogenic mutations detected in the 42 families in whom we identified a pathogenic or likely pathogenic mutation in a known CKD gene (i.e., families in whom we established a molecular genetic diagnosis). Each color represents a different molecular genetic diagnostic group (**b**): mutations in known cystic kidney disease including nephronophthisis genes (red); mutations in known syndromic congenital anomalies of the kidney and urinary tract (CAKUT genes; light blue); mutations in known isolated CAKUT genes (dark blue); mutations in known chronic glomerulonephritis (GN) genes (orange); mutations in known tubulointerstitial kidney disease (TIKD) genes (brown); mutations in known renal tubulopathy genes (purple); mutations in known nephrolithiasis/nephrocalcinosis (NLNC) genes (pink); mutations in known steroid-resistant nephrotic syndrome (SRNS) genes (green); mutations in known rare CKD genes (miscellaneous category) (cream) identified.

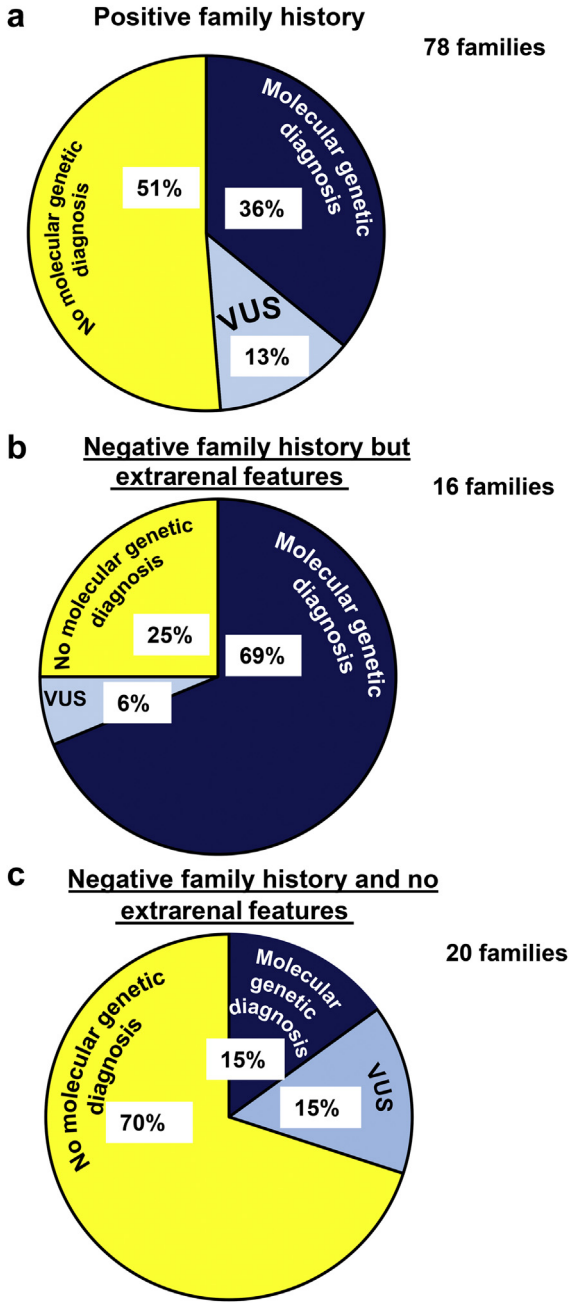

Figure 2 | Percentage of the 114 families in Ireland with chronic kidney disease (CKD) in whom whole exome sequencing established a molecular genetic diagnosis (i.e., a pathogenic or likely pathogenic monogenic mutation in a known CKD gene was detected post–whole exome sequencing [WES]), stratified by recruitment group. Navy blue indicates families in whom a pathogenic or likely pathogenic mutation in a known CKD gene was detected (i.e., molecular genetic diagnosis established post-WES). Light blue indicates families in whom we identified a variant of uncertain significance (VUS) in a known CKD gene post-WES. Yellow indicates that no meaningful genetic variant could be detected in a known CKD gene post-WES (i.e., no molecular genetic diagnosis established post-WES). Positive family history cohort indicates families with CKD who report CKD in either a first- or second-degree relative (78 of 144 families; **a**); negative family history but extrarenal features cohort (16 of 114 families; **b**); negative family history and no extrarenal features cohort (20 of 114 families; **c**).

FBN1). In 3 of the families in whom we detected mutations in syndromic CAKUT genes, extrarenal features present on clinical review were consistent with the corresponding molecular genetic diagnosis (Table 2, column 5). In 2 families, we identified mutations in non-CAKUT genes (Table 2, purple segment: B2457, *AQP2*; and orange segment:B2427, *COL4A3*). The molecular genetic diagnosis in these 2 families was inconsistent with the clinical diagnosis.

Detection of a molecular genetic diagnosis in families with an *a priori* clinical diagnosis of GN

In 2 of the 7 families referred with chronic GN (7 of 114), we detected mutations in genes known to cause focal segmental glomerulosclerosis (FSGS; Table 2, green segment). In both families (KF4 and P640), identification of a pathogenic mutation in the *INF2* gene resulted in correction of the clinical diagnosis from GN to FSGS.

Detection of a molecular genetic diagnosis in families with an *a priori* clinical diagnosis of TIKD

Within the TIKD cohort (7 of 114), we established a molecular genetic diagnosis in 2 of 7 families (29%). In family B2337, both siblings presented with CKD and gout at age 42 years. Examination of renal biopsy specimens showed evidence of tubulointerstitial nephritis in both. The molecular genetic diagnosis confirmed hyperuricaemic nephropathy with detection of a causative mutation in *UMOD* (Table 2, brown segment: B2337). In family B2342, the molecular genetic diagnosis facilitated a clinical review of 2 siblings presenting with CKD and diabetes mellitus in adulthood. Both affected siblings had renal biopsy findings of tubulointerstitial nephritis, and 1 sibling (B2342_44) had evidence of pancreatic exocrine dysfunction. Detection of a mutation in the gene *HNF1B* therefore facilitated reclassification of the clinical diagnosis to renal cyst and diabetes syndrome (Table 2, dark-blue segment: B2342).

No molecular genetic diagnosis established in families with an *a priori* clinical diagnosis of nephrotic syndrome

Of the 7 of 114 families referred with nephrotic syndrome, no molecular genetic diagnosis could be established post-WES.

Detection of a molecular genetic diagnosis in families with an *a priori* clinical diagnosis of renal tubulopathy

In 2 unrelated families with renal tubulopathies (Table 2, purple segment: B2350 and B2453), we detected a pathogenic homozygous mutation in *CLCNKB*, previously reported as being causative of Bartter syndrome.[13] Interestingly, B2453_80 presented with both features of Bartter syndrome and microscopic hematuria. After WES, we detected a second mutation in the Alport gene *COL4A5* (Table 2, purple and orange segments). Patients with this exact mutation are reported to develop late-onset microscopic hematuria and renal impairment.[14]

In summary, in 17 of the 42 solved families (40%), the molecular genetic diagnosis post-WES confirmed the *a priori* clinical diagnosis. The diagnostic yield varied depending on

Table 2 | Information on pre-WES *a priori* clinical diagnosis and post-WES molecular genetic diagnosis in the 42 families in whom pathogenic or likely pathogenic mutations in known monogenic chronic kidney disease genes were identified post-WES

