Personalized medicine in chronic kidney disease by detection of monogenic mutations

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ABSTRACT

A large fraction of early-onset chronic kidney disease (CKD) is known to be monogenic in origin. To date, ~450 monogenic (synonymous with single-gene disorders) genes, if mutated, are known to cause CKD, explaining ~30% of cases in pediatric cohorts and ~5–30% in adult cohorts. However, there are likely hundreds of additional monogenic nephropathy genes that may be revealed by whole-exome or -genome sequencing. Although the discovery of novel CKD-causing genes has accelerated, significant challenges in adult populations remain due to broad phenotypic heterogeneity together with variable expressivity, incomplete penetrance or age-related penetrance of these genes. Here we give an overview of the currently known monogenic causes for human CKD. We also describe how next-generation sequencing facilitates rapid molecular genetic diagnostics in individuals with suspected genetic kidney disease. In an era of precision medicine, understanding the utility of genetic testing in individuals with a suspected inherited nephropathy has important diagnostic and prognostic implications. Detection of monogenic causes of CKD permits molecular genetic diagnosis for patients and families and opens avenues for personalized treatment strategies for CKD. As an example, detection of a pathogenic mutation in the gene *HNF1B* not only allows for the formal diagnosis of CKD, but can also facilitate screening for additional extrarenal manifestations of disease, such as maturity-onset diabetes of youth, subclinical abnormal liver function tests, neonatal cholestasis and pancreatic hypoplasia. It also provides the driving force towards a better understanding of disease pathogenesis, potentially facilitating targeted new therapies for individuals with CKD.

Keywords: familial nephropathy, genetic kidney disease, Mendelian renal disease, monogenic disease, single-gene disorders

INTRODUCTION

Chronic kidney disease (CKD)

CKD is defined as abnormalities of kidney structure or function present for >3 months with evidence of either decreased glomerular filtration rate (GFR) of <60 mL/min/1.73 m² or the presence of one or more of the following markers of kidney damage: albuminuria, urine sediment abnormalities, electrolyte and other abnormalities due to tubular disorders, abnormalities detected by histology or structural abnormalities detected by imaging and/or a history of kidney transplantation [1]. End-stage kidney disease (ESKD) represents the final stage of CKD, culminating in the need for renal replacement therapy to sustain life. The specific etiology of ESKD differs in childhood- versus adult-onset CKD (Table 1). Advancing knowledge of etiologic causes of CKD is paramount for understanding the pathogenesis, for adequate classification, for prognosis and treatment and to provide a personalized medicine approach for patients with CKD.

Genetic kidney disease (GKD)

GKD encompasses a broad range of genetic causes of CKD. At one end of the spectrum is polygenic disease, which is characterized by a weak genotype–phenotype correlation (Figure 1). These diseases, which usually manifest in adulthood, have weak heritability patterns and are subject to modification through environmental factors. In this review we will place emphasis on monogenic, single-gene disorders versus polygenic disease. For this review we define GKD as Mendelian or monogenic disease (also known as a single-gene disorder), which results from a pathogenic mutation of a single causative gene. We will focus on autosomal recessive (AR) disease, where both parental alleles need to be mutated in order to cause disease, and autosomal dominant (AD) disease, where a mutation in one parental allele is sufficient to cause disease. Further discussions of X-linked,

Table 1. Frequency of clinical diagnostic subgroups in patients with childhood-onset ESKD versus adult-onset ESKD

Diagnostic group	Childhood-onset ESKD ^a		Adult-onset ESKD ^b	
	N	%	N	%
CAKUT		(39)	NR	
Aplasia/hypoplasia/dysplasia	1769	16		
Obstructive uropathy	1713	15		
Reflux nephropathy	576	5		
Prune belly	279	2		
Glomerulonephritis	1845	16	8802	8
FSGS	1308	12	–	
Congenital nephrotic syndrome	289	3	–	
Membranous nephropathy	51	<1	–	
Cystic kidney disease			2482	2
PKD	339	3		
Medullary cystic kidney disease	305	3		
Cystinosis	225	2	NR	
Oxalosis	58	1	NR	
Renal infarct	144	1	NR	
Diabetic nephropathy	11	<1	51 339	44
Hypertension	0	0	33 585	29
Other/unknown	2270	20	20 782	18
Total	11 182	100	116 990	100

Note that where there is a much higher occurrence of CAKUT (39%) in childhood-onset disease, diabetic nephropathy (44%) and hypertensive nephropathy (29%) predominate in adult-onset disease.

