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Diagnosis of Kidney Diseases of Unknown Etiology Through Biopsy-Genetic Analysis



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Introduction: Previous studies have suggested that genetic kidney diseases in adults are often overlooked, representing up to 10% of all cases of chronic kidney disease (CKD). We present data obtained from exome sequencing (ES) analysis of patients with biopsy-proven undetermined kidney disease (UKD).

Methods: ES was proposed during routine clinical care in patients with UKD from January 2020 to December 2021. We used *in silico* custom kidney genes panel analysis to detect pathological variations using American College of Medical Genetics guidelines in 52 patients with biopsy-proven UKD with histological finding reassessment.

Results: We detected 12 monogenic renal disorders in 21 (40.4%) patients. The most common diagnoses were collagenopathies (8/21, 38.1%), *COL4A3* and *COL4A4* accounting for 80% of these diagnoses, and ciliopathies (5/21, 23.8%). The diagnostic yield of ES was higher in female patients and patients with a family history of kidney disease (57.1% and 71%, respectively). Clinical nephropathy categories matched with the final genetic diagnoses in 72.7% of cases, whereas histological renal lesions matched with the final diagnoses in 92.3% of cases. The genetics diagnoses and histopathological findings were in complete agreement for both glomerular and tubulointerstitial cases. Interstitial inflammation without tubulitis was only observed in tubulopathies or ciliopathies. Isolated CKD, CKD with proteinuria or hematuria, and isolated proteinuria or hematuria yielded the highest diagnostic yields (54.6%, 52.6%, and 42.9%, respectively).

Conclusion: ES done in patients with biopsy-proven UKD should be considered as a first-line tool for CKD patients with a family history of kidney disease. Combination of ES and kidney biopsy may have major impacts on kidney disease ontology.

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KEYWORDS: alport disease; exome; inherited kidney disease; nephrogenomic; renal pathology; undetermined kidney disease

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CKD is a serious health concern for people all over the world, because it is estimated to affect more than 840 million individuals, representing more than 10% of the global population.¹ This disease is not only associated with significant risk of death and illness, but

is also projected to become the fifth leading cause of years of life lost by 2040.² Beyond just life loss, CKD has a serious impact on an individual's quality of life beyond its economic costs.³

In 2019, the Renal Epidemiology and Information Network registry reported a prevalence of 17.7% for UKD in France.⁴ Similar proportions of UKD were identified in other developed nations, such as 20% in Germany⁵ and 25% in the United States.⁶ It is likely that the prevalence of UKD in France could be higher if patients with hypertensive nephropathy are included, adding up to 24% more cases. UKD is diagnosed using the Kidney Disease Improving Global Outcomes CKD

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criteria, after excluding all possible etiologies and risk factors of CKD following thorough diagnostic investigations, such as physical examinations, blood tests, renal imaging, and kidney biopsies.

Familial clustering has been observed in patients with end-stage kidney disease (ESKD), suggesting that genetics ought to be considered as a diagnostic investigation.^{7,8} Proportionally, genetic nephropathies encompass approximately 10% of adult CKD⁹ cases and 70% of pediatric CKD cases.¹⁰ Genetic testing of pediatric patients has mainly been focused on gene panels targeting specific phenotypes, commonly referred to as phenotype-oriented approaches. A report by Groopman *et al.*⁸ shows that ES may be a useful diagnostic tool for adult patients. This work also suggests that the prevalence of genetic kidney diseases could be under-recognized in adults. Adult patients with UKD often present with silent evolution, poor clinical phenotypes, and multiple morbidities, making phenotypic approaches to identifying genetic diseases ineffective.¹¹ Gene panels can pinpoint limited diagnoses, whereas ES can provide a higher rate of diagnosis, with a cost similar to that of a gene panel.¹²

Kidney biopsy is considered one of the standard tools in nephrology with an 80% rate of diagnostic accuracy.^{13,14} However, it is not appropriate for all patients. Approximately 6700 renal biopsies are performed annually in France. In our local registry (NCT03305211), 20% of biopsies yielded inconclusive results (data not yet published).^{15,16} Kidney biopsy could result in complications including gross hematuria, perinephric hematoma, and blood transfusion as well as allo-sensitization. Noteworthy, a recent study demonstrated that major bleeding complications arising from kidney biopsy are associated with a 2-fold greater likelihood of death.¹⁵ Nevertheless, kidney biopsy represents the final step of the diagnostic work-up for majority of patients with UKD. In 2019, our institution implemented ES as part of the diagnostic work-up for patients with UKD, including those with inconclusive biopsy results. Nevertheless, the usefulness of ES in this population is yet to be evaluated. We here share our experience using ES in patients with biopsy-associated UKD.

METHODS

Patient Population and Phenotype Characterization

ES was proposed as part of routine clinical care in patients with UKD from January 2020 to December 2021. Clinical diagnoses of nephropathy were based on a combination of medical history, clinical data, laboratory results, and histological findings and classified as follows: unclassified nephropathy, undetermined

vascular nephropathy, undetermined glomerular nephropathy, and undetermined tubulointerstitial or cystic nephropathy.

UKD is defined as the absence of any of the following criteria: biopsy-proven diagnosis (e.g., IgA nephropathy), absence of a specific morphological renal diagnosis (e.g., polycystic kidney disease suspected to be autosomal or recessive polycystic kidney disease), or absence of a specific or plausible renal diagnosis (such as history of long-term insulin-dependent diabetes mellitus before the onset of CKD and ciclosporin-induced nephropathy). Because hypertensive nephropathy is a nonspecific diagnosis, and hypertension is also a very common consequence of CKD, patients with hypertensive nephropathy in the absence of a clear underlying disorder, such as renal artery stenosis, are considered to have unexplained CKD. Patients with renal hypoplasia, renal atrophy, and nonspecific histological conditions (such as secondary focal segmental glomerulosclerosis (FSGS), glomerulonephritis of unknown origin, or interstitial nephritis) are also considered to have UKD. We excluded patients with familial IgA nephropathy, patients with typical presentation of Gitelman or Bartter syndrome, or an established kidney-related genetic diagnosis in the family. If prior genetic analyses were performed, they were of the referring clinician's discretion. Patients with UKD both from our center and other tertiary care centers were referred for a nephrogenomic consultation with 1 of our adult nephrologists. Out of 288 index cases who had UKD and underwent an ES, 65 had an appropriate kidney biopsy. Of those 65 patients, 52 had pathology slides that were reviewed and used for the study. Phenotypes were acquired by using a standardized questionnaire and review of medical reports during consultation. Consanguinity was established during consultation either as reported by the patient or suspected by the clinician. Blood samples were collected after written informed consent from the patients or their legal guardians during the consultation. All patients gave their written informed consent for genetic testing.