Fam. ID	Ind. ID	*A priori* clinical Dx pre-WES (PRD code ₸	Reported clinical phenotype	Extrarenal features	Age at first Dx of CKD ESKD [years]	Sex	Inclusion criteria[a]	Molecular genetic Dx post-WES #OMIM[b]	Genotype (Inheritance)	WES confirms clinical Dx[c]	WES corrects clinical Dx[c]	WES establishes new clinical Dx[c]	c. change[d] p. change[e] [evolutionary conservation[f]]	Zygosity segregation	PP2[g] SIFT[h] MT[i]	gnom AD[j]	ACMG[k] HGMD[l] ClinVar[m]
Cystic kidney disease (Supplementary Table S6)																	
P13	65	NPHP (2836)	Small, cystic kidneys	Retinitis pigmentosa Mild learning disability	2 27	M	Fam Hx	Mainzer-Saldino syndrome # 266920	***IFT140* (AR)**	✓			c.634G>A p.Gly212Arg (*D.m.*)	hom Fa=het, Ma=het Aff sibs=hom Unaff	0.917 Del. D.C.	0/15/ 277150	Path. DM Path.
	60	NPHP (2836)	Small, cystic kidneys		5 12	F	Fam Hx	Mainzer-Saldino syndrome # 266920	***IFT140* (AR)**				c.634G>A p.Gly212Arg (*D.m.*)	sibs=het	0.917 Del. D.C.	0/15/ 277150	Path. DM Path.
P80	60	NPHP (2836)	Small, cystic kidneys	/	21 21	F	Fam Hx	Nephron-ophthisis 1, juvenile # 256100	***NPHP1* (AR)**	✓			c.555_556insA p.Pro186Thrfs*2	hom Fa=NA, Ma=NA Aff sibs=hom Unaff	/	/	Path. DM /
	61	NPHP (2836)	Small, cystic kidneys	/	11 12	M	Fam Hx	Nephron-ophthisis 1, juvenile # 256100	***NPHP1* (AR)**				c.555_556insA p.Pro186Thrfs*2	sib=het	/	/	Path. DM /
P389	23	NPHP (2836)	BL echogenic kidneys	Renal tubular acidosis post-transplant	8 16	M	Extrarenal	Nephron-ophthisis 1, juvenile # 256100	***NPHP1* (AR)**	✓			c.1027G>A p.Gly343Arg (*C.i.*)	hom Fa=NA, Ma=NA	1.00 Del. D.C.	0/32/ 276716	Path. DM Path.
P324	12	NPHP (2836)	BL echogenic kidneys	Intellectual disability Retinitis pigmentosa Diabetes mellitus Obesity	20 36	F	Extrarenal	Bardet-Biedl syndrome 9 # 615986	***BBS9* (AR)**	✓			c.542C>G p.Pro181Arg (*D.m.*)	hom Fa=NA, Ma=NA	0.99 Del. /	0/1/246048	Path. DM /
P231	62	Cystic KD (2794)	Small kidneys with subcortical cysts	/	8 40	M	Fam Hx	Polycystic kidney disease 4 # 263200	***PKHD1* (AR)**	✓			c.5221G>A p.Val1741Met (*C.e.*)	hom Fa=NA, Ma=NA Aff. sib=hom	0.76 Del. D.C.	0/9/276648	Path. DM Conflicting
	64			/	38 41	F	Fam Hx	Polycystic kidney disease 4 # 263200	***PKHD1* (AR)**				c.5221G>A p.Val1741Met (*C.e.*)		0.76 Del. D.C.	0/9/276648	Path. DM Conflicting
P317	48	Cystic KD (2794)	Normal size, cystic kidneys	**Congenital Hepatic Fibrosis**	46 52	F	Fam Hx	Polycystic kidney disease 4 # 263200	***PKHD1* (AR)**	✓			c.2702A>C p.Asn901Thr (*X.t.*)	Comp. het Fa=NA, Ma=NA Unaff. =single het	0.60 Del. P.M.	0/5/246108	VUS Gene /
													c.107C>T p.Thr36Met (*D.r.*)		0.97 Del. D.C.	0/142/ 277030	Path. DM Path.
P322	11	Unknown (3555)	BSK	Retinitis pigmentosa **Dextrocardia Cholestatic liver dysfunction**	62 70	F	Extrarenal	Short-rib thoracic dysplasia 3 with or without polydactyly # 613091	***DYNC2H1* (AR)**			✓	c.12431C>G p.Pro4144Arg (*C.i.*)	Comp. het Fa=NA, Ma=NA	0.97 Del. D.C.	0/3/276472	VUS Gene Likely Path. & uncertain /
									***DYNC2H1* (AR)**				c.10063+2T>G 100% ESS		/	0/3/227450	Path. DM at same position Path.
P105	58	Unknown (3555)	BSK	/	19 19	F	Fam Hx	Nephronophthisis 1, juvenile # 256100	***NPHP1* (AR)**			✓	c.555_556insA p.Pro186Hisfs*2	hom Fa=NA, Ma=NA	/	/	Path. DM Path.

Syndromic CAKUT (Supplementary Table S7)																	
B2330	12	CAKUT (1625)	R RHD Nephrectomy	**Seizure disorder**	0 14	M	Fam Hx	Syndromic CAKUT # 244200	***PROKR2* (AD)**	✓			c.332T>G p.Met111Arg *(X.t.)*	het Fa=NA, Ma=WT (Affection status Fa unknown)	0.88 Tol. D.C.	0/1/246266	Likely Path. DM /
B2481	83	CAKUT (1625)	Unilateral RA	Postaxial polydactyly **Inguinal hernia**	0 12	M	Extrarenal	Ulnar-mammary syndrome # 181450	***TBX3* (AD)**	✓			c.915del p.Asp305Glufs*18	het Fa=NA, Ma=WT (Affection status Fa unknown)	/	/	Path. Gene /
B2463	96	CAKUT (1673 & 1706)	BL hydro-nephrosis/ ureter, neurogenic bladder R kidney 22cm L kidney 35cm	Height 195cm Joint hypermotility **Saddle nose** **Gum hypertrophy** **Downslanting palpebral fissures High arch palate** **Hammer toes** **Pes planus**	0 CKD only	F	Extrarenal	Marfan syndrome (Syndromic CAKUT) # 154700	***FBN1* (AD)**	✓			c.4888C>T p.Gln1630*	het Fa=NA, Ma=NA	/	/	Path. DM Path.
B2328	44	NPHP (2836)	BL echogenic kidneys	Craniosynostosis Mild learning disability	0 18	F	Extrarenal	Greig cephalo-polysyndactyly # 175700	***GLI3* (AD)**		✓		c.539G>A p.Arg180Gln *(D.r.)*	het Fa=het, Ma=WT(Affection status Fa unknown)	0.89 Del. D.C	0/14/ 276690	Likely Path. Gene /
B2454	13	NPHP (2836)	Presumed NPHP Renal Bx - TIN	**Hypothyroid** Retinitis pigmentosa	30 CKD only	M	Extrarenal	Di George syndrome # 188400	***TBX1* (AD)**		✓		c.1309C>T p.Pro437Ser *(D.r.)*	het Fa=NA, Ma=NA	1.00 Tol. D.C.	0/19/ 211848	Likely Path. Gene /
P320	4	Cystic KD (2794)	Normal size cystic kidneys	Mild intellectual disability **Macrocephaly**	50 CKD only	M	Fam Hx	Cardiofacio-cutaneous syndrome # 615280	***MAP2K2* (AD)**				c.692G>T p.Arg231Leu *(D.m.)*	het Aff Fa=het, Unaff. Ma=NA	1.00 Del. D.C.	/	Likely Path. Gene Likely Path.
	73	Cystic KD (2794)	Normal size cystic kidneys plus VUR	**Hyperkeratosis Lentigines**	20 CKD only	F	Fam Hx		***MAP2K2* (AD)**		✓		c.692G>T p.Arg231Leu *(D.m.)*		1.00 Del. D.C.	/	Likely Path. Gene Likely Path.
P198	102	Unknown (3555)	CKD– aetiology unknown Renal Bx ND	**Hypertension** **Diabetes mellitus** **Depression**	36 CKD only	F	Fam Hx	Wolfram-like syndrome, autosomal dominant # 614296	***WFS1* (AD)**			✓	c.2654C>T p.Pro885Leu *(D.m.)*	het Fa=NA, Ma=NA Aff.=het	1.00 Del. D.C.	0/4/244868	Likely Path. Gene Likely Path.
B2479	75	Unknown (3564)	BL small kidneys (renal Bx FSGS query secondary)	Gout Retinitis Pigmentosa Anemia Diabetes mellitus **Pseudotumour cerebri**	2 15	M	Extrarenal	Fanconi anemia, complementation group I # 609053	***FANCI* (AR)**			✓	c.217A>T Ile73Phe *(D.r.[1])*	hom Fa=NA, Ma=NA	0.81 Del. D.C.	0/4/277214	Likely Path. Gene /
Isolated CAKUT (Supplementary Table S8)																	
P306	92	CAKUT (1618)	VUR R native nephrectomy	/	3 12	F	Fam Hx	Renal cysts and diabetes syndrome # 137920	***HNF1B* (AD)**	✓			c.1333_1334delGC p.Ala445fs*105	het Aff sibs=het Unaff. sibs=WT	/	/	Path. / /
B2482	98	CAKUT (1687 & 1618)	PUV BL VUR	/	0 9	M	Neither	CAKUT *611559	***UPK3A*(AD)**	✓			c.227C>A p.Ser76*	het Fa=NA, Ma=NA	/	0/21/ 246014	Path. ?DM /
P69	59	CAKUT (3517)	Unilateral RA	/	21 37	M	Fam Hx	Papillorenal syndrome # 120330	***PAX2* (AD)**	✓			c.491C>A p.Thr164Asn *(D.r.)*	het Fa=NA, Ma=NA	0.03 Del. D.C.	0/45/ 223294	Path. DM /
P307	77	CAKUT (1618)	VUR	/	42 46	M	Fam Hx	Papillorenal syndrome # 120330	***PAX2* (AD)**	✓			c.70G>C p.Gly24Arg *(D.m.)*	het Aff Fa=het Unaff. Ma=WT Aff.	1.00 Del.D.C	/	Path. DM /
	50	CAKUT (1618)	VUR	/	18 25	F	Fam Hx		***PAX2* (AD)**				c.70G>C p.Gly24Arg *(D.m.)*	sibs=het Unaff. sibs=WT	1.00 Del. D.C	/	Path. DM /
	94	CAKUT (3517)	Unilateral RA	/	29 29	F	Fam Hx		***PAX2* (AD)**				c.70G>C p.Gly24Arg *(D.m.)*		1.00 Del. D.C	/	Path. DM /

(*Continued on next page*)

Table 2 | (Continued)