^aAdapted from Contributions of the Transplant Registry: The 2014 Annual Report of the North American Pediatric Renal Trials and Collaborative Studies, <https://web.emmes.com/study/ped/annlrept/annualrept2014.pdf> (8 February 2019, date last accessed).

^bAdapted from the 2015 United States Renal Data System Annual Data Report detailing the primary cause of ESRD in incident cases of hemodialysis, peritoneal dialysis and transplantation in the US population, https://www.usrds.org/2015/download/vol2_USRDS_ESRD_15.pdf (8 February 2019, date last accessed).

NR, not reported; PKD, polycystic kidney disease.

mitochondrial and epigenetic mechanisms are beyond the scope of the current discussion.

Monogenic kidney disease

Monogenic kidney disease can be classified based on the mode of inheritance into AR and AD (Figure 1, red segment).

In recessive disease, both parental copies (alleles) of the monogenic disease gene need to be abnormal ('mutated') for disease to manifest. In recessive disease, there is often 'full penetrance'. That means that the disease manifests in early life in all individuals who carry mutations in both alleles [2]. Increasingly, particularly with the expansion of genetic analysis into adult cohorts, phenotypic variability is evident in CKD due to 'allelism'. An example of allelism in CKD is seen in basement membrane-associated Fraser complex (*FRAS1*, *FREM1*, *FREM2*, *GRIPI1*). We have previously demonstrated that truncating disease-causing alleles that result in loss of function of the corresponding protein give rise to a severe, early-onset phenotype, whereas missense, hypomorphic alleles cause milder, later-onset disease (Figure 2; Supplementary data, Figure S1) [3]. Due to the fact that in recessive inheritance heterozygous individuals, as a rule do not manifest with disease, the family history may be negative because ancestors of the affected individual are heterozygous (and thereby healthy) carriers.

In AD disease, mutations of one of the two parental alleles are sufficient to cause disease. Therefore AD diseases tend to segregate through multiple generations in a single family. AD disease-causing genes may have features such as variable expressivity and incomplete penetrance, resulting in a weakened genotype–phenotype correlation. The term variable expressivity

refers to the phenomenon that individuals carrying a mutation in a disease gene can present with a phenotype that differs from the phenotypic manifestation of other individuals with an identical mutation. The term incomplete penetrance refers to the situation where, in a pedigree with dominant segregation of the disease phenotype, an individual, even though carrying a mutation, does not exhibit a disease phenotype at all (Figure 2). Dominant disease genes may also manifest by *de novo* mutations (i.e. noninherited). This phenomenon is virtually absent from recessive disease genes because it is extremely unlikely that two *de novo* mutations occur in the same gene spontaneously [4].

Epidemiology of GKD

In the last 15 years, next-generation sequencing combined with biological studies and generation of animal models of human disease has led to an exponential rate of discovery of monogenic disease genes that cause childhood-onset CKD. Historically, CKD in adults was not considered to be monogenic in origin. A recent epidemiological study of almost 3000 Australian patients suggested that GKD may account for 9.8% of adult-onset CKD, increasing to 17.5% in incident adult patients on renal replacement therapy [5]. Other studies suggest that CKD aggregates within families. For example, in a Norwegian population, the existence of a first-degree relative with ESKD conferred a 7-fold increased risk of ESKD [6]. Furthermore, Freedman *et al.* [7] demonstrated that 23% of incident dialysis patients have close relatives with ESRD, while in an Irish cohort of adult patients with CKD, 34% report a

FIGURE 1: Disease causation hypothesis. Disease causation is displayed ranging from almost exclusive genetic causes in the case of single-gene disorders (monogenic, recessive and dominant; top left corner, red), to diseases with a strong genetic component (metabolic, developmental, polygenic), to damage that is primarily inflicted by the environment with some component of genetic susceptibility (degenerative, infectious, toxic and trauma; bottom right corner, blue).

positive family history of CKD in either a first- or second-degree relative, which again suggests genetic contribution [8].

