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Monogenic causes of chronic kidney disease in adults

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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.

DISCLOSURE

No conflict of interests

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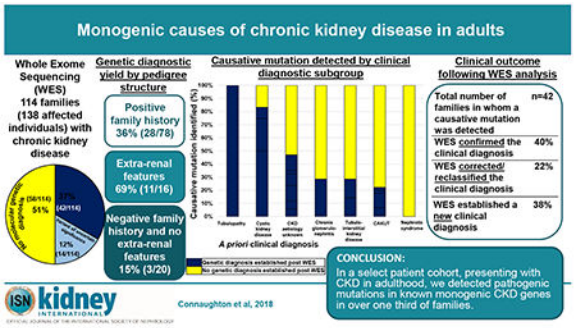
Abstract

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.

Abstract



Keywords

chronic kidney disease; genetic kidney disease; whole exome sequencing

INTRODUCTION

The estimated global prevalence of chronic kidney disease (CKD) is 11 to 13%.[1] CKD is associated with high morbidity and resource utilisation.[2] Mounting evidence highlights the urgency for early diagnosis and intervention, to stem the sequelae of elevated cardiovascular risk and to delay progression to end stage kidney disease (ESKD).[3] Monogenic causes of CKD in childhood are well established[4], whereas very little data exists on monogenic causation of CKD in adults. In 34% of adults with CKD a positive family history is reported which suggests genetic causation.[5–7] However, genetic testing for adults is still not routinely performed in clinical practice. Panel sequencing of CKD genes directed towards specific diagnostic groups has revealed a genetic disorder in up to 43% of patients.[8] Using whole exome sequencing (WES), a single centre study demonstrated that a monogenic disease-causing gene can be identified in 24% of adults with CKD.[9]

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

The estimated prevalence of CKD – aetiology unknown (CKDU) is 10-36% 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 can still be uninformative, as advanced CKD can result in histological findings that are indistinguishable between multiple diseases.[11] We also hypothesise that WES may be especially useful in patients with CKDU. Establishing a molecular diagnosis in patients with CKDU can therefore have resulting consequences 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 CKD (138 affected individuals). The median age at time of recruitment was 48 years [range 180-85 years], with a slight male predominance (70/138, 51%, Table 1). We detected a molecular genetic diagnosis in 42 of the 114 families (37%) (Figure 1 A, navy blue segment). The genetic diagnostic rate varied by recruitment group (Figure 2). We detected mutations across a diverse spectrum of known monogenic CKD genes encompassing mutations in 29 different genes (Table 2, Figure 3). These categories included cystic kidney disease genes (8/42 families, Figure 1 B, red segment), syndromic congenital anomalies of the kidney and urinary tract (CAKUT) genes (8/42 families, Figure 1 B, light blue segment), isolated CAKUT genes (6/42 families, Figure 1 B, dark blue segment), chronic glomerulonephritis (GN) genes (5/42 families, Figure 1 B, orange segment), tubulo-interstitial kidney disease (TIKD) genes (4/42 families, Figure 1 B, brown segment), renal tubulopathy genes (4/42, Figure 1 B, purple segment), nephrolithiasis/ nephrocalcinosis (NLNC) genes (4/42 families, Figure 1 B, pink segment),

steroid resistant nephrotic syndrome (SRNS) genes (2/42 families, Figure 1 B, green segment), and Fabry disease genes (1/42 families, Figure 1 B, cream segment).

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

In families with *a priori* clinical diagnosis of cystic kidney disease (12/114), we detected a pathogenic mutation in ten of 12 families (83%). In six 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*; P231and P317, *PKHD1*). In four families, we detected mutations in CKD genes known to phenocopy cystic kidney disease. This 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 one family, WES identified a likely pathogenic mutation in the gene *GLA*, previously reported in patients with Fabry disease (Table 2, B2327, cream segment).[12]

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

For families with CAKUT (45/114 families), we detected mutations in ten of 45 families (22%). Five families had mutations in isolated CAKUT genes (Table 2, dark blue segment, P306, *HNF1B*; B2482, *UPK3A*; P69 and P307, *PAX2*; P162, *FREM2*), while three families had mutations in syndromic CAKUT genes (Table 2, light blue segment, B2330, *PROKR2*; B2481, *TBX3*; B2463, *FBN1*). In three of the families in whom we detected mutations in syndromic CAKUT genes, extra-renal features were present on clinical review that were concordant with the corresponding molecular genetic diagnosis (Table 2, column 5). In two families, we identified mutations in non-CAKUT genes (Table. 2 B2457, *AQP2,* purple segment and B2427, *COL4A3,* orange segment). The molecular genetic diagnosis in these two families was discordant with the clinical diagnosis.

Detection of a molecular genetic diagnosis in families with an *a priori* clinical diagnosis of chronic glomerulonephritis (GN)

In two of the seven families referred with chronic GN (7/114), we detected mutations in genes known to be causative of 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 the correction of the clinical diagnosis from GN to FSGS.