Fam. ID	Ind. ID	*A priori* clinical Dx pre-WES (PRD code ₸	Reported clinical phenotype	Extrarenal features	Age at first Dx of CKD ESKD [years]	Sex	Inclusion criteria[a]	Molecular genetic Dx post-WES #OMIM[b]	Genotype (Inheritance)	WES confirms clinical Dx[c]	WES corrects clinical Dx[c]	WES establishes new clinical Dx[c]	c. change[d] p. change[e] [evolutionary conservation[f]]	Zygosity segregation	PP2[g] SIFT[h] MT[i]	gnom AD[j]	ACMG[k] HGMD[l] ClinVar[m]
P162	99	CAKUT (1618)	VUR	/	50 51	M	Fam Hx	Fraser Syndrome # 617666	***FREM2* (AR)**	✓			c.3661C>T p.Pro1221Ser *(C.i.)*	Comp. het Aff sib = comp. het, Unaff sib=single	1.00 Del.D.C.	0/16/ 277168	Likely Path. Gene /
									***FREM2* (AR)**				c.2533C>T p.His845Tyr *(D.r.)*	het	0.01 Del./	/	Likely Path. Gene /
B2342	44	TIKD (1897)	Renal Bx - TIN	**Diabetes mellitus** **Annular pancreas**	37 40	F	Fam Hx	Renal cysts and diabetes syndrome # 137920	***HNF1B* (AD)**		✓		c.544+3_544+6 del 75% ESS	het Aff sib = het Unaff sib= WT	/	/	Likely Path. Gene /
	63	TIKD (1897)	Renal Bx - TIN	**Diabetes mellitus**	42 CKD only	M	Fam Hx		***HNF1B* (AD)**				c.544+3_544+6 del 75% ESS		/	/	Likely Path. Gene /
Chronic glomerulonephritis (Supplementary Table S9)																	
B2427	56	CAKUT (1625)	Haematuria BL RHD	/	3 CKD only	M	Neither	Alport syndrome # 104200	***COL4A3* (AD)**		✓		c.4981C>T p.Arg1661Cys *(D.m.)*	het Fa = NA, Unaff Ma=het	1.00 Del. D.C.	1/103/ 277100	Path. DM Path. & Likely Path.
B2347	17	Unknown (3564)	Hematuria Renal Bx indeterminate	/	12 48	F	Fam Hx	Alport Syndrome # 104200	***COL4A3* (AD)**			✓	c.2452G>A p.Gly818Arg *(D.m.)*	het Fa=NA, Ma=NA	1.00 Del. D.C	/	Likely Path. DM Path.
P241	63	Unknown (3564)	Renal Bx indeterminate	/	33 CKD only	F	Fam Hx	Alport Syndrome # 301050	***COL4A5* (XLD)**			✓	c.2396G>A p.Gly799Asp *(C.e.)*	het Fa=NA, Ma=NA	1.00 Del. D.C.	/	Likely Path. Gene /
P58	86	Unknown (3564)	Renal Bx indeterminate, HTN	/	23 52	M	Fam Hx	Alport Syndrome # 301050	***COL4A5* (XLD)**			✓	c.1423+1G>T 100% ESS	hemi Fa=NA, Ma=NA	/	/	Path. DM Path.
P100	30	Unknown (3564)	Renal Bx indeterminate	**Deafness** **Glaucoma** **Recurrent pneumonia**	30 40	F	Fam Hx	Alport Syndrome # 301050	***COL4A5* (XLD)**			✓	c.2605G>A p.Gly869Arg *(C.e.)*	het/ hemi Fa=NA, Aff Ma=het Aff sib = hemi	1.00 Del. D.C.	/	Path. DM Path.
	16	Unknown (3564)	Renal Bx indeterminate	**Hearing impairment**	17 20	M	Fam Hx		***COL4A5* (XLD)**				c.2605G>A p.Gly869Arg *(C.e.)*		1.00 Del. D.C.	/	Path. DM Path.
B2453 **	80	Micro-scopic haem-aturia (3712)	Hypokalemic metabolic alkalosis/ Bartter syndrome (3085)	/	3 CKD only	M	Extrarenal	Alport Syndrome # 301050	***COL4A5* (XLD)**	2 molecular Dx – see purple segment below			c.2692A>G p.Met898Val *(D.r.)*	hemi Fa=NA, Ma=NA	0.01 Tol. D.C.	0/28/57/ 198228	Path. DM /
P640 **	2008	Chronic GN (1377)	Microscopic hematuria Normal renal Bx	Low complement (C3) levels	20 Normal renal function	M	Fam Hx	Susceptibility to atypical hemolytic uremic syndrome # 612925	***C3* (AD)**	2 molecular Dx – see green segment below			c.4534C>T p.Arg1512Cys *(M.m.)*	het Aff. Fa=het Aff. sib=het	0.65 Del. D.C.	0/2/ 246248	Likely Path. Gene /
	82	Chronic GN (3749)	No renal Bx performed	Low complement (C3) levels	20 30	M	Fam Hx		***C3* (AD)**				c.4534C>T p.Arg1512Cys *(M.m.)*	Unaff. Ma=WT Unaff. sib=WT	0.65 Del. D.C.	0/2/246248	Likely Path. Gene /
Tubulointerstitial kidney disease (Supplementary Table S6)																	
B2337	52	TIKD (1897)	Renal Bx - TIN	Uveitis Mucosal ulcers	42 CKD only	M	Fam Hx	Hyperuricemic nephropathy # 162000	***UMOD* (AD)**	✓			c.317G>A p.Cys106Tyr *(X.t.)*	het Fa=NA, Ma=NA Aff. sib= het	1.00 Del. D.C.	/	Path. DM Uncertain
	53	TIKD (1897)	Renal Bx - TIN	Hyperuricemia	42 CKD only	M	Fam Hx	Hyperuricemic nephropathy # 162000	***UMOD* (AD)**				c.317G>A p.Cys106Tyr *(X.t.)*		1.00 Del. D.C.	/	Path. DM Uncertain

P193	83	Unknown (3555)	BSK	/	20 CKD only	F	Fam Hx	Hyperuricemic nephropathy # 162000	***UMOD* (AD)**			✓	c.280T>C p.Cys94Arg *(D.r.)*	het Fa=NA, Ma=NA	1.00 Del. D.C.	/	Likely Path. Gene /
P232	60	Unknown (3564)	Renal Bx indeterminate	Hyperuricemia	8 18	M	Fam Hx	Hyperuricemic nephropathy # 162000	***UMOD* (AD)**			✓	c.1382C>A p.Ala461Glu *(X.t.)*	het Fa=NA, Ma=NA	1.00 Tol. D.C.	/	Path. DM Likely Path.
P88	47	Unknown (3555)	BSK Renal Bx – insufficient tissue	Bronchiectasis Liver dysfunction Non-melanomatous cancers	44 45	M	Fam Hx	Karyomegalic interstitial nephritis # 614817	***FAN1* (AR)**			✓	c.2590C>T p.Gln864*	Comp. het Fa=het, Ma=het Aff. sibs=comp het	/	0/1/246242	Path. Gene /
													c.2774_2775delTT p.Leu925fs	Unaff. sibs=het	/	0/10/ 267846	Path. Gene /
	38	Unknown (3555)	BSK Renal Bx – insufficient tissue	Metastatic lung cancer	33 38	F	Fam Hx		***FAN1* (AR)**				c.2590C>T p.Gln864*		/	0/1/246242	Path. Gene /
													c.2774_2775delTT p.Leu925fs		/	0/10/ 267846	Path. Gene /
Renal tubulopathy (Supplementary Table S10)																	
B2457	78	CAKUT (1625 & 2476)	BL RHD **BL renal vein thrombosis Hyper-natremia/ Dehydration**	/	0 10	M	Neither	Nephrogenic diabetes insipidus # 125800	***AQP2* (AD)**		✓		c.782C>T p.Ser261Leu *(X.t.)*	het Fa=NA, Ma=NA	0.99 Del. D.C.	0/4/228000	Likely Path. Gene /
B2467	35	Unknown (3564)	Hypertension CKD Renal Bx indeterminate	/	26 CKD only	F	Fam Hx	Pseudohypo-aldosteronism - hypertensive CKD # 614491	***WNK4* (AD)**			✓	c.506C>T p.Pro169Leu*(D .r.)*	het Fa=NA, Ma=NA	0.69 Del. D.C.	/	Path. DM /
B2350 **	32	Bartter Syndrome (3085)	Hypokalemic metabolic alkalosis	**Gout**	17 38	F	Fam Hx	Bartter Syndrome # 607364	***CLCNKB* (AR)**	✓			c.226C>T p.Arg76*	hom Fa=NA, Ma=NA	/	0/3/246064	Path. DM /
B2453 **	80	Bartter syndrome (3085)	Hypokalemic metabolic alkalosis	**Microscopic hematuria**	3 CKD only	M	Extrarenal	Bartter syndrome# 607364	***CLCNKB* (AR)**	✓			c.226C>T p.Arg76*	hom Fa=NA, Ma=NA	/	0/3/246064	Path. DM /
Nephrolithiasis/nephrocalcinosis (Supplementary Table S11)																	
B2344	78	Unknown (3564)	Renal Bx indeterminate	Gout	25 CKD only	M	Extrarenal	Cystinuria # 220100	***SLC3A1* (AD)**			✓	c.1799G>A p.Gly600Glu *(D.m.)*	het Fa=NA, Ma=NA Aff. = het	1.00 Del D.C.	0/21/ 276676	Likely Path. DM /
P318	50	Unknown (3555)	BSK	**Intellectual disability Finger and wrist swelling Seizure disorder**	41 CKD only	M	Fam Hx	Lowe Syndrome # 309000	***OCRL* (XL)**			✓	c.1567G>A p.Asp523Asn *(S.c.)*	Hemi Unaff. Fa=NA Unaff. Ma=het	1.00 Del. D.C.	/	Path. DM /
P182	602	Unknown (3555)	BSK	**Hypophos-phatemia Bony pain multiple fractures Bony spurs & sclerosis**	51 55	M	Fam Hx	Dent disease # 300009	***CLCN5* (XL)**			✓	c.1938del p.Phe646Leufs*10	hemi Fa=NA, Ma=NA	/	/	Likely Path. Gene /
B2340	15	Unknown (3555)	BSK	**(Son has nephrolithiasis)**	60 CKD only	F	Fam Hx	Dent disease # 300009	***CLCN5* (XL)**			✓	c.925C>T p.Arg309Cys *(D.r.)*	het Aff. son=hemi Fa=NA, Ma=NA	0.92 Del. D.C.	0/1/6/ 193605	Likely Path. Gene /
Steroid-resistant nephrotic syndrome (Supplementary Table S12)																	
KF4	16	Chronic GN (1377)	Renal Bx indeterminate	/	74 78	F	Fam Hx	Focal segmental glomerulo-sclerosis # 613237	***INF2* (AD)**		✓		c.653G>A p.Arg218Gln *(X.t.)*	het Unaff. Fa=NA, Aff. Ma=het	1.00 Del. D.C.	/	Path. DM Path.
	15	Chronic GN (1377)	Renal Bx indeterminate	Gout	52 55	F	Fam Hx		***INF2* (AD)**				c.653G>A p.Arg218Gln *(X.t.)*		1.00 Del. D.C.	/	Path. DM Path.