Monogenic contribution in GKD

Currently, ~30% of CKD, with onset <25 years, can be attributed to an established monogenic cause; however, there is significant variability in the reported prevalence of monogenic causation depending on the underlying CKD phenotype (Figure 3). Data is now emerging which indicates that genetically inherited CKD is more prevalent than previously thought, particularly in adult cohorts with a positive family history of CKD. Currently, three large studies have evaluated the contribution of monogenic inheritance to adult-onset CKD; however, as shown in Figure 3, most adult cohorts analyzed to date have been enriched for familial cases of CKD, therefore diagnostic yield is likely higher compared with the general CKD population.

First, Mallett *et al.* [20] demonstrated, using a gene panel sequencing approach, that in patients with familial nephropathy, monogenic causation can be detected in 43% (58 of 135 families). Using a curated gene panel encompassing 207 genes known to cause CKD, they assessed 135 families referred to a renal genetic service over a 2-year period for pathogenic mutations across 10 different diagnostic categories [autosomal dominant tubulointerstitial kidney disease (ADTKD), atypical hemolytic uremic syndrome (aHUS) C3 glomerulonephritis (aHUS-C3GN), Alport syndrome (AS)/thin basement membrane nephropathy, Bardet-Biedl syndrome (BBS), congenital anomalies of the kidney and urinary tract (CAKUT), cystinosis, nephrotic syndrome, nephronophthisis-related disorder (NPHP-RD) and tubular disorders]. The genetic diagnostic rate varied depending on the underlying diagnostic category, ranging from 81% (22/27) for renal phenotypes such as AS to as low

FIGURE 2: Age-related penetrance of monogenic alleles (mutations). The x-axis shows the age of disease manifestation in years. Characteristic features of age-related penetrance are shown for AR versus AD CKD genes. For example, the median age of onset for a homozygous truncating mutation ('strong allele') in an AR gene may occur at 10 years of age (dark blue curve). For another individual with a homozygous missense mutation in the same disease gene ('mild allele'), the age-of-onset curve may be skewed to a later median age of onset (light blue curve). Similar to AR genes, AD genes show age-related penetrance. As a tendency, AD disease genes manifest later in life. In addition, AD disease genes feature effects such as incomplete penetrance (i.e. a mutation carrier never manifesting with disease) and variable expressivity (i.e. varied organ involvement between carriers of an identical mutation), which are usually not seen in AR acting genes. Exposure to environmental risk factors can hasten disease onset (red arrow), while treatment or prophylaxis can delay the onset of disease (green arrow).

as 8% (1/13) for CAKUT. Interestingly, the overall diagnostic rates in families with childhood-onset disease (<18 years) and adult-onset disease (≥ 18 years) were not significantly different (46% versus 40%, $P = 0.44$; [Supplementary data, Table S1](#)); however, variability between disease subgroups was evident.

Second, using whole-exome sequencing (WES) analysis, Lata *et al.* [30] demonstrated that in 20% (19 of 92 families) of adults with CKD, a causative mutation in a known monogenic CKD gene could be identified. Their cohort consisted of 92 patients with CKD attending a tertiary referral nephrology center in the USA. Adults presenting with CKD with one of the following five disease entities were submitted for WES analysis: glomerular disease (50/92), tubulointerstitial disease (10/92), developmental disorders (11/92), hypertension (5/92) and undiagnosed disease/other (16/92). The cohort was enriched for patients with either a family history of CKD, undiagnosed CKD or clinical suspicion of a GKD such a childhood-onset disease. Overall, WES confirmed the clinical diagnosis in 6 of the 19 families. Interestingly, WES led to reclassification of the diagnosis in 6 of 19 families, while establishment of a clinical diagnosis in patients with undiagnosed CKD was possible in a further 7 of the 19 families ([Supplementary data, Table S2](#)).

Third, using WAS, we recently demonstrated a genetic diagnosis rate of 37% (42 of 114) in families with CKD presenting as adults to nephrology services in Ireland [12]. As observed by