Renal Histology

For the purpose of this study, all patient biopsy samples were systematically analyzed and scored for pre-defined features by one renal pathologist (JT) (Supplementary Table S1).

Light microscopy analysis was conducted to reclassify the patients based on the dominant histologic finding. The findings were divided into 3 categories: vascular disease, glomerular disease, and tubulointerstitial disease. In cases where no dominant histologic lesion was present, the pathologist concluded that the patient had a global chronic lesion, which was deemed

nonspecific. We evaluate the genetic-renal biopsy agreement between the dominant lesion from light microscopy analysis and the genetic diagnoses category identified by ES (podocytopathies, collagenopathies, tubulopathies, ciliopathies, and vasculopathies)

ES and Sequence Interpretation

DNA was extracted from peripheral blood using the QIAasympyphony DSP DNA Mini Kit on a QIAasympyphony instrument following the manufacturer's (QIAGEN N.V., Hulsterweg, Netherlands) guidelines. From 50 ng of fragmented DNA, indexed libraries were prepared and hybridized with a biotinylated probe from Twist Human Core Exome (33 Mb) and, from April 2019, Twist Human Comprehensive Exome (37 Mb). ES was performed on the Illumina NextSeq 500 (Illumina, Inc, San Diego, CA) in paired-end mode (2×75 bp reads) then, from March 2021, NextSeq 2000 platform in paired-end mode (2×150 bp reads) on FlowCell P3. Raw data (bcl format) were converted to FASTQ format using the Dragen software sequencer (Illumina). Reads were aligned to the human reference genome (UCSC Genome Browser build hg19). Sequences were analyzed according to GATK Broad Institute good practice with 2 pipelines: Intern pipeline (BWA-MEM, GATK v3.6-44ge7d1cd2) and SeqOne pipeline (v1.2, 2018). Copy number variants calls were performed using the GATK4 copy number variants calling module and were validated using Multiplex Ligation-dependent Probe Amplification.¹⁷

We use an *in silico* gene panel analysis of known genes related to kidney diseases (Supplementary Table S2). To identify diagnostic variants, we assessed the pathogenicity of the variant using American College of Medical Genetics guidelines.¹⁸ Variants were filtered according to coverage level (DP >10), allelic fraction (>20%) and having effect on the protein. Frequency of variants in GnomAd was also considered: for the analysis of *de novo*, autosomal dominant, autosomal recessive, and X-linked variants, only variants with a minor allele frequency <1% in the GnomAd database were eventually considered. For the analysis of autosomal recessive and X-linked variants (homozygous, hemizygous, or putative compound heterozygous) in unsolved cases, additional research with a minor allele frequency up to 3% was considered. In addition, we assessed the APOL1 genotype (G1 [rs73885139 and rs60910145]) and G2(rs71785313) as forms of nephropathy when 2 copies were present as follows: G1/G1, G1/G2 or G2/G2.¹⁹ All identified variants were compared with available databases for pathogenic variants such ClinVar, the Human Gene Mutation Database,²⁰ the Leiden Open Variation Database and databases for pathogenic copy number

variants, such as DECIPHER. Only variants rated as “likely pathogenic” or “pathogenic” according to the American College of Medical Genetics classification, and with a genotype in agreement with the mode of inheritance and the phenotype, led to a diagnostic ES result. Patients with variants classified as benign, likely benign, or of unknown significance according to American College of Medical Genetics classification led to a nondiagnostic exome test. ES results were communicated to the patients by the same nephrologist with whom they had had the initial nephrogenomic consultation.

Statistical Analysis and Graphical Visualization

Baseline characteristics were expressed as frequencies (*n*, %), means, standard deviations, and medians (range). Fisher's exact test was performed for categorical data. Diagnostic yield was calculated based on counts of variants classified as “pathogenic” or “likely pathogenic.” To compare 2 continuous variables, we used normality tests and then an unpaired *t* test or unpaired Mann-Whitney nonparametric test if values were not sampled from Gaussian distribution. *P*-values <0.05 were considered statistically significant. Two-tailed *P*-values <0.05 were regarded as statistically significant. Statistical analyses were performed using Prism 9 (GraphPad Software) software.

RESULTS

Characteristics of the Population

Fifty-two cases of unsolved kidney biopsies were analyzed (32 from males). Median age of the patients was 39.5 years (interquartile range 25.8–49.5 years) and 67% identified as Caucasian and 21% North African. Consanguinity was reported in 6 patients and suspected in 2. Thirty-seven patients reported a family history of kidney disease. At the time of biopsy, one-third of the patients had ESKD and 58% have evolved to ESKD at the time of ES (27% on dialysis, 21% with kidney transplantation and 10% on conservative management). The median delay between biopsy and ES was 1 years (0,5–5). A gene panel has been previously performed on 2 cases (Table 1). Glomerular nephropathy (42%) was the most common clinical subgroup of UKD.

First indications for kidney biopsy are isolated CKD or CKD associated with proteinuria, hematuria, and/or nephrotic syndrome (Table 1). Biopsy analysis revealed that prevalent histologic conclusion was glomerular disease (48.1%), followed by vascular disease (23.1%) and tubulointerstitial disease (7.7%). Eleven (21.2%) patients had nonspecific histologic lesions (Table 1).