Detection of a molecular genetic diagnosis in families with an *a priori* clinical diagnosis of tubulo-interstitial kidney disease (TIKD)

Within the TIKD cohort (7/114), we established a molecular genetic diagnosis in two of seven families (29%). In family B2337, both siblings presented with CKD and gout at 42 years. Examination of renal biopsy specimens in both showed evidence of tubulo-interstitial nephritis. The molecular genetic diagnosis confirmed hyperuricaemic nephropathy with detection of a causative mutation in *UMOD* (Table 2 B2337, brown segment). In family

B2342, the molecular genetic diagnosis facilitated a clinical review of two siblings presenting with CKD and diabetes mellitus in adulthood. Both affected siblings had renal biopsy findings of tubulo-interstitial nephritis, while one 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 B2342, dark blue segment).

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

Of the seven 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 two 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 both with features of Bartter syndrome and microscopic hematuria. Following WES, we detected a second mutation in the Alport gene *COL4A5* (Table 2, purple and orange segment). 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 the *a priori* clinical diagnosis (Figure 2). In nine of the 42 families (22%), the molecular genetic diagnosis resulted in correction of the clinical diagnosis, while in 16 families with CKD – aetiology unknown" (38%), WES established a new molecular genetic diagnosis (Table 3).

WES corrected the *a priori* clinical diagnosis

In nine of 42 solved families (22%), WES corrected the clinical diagnosis (Table 3). As an 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. Following WES, we detected a heterozygous *AQP2* mutation (Table 2, purple segment). On review post WES, the patient had initially presented as an infant in the 1970s with polyuria, vomiting and hypernatremia and subsequent bilateral renal vein thrombosis. This reverse phenotyping confirmed the molecular genetic diagnosis of nephrogenic diabetes insipidus by WES.

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). Due to the lack of a family history and absence of a renal biopsy specimen, the clinical diagnosis of autosomal dominant Alport syndrome had not been suspected initially. This 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 segment, Supplementary 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. Following WES, the molecular genetic diagnosis of FSGS due to a dominant heterozygous mutation in *INF2* was established in P640_82 and P640_83. Interestingly, an additional finding of a dominant heterozygous variant in *C3* was also identified in P640_82 with ESKD and P640_2008 without ESKD, both of whom were hypocomplementaemic. Mutations in this gene can result in complement dysregulation characterised by low C3 levels thereby increasing susceptibility to atypical haemolytic uraemic syndrome.[15]

WES established a new clinical diagnosis families with "CKD – aetiology unknown"

In families referred with CKDU (34 of 114 families, 30%), we detected a pathogenic mutation in 16 of 34 families (47%). This represents 38% of the solved cohort (16 of 42 solved families) (Table 4). The molecular genetic diagnoses in these families included cystic kidney disease or NPHP (P322, *DYNC2H1;* P105, *NPHP1;* Table 2, red segment), syndromic CAKUT (P198, *WFS1 and* B2479, *FANC1*, Table 2, light blue segment), Alport syndrome (B2347 *COL4A3*, and P241, P58, P100, *COL4A5,* Table 2, orange segment), TIKD (P193 & P232, *UMOD;* P88, *FAN1,* Table 2, brown segment), hypertensive renal disease (B2467, *WNK4,* Table 2, purple segment), and nephrocalcinosis/nephrolithiasis (B2344, *SLC3A1;* P318, *OCRL*; P182 and B2340, *CLCN5*, Table 2, pink segment). None of the above disease–causing mutations were 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 (VUS)

In 12% of families (14/114) 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, either due to lack of clinical evidence to perform adequate genotype-phenotype correlation or lack 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 extra-renal features (11/16 families, 69%). In families with a positive family history, we obtained a molecular genetic diagnosis in 36% (28/78 families). In families with a negative family history and no extra-renal features, monogenic causation was observed in 15% (3/20 families) (Figure 2). No significant difference was observed in the median age of reaching ESKD in individuals in whom we established a molecular diagnosis (33 years, range 6-78 years, Table 4) versus individuals in whom no molecular diagnosis was established (31 years, range 5-68 years, p=0.955).

DISCUSSION

In this large multicentre study, we systematically evaluated the utility of WES in a cohort of adults with CKD. We established a molecular genetic diagnosis following WES in 42 of 114 (37%) families with CKD attending nephrology services in Ireland. A genetic diagnosis was established in 69% (11/16) of families with extra-renal features, 36% (28/78) of families with familial nephropathy, while in families negative for both family history and extra-renal features, monogenic causation was observed in 15% (3/20). It has previously been estimated that ~10% of all adults with CKD have an underlying genetic aetiology.[16] Recently, a higher prevalence of 24% for monogenic causation was reported following WES.[9] In this single centre study, Lata et al. recruited 92 patients with either a family history of CKD, undiagnosed CKD or a clinical suspicion of genetic kidney disease due to childhood onset CKD. We observed comparable rates of confirmation, correction, and establishment of a new clinical diagnosis post WES (Table 3). More recently, Mallett demonstrated, using targeted exomic sequencing, a genetic diagnostic rate of 43% in patients with familial renal disease.[8] Akin to our findings, the genetic diagnostic rate was similar in those with pediatric onset disease and adult onset disease (Supplementary Table S2). Together, these data provide compelling evidence that monogenic disease causation is under-recognised in adults with CKD, and WES can be utilised to provide a monogenic aetiologic diagnosis in adults with CKD.