CKD subtype	Childhood onset CKD < 18 years	Adult onset CKD ≥ 18 years^a
CAKUT	Median 17% 14% ^b (32/232) 17% ^c (17/99) 20% ^d (7/56)	Median 22%^{a,e} (10/45)
SRNS	Median 26% 6.3% ^f (6/95) 25% ^g (75/300) 26% ^h (49/187) 29.5% ⁱ (526/1783) 25-100% ^j (30/106) [†]	Median 14% 0% ^e (0/7) 1.4% ^g (3/217) 14% ⁱ (7/48) 22% ^k (30/135)
Chronic glomerulopathies	Median 67% 14% ^l (51/362) 67% ^{a,m} (6/9) 88% ^{a,m} (7/8)	Median 79% 17% ^{a,m} (4/24) 79% ^{a,m} (15/19) 82% ⁿ (83/101)
Cystic Kidney Disease	Median 50% 36% ^{a,m} (6/12) 63% ^o (50/79)	Median 17% 0.5% ^p (26/5606) 17% ^{a,m} (1/6) 83% ^{a,e} (10/12)
ADTKD	NA	Median 45% 25% ^{a,m} (1/4) 29% ^{a,e} (2/7) 45% ^q (25/56) 78% ^r (7/9) 82% ^s (37/45)
Nephrolithiasis / Nephrocalcinosis	Median 25% 21% ^t (22/106) 29% ^u (15/51)	Median 11%^t (19/166)
Renal Tubulopathies	Median 70% 64% ^v (245/384) 75% ^{a,m} (3/4)	Median 83%^{a,m} (5/6)
CKD etiology unknown	NA	Median 40% 32% ^w (7/22) 47% ^{a,e} (16/34)
Total solve rate per 1000 families (%)	299/1000 (30%)	47-294/1000* (5-30%)

FIGURE 3: Diagnostic yield from reported studies using molecular genetic analysis in each disease subgroup comparing childhood-onset (<18 years) versus adult-onset (≥18 years) CKD. Molecular genetic analysis was performed by WES or gene panel sequencing. For disease subgroups with more than two studies, the median diagnostic yield is reported. For disease subgroups with two or fewer studies, the average diagnostic yield is calculated as a marker of the median. Median, median diagnostic yield across all reported studies for each specific disease subcategory; NA, not available. Note: *n* indicates the number of families included in the study. If the number of families was not available, then the number of unrelated patients was included. [†]Indicates the diagnostic rate varied depending on the age of onset of disease. *Indicates results are quoted including and excluding the Snoek *et al.* study [23]. ^aMost adult cohorts analyzed to date have been enriched for familial cases of CKD, therefore diagnostic yield is likely higher compared with the general CKD population; ^b[9]; ^c[10]; ^d[11]; ^e[12]; ^f[13]; ^g[14]; ^h[15]; ⁱ[16]; ^j[17]; ^k[18]; ^l[19]; ^m[20]; ⁿ[21]; ^o[22]; ^p[23]; ^q[24]; ^r[25]; ^s[26]; ^t[27]; ^u[28]; ^v[29] and ^w[30].

other groups, the likelihood of obtaining a genetic diagnosis varied depending on the renal phenotype (Figure 3). However, despite our *a priori* hypothesis that monogenic disease causation would be more likely in patients with childhood-onset (<18 years) versus adult-onset (≥18 years) CKD [2], no significant difference was observed either for age of onset of CKD [20/

50 (40%) childhood onset versus 35/85 (41%) adult onset, *P* = 0.893] or age of onset of ESKD [10/21 (48%) childhood onset versus 29/69 (42%) adult onset, *P* = 0.459] (Supplementary data, Table S1) [12]. In 9 of the 42 families, the initial clinical diagnosis was corrected post-WES based on the genetic mutation detected, while in a further 16 of 34 families with ‘CKD–

etiology unknown' (CKDu), we were able to provide a definitive genetic diagnosis post-WES ([Supplementary data, Table S2](#)).

Despite these data, only recently have investigators begun to unravel the genetic basis of adult-onset CKD, and routine genetic testing for adults is still not widely available in clinical practice. However, detection of monogenic causes of CKD in these patients, particularly patients with CKDu, can facilitate a personalized medicine strategy for patients who would otherwise remain without a formal diagnosis. Below, we summarize the prevalence of monogenic contribution in both pediatric and adult cohorts for eight subcategories of CKD as a guide for potential diagnostic yield following molecular genetic analysis in each disease subcategory ([Figure 3](#)).