(*Continued on next page*)

Table 2 | (Continued)

Fam. ID	Ind. ID	*A priori* clinical Dx pre-WES (PRD code ₸	Reported clinical phenotype	Extrarenal features	Age at first Dx of CKD ESKD [years]	Sex	Inclusion criteria[a]	Molecular genetic Dx post-WES #OMIM[b]	Genotype (Inheritance)	WES confirms clinical Dx[c]	WES corrects clinical Dx[c]	WES establishes new clinical Dx[c]	c. change[d] p. change[e] [evolutionary conservation[f]]	Zygosity segregation	PP2[g] SIFT[h] MT[i]	gnom AD[j]	ACMG[k] HGMD[l] ClinVar[m]
P640 **	83	Chronic GN (1349)	Renal Bx - indeterminate	Normal complement (C3) levels	20 23	F	Fam Hx	Focal segmental glomerulo-sclerosis # 613237	***INF2* (AD)**		✓		c.353T>A p.Ile118Asn *(D.r.)*	het Aff. Fa=het Aff. sib=het Unaff.	1.00 Del. D.C.	/	Likely Path. Gene /
	82	Chronic GN (3749)	Renal Bx ND	Low complement (C3) levels	20 30	M	Fam Hx		***INF2* (AD)**				c.353T>A p.Ile118Asn *(D.r.)*	Ma=WT Unaff. sib=WT	1.00 Del. D.C.	/	Likely Path. Gene /
Rare monogenic CKD genes (miscellaneous category) (Supplementary Table S13)																	
B2327	66	Cystic KD (2794)	Normal size cystic kidneys	Liver dysfunction **Unexplained seizures, L facial weakness without definite pathology noted on brain imaging, unexplained abdominal symptoms, photophobia**	1 6	F	Extrarenal	Fabry Disease # 301500	***GLA* (XL)**		✓		c.352C>T p.Arg118Cys het *(X.t.[2])*	het Fa=NA, Ma=NA	0.99 Del.P.M.	0/15/43/ 200247	Likely Path. DM Conflicting

A, adenine; AD, autosomal dominant; Aff., affected; AR, autosomal recessive; BL, bilateral; BSK, bilateral small kidneys; Bx, biopsy; C, cytosine; c. change, nucleotide change; CAKUT, congenital anomalies of the kidney and urinary tract; CKD, chronic kidney disease; cm, centimeter; comp, compound; conflicting, multiple submitters have provided assertion criteria to the ClinVar database, but there are conflicting interpretations; del, deletion; delins, deletion insertion; Del., deleterious; D.C., disease-causing; DM, disease mutation; Dx, diagnosis; ESKD, end-stage kidney disease; ESS, essential splice site; F, female; Fa, father; Fam. ID, unique family identifier; fs, frameshift mutation; FSGS, focal segmental glomerulosclerosis; G, guanine; GN, glomerulosclerosis; hemi, hemizygous; het, heterozygous; hom, homozygous; HTN, hypertension; Ind. ID, unique individual identifier; ins, insertion; KD, kidney disease; L, left; M, male; Ma, maternal; NA, not available; ND, not done; NPHP, nephronophthisis; p. change, amino acid change; Path., pathogenic; P.M., polymorphism; PRD, primary renal diagnosis; PUV, posterior urethral valve; R, right; RA, renal agenesis; RHD, renal hypodysplasia; sibs, sibling(s); T, thymine; TIKD, tubulointerstitial kidney disease; TIN, tubulointerstitial nephritis; Tol., Tolerated; Unaff., unaffected; unknown, CKD etiology unknown; VUR, vesico-ureteric reflux; VUS, variant of uncertain significance; WES, whole exome sequencing; WT, wild type; XL, X-linked; XLD, X-linked dominant.

All families with a molecular genetic diagnosis were classified as pathogenic or likely pathogenic per the ACMG guidelines (last column). In families in whom segregation was not possible (column 12) AND the exact mutation was not previously described (last column), the variant was considered a variant of uncertain significance (Supplementary Table S1). In the case of a compound heterozygous mutation, at least one of the alleles was classified as either pathogenic or likely pathogenic. *A priori* clinical diagnosis is clinical diagnosis of chronic kidney disease pre-WES per referral by the primary nephrologist. Additional clinical features established post-WES, following clinical re-review in full cognizance of the molecular genetic diagnosis are highlighted with bold, underlined text in columns 4 and 5.

**Indicates additional finding in another category; *nonsense mutation; ₸, ERA-EDTA primary renal disease codes (https://www.era-edta-reg.org/prd.jsp); / indicates data not available.

[a]Inclusion criteria: Fam Hx indicates positive family history; Extrarenal indicates CKD with extrarenal features; "Neither" indicates no family history and no extrarenal features.

[b]# OMIM, Online Mendelian Inheritance in Man (https://www.omim.org).

[c]Outcome post-WES: WES confirms clinical Dx indicates that WES confirmed the clinical diagnosis; WES corrects clinical Dx indicates that WES resulted in reclassification/ correction of the clinical diagnosis; WES establishes new clinical Dx indicates that WES resulted in establishment of a molecular diagnosis in families with CKD—etiology unknown.

[d]Impact of variant on cDNA level.

[e]Impact of variant on the amino acid or protein level.

[f]Evolutionary conservation was assessed across phylogeny over eight species: *M.m., Mus musculus; G.g., Gallus gallus; X.t., Xenopus tropicalis; D.r., Danio rerio; C.e., elegans; C.i., Ciona intestinalis; D.m., Drosphilia melanogaster; S.c., Saccharomyces cerevisiae*. If conservation is interrupted in one species but otherwise preserved across phylogeny a numerical reference is provided: [1]Valine in *G. gallus*; [2]Lysine in *M. musculus*.

[g]PP2, PolyPhen 2 (http://genetics.bwh.harvard.edu/pph2).

[h]SIFT, Sorting intolerant from tolerant (http://sift.jcvi.org/).

[i]MT, Mutation Taster (http://www.mutationtaster.org).

[j]gnomAD, variant frequencies listed for homozygous/ hemizygous (if applicable)/ heterozygous/ total alleles(http://gnomad.broadinstitute.org/).

[k]ACMG, American College of Medical Genetics and Genomics Standards and Guidelines Classification as pathogenic, likely pathogenic or VUS (Richards Genet Med 17(5):405, 2015).

[l]HGMD, Human Gene Mutation Database; https://portal.biobaseinternational.com/hgmd. If the exact variant has been reported previously on HGMD® Professional 2017.2 for the reported phenotype and classified as a disease-causing pathogenic mutation, the variant is denoted as "DM." The variant is denoted as "?DM" if the variant is likely a disease-causing pathogenic mutation, but either the author indicated some doubt or subsequent evidence calls the deleterious nature of the variant into question. If the gene, but not the exact variant, has been reported for the corresponding phenotype, then "Gene" is indicated in this column.

[m]ClinVar classification indicates that the variant has been submitted to the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar).