Our data highlights that mutations in genes classically described as “childhood” CKD genes can also be identified in adults. We hypothesise 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 (ARPKD) has classically been characterised as a childhood onset nephropathy, with few cases of ESKD observed beyond 40 years.[18] We identified recessive missense mutations in the *PKHD1* gene in two unrelated families with onset of ESKD >40 years (Table 2, red segment, P231 and P317). Patient P317_48, presented with “CKD – aetiology unknown” age 46 years, progressing to ESKD at 52 years. A compound heterozygous missense mutation in *PKHD1* was identified by WES (a novel c.2702A>C, p.Asn901Thr variant and a previously reported c.107C>T p.Thr36Met variant[18]). Recently, it has been shown that compound heterozygous mutations that involve at least one 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 with CKD.

The estimated prevalence of CKDU is 10-36% in adults,[7, 10] with a prevalence of 30% (34/114) in the current cohort. By employing WES, we were able to establish a molecular genetic diagnosis in almost half of families with CKDU (16/34, 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 where renal ultrasound and kidney biopsies were uninformative, we detected pathogenic mutations across a diverse spectrum of known monogenic causes of CKD including cystic kidney disease (2/16 families, Table 2, red segment), CAKUT (2/16 families, Table 2, blue segment), chronic GN (4/16 families, Table 2, orange segment), TIKD (3/16 families, Table 2, brown segment), renal tubulopathy (1/16, Table 2, purple segment), and nephrolithiasis/nephrocalcinosis

(4/16 families, Table 2, pink segment) (Figure 3). Given that all these patients would have remained without a clinical diagnosis, these findings are significant.

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 extra-renal manifestations (69%).[21] As demonstrated in our cohort, mild extra-renal features are commonly unrecognised 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 in childhood cohorts[23], and our data shows that this holds relevance in adults with CKD. Identification of specific pathogenic mutations can also facilitate screening for otherwise undetected extra-renal features. For example, identifications of mutations in the gene *HNF1B,* has allowed for screening for associated extra-renal features such as diabetes, facilitating early lifestyle intervention strategies and avoidance of pro-diabetic medications in the post-transplant period (P306 and B2342, Table 2, blue segment).

Unnecessary diagnostic interventions can, in certain cases, be avoided following establishment of a molecular genetic diagnosis. This was particularly evident in cases where the pre-test probability of obtaining a diagnosis is low such as occurs in patients presenting with bilateral small kidneys not amenable to biopsy. This was the case for family P88 in whom we detected a pathogenic mutation in the gene *FAN1,* where 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 anti-proteinuric medication, avoidance of systemic immunosuppression and negate the need for a non-diagnostic kidney biopsy.

Employing WES can allow for establishment of a more precise molecular genetic diagnosis in families with 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), while detection of a second variant in the gene *C3* may explain the observation of complement dysregulation in other family members (Table 2, P640_2008 and P640_82). In patient B2453_80 with Bartter syndrome, clinical heterogeneity was evident at clinical presentation which remained unresolved prior to WES. This patient presented with microscopic hematuria, a finding which was resolved following 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 anti-proteinuric 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 when 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 predominantly Irish ethnicity, thereby reducing generalisability to other populations. Second, our cohort had a higher prevalence of familial CKD (78/114, 68%, Figure 2)

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

In 58 of 114 families (51%) no molecular genetic diagnosis was established following 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 might explain why some remain without a molecular diagnosis. Furthermore, WES might miss a subset of causative variants due 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, which did not reach 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 over one third of families. Detection of monogenic causes of CKD permit molecular genetic diagnosis for patients and families, and open avenues for personalised treatment strategies for CKD.

METHODS

Human subjects

This multi-centre study enroled 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 approved by the medical ethics boards at each recruitment site in Ireland. Patients with CKD who had either a positive family history of CKD (Supplementary Figure S2 A, 78/114 families) or extra-renal features (Supplementary Figure S2 B, 16/114 families) were recruited. To assess the effect of familial diagnosis and extra-renal features on detection rate of a molecular genetic diagnosis, families with CKD with a negative family history and no extra-renal features were

also recruited (Supplementary Figure S2 C, 20/114 families). The clinical diagnosis of CKD was defined pre-WES as per the primary nephrologist's referral into one of the following *a priori* clinical diagnoses[26]:

- Cystic kidney disease including nephronophthisis (NPHP), medullary cystic disease, or other renal cystic ciliopathies (12/114 families). Patients with autosomal dominant polycystic kidney disease (ADPKD) were excluded.
- Congenital anomalies of the kidney and urinary tract (CAKUT) (45/114 families) defined as any abnormality of number, size, shape, or anatomical position within the kidneys or urinary tract.
- Chronic glomerulonephritis (GN) encompassing membranoproliferative GN (MPGN), crescentic GN, and haemolytic uraemic syndrome (7/114 families). Patients with genetically confirmed Alport syndrome and CKD due to systemic vasculitis were excluded.
- Tubulo-interstitial kidney disease (TIKD) with biopsy findings of chronic tubulo-intersititat nephritis without an obvious precipitating cause (7/114 families). Patients with confirmed mutations in *MUC1* and *UMOD* were excluded.
- Steroid resistant nephrotic syndrome (SRNS), or nephrotic syndrome with biopsy findings of focal segmental glomerulosclerosis (FSGS) (7/114 families).
- Renal tubulopathies (2/114 families)
- "CKD – aetiology unknown" (CKDU) (34/114 families) where patients had small kidneys bilaterally and/or lacked an informative kidney biopsy.