Table 1. Population characteristics

| Variable ^a | Patients (n = 52) |
|--|-------------------|
| Age at biopsy (yrs) | 28.5 (21.3; 46.1) |
| Age at ES (yrs) | 39.5 (25.3; 50.5) |
| Male | 32 (61.5) |
| Geographic origin: | |
| • Europe | 35 (67.3) |
| • North Africa | 11 (21.3) |
| • Sub-Saharan Africa | 2 (3.8) |
| • French Antilles | 2 (3.8) |
| • Asia | 2 (3.8) |
| Consanguinity | 8 (15.4) |
| Kidney disease onset before 35 years old | 33 (63.5) |
| • With familial history | 15 (45) |
| Familial history of kidney disease | 27 (51.9) |
| Prior negative genetic exploration with gene panel | 2 (3.8) |
| Delay between kidney biopsy and WES (yrs) | 1 (0.5–5) |
| Undetermined Clinical Nephropathy Subgroup | |
| • Glomerular | 22 (42.3) |
| • Tubulointerstitial/Cyst | 6 (1.5) |
| • Vascular | 8 (15.4) |
| • Unclassified | 16 (30.8) |
| IVV stage of kidney disease at time of | |
| • Kidney biopsy | 23 (44.2) |
| • ES | 34 (65.4) |
| Patient with transplantation project ^b | 19 (36.5) |
| Indication of kidney biopsy | |
| • Isolated CKD | 11 (21.2) |
| • CKD ± proteinuria ± hematuria | 19 (36.5) |
| • Proteinuria ± hematuria | 7 (13.5) |
| • Nephrotic syndrome | 7 (13.5) |
| • Hypertensive emergencies | 4 (7.7) |
| • Rapidly progressive glomerulonephritis | 2 (3.8) |
| • Acute kidney injury | 1 (1.9) |
| • Thrombotic microangiopathy | 1 (1.9) |
| Dominant findings in kidney biopsy | |
| • Glomerular | 14 (26.9) |
| • Tubulointerstitial | 4 (7.7) |
| • Vascular | 12 (23.1) |
| • Non-significant lesions | 11 (21.1) |
| • Non-specific | 11 (21.1) |
| Number of glomeruli | 10 (7–16) |

CKD, chronic kidney disease; ES, exome sequencing.

^aFor quantitative variables, values are expressed as median [interquartile ranges]. For qualitative variables, values are expressed as n (%).

^bPatient active on the waiting list or patient's evaluation for waiting list registration is ongoing.

Genetic Findings and Diagnostic Yield

We detected 13 monogenic renal disorders in 21 patients carrying either pathogenic or likely pathogenic variants (single nucleotide variants/small indels; $n = 19$) or copy number variants ($n = 2$) among 52 patients (40.4%) (Supplementary Table S3). Glomerular diseases were the most common, accounting for 57.1% (12/21) of cases, mainly attributed to collagenopathies (COL4A3 [$n = 4$], COL4A4 [$n = 3$], COL4A5 [$n = 1$]). Ciliopathies (NPHPI

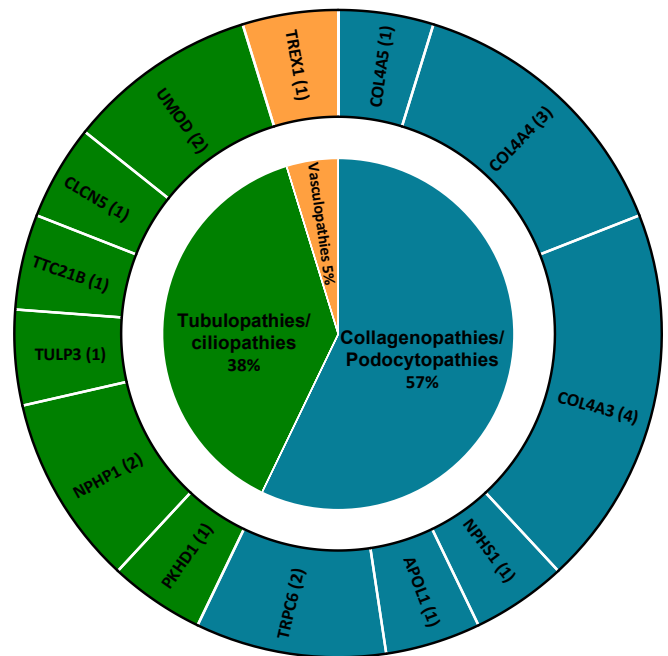


Figure 1. Distribution of genetic results. The inner circle represents the categories of genetic kidney disease and the outer circle gene reported with a pathogenic variant in patients, numbers in brackets represent the number of patient's carrier. Genes have been clustered according to their associated nephropathy category (blue, glomerular disease; green, tubulointerstitial disease and cystic disease; yellow, vasculopathies).

[$n = 2$], *PKHD1* [$n = 1$], *TTC21B* [$n = 1$], *TULP3* [$n = 1$]) and tubulopathies (*UMOD* [$n = 2$], *CNCL5* [$n = 1$]) diseases were the second largest diagnostic subgroup, representing 38%. Lastly, a single instance of vasculopathy was detected, linked to a pathogenic variation in the *TREX1* gene (Figure 1).

There was a significantly higher familial history of kidney disease among the ES-solved group than the ES-unsolved group (71.4% vs. 38.7%, $P = 0.026$). In addition, more female patients were present in the ES-solved group compared to the ES-unsolved group (57.1% vs. 25.8%, $P = 0.04$). However, no statistically significant differences were observed in regard to the age of onset ($P = 0.26$), consanguinity ($P = 0.24$) or age at the kidney biopsy ($P = 0.8$) (Supplementary Table S4).

Genetic Diagnoses According to the Clinical Nephropathy and the Kidney Biopsy Indication

Of the 11 patients with a classifiable nephropathy, 8 (72.7%) had a genetic diagnosis that matched with their clinical phenotype. Two cases of glomerular nephropathy (*TTC21B*, $n = 1$; *TREX1*, $n = 1$) and 1 case of vascular nephropathy (*COL4A3*, $n = 1$) were reclassified based on the genetic findings. Tubulointerstitial nephropathies (*NPHPI*, $n = 1$; *TULP3*, $n = 1$; *CLCN5*, $n = 1$) were accurately classified based on the genetics findings (Figure 2B).