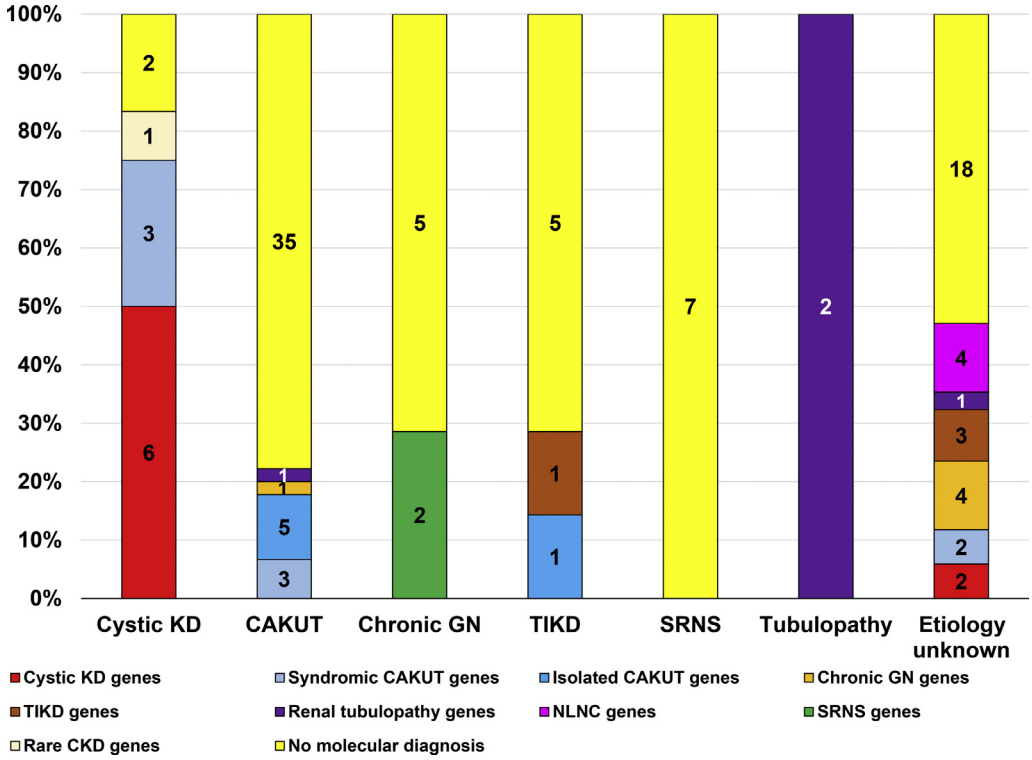

Figure 3 | Specific mutation category identified by whole exome sequencing in 114 families with chronic kidney disease (CKD). The x-axis displays the 7 *a priori* clinical diagnostic groups listed horizontally and includes cystic kidney disease (KD), congenital anomalies of the kidney and urinary tract (CAKUT), chronic glomerulonephritis (GN), tubulointerstitial kidney disease (TIKD), steroid-resistant nephrotic syndrome (SRNS), renal tubulopathy (tubulopathy), and CKD—etiology unknown. The y-axis displays the molecular genetic diagnosis established post–whole exome sequencing and includes the following: mutations in known cystic kidney disease including nephronophthisis genes (red) identified; mutations in known syndromic CAKUT genes (light blue) identified; mutations in known isolated CAKUT genes (dark blue) identified; mutations in known GN genes (orange) identified; mutations in known TIKD genes (brown) identified; mutations in known renal tubulopathy genes (purple) identified; mutations in known SRNS genes (green) identified; mutations in known nephrolithiasis/nephrocalcinosis (NLNC) genes (pink) identified; mutations in known rare CKD genes (miscellaneous category) (cream) identified; no molecular genetic diagnosis established post-WES (yellow).

the *a priori* clinical diagnosis (Figure 2). In 9 of the 42 families (22%), the molecular genetic diagnosis resulted in correction of the clinical diagnosis, whereas in 16 families with CKDU (38%), WES established a new molecular genetic diagnosis (Table 3).

WES corrected the *a priori* clinical diagnosis

In 9 of 42 solved families (22%), WES corrected the clinical diagnosis (Table 3). For example, patient B2457_78, with an *a priori* clinical diagnosis of CAKUT, presented with ESKD and a renal ultrasound showing bilateral small kidneys presumed to be due to bilateral renal hypodysplasia. After WES, we detected a heterozygous *AQP2* mutation (Table 2, purple segment). A review post-WES indicated that the patient had initially presented as an infant in the 1970s with polyuria, vomiting, and hypernatremia, with subsequent bilateral renal vein thrombosis. This reverse phenotyping confirmed the molecular genetic diagnosis, by WES, of nephrogenic diabetes insipidus.

In patient B2427_56, with an *a priori* clinical diagnosis of CAKUT, we detected a heterozygous mutation in the *COL4A3* gene[11] (Table 2, orange segment). Owing to the lack of a family history and the absence of a renal biopsy specimen, the clinical diagnosis of autosomal dominant Alport syndrome had not been suspected initially. This finding demonstrates the utility of WES in establishing a definitive clinical diagnosis in patients with atypical or indistinct phenotypes.

Family P640 had an initial diagnosis of C3 glomerulonephritis (Table 2, green and orange segments; Supplementary

Table 3 | Comparison between the outcomes in the current study and a recent publication

Outcome	Current study n (%)	Lata *et al.* study[9] n (%)
WES confirmed the clinical diagnosis	17 (40)	6 (27)
WES corrected/reclassified the clinical diagnosis	9 (22)	6 (27)
WES established a new clinical diagnosis	16 (38)	7 (32)
Novel candidate gene identified following WES	NA	3 (14)
Total in whom WES confirmed, corrected, reclassified or established a genetic diagnosis	42 (100)	22 (100)

NA, not applicable; WES, whole exome sequencing.

Figure S1). Both affected individuals (P640_82 and P640_83) presented with advanced proteinuric CKD in their twenties. Multiple family members were noted to have low C3 levels, but all had normal renal function. After WES, the molecular genetic diagnosis of FSGS owing to a dominant heterozygous mutation in *INF2* was established in P640_82 and P640_83. An additional finding of a dominant heterozygous variant in *C3* was also identified in P640_82, who had ESKD, and P640_2008, who did not have ESKD, both of whom were hypocomplementemic. Mutations in this gene can result in complement dysregulation characterized by low C3 levels, thereby increasing susceptibility to atypical hemolytic uremic syndrome.[15]

WES established a new clinical diagnosis in families with CKDU

In families referred with CKDU (34 of 114; 30%), we detected a pathogenic mutation in 16 of 34 families (47%), which represents 38% of the solved cohort (16 of 42 solved families; Table 4). The molecular genetic diagnoses in these families included the following: cystic kidney disease or NPHP (Table 2, red segment: P322, *DYNC2H1;* P105, *NPHP1*); syndromic CAKUT (Table 2, light-blue segment: P198, *WFS1* and B2479, *FANC1*); Alport syndrome (Table 2, orange segment: B2347, *COL4A3*; P241, P58, & P100, *COL4A5*); TIKD (Table 2, brown segment: P193 & P232, *UMOD;* P88, *FAN1*); hypertensive renal disease (Table 2, purple segment: B2467, *WNK4*); and nephrocalcinosis/nephrolithiasis (Table 2, pink segment: B2344, *SLC3A1*; P318, *OCRL*; P182 & B2340, *CLCN5*). None of the mutations causing the above diseases was suspected on clinical grounds before this study, and affected patients were not clinically distinguished from other patients with CKDU. WES therefore facilitated establishment of a molecular genetic diagnosis in families who otherwise would have remained without a formal diagnosis.

Identification of variants of uncertain significance

In 12% of families (14 of 114), we detected a potentially pathogenic mutation in a gene known to cause CKD (Figure 1, light-blue segment; Supplementary Table S1). However, the identified variants did not meet our *a priori* criteria for definite confirmation of pathogenicity, because of a lack either of clinical evidence to perform adequate genotype–phenotype correlation or of additional familial DNA to perform segregation analysis.

Factors associated with obtaining genetic diagnosis

The highest yield in terms of establishing a molecular genetic diagnosis was in families with CKD and extrarenal features (11 of 16; 69%). In families with a positive family history, we obtained a molecular genetic diagnosis in 36% (28 of 78). In families with a negative family history and no extrarenal features, monogenic causation was observed in 15% (3 of 20; Figure 2). No significant difference was observed in the median age of reaching ESKD among individuals in whom we established a molecular diagnosis (33 years [range: 6–78 years]; Table 4) versus those in whom no molecular diagnosis was established (31 years [range: 5–68 years; $P = 0.955$).