Whole exome sequencing and variant calling

WES was performed as previously described.[23, 27] Genomic DNA was isolated from blood lymphocytes or saliva samples as per standard protocols and subjected to exome capture using Agilent SureSelect™ human exome capture arrays (Life technologies™) followed by next generation sequencing on the Illumina HiSeq™ sequencing platform. Sequence reads were mapped to the human reference genome assembly (NCBI build 37/hg19) using CLC Genomics Workbench™ (version 6.5.2, CLC bio, Aarhus, Denmark). Variants with minor allele frequencies >1% in either dbSNP (version 147), 1,000 Genomes Project, EVS and 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, since this allele occurs at a frequency of >1%.[28] Synonymous and intronic variants not located within splice site regions were excluded. Retained variants, which included non-synonymous and splice site variants, were then analysed (Supplementary Figure 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 (i.e. *a priori* clinical diagnosis of chronic GN, we examined for mutations in known chronic GN genes, Supplementary Table S6–S13). If the family remained unsolved 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 and assembled using Clustal Omega, as well as the web-based prediction programmes PolyPhen-2, SIFT, and MutationTaster (Supplementary Table S14). Following filtering using our *a priori* criteria (Supplementary Figure S3 and S4), each mutation was then classified as per the American College of Medical Genetics and Genomics (ACMG) guidelines.[29] In each family in whom we identified a likely causative monogenic mutation, clinical review with the referring physician was conducted to confirm that the phenotype was concordant with previously reported phenotypes, so called "reverse phenotyping". Each likely causative monogenic mutation was classified as per the ACMG guidelines as pathogenic or likely pathogenic. Variants were classified as variants of uncertain significance (VUS) 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, 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 1st presentation to a nephrology service with CKD, while age at ESKD was defined as age of commencement of renal replacement therapy (i.e. date of receipt of 1st kidney transplant or date of commencement of dialysis).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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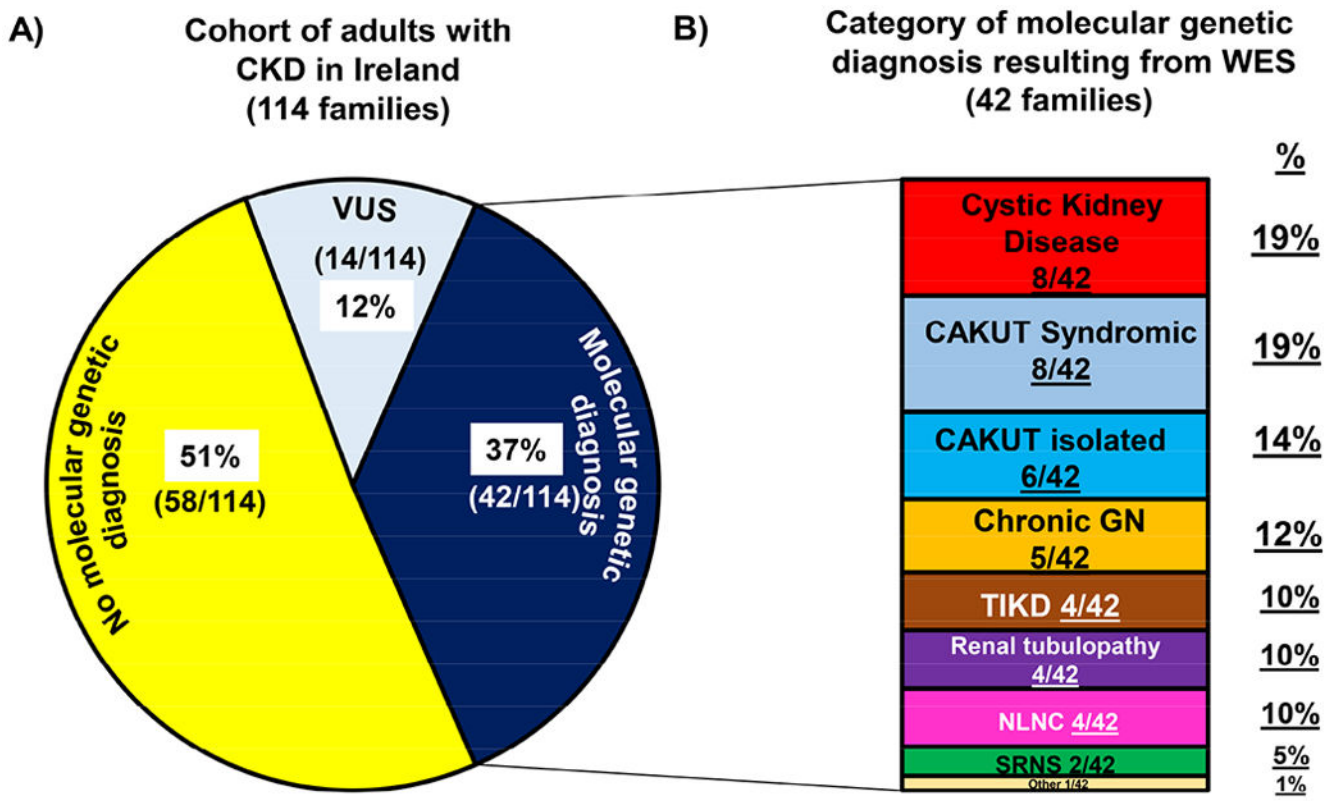