CAKUT

CAKUT is the most common cause of CKD in the first three decades of life ([Table 1](#)). In adults with CKD, the prevalence is less clear, with an estimated prevalence of 5–10% in the general CKD population and 1.3% in an incident renal replacement therapy population [8, 11, 31]. Clinically, CAKUT represents a spectrum of pathologies involving the kidney, ureters, bladder, urethra and/or distal genitourinary tract. To date, 40 monogenic causes of isolated CAKUT ([Supplementary data, Table S3](#)) and 153 monogenic causes of syndromic CAKUT ([Supplementary data, Table S4](#)) have been described. The prevalence of monogenic causation within pediatric cohorts with CAKUT ranges from 14 to 20% depending on study inclusion criteria and methods of analysis. For example, Rasmussen *et al.* [11] applied a gene panel approach in combination with WES to samples from 62 fetuses on 56 families with prenatal CAKUT, identifying likely deleterious variants in 11 of 56 (20%) families. Similarly, Weber *et al.* [10] demonstrated that, using gene panel sequencing in a cohort of 100 children with renal hypodysplasia, a monogenic disease-causing gene could be identified in 17%. We recently observed monogenic causation in 14% in a pediatric cohort ($n = 232$) with CAKUT [9]. Recent studies have also demonstrated that copy-number variants (CNVs) contributed to the genetic architecture of CAKUT, with up to 16.6% of patients with renal hypodysplasia having a molecular diagnosis attributable to CNVs [32].

Steroid-resistant nephrotic syndrome (SRNS)

Nephrotic syndrome is defined by the presence of proteinuria ($>40 \text{ mg/m}^2/\text{h}$), hypoalbuminemia, edema and hyperlipidemia. Patients are further subcategorized depending on their response to steroid therapy into steroid-sensitive nephrotic syndrome and SRNS. We have previously shown by targeted panel sequencing of 27 known SRNS genes that in 29.5% of SRNS cases, with onset before 25 years, a causative mutation can be detected [16]. Using a gene panel approach in a single-center study in patients with childhood-onset SRNS, we detected a disease-causing mutation in 8 of 72 (11.1%) families [33]. Later, using WES, we demonstrated that a mutation in 1 of the 59 known SRNS genes can be identified in 25% of patients with childhood-onset disease ([Supplementary data, Table S5](#)) [19]. Recently, in 187 pediatric patients with SRNS, a causative mutation was detected in 1 of 53 SRNS genes in 26% of individuals

[15]. However, diagnostic yield can vary depending on the cohort analyzed and the methods of analysis employed. For example, Sampson *et al.* [13] demonstrated that using targeted sequencing of 21 genes known to cause monogenic nephrotic syndrome in combination with a pathogenicity filtering strategy, a pathogenic mutation was detected in 6.3% (6/95) of pediatric patients and 1.4% (3/217) of adult patients with sporadic nephrotic syndrome. The diagnostic yield in patients with adult-onset SRNS is lower and the prevalence of mutations inversely correlates with age of onset, in that a detection rate of 100% was found for congenital-onset versus 14% for adult-onset disease [17]. The predominant histological finding in patients with SRNS is focal segmental glomerulosclerosis (FSGS). However, a poor correlation between the histological findings and the underlying genotype has been reported [15]. This was particularly evident in adult cohorts where phenotypic variability can be attributed to the presence of mutations in the collagen genes (*COL4A3*, *COL4A4* and *COL4A5*), which in fact represent a form of nephritis that may phenocopy nephrosis. For example, Sen *et al.* [34] demonstrated that in five patients with an SRNS phenotype, pathogenic mutations in collagen genes were identified. Equally, Mallet *et al.* [20] found a high diagnostic rate in patients with adult-onset glomerular disease [22 of 27 families (81%)]. However, on further inspection of this Australian cohort, most of the mutations identified within the glomerular disease category were mutations in genes known to be causative of AS. These findings are consistent with findings by other groups that pathogenic mutations in collagen genes (*COL4A3–5*) can be detected in large proportions of patient with an underlying diagnosis of FSGS [35]. Recently, Gribouval *et al.* [18] showed that even in patients with adult-onset SRNS without a positive family history, a pathogenic mutation can be detected in $>20\%$, indicating that a monogenic contribution may be higher in adult cohorts than previously considered.