DISCUSSION

In this large, multicenter study, we systematically evaluated the utility of WES in a cohort of adults who had CKD. We established a molecular genetic diagnosis after WES in 42 of 114 families (37%) who had CKD and were utilizing nephrology services in Ireland. A genetic diagnosis was established in 69% (11 of 16) of families exhibiting extrarenal features, and in 36% (28 of 78) of families with familial nephropathy, whereas in families negative for both family history and extrarenal features, monogenic causation was observed in 15% (3 of 20). Previous estimates indicate that in ~10% of all adults who have CKD, the disease has an underlying genetic etiology.[16]

Recently, a higher prevalence of 24% for monogenic causation was reported post-WES.[9] In that single-center study, Lata *et al.*[9] recruited 92 patients with either a family history of CKD, undiagnosed CKD or a clinical suspicion of genetic kidney disease owing to childhood-onset CKD. We observed comparable rates of confirmation, correction, and

Table 4 | Comparison of the clinical characteristics of the 138 affected patients with chronic kidney disease by molecular genetic diagnostic category post-WES

Molecular genetic diagnostic category post-WES	Median age at onset of ESKD, years (range)	CKD only in adulthood[a]	ESKD in adulthood[b]	ESKD in childhood[c]	Total "solved" individuals
Cystic kidney disease	27 (12–70)	0	8 (73)	3 (27)	11 (100)
CAKUT	21.5 (9–51)	6 (33)	7 (39)	5 (28)	18 (100)
Chronic GN	40 (20–52)	3 (43)	4 (57)	0	7 (100)
TIKD	38 (18–45)	3 (50)	3 (50)	0	6 (100)
Renal tubulopathy	24 (10–38)	2 (50)	1 (25)	1 (25)	4 (100)
Nephrolithiasis/nephrocalcinosis	55	3 (75)	1 (25)	0	4 (100)
Steroid-resistant nephrotic syndrome	37.5 (20–78)	0	4 (100)	0	4 (100)
Other disease category	6	0	0	1 (100)	1 (100)
All molecular diagnostic categories	33 (6–78)	17 (31)	28 (51)	10 (18)	55 (100)

Values are n(%), unless otherwise indicated. "Solved" indicates that a pathogenic or likely pathogenic monogenic mutation in a known CKD gene was detected post-WES. CAKUT, congenital anomalies of the kidney and urinary tract; CKD, chronic kidney disease; ESKD, end-stage kidney disease; GN, glomerulonephritis; TIKD, tubulointerstitial kidney disease.
[a]Adult patients who had CKD at time of analysis (i.e., had not yet progressed to ESKD in adulthood /at an age ≥18 years).
[b]Patients who developed ESKD at age ≥18 years.
[c]Patients who developed ESKD at age <18 years.

establishment of a new clinical diagnosis post-WES (Table 3). More recently, Mallett *et al.*[8] demonstrated, using targeted exomic sequencing, a genetic diagnostic rate of 43% in patients who have familial renal disease. As in our findings, the genetic diagnostic rate was similar in those who had pediatric-onset disease compared with those who had adult-onset disease (Supplementary Table S2). Together, these findings provide compelling evidence that monogenic disease causation is under-recognized in adults with CKD and that WES can provide a monogenic etiologic diagnosis in adults who have CKD.

Our data highlight the fact that mutations in genes classically described as "childhood" CKD genes can also be identified in adults. We hypothesize that later-onset disease is due to allelic heterogeneity, with "milder" phenotypes likely attributable to "milder" missense mutations.[17] For example, autosomal recessive polycystic kidney disease typically has been characterized as a childhood-onset nephropathy, with few cases of ESKD observed in those aged >40 years.[18] We identified recessive missense mutations in the *PKHD1* gene in 2 unrelated families in which onset of ESKD occurred at ages >40 years (Table 2, red segment: P231 and P317). Patient P317_48 presented with CKDU at age 46 years, which progressed to ESKD at age 52 years. A compound heterozygous missense mutation in *PKHD1* was identified using WES (a novel c.2702A>C, p.Asn901Thr variant and a previously reported c.107C>T p.Thr36Met variant[18]). Recent findings show that compound heterozygous mutations that involve at least 1 missense mutation of *PKHD1* can lead to adult-onset disease.[19,20] These data add to the mounting evidence supporting a monogenic causation hypothesis in adults and highlight the utility of WES in the investigation of adults who have CKD.

The estimated prevalence of CKDU is 10% to 36% in adults,[7,10] with a prevalence of 30% (34 of 114) in the current cohort. By employing WES, we established a molecular genetic diagnosis in almost half of the families with CKDU (16 of 34; 47%), confirming our hypothesis that CKDU may have a monogenic component. These data are consistent with findings from other groups (Table 3). By employing WES in cases in which renal ultrasound and kidney biopsies were uninformative, we detected pathogenic mutations across a diverse spectrum of known monogenic causes of CKD including the following: cystic kidney disease (2 of 16 families; Table 2, red segment); CAKUT (2 of 16 families; Table 2, blue segment); chronic GN (4 of 16 families; Table 2, orange segment); TIKD (3 of 16 families; Table 2, brown segment); renal tubulopathy (1 of 16; Table 2, purple segment); and nephrolithiasis/nephrocalcinosis (4 of 16 families; Table 2, pink segment; Figure 3). Given that all these patients would have remained without a clinical diagnosis had WES not been used, these findings are notable.

Consistent with prior literature on genetic CKD in childhood, we demonstrated that the likelihood of obtaining a molecular genetic diagnosis in adults increased with the recognition of extrarenal manifestations (69%).[21] As demonstrated in our cohort, mild extrarenal features commonly go unrecognized until clinical re-review is performed in full cognizance of the molecular genetic diagnosis (Table 2; Supplementary Table S1).[22] This strategy of "reverse phenotyping" has been described extensively for childhood cohorts,[23] and our data show that this finding holds relevance for adults who have CKD. Identification of specific pathogenic mutations can also facilitate screening for otherwise undetected extrarenal features. For example, identification of mutations in the gene *HNF1B* has allowed for screening for associated extrarenal features such as diabetes, facilitating early lifestyle intervention strategies and avoidance of prodiabetic medications in the posttransplant period (Table 2, blue segment: P306 and B2342,).

Unnecessary diagnostic interventions can, in certain cases, be avoided following establishment of a molecular genetic diagnosis. This finding was particularly evident in cases in which the pretest probability of obtaining a diagnosis is low, as in patients presenting with bilateral small kidneys not amenable to biopsy. Such was the case for family P88, in whom we detected a pathogenic mutation in the gene *FAN1*, and for whom multiple attempts to obtain a kidney biopsy were futile (Table 2**,** brown segment). On retrospective review, WES could have provided an earlier, more precise molecular diagnosis, thereby facilitating early institution of antiproteinuric medication, avoiding systemic immunosuppression, and negating the need for a nondiagnostic kidney biopsy.

Employing WES can allow for establishment of a more precise molecular genetic diagnosis in families that have complex clinical presentations. As seen in family P640, with a presumed diagnosis of C3 glomerulonephritis, identification of a pathogenic mutation in *INF2* permitted the diagnosis of FSGS (Table 2: P640_83, P640_82; Supplementary Figure S1), and detection of a second variant in the gene *C3* may explain the complement dysregulation observed in other family members (Table 2: P640_2008 and P640_82). In patient B2453_80, who had Bartter syndrome, clinical heterogeneity that was evident at clinical presentation remained unresolved prior to WES. This patient presented with microscopic hematuria, explained by identification of a hemizygous mutation in the Alport gene *COL4A5* (Table 2, orange segment). Patients with this exact mutation are reported to develop late-onset microscopic hematuria and renal impairment.[14] Findings such as these can facilitate early intervention with antiproteinuric medication to stem the progression of CKD. Additionally, given the emerging evidence of increased risk of ESKD in both donors and recipients in families with Alport syndrome,[24] a molecular genetic diagnosis can help guide physicians in performing risk stratification of potential related donors at live-donor assessment.

This study is not without limitations. First, the study was performed on a select population of those of predominantly Irish ethnicity, thereby reducing generalizability of the findings to other populations. Second, our cohort had a higher prevalence of familial CKD (78 of 114; 68%; Figure 2)

compared with the reported prevalence in the general Irish CKD population (629 of 1840; 34%).[7] Finally, although all patients were recruited as adults (median age at recruitment 48 years [range: 18–85 years]), some developed ESKD in childhood (21 of 138; 15%; Table 1). Given that prior reports suggest a higher prevalence of monogenic causation in childhood,[4] these findings should be considered when extrapolating to the general CKD population. A comparison of the rate of molecular genetic diagnosis for childhood-onset versus adult-onset CKD revealed no significant difference (20 of 50 or 40% with childhood-onset versus 35 of 85 or 41% with adult-onset CKD; $P = 0.893$; Supplementary Table S3). Similarly, no significant difference was observed in the median age of ESKD onset in patients in whom we established a genetic diagnosis (median age 33 years [range: 6–78 years]; Table 4) versus those who remained genetically unsolved after WES (median age 31 years [range: 5–68 years; $P = 0.651$). These findings are further supported by other groups, who have found no significant difference in genetic diagnosis rates in those with pediatric versus adult-onset disease (46% vs. 40%; Supplementary Table S2).[8]

In 58 of 114 families (51%), no molecular genetic diagnosis was established post-WES (Supplementary Table S4). In monogenic diseases, about 85% of all causative mutations are located within the coding sequence or the adjacent splice sites.[25] The remaining 15% are complex deletion–insertion, copy-number variants, or reside within a promotor or other intronic region. As none of these variants can be detected by WES, this technical limitation may explain why some remain without a molecular diagnosis. Furthermore, WES might miss a subset of causative variants, owing to low coverage in the respective target region. In the current cohort, a mean depth of coverage of 48× was achieved. Specific exonic regions in the 478 known CKD genes not reached by this depth of coverage are outlined in Supplementary Table S5. Mutations in these regions may have been missed by WES analysis.