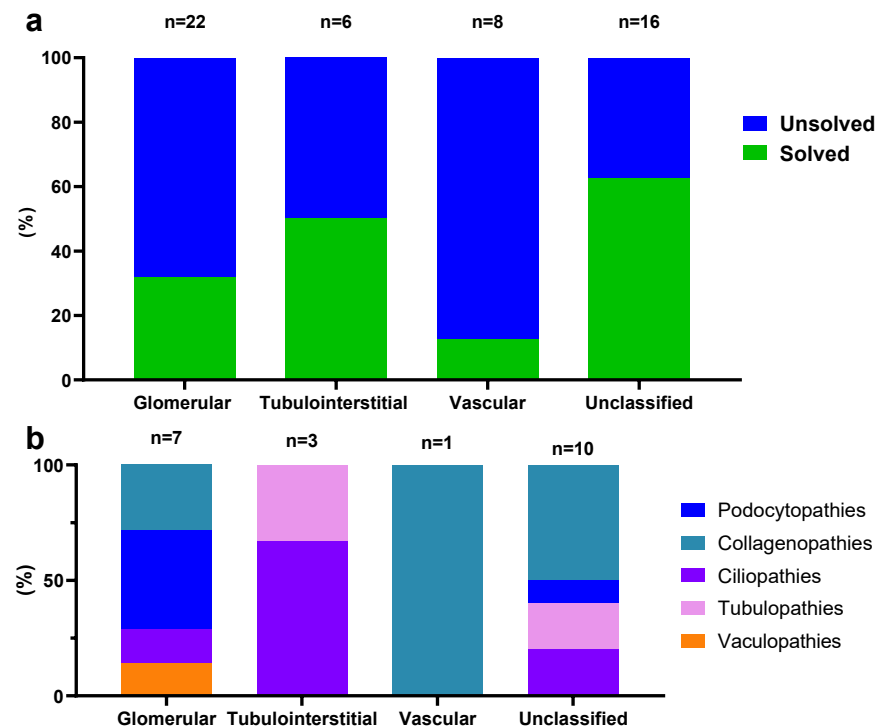


Figure 2. Exome sequencing yield and associated genetic diagnoses according to clinical nephropathy classification.

Out of the 16 patients with unclassifiable nephropathy (Figure 2A), 10 (62.5%) cases were successfully resolved through ES. Collagenopathies, *COL4A4* ($n = 3$) and *COL4A3* ($n = 2$), were the most prevalent genetic findings followed by tubulopathies (*UMOD*, $n = 2$) and ciliopathies (*PKHD1*, $n = 1$; *NPH1*, $n = 1$) (Figure 2B).

The 3 kidney biopsy indications with the highest diagnostic yield in ES were isolated CKD, CKD with proteinuria or hematuria, or both, and isolated proteinuria or hematuria, or both, with rates of 54.6% (6/11), 52.6% (10/19), and 42.9% (3/7), respectively. Among the 7 cases of nephrotic syndrome, 2 (28.6%) were resolved after ES (Supplementary Figure S1A). Patients with nephrotic syndrome, isolated proteinuria and/or hematuria are diagnosed as either having collagenopathy or a podocytopathy (Supplementary Figure S1B). In the group of patients with isolated CKD, 50% were diagnosed with a ciliopathy, whereas 33.3% were found to have a tubulopathy (Supplementary Figure S1B).

Genetic Diagnoses and Histological Findings

Out of the 13 genetic diagnoses, 12 (92.3%) were found to be consistent with the histologic dominant lesion. One patient initially diagnosed with a vascular dominant lesion was later reclassified as an *UMOD*-linked autosomal dominant tubulointerstitial kidney disease. The 3 groups with the highest diagnostic yield through ES were nonspecific histologic lesions, tubulointerstitial lesions, and nonsignificant lesions, with success rates of 72.7% (8/11), 75% (3/4), and 45.5% (5/11) respectively

(Supplementary Figure S2A). Among patients with nonspecific histologic lesion, ES identified ciliopathies ($n = 3$), tubulopathy ($n = 1$), collagenopathies ($n = 2$), and podocytopathies ($n = 2$) (Supplementary Figure S2B).

We conducted a comparison of histological findings between the collagenopathies/podocytopathies group and the tubulopathies/ciliopathies group, excluding patients with nonsignificant lesions on kidney biopsies. The results revealed a significantly higher extent of interstitial fibrosis in the tubulopathies/ciliopathies group compared to the collagenopathies/podocytopathies group ($P = 0.04$). Furthermore, within the tubulopathies/ciliopathies group, 5 of 8 cases (62.5%) exhibited interstitial infiltrate without tubulitis, whereas none of the 12 cases (0%) in the collagenopathies/podocytopathies group showed this pattern ($P = 0.02$) (Supplementary Table S5). The collagenopathies/podocytopathies group exhibited significantly higher glomerular deposits (5/7, 71.4%) compared to the tubulopathies/ciliopathies group (1/8, 12.5%) ($P = 0.04$) (Supplementary Table S5). Interstitial foamy macrophages were observed in 4 renal biopsies with nonsignificant lesions or glomerular-dominant lesions. Among these cases, 3 patients were diagnosed with collagenopathies. No significant differences were observed between the nondiagnostic and diagnostic exome groups (Supplementary Table S6).

Genetic Findings Impact

The impact of ES according to patient's medical history is summarized in Table 2. In 2 of 21 (10%) index cases

Table 2. Genetic impact according patient's history

| Impact Items ^a | Patients (n = 21) |
|---|-------------------|
| Diagnostic algorithm outcome (n = 21; 100%) | |
| Reclassification of the clinical nephropathy ^b | 3/11 (27.3) |
| Reclassification of the dominant histologic lesion ^b | 1/13 (7.7) |
| Prevent unnecessary second kidney biopsy | 1 (4.7) |
| Prevent unnecessary second kidney biopsy | 2 (9.5) |
| Establishing kidney diagnosis in relatives with known CKD | 11 (52.4) |
| o < second degree | 5 (23.4) |
| o > second degree | |
| Non-renal Disease identification | 3 (14.3) |
| Therapeutic guided decision-making (n = 4; 19%) | |
| Prevent unnecessary immunosuppressive treatment | 2 (9.5) |
| Prevent unnecessary immunosuppressive treatment | 2 (9.5) |
| Screening of asymptomatic at-risk family members (n = 12; 57%) | |
| Younger asymptomatic sibling or children | 11 (52.4) |
| Younger asymptomatic potential kidney donor | 5 (23.8) |
| Risk information (n = 15; 71%) | |
| Absence of post transplantation kidney disease recurrence | 6 (28.6) |
| Clarification between dominant or recessive transmission | 11 (52.4) |
| Genetic counseling | 3 (14.3) |
| Extrarenal manifestation screening or monitoring (n = 14; 66%) | |
| Eye | 11 (52.4) |
| Ear | 8 (38.1) |
| Liver | 4 (19) |
| Central nervous system | 1 (4.8) |
| Heart | 1 (4.8) |
| Bone | 1 (4.8) |

CKD, chronic kidney disease.

^aFor qualitative variables, values are expressed as n (%).

^bGenetic counseling is considered when it implies counseling regarding consanguinity or prenatal diagnosis, including prenatal and preimplantation diagnostics in future pregnancies.

^cUnclassified clinical nephropathy and global fibrous lesions were not included in these analysis.