Chronic glomerulopathies

Chronic glomerulopathies are defined by persistent low-grade proteinuria ($>300 \text{ mg/day}$) and hematuria. AS, an inherited hematuric nephropathy, falls within the chronic glomerulopathy disease spectrum and accounts for $\sim 0.5\text{--}1.5\%$ of all cases of CKD [5, 8, 36, 37]. Mutations have been identified in the *COL4A3*, *COL4A4* and *COL4A5* genes as causative of AS ([Supplementary data, Table S6](#)) [38–40]. aHUS is another rare hematuric nephropathy, characterized by microangiopathic hemolytic anemia, thrombocytopenia and acute kidney injury, accounting for $\sim 1\text{--}2\%$ of all cases of CKD [8, 36, 37]. Mutations in nine genes have been identified as causative for aHUS ([Supplementary data, Table S6](#)) [36, 37, 41–44]. We have previously shown that in heterogeneous, pediatric patient populations with persistent proteinuria and hematuria, the likelihood of detecting mutations in monogenic disease-causing genes is 14% (51 of 362 families). Similar to observations within SRNS cohorts, there was evidence of genetic heterogeneity with detection of pathogenic mutations in AS-, aHUS- and SRNS-causing genes [19]. Screening more homogeneous cohorts with suspected AS, however, has revealed a higher diagnostic yield. For example, using next-generation sequencing analysis of

COL4A3, *COL4A4* and *COL4A5* genes in 101 unrelated patients with AS, Morinière *et al.* [21] demonstrated a diagnostic yield of 80% (81/101). Similar findings were observed by another group, where a genetic diagnosis was made in 22 of 27 families with AS (81%) [20]. A lower overall prevalence of 30% for monogenic contribution has been observed in patients referred with aHUS. For example, in 33 families referred with aHUS, 10 had a pathogenic mutation identified in a gene known to be causative of aHUS [20].

Cystic kidney disease

Cystic kidney disease encompasses a broad spectrum of diseases, including polycystic kidney disease, NPHP, medullary cystic disease, BBS and other so-called renal cystic ciliopathies. Most cystic kidney disease is inherited in an AR manner [45], with the major exception of autosomal dominant polycystic kidney disease (ADPKD). The phenotype observed in cystic kidney diseases entails cystic fibrotic kidney disease with renal enlargement in ADPKD and small or normal-sized kidneys in NPHP-related ciliopathies. Extrarenal features include neurodevelopmental defects, retinal degeneration, liver fibrosis, skeletal deformities, facial dysmorphism and congenital heart disease [46]. For the purposes of this review, we have not included further discussion on monogenic causation in ADPKD. To date, mutations in almost 100 genes have been described as causative of monogenic renal cystic ciliopathies (Supplementary data, Table S7). In fact, the highest yield in terms of obtaining a genetic diagnosis for pediatric patients with CKD is currently seen in cystic kidney disease, where a genetic diagnostic yield of 63% has been observed [22]. Although direct comparison between studies can be problematic due to the use of different classification systems and inclusion criteria, Mallet *et al.* [20] demonstrated similarly high diagnostic rates in certain subcategories of cystic kidney disease: a genetic diagnostic rate of 100% was observed for BBS and 29% for NPHP. NPHP has classically been described as a childhood-onset disease, with a low index of clinical suspicion among patients with adult-onset disease. Interestingly, Snoek *et al.* [23] recently reported a prevalence of NPHP of 0.5% among a cohort with adult-onset ESKD (≥ 18 years). Using a large renal transplant recipient cohort ($n = 5606$), they found that 26 patients had a homozygous *NPHP1* deletion. Interestingly, only 3 of the 26 patients had been diagnosed clinically with NPHP prior to genetic analysis. These data add to the mounting evidence that monogenic cystic kidney disease may be an underrecognized cause of adult-onset CKD.

ADTKD

ADTKD is a newly defined subclassification of genetic CKD characterized by an AD pattern of inheritance, tubulointerstitial pattern of injury on kidney biopsy and progressive renal impairment in association with bland urinary sediment with minimal blood and protein [47]. This progressive disease leads to gradual deterioration in renal function and ESKD in adulthood. To date, pathogenic mutations in five genes have been identified in patients with ADTKD (Supplementary data, Table S8). In terms of prevalence, Ekici *et al.* [25] demonstrated

that in 7 of 10 families with ADTKD, mutations in the genes *MUC1* or *UMOD* could be detected. In a larger study, assessing the prevalence of *UMOD* mutations among 109 unrelated patients with a clinical phenotype consistent with ADTKD, a mutation was detected in 37 patients (34%) [26]. The predominant pathogenic mutation in the *MUC1* gene is a cytosine duplication within the variable number of tandem repeats (VNTRs) region of the *MUC1* gene. Mutational analysis of *MUC1* has been complicated by difficulties sequencing this region, due to both the high guanosine/cytosine content and repetitive nature of the VNTR region [48]. Despite these technical difficulties, Ayasreh *et al.* [24] found in a cohort of 56 families with presumed ADTKD that a genetic diagnosis was established in 25 families (45%). The majority carried the pathogenic 'c duplication' mutation within the VNTR region in *MUC1*, while nine carried a pathogenic mutation in *UMOD*. No mutations were identified in *REN* or *HNF1B*. With ongoing advances in analysis techniques [48], it is likely that further mutations will be identified in patients with ADTKD.