CONCLUSION

In a select patient cohort presenting with CKD in adulthood, we detected pathogenic mutations in known monogenic CKD genes in more than one-third of families. Detection of monogenic causes of CKD permit molecular genetic diagnosis for patients and families and open avenues for personalized treatment strategies for CKD.

METHODS

Human subjects

This multicenter study enrolled adult patients with CKD presenting to nephrology services in Ireland in a consecutive manner from January 2014 to July 2017, as previously described.[7] Consent for WES was obtained from each individual recruited and was approved by the medical ethics boards at each recruitment site in Ireland. Patients with CKD who had either a positive family history of CKD (78 of 114 families; Supplementary Figure S2A) or extrarenal features (16 of 114 families; Supplementary Figure S2B) were recruited. To assess the effect of familial diagnosis and extrarenal features on the detection rate of a molecular genetic diagnosis, families with CKD that had a negative family history and no extrarenal features were also recruited (20 of 114 families; Supplementary Figure S2C). The clinical diagnosis of CKD was defined pre-WES per the primary nephrologist's referral into one of the following *a priori* clinical diagnosis categories[26]:

- Cystic kidney disease including NPHP, medullary cystic disease, and other renal cystic ciliopathies (12 of 114 families). Patients with autosomal dominant polycystic kidney disease were excluded.
- CAKUT (45 of 114 families), defined as any abnormality of number, size, shape, or anatomic position within the kidneys or urinary tract.
- Chronic GN encompassing membranoproliferative GN, crescentic GN, and hemolytic uremic syndrome (7 of 114 families). Patients with genetically confirmed Alport syndrome and CKD resulting from systemic vasculitis were excluded.
- TIKD with biopsy findings of chronic tubulointersititial nephritis without an obvious precipitating cause (7 of 114 families). Patients with confirmed mutations in *MUC1* and *UMOD* were excluded.
- SRNS, or nephrotic syndrome with biopsy findings of FSGS (7 of 114 families).
- Renal tubulopathies (2 of 114 families).
- CKDU (34 of 114 families) for patients who had small kidneys bilaterally and/or lacked an informative kidney biopsy.

WES and variant calling

WES was performed as previously described.[23,27] Genomic DNA was isolated from blood lymphocytes or saliva samples per standard protocols and subjected to exome capture using SureSelect human exome capture arrays (Agilent Technologies, Santa Clara, CA) followed by next generation sequencing on the Illumina HiSeq sequencing platform (San Diego, CA). Sequence reads were mapped to the human reference genome assembly (NCBI build 37/hg19) using CLC Genomics Workbench (version 6.5.2, CLC bio [https://www.qiagenbioinformatics.com/], Aarhus, Denmark). Variants with minor allele frequencies >1% in the dbSNP (version 147), 1000 Genomes Project, EVS (Exome Variant Server), or gnomAD databases were excluded. For patients referred with an *a priori* clinical diagnosis of nephrotic syndrome, we manually searched for the p.Arg229Gln mutation in the *NPHS2* gene, because this allele occurs[28] at a frequency of >1%. Synonymous and intronic variants not located within splice-site regions were excluded. Retained variants, which included nonsynonymous and splice-site variants, were then analyzed (Supplementary Figures S3 and S4).

Depending on the *a priori* clinical diagnosis, we evaluated WES data for mutations in known CKD genes in the matching disease category (e.g., for *a priori* clinical diagnosis of chronic GN, we examined for mutations in known chronic GN genes; Supplementary Tables S6–S13). If the family remained unsolved for genetic diagnosis or the *a priori* clinical diagnosis was CKDU, we evaluated for mutations in all 478 known CKD genes (Supplementary Figure S5; Supplementary Tables S6–S13). Remaining variants were ranked based on their probable impact on the function of the encoded protein, considering evolutionary conservation among orthologues across phylogeny using ENSEMBL Genome Browser (European Bioinformatics Institute and Wellcome Sanger Institute [Berlin, Germany]) and assembled using Clustal Omega (Conway Institute, Dublin, Ireland), as well as the web-based prediction programs PolyPhen-2 (polymorphism phenotyping v2), SIFT (Sorting intolerant from tolerant), and MutationTaster (Berlin, Germany) (Supplementary Table S14). After filtering using our *a priori* criteria (Supplementary Figures S3 and S4), each mutation was classified per the American

College of Medical Genetics and Genomics guidelines.[29] In each family in which we identified a likely causative monogenic mutation, clinical review was conducted with the referring physician to confirm that the phenotype was concordant with previously reported phenotypes, so-called "reverse phenotyping." Each likely causative monogenic mutation was classified per the guidelines as pathogenic or likely pathogenic. Variants were classified as variants of uncertain significance if there was discordance with previously published phenotypes, the exact variant was not previously reported, and additional familial DNA was not available to perform segregation analysis. Remaining mutations were confirmed in original patient DNA by Sanger Sequencing, with segregation whenever familial DNA was available.

Statistical analysis

Descriptive statistics were expressed using frequencies and proportions. Age at diagnosis of CKD was defined as age of first presentation to a nephrology service with CKD, while age at diagnosis of ESKD was defined as age of commencement of renal replacement therapy (i.e., date of receipt of first kidney transplant or date of commencement of dialysis).

DISCLOSURE

FH is a cofounder and Scientific Advisory Commitee member and holds stock in Goldfinch-Bio. All the other authors declared no competing interests.

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AUTHOR CONTRIBUTIONS

DMC, CK, SLM, PAW, EC, BM, CPM, JSS, MD, CM, CMOS, WDP, MDG, AA, CS, BG, SL, LC, DGdF, JH, PJL, MAL, PJC, and FH recruited patients and gathered detailed clinical information for the study. DMC, SS, NM, MN, ATvdV, HI, FK, CMK, RD, AV, DAB, and FH performed whole exome evaluation and mutation analysis. DMC, CK, SS, NM, RS, TMK, AD, BD, PJC, and FH performed genotype–phenotype correlation. DMC, CK, SLM, AK, KB, and CS recruited patients and extracted DNA for analysis. SS, SMM, and RPL performed whole exome sequencing and downstream data analysis. MAL, PJC, and FH conceived of and directed the study with DMC. DMC, with PJC and FH, wrote the manuscript. All authors approved the final version.

SUPPLEMENTARY MATERIAL

Table S1. Information on pre–whole exome sequencing *a priori* clinical diagnosis in the 14 families in whom we identified a variant of uncertain significance (VUS) in a known monogenic chronic kidney disease gene.
Table S2. Comparison between the outcomes in the current study and those reported in a recent publication.
Table S3. Age of onset of end-stage kidney disease and chronic kidney disease in the solved cohort (i.e., molecular genetic diagnosis established pre–whole exome sequencing) versus the unsolved cohort (i.e., no molecular genetic diagnosis established post–whole exome sequencing).
Table S4. Information on pre–whole exome sequencing *a priori* clinical diagnosis in the 58 families in whom no variants in known monogenic chronic kidney disease genes were identified.
Table S5. Information on 16 of 478 known chronic kidney disease genes that did not achieve a mean coverage of at least 30X.
Table S6. The 96 genes that represent monogenic causes of human cystic kidney disease or nephronophthisis, if mutated, and that were evaluated in the WES data of this study.
Table S7. The 165 genes that represent monogenic causes of human syndromic CAKUT, if mutated, and that were evaluated in the WES data of this study.
Table S8. The 40 genes that represent monogenic causes of human isolated CAKUT, if mutated, and that were evaluated in the WES data of this study.
Table S9. The 20 genes that represent monogenic causes of human chronic glomerulonephritis, if mutated, and that were evaluated in the WES data of this study.
Table S10. The 51 genes that represent monogenic causes of monogenic human renal tubulopathies, if mutated, and that were evaluated in the WES data of this study.
Table S11. The 38 genes that represent monogenic causes of human nephrolithiasis or nephrocalcinosis, if mutated, and that were evaluated in the WES data of this study.
Table S12. The 59 genes that represent monogenic causes of human nephrotic syndrome, if mutated, and that were evaluated in the WES data of this study.
Table S13. The 9 genes that represent rare monogenic causes of human chronic kidney disease (miscellaneous category), if mutated, and that were evaluated in the WES data of this study.
Table S14. Web resources.
Table S15. A. Information on the 14 families in whom pathogenic or likely pathogenic mutations were identified in known monogenic CAKUT genes that were identified post-WES. **B.** Distribution of missense and null mutations, stratified by age at first diagnosis of CKD.
Figure S1. Genetic and clinical data on family P640. Extended family pedigree of family P640—males are indicated by a square; females are indicated by a circle (**A**). Focus of whole exome sequencing analysis—4 individuals were submitted for whole exome analysis (**B**). Final evaluation table post–WES analysis for family P640 (**C**). Kidney biopsy of patient P640_83. Low-power light microscopy showing 3 sclerosed glomeruli, 1 partially sclerosed, and 1 proliferative glomeruli. Direct immunofluorescence demonstrating a glomerulus with granular, mesangial C3. Electron microscopy demonstrating loss of podocytes—red arrow indicates granular subendothelial dense deposits (**D**).
Figure S2. Selection process for the 114 families with CKD for the whole exome sequencing study. *CKD defined for initial enrollment per