(66 and 179) with histologically confirmed FSGS, inappropriate immunosuppressive therapy observed-corticosteroid and anticalcineurin immunosuppressants for cases 66 and 179, with an additional anti-CD20 therapy for case 179. These cases were subsequently diagnosed with *NPHS1* and *APOL1* gene pathogenic variation. In 2 additional cases of FSGS (index cases 29 and 97), the identification of pathogenic variants in *COL4A3* and *TTC21B* genes, respectively, obviated the need for immunosuppressive therapy. For index case 119, a pathogenic variant in the *TRPC6* gene was identified, enabling a meaningful reassessment of the patient's relative. His son was diagnosed with corticosteroid resistant nephrotic syndrome caused by IgA nephropathy exhibiting overt FSGS lesions. Confirmation of the transmission of this *TRPC6* variation from the father enabled a specific diagnosis, eliminating the requirement of a second renal biopsy and the intensification of immunosuppressive therapy. Moreover, for index case 25, the identification of an intermediate-effect size variant in the *UMOD* gene enabled the exclusion of a living donor project with the patient's son who showed normal renal function upon assessment and screening of the sibling, thereby facilitating

the process of screening for an unaffected familial living donor.²¹ Furthermore, in the past medical history of index case 25, biotherapy with antitumor necrosis factor for axial spondyloarthritis has been implicated as a cause of the kidney disease and was thus discontinued, resulting in an exacerbation of symptoms related to axial spondyloarthritis. In index case 72, the identification of X-linked Alport syndrome enabled screening of the family, ultimately revealing that the sister who had donated a kidney was a carrier of the same mutation. The kidney function assessment for the living donor project showed no abnormalities apart from microscopic hematuria, with no proteinuria present at the time of the donation. Case 71 demonstrated an incidental diagnosis of familial MYL2-associated hypertrophic cardiomyopathy, characterized by left ventricular hypertrophy in the absence of any predisposing cardiac conditions or arterial hypertension.

This diagnosis facilitated the discontinuation of the ongoing living donor project involving an asymptomatic and affected brother who had not been identified with hypertrophic cardiomyopathy upon initial assessment. However, upon further specific cardiac evaluation in the light of MYL2 genotype, hypertrophic cardiomyopathy was confirmed. Index case 149 provided adequate genetic counseling to its 2 sisters (dent disease), allowing them to grasp the potential implications of mutation transmission in the context of future pregnancies. The dent disease diagnosis of index case 149 provided adequate genetic counseling to its 2 sisters, enabling them to be aware of the potential implications of mutation transmission in the context of future pregnancies. In index case 230, we identified a second X-linked dominant disorder caused by a pathogenic variation in the WAS gene. This disorder explains the maternally transmitted thrombopenia, thus providing an indication for prenatal diagnosis, because carrier women have a 50% risk of transmitting the disease to their male offspring.

DISCUSSION

This study represents the first evaluation of ES in the diagnostic process of adult patients with UKD who have undergone kidney biopsy. Our findings demonstrate a diagnostic yield rate of approximately 40%, which has significant implications for both patients and their families. Our study observed a higher yield rate compared to Groopman *et al.*⁸ study, possibly because of a larger proportion of patients with a positive family history of kidney disease and a younger population in our cohort, which may have contributed to this disparity. Our results confirm the underestimation of

the prevalence of genetic kidney disease in adult patients with UKD, with collagen IV-related nephropathy being the most commonly identified genetic finding. UKD accounts for approximately 20% of all CKD cases in developed countries, and globally, 11% to 16% of the population is estimated to be affected by CKD. These statistics highlight the importance of increasing access to genetic diagnosis, including patient with inconclusive kidney biopsy, because it has the potential to significantly improve patient care.²²

We have developed a specialized nephrogenomic consultation specifically tailored to a population where an underlying genetic cause is not specifically suspected in majority of the case. This is supported by the low rates of prior genetic testing among this population, which stand at only 3.8%. Furthermore, it is important to emphasize that a high proportion of family history of kidney disease is a characteristic of our UKD cohort, rather than a selection criterion for referring patients to the nephrogenomic consultation. This distinction ensures that our study captures a wide range of cases and avoids potential bias in patient selection.

Among our cohort of adults, glomerular disease, particularly collagen IV-related nephropathy, was the most common genetic diagnosis. This contrasts with the prevalence of congenital anomalies of the kidney and urinary tract in children and young adults, which accounted for 50% of cases.²³ Genetic kidney conditions appear to be much more prevalent than previously believed. Studies indicate that approximately 25% of individuals with CKD have a family history of CKD.^{8,9,24} McClellan *et al.*²⁴ found that approximately 20% of individuals on dialysis have a family member with ESKD. The presence of a first-degree relative with ESKD has been associated with an increased risk of developing kidney disease. In a study involving the Irish population, which included both CKD and ESKD patients, Connaughton *et al.*²⁵ discovered that the most common cause of kidney disease in patients with a positive family history, excluding polycystic kidney disease, was CKD of unknown or uncertain etiology.²¹ In our study, we found that a significant proportion (71.4%) of patients with a genetic kidney disorder had a family history of kidney disease, indicating a strong familial association. This rate was higher compared to cases where the cause of the renal disease remained inconclusive after genetic testing (38.7%). The presence of a positive familial history emerged as a crucial factor in successfully diagnosing genetic kidney disorders. Therefore, it is important for nephrologists to systematically inquire about a patient's family history during the initial interview as part of routine clinical nephrology practices. This approach becomes especially

valuable when the underlying cause of the kidney disease is uncertain. A comprehensive family history should include information about close relatives who have required renal replacement therapy or have been diagnosed with kidney-related conditions requiring ongoing nephrology care. Gathering this information during the initial visit can provide vital insights into a patient's medical background, guide the diagnostic process, and facilitate genetic assessment.

Accurate classification and diagnosis of kidney disease require identification of the primary renal lesion. Clinical syndromes, laboratory abnormalities, and imaging findings serve as valuable initial indicators in this regard. However, it is important to emphasize that kidney biopsy continues to be the gold standard for diagnosing renal disease and plays a vital role in predicting both diagnosis and prognosis for patients with CKD. Kidney biopsies have an estimated diagnostic yield of 80% and provide valuable clinical information, including primary and secondary findings, which greatly assist in guiding patient management.¹⁴ Primary glomerular lesions typically present with specific syndromes, whereas diseases affecting other compartments may be less specific. Identifying the primary renal lesion can be challenging when secondary lesions overshadow glomerular lesions or when no specific lesion is evident. Genetic kidney diseases often exhibit nonspecific histopathological characteristics, except for Alport syndrome, which shows distinctive basement membrane abnormalities visible only through electron microscopy.^{26,27} Our observations indicate that 45% of patients with nonsignificant lesions at kidney biopsy were successfully resolved through ES, with collagen IV-related nephropathies being the predominant findings. Based on these findings, we suggest that patients with nonsignificant lesions at kidney biopsy should undergo systematic ultrastructural analysis of the glomerular basement membrane using electron microscopy.