Figure 1. Percentage of the 114 families in Ireland with CKD in whom whole exome sequencing (WES) established a molecular genetic diagnosis (i.e. a pathogenic or likely pathogenic monogenic mutation in a known CKD gene was detected following WES)

(A) The 37% of families (42/114) in whom a pathogenic or likely pathogenic mutation in a known CKD disease gene was detected (i.e. molecular genetic diagnosis established following WES) is denoted by a navy blue colour. The 12% of families (14/114) in whom a variant of uncertain significance (VUS) in a known CKD gene was detected, is denoted by the light blue colour. Yellow colour indicates that no meaningful genetic variant could be detected in a known CKD gene following WES (i.e. no molecular genetic diagnosis established following WES).

(B) 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 colour represents a different molecular genetic diagnostic group.

i) Mutations in known cystic kidney disease including nephronophthisis genes (red)
ii) Mutations in known syndromic CAKUT genes (light blue)
iii) Mutations in known isolated CAKUT genes (dark blue)
iv) Mutations in known chronic glomerulonephritis (GN) genes (orange)
v) Mutations in known tubulo-interstitial kidney disease (TIKD) genes (brown)
vi) Mutations in known renal tubulopathy genes (purple)
vii) Mutations in known nephrolithiasis/nephrocalcinosis (NLNC) genes (pink)
viii) Mutations in known steroid resistant nephrotic syndrome (SRNS) genes (green)

ix) Mutations in known rare chronic kidney disease genes (miscellaneous category) (cream) identified

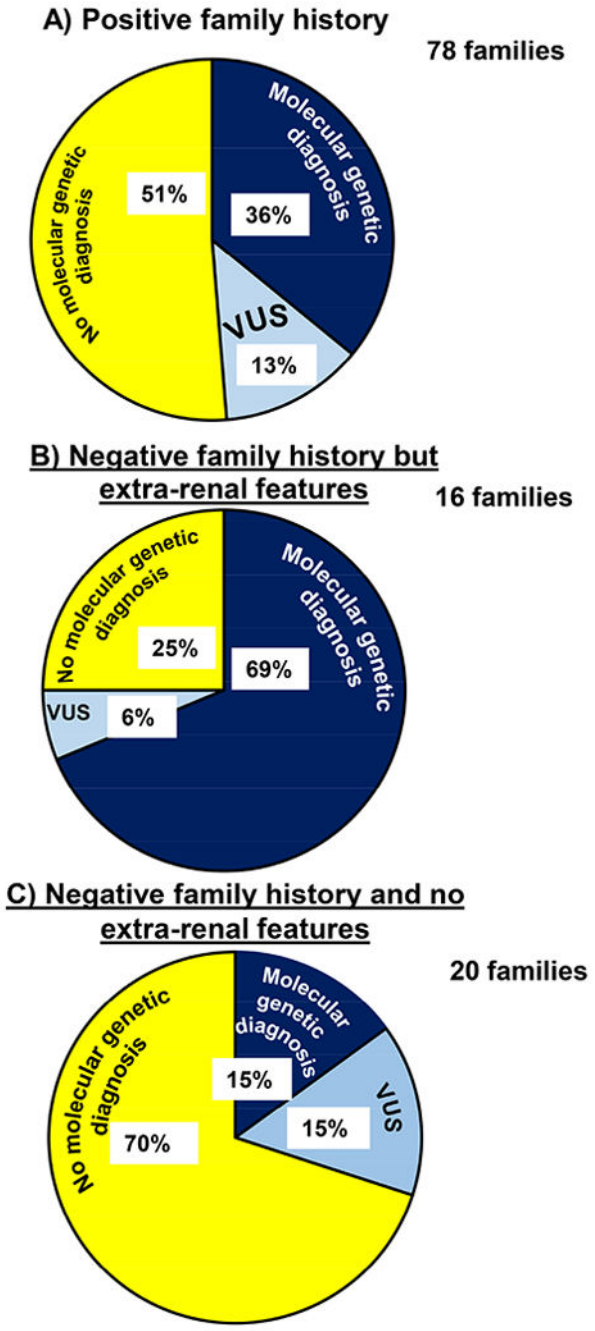

Figure 2. Percentage of the 114 families in Ireland with CKD in whom whole exome sequencing established a molecular genetic diagnosis (i.e. a pathogenic or likely pathogenic monogenic mutation in a known CKD gene was detected following WES) stratified by recruitment group

Navy blue colour denotes families in whom a pathogenic or likely pathogenic mutation in a known CKD gene was detected (i.e. molecular genetic diagnosis established following WES). Light blue colour denotes families in whom we identified a variant of uncertain significance (VUS) in a known CKD gene following WES. Yellow colour indicates that no meaningful genetic variant could be detected in a known CKD gene following WES (i.e. no molecular genetic diagnosis established following WES).