NKF KDOQI (National Kidney Foundation Kidney Disease Outcomes Quality Initiative) guidelines as CKD stage 3 or higher (i.e., decreased glomerular filtration rate $<$60 ml/min per 1.73 m^2 for $\geq$3 months) OR renal imaging showing any abnormality in number, shape, size, or location within the kidneys or genitourinary tract (CAKUT) OR renal imaging showing chronically increased echogenicity, loss of corticomedullary differentiation, and/or $\geq$2 renal cysts (cystic kidney disease or NPHP) OR tubulointerstitial pattern of injury on renal biopsy without obvious precipitating cause (TIKD) OR chronic proteinuria resistant to steroid treatment and/or evidence on renal biopsy of FSGS (nephrotic syndrome) OR chronic hematuria and renal biopsy with a pattern of injury consistent with chronic glomerulonephritis OR evidence of biochemical abnormalities indicative of renal tubular dysfunction. CKD defined per the ERA-EDTA (European Renal Association—European Dialysis and Transplant Association) primary renal diagnosis coding system (https://www.era-edta-reg.org).

Figure S3. Variant filtering process for the identification of pathogenic mutations in genes known to cause chronic kidney disease (CKD). Schematic overview of the workflow used for filtering of whole exome sequencing data: (i) keep rare variants present with a minor allele frequency (MAF) $<$1% in healthy control cohorts dbSNP147 (https://www.ncbi.nlm.nih.gov/projects/SNP); (ii) keep nonsynonymous variants and intronic variants that are located within splice sites; (iii) apply known gene approach by selecting all variants detected in known CKD genes (Supplementary Tables S6–S13); (iv) rank remaining variants based on their predicted likelihood of being deleterious for the function of the encoded protein, using Polyphen 2 (http://genetics.bwh.harvard.edu/pph2), SIFT (http://sift.jcvi.org/), and Mutation Taster (http://www.mutationtaster.org); (v) review literature and review with referring physician delineating whether the detected mutation matches the phenotype; (vi) cross-reference with the ACMG (American College of Medical Genetics and Genomics) guidelines to determine if variants are pathogenic, likely pathogenic, or a variant of uncertain significance.

Figure S4. Decision-making strategy to pathogenicity of a variant identified by whole exome sequencing.

Figure S5. Evaluation of whole exome sequencing (WES) data in 114 families with CKD. WES output of the 138 affected individuals was interrogated for mutations in known CKD genes matching the initial referral phenotype in all 114 families with CKD (Supplementary Tables S6–S13). If no likely causative mutation was identified in a known CKD matching the referral phenotype, evaluation for mutations in remaining known CKD genes not matching the referral phenotype was performed. If the *a priori* clinical diagnosis was CKD of unknown etiology, evaluation for mutations in all 478 genes known to cause CKD in humans was performed for the presence of pathogenic or likely pathogenic monogenic mutations (Supplementary Tables S6–S13).

Supplementary material is linked to the online version of the paper at www.kidney-international.org.

REFERENCES

1. Hill NR, Fatoba ST, Oke JL, et al. Global prevalence of chronic kidney disease—a systematic review and meta-analysis. *PLoS One*. 2016;11:e0158765.
2. Go AS, Chertow GM, Fan D, et al. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N Engl J Med*. 2009;351:1296–1305.
3. Gansevoort RT, Correa-Rotter R, Hemmelgarn BR, et al. Chronic kidney disease and cardiovascular risk: epidemiology, mechanisms, and prevention. *Lancet*. 2013;382:339–352.
4. Vivante A, Hildebrandt F. Exploring the genetic basis of early-onset chronic kidney disease. *Nat Rev Nephrol*. 2016;12:133–146.
5. Skrunes R, Svarstad E, Reisaeter AV, et al. Familial clustering of ESRD in the Norwegian population. *Clin J Am Soc Nephrol*. 2014;9:1692–1700.
6. McClellan WM, Satko SG, Gladstone E, et al. Individuals with a family history of ESRD are a high-risk population for CKD: implications for targeted surveillance and intervention activities. *Am J Kid Dis*. 2009;53(suppl 3):S100–S106.
7. 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;130:293–301.
8. Mallett AJ, McCarthy HJ, Ho G, et al. Massively parallel sequencing and targeted exomes in familial kidney disease can diagnose underlying genetic disorders. *Kid Int*. 2017;92:1493–1506.
9. Lata S, Marasa M, Li Y, et al. Whole-exome sequencing in adults with chronic kidney disease: a pilot study. *Ann Intern Med*. 2018;168:100–109.
10. Neild GH. Primary renal disease in young adults with renal failure. *Nephrol Dial Transplant*. 2010;25:1025–1032.
11. Malone AF, Phelan PJ, Hall G, et al. Rare hereditary COL4A3/COL4A4 variants may be mistaken for familial focal segmental glomerulosclerosis. *Kid Int*. 2014;86:1253–1259.
12. Spada M, Pagliardini S, Yasuda M, et al. High incidence of later-onset Fabry disease revealed by newborn screening. *Am J Hum Genet*. 2006;79:31–40.
13. Nozu K, Iijima K, Kanda K, et al. The pharmacological characteristics of molecular-based inherited salt-losing tubulopathies. *J Clin Endocrinol Metab*. 2010;95:E511–E518.
14. Barker DF, Denison JC, Atkin CL, Gregory MC. Efficient detection of Alport syndrome COL4A5 mutations with multiplex genomic PCR-SSCP. *Am J Med Genet*. 2001;98:148–160.
15. Frémeaux-Bacchi V, Miller EC, Liszewski MK, et al. Mutations in complement C3 predispose to development of atypical hemolytic uremic syndrome. *Blood*. 2008;112:4948–4952.
16. Mallett A, Patel C, Salisbury A, et al. The prevalence and epidemiology of genetic renal disease amongst adults with chronic kidney disease in Australia. *Orphanet J Rare Dis*. 2014;9:98.
17. Kohl S, Hwang DY, Dworschak GC, et al. Mild recessive mutations in six Fraser syndrome-related genes cause isolated congenital anomalies of the kidney and urinary tract. *J Am Soc Nephrol*. 2014;25:1917–1922.
18. Bergmann C, Senderek J, Windelen E, et al. Clinical consequences of PKHD1 mutations in 164 patients with autosomal-recessive polycystic kidney disease (ARPKD). *Kid Int*. 2005;67:829–848.
19. Ward CJ, Hogan MC, Rossetti S, et al. The gene mutated in autosomal recessive polycystic kidney disease encodes a large, receptor-like protein. *Nat Genet*. 2002;30:259.
20. Adeva M, El-Youssef M, Rossetti S, et al. Clinical and molecular characterization defines a broadened spectrum of autosomal recessive polycystic kidney disease (ARPKD). *Medicine (Baltimore)*. 2006;85:1–21.
21. Vivante A, Hwang DY, Kohl S, et al. Exome sequencing discerns syndromes in patients from consanguineous families with congenital anomalies of the kidneys and urinary tract. *J Am Soc Nephrol*. 2017;28:69–75.
22. van der Ven AT, Shril S, Ityel H, et al. Whole-exome sequencing reveals FAT4 mutations in a clinically unrecognizable patient with syndromic CAKUT: a case report. *Mol Syndromol*. 2018;8:272–277.
23. van der Ven AT, Connaughton DM, Ityel H, et al. Whole-exome sequencing identifies causative mutations in families with congenital anomalies of the kidney and urinary tract. *J Am Soc Nephrol*. 2018;29:2348–2361.
24. Gross O, Weber M, Fries JW, Müller GA. Living donor kidney transplantation from relatives with mild urinary abnormalities in Alport syndrome: long-term risk, benefit and outcome. *Nephrol Dial Transplant*. 2008;24:1626–1630.
25. Lupski JR, Reid JG, Gonzaga-Jauregui C, et al. Whole-genome sequencing in a patient with Charcot–Marie–Tooth neuropathy. *N Engl J Med*. 2010;362:1181–1191.
26. Postorino M, Limido A, Teatini U, et al. The new ERA-EDTA codes for primary kidney diseases. *G Ital Nefrol*. 2013;30:pii:gin/30.3.15.
27. Gee HY, Otto EA, Hurd TW, et al. Whole exome resequencing distinguishes cystic kidney diseases fromphenocopies in renal ciliopathies. *Kid Int*. 2014;85:880–887.
28. Machuca E, Hummel A, Nevo F, et al. Clinical and epidemiological assessment of steroid-resistant nephrotic syndrome associated with the NPHS2 R229Q variant. *Kid Int*. 2009;75:727–735.
29. Richards S, Aziz N, Bale S, et al. 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;17:405.