Nephrolithiasis and nephrocalcinosis

Renal stone disease, encompassing both nephrolithiasis and nephrocalcinosis (NLNC), is a prevalent condition, with approximately 1 in 10 people affected worldwide [49]. Although renal stone disease can be associated with a preserved GFR, and despite the obvious environmental contributions, there is increasing awareness of single-gene mutations causing NLNC (Supplementary data, Table S9). We previously demonstrated, using gene panel sequencing of 30 genes known to cause NLNC, a prevalence of single-gene disorders in 11% with adult-onset disease and between 17 and 21% with childhood onset [27, 50]. Using WES, we later established a rate of 29% of monogenic contribution in patients <25 years of age presenting with either nephrolithiasis or nephrocalcinosis [28].

Renal tubulopathies

Renal tubulopathies are defined as a dysfunction in the specialized transporters and channels in the renal tubules [29], with >50 monogenic disease-causing genes identified to date (Supplementary data, Table S10). Although renal tubulopathies can be associated with a preserved GFR, the clinical impact of genetic causes in this group of disorders is high and often highly related to the clinical phenotype. Most studies thus far have focused on characterization of specific disorders. Recently the working group for tubulopathies in the European Consortium for High-Throughput Research in Rare Kidney Diseases established a large cohort of 410 patients with renal tubulopathies from 384 families. They demonstrated by gene panel sequencing of 37 genes in this pediatric cohort that a causative mutation can be identified in 64% (245 of 384 families) [29]. This high diagnostic rate has been replicated in smaller cohorts. For example, Mallet *et al.* [20] demonstrated that in 10 families referred with a renal tubulopathy phenotype, 8 had pathogenic mutations in a gene known to be causative of a renal tubulopathy.

CKDu

CKDu is frequently seen among CKD cohorts, accounting for 6% at 12–15 years, 21% at 18–21 years and up to 36% of all cases with adult-onset CKD [51]. With the increasing availability of WES, identification of monogenic causes of CKDu may provide alternative diagnostic strategies to obtain a definitive diagnosis for these patients and families. For example, using WES in a cohort of 92 patients with CKD, Lata *et al.* [30] recently demonstrated that a molecular diagnosis could be established in 9 patients with CKDu. Similarly, in a cohort of 114 Irish families, we established a definitive diagnosis in 16 of 34 families (47%) referred with a clinical diagnosis of CKDu (Supplementary data, Table S2) [12]. An important lesson from these studies is that in many patients, the features attributable to the underlying genetic diagnosis were not recognized prior to genetic analysis. Indeed, in adults, recognition of features associated with the respective genetic disease may be delayed due to a low index of clinical suspicion or the presence of subtle extrarenal features of disease that are often not recognized until a clinical review is performed in full cognizance of the molecular genetic diagnosis [12]. Taken together, these data suggest that performing WES may be particularly beneficial in adults with CKDu to facilitate establishment of an unequivocal genetic diagnosis in patients and families who would otherwise remain without a definitive diagnosis.

Clinical consequences of establishing a genetic diagnosis

In summary, the significance of identifying a genetic nephropathy is multifold. First, in the era of precision medicine, molecular genetic testing can provide an accurate diagnosis, thereby facilitating personalized treatment and prognostication. Second, receiving a molecular diagnosis can in some cases obviate the need for invasive kidney biopsies, particularly in cases where the kidney biopsy was uninformative. Third, in cases of familial nephropathy, molecular genetics can allow for genetic counseling and screening of at-risk family members, which is essential to allow for informed decision-making strategies, particularly in the setting of live related kidney donation. Finally, establishing monogenic causation in patients with CKD can not only provide insights into the pathways involved in disease pathogenesis, but also enables the generation of gene-specific animal models.

SUPPLEMENTARY DATA

Supplementary data are available at [ndt online](https://www.ndt-online.org).

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CONFLICT OF INTEREST STATEMENT

F.H. is a cofounder and SAC member and holds stock in Goldfinch-Bio.

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