A) Positive family history cohort denotes families with CKD who report CKD in either a 1st or 2nd degree relative (78/144 families)

B) Negative family history but extra-renal features cohort (16/114 families)

C) Negative family history and no extra-renal features cohort (20/114 families)

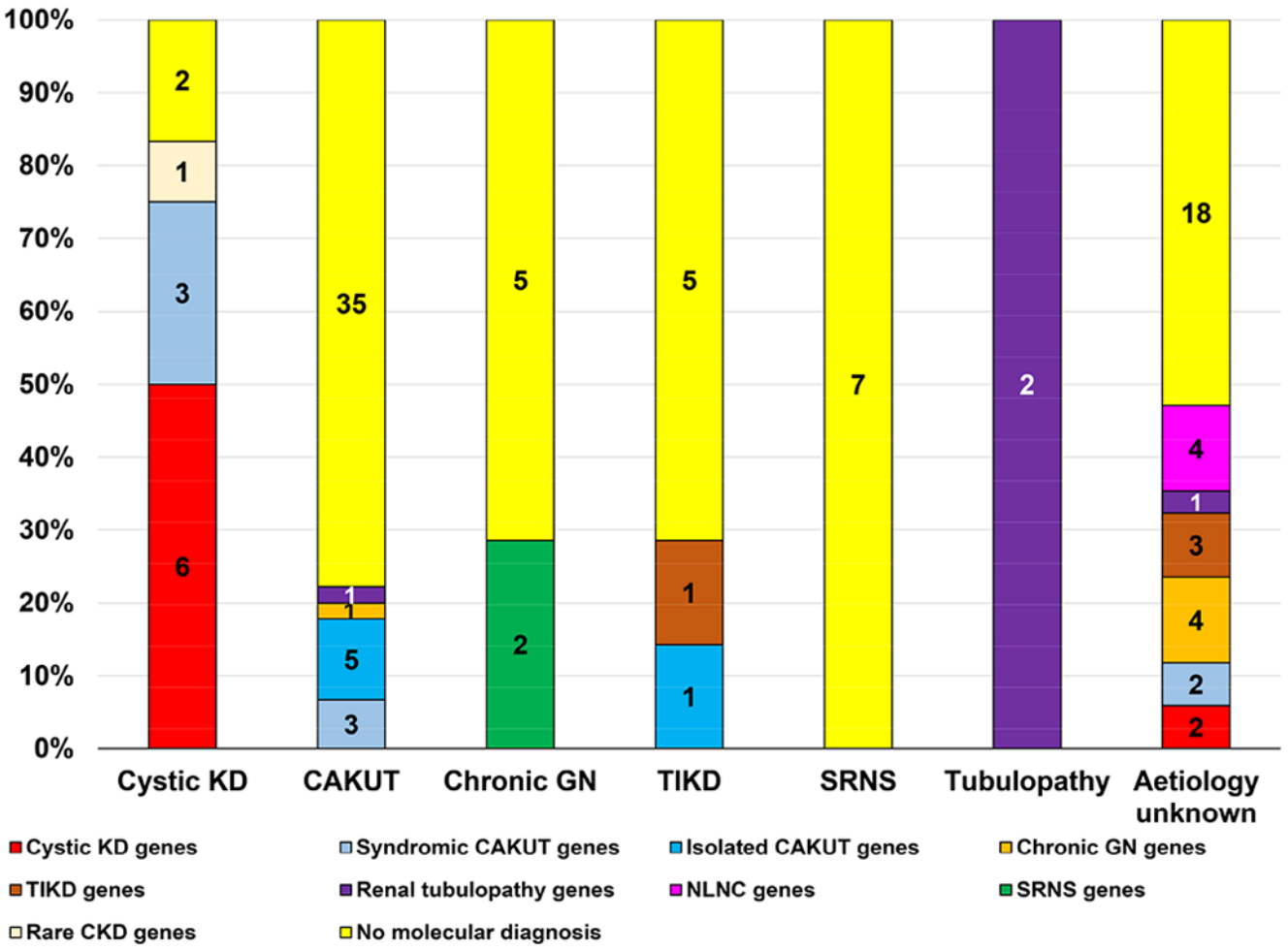

Figure 3. Specific mutation category identified by whole exome sequencing in 114 families with chronic kidney disease

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), tubulo-interstitial kidney disease (TIKD), steroid resistant nephrotic syndrome (SRNS), renal tubulopathy (tubulopathy) and CKD - aetiology unknown. The Y-axis displays the molecular genetic diagnosis established following whole exome sequencing and includes the following:

i) Mutations in known cystic kidney disease including nephronophthisis genes (red) identified

ii) Mutations in known syndromic CAKUT genes (light blue) identified

iii) Mutations in known isolated CAKUT genes (dark blue) identified

iv) Mutations in known chronic glomerulonephritis (GN) genes (orange) identified

v) Mutations in known tubulo-interstitial kidney disease (TIKD) genes (brown) identified

vi) Mutations in known renal tubulopathy genes (purple) identified

vii) Mutations in known steroid resistant nephrotic syndrome (SRNS) genes (green) identified

viii) Mutations in known nephrolithiasis/nephrocalcinosis (NLNC) genes (pink) identified

ix) Mutations in known rare chronic kidney disease genes (miscellaneous category) (cream) identified

x) No molecular genetic diagnosis established following WES (yellow)

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 but extra-renal features		Negative family history and no extra-renal features	
Individuals (Families)	**138 (114)**		**102 (78)**		**16 (16)**		**20 (20)**	
***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 aetiology 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%**
Onset of CKD[a] (years)								
<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%**
Onset of ESKD[b] (years)								
<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,** the clinical diagnosis of chronic kidney disease defined pre-WES as per the primary nephrologist's referral; **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**, tubulo-interstitial kidney disease

[a] age of 1st presentation to medical services with evidence of CKD

[b] age at commencement of renal replacement therapy i.e. dialysis or kidney transplant

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Table 2.