In our study, one-third of the genetic findings were related to collagen IV-related nephropathies, indicating potential underdiagnosis due to limited use of electron microscopy. Notably, mutations in the *COL4A3* and *COL4A4* genes accounted for a significant proportion (87.5%) of the identified collagenopathies in our cohort. This deviates from the traditional understanding of Alport syndrome, where *COL4A5* mutations were believed to be predominant and heterozygous variations in *COL4A3* or *COL4A4* were considered benign.²⁸ Our findings challenge previous assertions and recent position paper from experts and working group on Alport syndrome of low kidney failure risk estimated below 1% in individuals with heterozygous *COL4A3* or *COL4A4* variations.²⁹ We reported a higher

proportion of end-stage renal disease in a larger cohort of patients with such variations.^{30,31} These findings support the concept of grouping kidney diseases related to basement membrane disorders, encompassing various phenotypes from classical X-linked Alport syndrome to isolated FSGS and thin basement membrane nephropathy, under the spectrum of collagen IV-related nephropathy.

In our study, we aimed to determine if the primary histologic lesion observed in patients with UKD could provide information about their genetic diagnoses. Through a thorough analysis of kidney biopsies, we found a strong correlation (>90%) between the histologic findings and the underlying genetic causes when a dominant lesion was present. To streamline the prioritization of variants from ES data which is time-intensive, a dominant histologic lesion-based approach may help to select *in silico* genes panel. Furthermore, the UKD nephropathy classification should incorporate the dominant histological lesion as a crucial factor in accurately classifying patients during the phenotype process. We achieved a diagnostic yield of 72.5% using ES in cases with nonspecific histologic lesions, highlighting its value in enhancing the diagnostic work-up after inconclusive kidney biopsy, especially for patients with UKD who have a family history of kidney disease. This gene-oriented approach, known as the “reverse phenotype,” proves particularly beneficial for patients and their families in the context of biopsied patients with UKD who have nonspecific or vascular dominant lesions.^{32–36} Our findings suggest that ES provides valuable insights into the underlying causes of kidney disease, offering a new avenue for a deeper understanding of the disease mechanisms and aiding in informed treatment decisions. We firmly believe that ES and genome sequencing will play a pivotal role in the diagnostic work-up of kidney disease, complementing kidney biopsy and providing comprehensive information beyond biopsy alone. This advancement has the potential to significantly enhance our understanding of genetic kidney diseases in adults.

Considering the growing cost-effectiveness and decreased time required for ES, it holds the potential to serve as a first-line diagnostic tool when a kidney biopsy is indicated for isolated CKD with or without hematuria or proteinuria and a family history of kidney disease, especially in at-risk kidney biopsy patients.³⁷ This patient category demonstrates a diagnostic yield exceeding 50% in our study. This shift toward using ES as a noninvasive diagnostic tool has the potential to improve patient care and reduce the risks associated with invasive procedures. An exome-first approach may be thoroughly discussed in challenging situations, such as pregnancy or atrophic kidneys, when there is a familial kidney disease.

Genetic testing of individuals with UKD can offer a multitude of benefits for both the affected individual and their family. First, obtaining a precise genetic diagnosis enables personalized and targeted treatment strategies that are tailored to the individual’s specific needs. Furthermore, identifying the genetic mutation allows for the screening of at-risk relatives, enabling early detection and intervention in at-risk individuals. This facilitates timely medical intervention and appropriate monitoring for potential kidney-related complications. Genetic counseling plays a crucial role by providing valuable information on the inheritance pattern of the genetic disorder, empowering individuals and families to make informed decisions regarding family planning and reproductive options in the presence of severe disease. Our observations have highlighted the significant medical implications of genetic testing for patients and their families. For example, in conditions such as *UMOD* nephropathy that typically manifest later in life, early detection is essential to identify suitable healthy donors for transplantation, particularly when young living donors are available. In the case of FSGS, traditional immunosuppression is effective, but genetic causes of FSGS may require different management approaches. Genetic testing and identification of the specific genetic disorder are vital for guiding appropriate management decisions and optimizing patient outcomes. Testing patients for genetic diseases initially allows for tailored and effective therapeutic strategies, minimizing unnecessary exposure to immunosuppressive therapy or repeat kidney biopsies.³⁸ Diagnosing a kidney disorder caused by an X-linked mutation can have profound implications for the patient’s family, especially for female members who may be carriers without exhibiting symptoms. It empowers them to make informed decisions considering options such as genetic counseling, prenatal testing, or preimplantation genetic diagnosis based on the disease’s severity.³⁹

In our study, we found that there was a higher rate of positive ES results in females compared to males. This observation is consistent with a previous study conducted by the Irish Kidney Gene Project, which aims to investigate adult kidney disease in Ireland and explore the occurrence of familial clustering of kidney disease within the population and reported a link between the female gender and a positive family history of kidney disease.²⁵ It is possible that other genetic factors or mechanisms contribute to this disparity. Further research and exploration are warranted to better understand the underlying reasons for this finding.

Our study has some limitations. This was a single center approach with a modest cohort size.

Failure to identify age of first kidney manifestation as risk factor potentially suggests lack of statistical power because studies have demonstrated link between early-onset CKD and genetic kidney disease.⁴⁰ Lack of double-blind reviewing of the pathology slide is another limitation of the study. The lack of systematic electron microscopy may lead to an underestimation of collagenopathies with deep intronic variations, a limitation which should be considered. One important limitation of our study is the analysis of variable number tandem repeat of the *MUC1* gene which is not actually possible despite it being the dominant gene associated with tubulointerstitial disease.⁴¹ ES presents suboptimal coverage of regions of interest such as mitochondrial genome and difficulties to detect deep intronic variation. This leads to underestimation of genetic diagnosis.⁴²

CONCLUSION

ES has demonstrated a high diagnostic yield in biopsied patients with UKD, including those with nonspecific histologic lesions, highlighted the significant underestimation of genetic kidney diseases in the adult population. Collagen IV-related nephropathy, especially linked to heterozygous variation on *COL4A3* or *COL4A4* gene, is frequently overlooked in adult population. In patients presenting with advanced CKD and a family history of kidney disease, ES should be considered as a potential first-line diagnostic tool. The classification of kidney disease based on molecular diagnosis, in addition to clinical and kidney biopsy features, is a major challenge in nephrology, and ES in combination with kidney biopsy will impact kidney diseases ontology.