Information on pre-whole exome sequencing a priori clinical diagnosis and post- whole exome sequencing 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. All families with a molecular genetic diagnosis were classified as pathogenic or likely pathogenic as 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 were classified as either pathogenic or likely pathogeni

Fam. ID	Ind. ID	*A priori* clinical Dx pre-WES (PRD code[T])	Reported clinical phenotype	Extra-renal features	Age at 1st Dx of CKD ESKD [years]	Sex	Inclusion criteria[a]	Molecular genetic Dx post WES #OMIM[b]	Genotype (Inheritance)	WES confirm clinical Dx[c]	WES correct clinical Dx[c]	WES establish 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 sibs=het	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.*)		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 sib=het	/	/	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		/	/	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.5221 G>A p.Val1741 Met (*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.5221 G>A p.Val1741 Met (*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	Nephronophthisi s 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.Met111 Arg (*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 hydronephrosis/ureter, neurogenic bladder R kidney 22cm L kidney 35cm	Height 195cm Joint hypermotility **Saddle nose Gum hypertrophy Downslantina palpebral fissures Hiah arch palate Hammer toes Pes planus**	0 CKD only	F	Extrarenal	Marfan syndrome (Syndromic CAKUT) # 154700	***FBN1* (AD)**	✓			c.4888C>T p.Gin 1630*	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/

Fam. ID	Ind. ID	*A priori* clinical Dx pre-WES (PRD code[PP])	Reported clinical phenotype	Extra-renal features	Age at 1st Dx of CKD ESKD [years]	Sex	Inclusion criteria[a]	Molecular genetic Dx post WES #OMIM[b]	Genotype (Inheritance)	WES confirm clinical Dx[c]	WES correct clinical Dx[c]	WES establish 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]
B2454	13	NPHP(2836)	PresumedNPHP 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 intellectualdisability **Macrocephaly Hyperkeratosis Lentiaines**	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		20 CKD only	F	Fam Hx		***MAP2K2* (AD)**				c.692G>T p.Arg231 Leu (*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 FSGSquery secondary)	Gout Retinitis Pigmentosa Anemia Diabetes mellitus **Pseudotumour cerebri**	2 15	M	Extrarenal	Fanconi anemia, complementation group I # 609053	***FANCI* (AR)**			✓	c.217A>T p.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.491 C>A p.Thrl 64Asn (*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 sibs=het Unaff sibs=WT	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.*)		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/
P162	99	CAKUT (1618)	VUR	/	50 51	M	Fam Hx	Fraser Syndrome # 617666	***FREM2* (AR)**	✓			c.3661 C>T p.Pro1221Ser (*C.i.*)	Comp. het Aff sib = comp. het, Unaff sib=single het	1.00 Del. D.C.	0/16/277168	Likely Path. Gene/
									FREM2 **(AR)**				c.2533C>T p.His845Tyr (*D.r.*)		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 BLRHD	/	3 CKD only	M	Neither	Alport syndrome # 104200	***COL4A3* (AD)**		✓		c.4981 C>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)	Haematuria 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+1 G>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	**Hearina 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	Microscopic haematuria (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	ChronicGN (1377)	Microscopic hematuria Normal renal Bx	Low complement (C3) levels	20 Normal renal function	M	Fam Hx	Susceptibility to atypical haemolytic uraemic 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 Unaff. Ma=WT Unaff. sib=WT	0.65 Del. D.C.	0/2/246248	Likely Path. Gene/

Kidney Int. Author manuscript; available in PMC 2020 April 01.

Fam. ID	Ind. ID	*A priori* clinical Dx pre-WES (PRD code[a])	Reported clinical phenotype	Extra-renal features	Age at 1st Dx of CKD ESKD [years]	Sex	Inclusion criteria[a]	Molecular genetic Dx post WES #OMIM[b]	Genotype (Inheritance)	WES confirm clinical Dx[c]	WES correct clinical Dx[c]	WES establish 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]
	82	ChronicGN (3749)	No renal Bx performed	Low complement (C3) levels	20 30	M	Fam Hx		***C3* (AD)**				c.4534C>T p.Arg1512Cys (*M.m.*)		0.65 Del. D.C.	0/2/246248	Likely Path. Gene/
TUBULO-INTERSTITIAL 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.Ala461 Glu (*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 dysfunctionNon-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 Unaff. sibs=het	/	0/1/246242	Path. Gene/
													c.2774_2775delTT p.Leu925fs		/	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)	BLRHD **BL renal vein thrombosis Hypernatremia/Dehydration**	/	0 10	M	Neither	Nephrogenic diabetes insipidus # 125800	***AQP2* (AD)**		✓		c.782C>T p.Ser261 Leu (*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 (SuDDlementary 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 Finaer and wrist swell ing 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	**Hypophosphatemia 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 (Supplemental Table S12)																	
KF4	16	Chronic GN (1377)	Renal Bx indeterminate	/	74 78	F	Fam Hx	Focal segmental glomerulosclerosis # 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.
P640**	83	Chronic GN (1349)	Renal Bx-indeterminate	Normal complement (C3) levels	20 23	F	Fam Hx	Focal segmental glomerulosclerosis # 613237	***INF2* (AD)**		✓		c.353T>A p.Ile1 18Asn(*D.r.*)	het Aff. Fa=het Aff. sib=het Unaff. Ma=WT Unaff. sib=WT	1.00 Del. D.C.	/	Likely Path. Gene/
	82	ChronicGN (3749)	Renal Bx ND	Low complement (C3) levels	20 30	M	Fam Hx		***INF2* (AD)**				c.353T>A p.Ile1 18Asn (*D.r.*)		1.00 Del. D.C.	/	Likely Path. Gene/
RARE MONOGENIC CKD genes (MISCELLANEOUS CATEGORY) (Supplementary Table S13)																	