DISCLOSURE

All the authors have declared no competing interests.

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SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Figure S1. Exome yield (a) and genetics diagnosis (b) according to the kidney biopsy indication. (a) Exome yield according to kidney biopsy indication. Others are thrombotic microangiopathy ($n = 1$), hypertensive urgencies ($n = 4$), acute kidney injury ($n = 1$), rapidly

progressive glomerulonephritis ($n = 2$); (b) Genetic diagnoses according to kidney biopsy indication.

Figure S2. Exome yield (a) and genetics diagnosis (b) according to the dominant lesion in kidney biopsy. (a) Exome yield according to the dominant lesion histological lesion; (b) Genetic diagnoses according to the dominant lesion histological lesion.

Table S1. Light microscopic findings in kidney biopsy.

Table S2. Gene panel.

Table S3. Exome-solved cases with likely pathogenic or pathogenic variations.

Table S4. Characteristics comparison according to exome sequencing results.

Table S5. Description of Histological Findings (excluding patient with non-significant lesions) between collagenopathies/podocytopathies and Ciliopathies/tubulopathies.

Table S6. Histological findings and comparison according exome test results (excluding patient with non-significant lesions).

REFERENCES

- Jager KJ, Kovesdy C, Langham R, Rosenberg M, Jha V, Zoccali C. A single number for advocacy and communication-worldwide more than 850 million individuals have kidney diseases. *Kidney Int.* 2019;96:1048–1050. <https://doi.org/10.1016/j.kint.2019.07.012>
- Kovesdy CP. Epidemiology of chronic kidney disease: an update 2022. *Kidney Int Suppl.* 2011;12:7–11. <https://doi.org/10.1016/j.kisu.2021.11.003>
- Nguyen NTQ, Cockwell P, Maxwell AP, Griffin M, O'Brien T, O'Neill C. Chronic kidney disease, health-related quality of life and their associated economic burden among a nationally representative sample of community dwelling adults in England. *PLoS One.* 2018;13:e0207960. <https://doi.org/10.1371/journal.pone.0207960>
- Couchoud C, Stengel B, Landais P, et al. The renal epidemiology and information network (REIN): a new registry for end-stage renal disease in France. *Nephrol Dial Transplant.* 2006;21:411–418. <https://doi.org/10.1093/ndt/gfi198>
- Titze S, Schmid M, Köttgen A, et al. Disease burden and risk profile in referred patients with moderate chronic kidney disease: composition of the German Chronic Kidney Disease (GCKD) cohort. *Nephrol Dial Transplant.* 2015;30:441–451. <https://doi.org/10.1093/ndt/gfu294>
- Saran R, Li Y, Robinson B, et al. US renal data system 2015 annual data report: epidemiology of kidney disease in the United States. *Am J Kidney Dis.* 2016;67(suppl 1):S1–S305. <https://doi.org/10.1053/j.ajkd.2015.12.014>
- Cocchi E, Nestor JG, Gharavi AG. Clinical genetic screening in adult patients with kidney disease. *Clin J Am Soc Nephrol.* 2020;15:1497–1510. <https://doi.org/10.2215/CJN.15141219>
- Groopman EE, Marasa M, Cameron-Christie S, et al. Diagnostic utility of exome sequencing for kidney disease. *N Engl J Med.* 2019;380:142–151. <https://doi.org/10.1056/NEJMoa1806891>
- Wühl E, van Stralen KJ, Wanner C, et al. Renal replacement therapy for rare diseases affecting the kidney: an analysis of

- the ERA-EDTA Registry. *Nephrol Dial Transplant*. 2014;29(suppl 4):iv1–iv8. <https://doi.org/10.1093/ndt/gfu030>
10. Vivante A, Hildebrandt F. Exploring the genetic basis of early-onset chronic kidney disease. *Nat Rev Nephrol*. 2016;12:133–146. <https://doi.org/10.1038/nrneph.2015.205>
 11. Hays T, Groopman EE, Gharavi AG. Genetic testing for kidney disease of unknown etiology. *Kidney Int*. 2020;98:590–600. <https://doi.org/10.1016/j.kint.2020.03.031>
 12. Knoers N, Antignac C, Bergmann C, et al. Genetic testing in the diagnosis of chronic kidney disease: recommendations for clinical practice. *Nephrol Dial Transplant*. 2022;37:239–254. <https://doi.org/10.1093/ndt/gfab218>
 13. Tøndel C, Vikse BE, Bostad L, Svarstad E. Safety and complications of percutaneous kidney biopsies in 715 children and 8573 adults in Norway 1988–2010. *Clin J Am Soc Nephrol*. 2012;7:1591–1597. <https://doi.org/10.2215/CJN.02150212>
 14. Scheckner B, Peyser A, Rube J, et al. Diagnostic yield of renal biopsies: a retrospective single center review. *BMC Nephrol*. 2009;10:11. <https://doi.org/10.1186/1471-2369-10-11>
 15. Halimi JM, Gatault P, Longuet H, et al. Major bleeding and risk of death after percutaneous native kidney biopsies: a French nationwide cohort study. *Clin J Am Soc Nephrol*. 2020;15:1587–1594. <https://doi.org/10.2215/CJN.14721219>
 16. Halimi JM, Gatault P, Longuet H, et al. Major bleeding of transjugular native kidney biopsies. A French nationwide cohort study. *Kidney Int Rep*. 2021;6:2594–2603. <https://doi.org/10.1016/j.ekir.2021.07.011>
 17. Testard Q, Vanhoye X, Yauy K, et al. Exome sequencing as a first-tier test for copy number variant detection: retrospective evaluation and prospective screening in 2418 cases. *J Med Genet*. 2022;59:1234–1240. <https://doi.org/10.1136/jmg-2022-108439>
 18. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17:405–424. <https://doi.org/10.1038/gim.2015.30>
 19. Kopp JB, Nelson GW, Sampath K, et al. APOL1 genetic variants in focal segmental glomerulosclerosis and HIV-associated nephropathy. *J Am Soc Nephrol*. 2011;22:2129–2137. <https://doi.org/10.1681/ASN.2011040388>
 20. Stenson PD, Mort M, Ball EV, et al. The Human Gene Mutation Database: towards a comprehensive repository of inherited mutation data for medical research, genetic diagnosis and next-generation sequencing studies. *Hum Genet*. 2017;136:665–677. <https://doi.org/10.1007/s00439-017-1779-6>
 21. Olinger E, Schaeffer C, Kidd K, et al. An intermediate-effect size variant in UMOD confers risk for chronic kidney disease. *Proc Natl Acad Sci U S A*. 2022;119:e2114734119. <https://doi.org/10.1073/pnas.2114734119>
 22. Hill NR, Fatoba ST, Oke JL, et al. Global prevalence of chronic kidney disease—a systematic review and meta-analysis. *PLoS One*. 2016;11:e0158765. <https://doi.org/10.1371/journal.pone.0158765>
 23. Smith JM, Stablein DM, Munoz R, Hebert D, McDonald RA. Contributions of the transplant registry: the 2006 annual report of the North American pediatric renal trials and collaborative studies (NAPRTCS). *Pediatr Transplant*. 2007;11:366–373. <https://doi.org/10.1111/j.1399-3046.2007.00704.x>
 24. McClellan WM, Satko SG, Gladstone E, Krisher JO, Narva AS, Freedman BI. Individuals with a family history of ESRD are a high-risk population for CKD: implications for targeted surveillance and intervention activities. *Am J Kidney Dis*. 2009;53(Suppl 3):S100–S106. <https://doi.org/10.1053/j.ajkd.2008.07.059>
 25. Connaughton DM, Bukhari S, Conlon P, et al. The Irish Kidney gene project—prevalence of family history in patients with kidney disease in Ireland. *Nephron*. 2015;130:293–301. <https://doi.org/10.1159/000436983>
 26. Onoe T, Hara S, Yamada K, et al. Significance of kidney biopsy in autosomal dominant tubulointerstitial kidney disease—UMOD: is kidney biopsy truly nonspecific? *BMC Nephrol*. 2021;22:1. <https://doi.org/10.1186/s12882-020-02169-x>
 27. Ben Moshe Y, Bekheirnia N, Smith RJH, Hicks J, Braun MC, Bekheirnia MR. Genetic diagnosis and renal biopsy findings in the setting of a renal genetics clinic. *Am J Med Genet C Semin Med Genet*. 2022;190:302–308. <https://doi.org/10.1002/ajmg.c.32009>
 28. Quinlan C, Rheault MN. Genetic basis of Type IV collagen disorders of the kidney. *Clin J Am Soc Nephrol*. 2021;16:1101–1109. <https://doi.org/10.2215/CJN.19171220>
 29. Savige J, Lipska-Zietkiewicz BS, Watson E, et al. Guidelines for genetic testing and management of Alport syndrome. *Clin J Am Soc Nephrol*. 2022;17:143–154. <https://doi.org/10.2215/CJN.04230321>
 30. Robert T, Raymond L, Dancer M, et al. Beyond the kidney biopsy: genomic approach to undetermined kidney diseases. *Clin Kidney J*. 2023. <https://doi.org/10.1093/ckj/sfad099>. Forthcoming.
 31. Kashtan CE, Ding J, Garosi G, et al. Alport syndrome: a unified classification of genetic disorders of collagen IV α 345: a position paper of the Alport Syndrome Classification Working Group. *Kidney Int*. 2018;93:1045–1051. <https://doi.org/10.1016/j.kint.2017.12.018>
 32. KDIGO conference participants. Genetics in chronic kidney disease: conclusions from a Kidney Disease: Improving Global Outcomes (KDIGO) Controversies Conference. *Kidney Int*. 2022;101:1126–1141. <https://doi.org/10.1016/j.kint.2022.03.019>
 33. Dryja TP. Gene-based approach to human gene-phenotype correlations. *Proc Natl Acad Sci U S A*. 1997;94:12117–12121. <https://doi.org/10.1073/pnas.94.22.12117>
 34. Ahram DF, Aggarwal VS, Sanna-Cherchi S. Phenocopies, phenotypic expansion, and coincidental diagnoses: time to abandon targeted gene panels? *Am J Kidney Dis*. 2020;76:451–453. <https://doi.org/10.1053/j.ajkd.2020.07.003>
 35. Schrezenmeier E, Kremerskothen E, Halleck F, et al. The underestimated burden of monogenic kidney disease in adults waitlisted for kidney transplantation. *Genet Med*. 2021;23:1219–1224. <https://doi.org/10.1038/s41436-021-01127-8>
 36. Ottlewski I, Münch J, Wagner T, et al. Value of renal gene panel diagnostics in adults waiting for kidney transplantation due to undetermined end-stage renal disease. *Kidney Int*. 2019;96:222–230. <https://doi.org/10.1016/j.kint.2019.01.038>
 37. Kalatharan V, Lemaire M, Lanktree MB. Opportunities and challenges for genetic studies of end-stage renal disease in Canada. *Can J Kidney Health Dis*. 2018;5:2054358118789368. <https://doi.org/10.1177/2054358118789368>
 38. Mann N, Braun DA, Amann K, et al. Whole-exome sequencing enables a precision medicine approach for kidney transplant recipients. *J Am Soc Nephrol*. 2019;30:201–215. <https://doi.org/10.1681/ASN.2018060575>

39. Jayasinghe K, Stark Z, Kerr PG, et al. Clinical impact of genomic testing in patients with suspected monogenic kidney disease. *Genet Med*. 2021;23:183–191. <https://doi.org/10.1038/s41436-020-00963-4>
40. Domingo-Gallego A, Pybus M, Bullich G, et al. Clinical utility of genetic testing in early-onset kidney disease: seven genes are the main players. *Nephrol Dial Transplant*. 2022;37:687–696. <https://doi.org/10.1093/ndt/gfab019>
41. Murray SL, Fennelly NK, Doyle B, Lynch SA, Conlon PJ. Integration of genetic and histopathology data in interpretation of kidney disease. *Nephrol Dial Transplant*. 2020;35:1113–1132. <https://doi.org/10.1093/ndt/gfaa176>
42. Wang X, Zhang Y, Ding J, Wang F. mRNA analysis identifies deep intronic variants causing Alport syndrome and overcomes the problem of negative results of exome sequencing. *Sci Rep*. 2021;11:18097. <https://doi.org/10.1038/s41598-021-97414-0>