Fam. ID	Ind. ID	*A priori* clinical Dx pre-WES (PRD code[Ŧ])	Reported clinical phenotype	Extra-renal features	Age at 1st Dx of CKD ESKD [years]	Sex	Inclusion criteria[a]	Molecular genetic Dx post WES #OMIM[b]	Genotype (Inheritance)	WES confirm clinical Dx[c]	WES correct clinical Dx[c]	WES establish 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]
B2327	66	Cystic KD (2794)	Normal size cystic kidneys	Liver dysfunction **Unexplained seizures, L facial weakness without definite patholoav noted on brain imaaina, unexplained abdominal symptoms, photophobia**	1 6	F	Extrarenal	Fabry Disease # 301500	***GLA* (XL)**		✓		c.352C>T p.Argl 18Cys het($X.t.^2$)	het Fa=NA, Ma=NA	0.99 Del. P.M.	0/15/43/200247	Likely Path. DM Conflicting

***A priori* clinical diagnosis,** the clinical diagnosis of chronic kidney disease defined pre-WES as per the primary nephrologist's referral; **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); **unknown**, CKD - aetiology unknown; **T**, thymine; **TIKD**, tubulo-interstitial kidney disease; **TIN**, tubulo-interstitial nephritis; **Tol.**, Tolerated; **VUR**, vesico-ureteric reflux; **VUS**, variant of uncertain significance; **WES**, whole exome sequencing; **WT**, wild type; **XL**, X-linked; **XLD**, X-linked dominant.

Additional clinical features established post WES, following clinical re-review in full cognizance of the molecular genetic diagnosis are highlighted in **bold, underlined text** in columns 4 & 5.

** indicates additional finding in another category;

* nonsense mutation;

Ŧ, ERA-EDTA primary renal disease codes (https://www.era-edta-reg.org/prd.jsp); /, data not available.

[a] Inclusion criteria; Fam Hx, positive family history; Extra-renal, CKD with extra-renal features; Neither, no family history and no extra-renal features

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

[c] Outcome following WES: WES confirm clinical Dx, WES confirmed the clinical diagnosis; WES correct clinical Dx, WES resulted in reclassification/ correction of the clinical diagnosis; WES establish new clinical Dx, WES resulting in establishment of a molecular diagnosis in families with CKD - aetiology 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] gnom AD, variant frequencies listed for homozygous/ hemizygous (if applicable)/ heterozygous/ total alleles(http://gnomad.broadinstitute.org/)

[k] ACMG, American College of Human Genetics 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 previously been reported on HGMD Professional 2017.2 for the reported phenotype and classified as a pathogenic mutations to be disease causing, variant denoted as "DM". Variant denoted as "?DM" if the variant is a likely pathogenic mutation to be disease causing but where the author indicated some degree of 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 "Gene" is indicated in this column.

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

Table 3.

Comparison between the outcomes in the current study and a recent publication

	Current Study n (%)	Lata Study[1] 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%)

CKD, chronic kidney disease; **WES**, whole exome sequencing; NA, not applicable.

[1]Lata S *et al.* Whole-Exome Sequencing in Adults With Chronic Kidney Disease: A Pilot Study. *Annals of Internal Medicine* 2018; **168:** 100–109.

Table 4.

Comparison of the clinical characteristics of the 138 affected patients with chronic kidney disease by molecular genetic diagnostic category following whole exome sequencing

Molecular genetic diagnostic category post WES	Median age in years of onset of ESKD [years] (range)	CKD only in adulthood[a] (%)	ESKD in adulthood[b] n (%)	ESKD in childhood[c] n (%)	Total numbers of "solved" individual s n (%)
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	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)**

CAKUT, congenital anomalies of the kidney and urinary tract; **CKD**, chronic kidney disease; **ESKD**, End Stage Kidney Disease; **GN**, glomerulonephritis; **solved**, a pathogenic or likely pathogenic monogenic mutation in a known CKD gene was detected following WES; **TIKD**, tubulo-interstitial kidney disease.

[a]Adult patients who had CKD at time of analysis (i.e. Not yet progressed to ESKD in adulthood i.e. ≥ 18 years)

[b]Patients who developed ESKD ≥ 18 years of age

[c]Patients who developed ESKD < 18 years of age