





# Genetic testing in the diagnosis of chronic kidney disease: recommendations for clinical practice

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## ABSTRACT

The overall diagnostic yield of massively parallel sequencing-based tests in patients with chronic kidney disease (CKD) is 30% for paediatric cases and 6–30% for adult cases. These figures should encourage nephrologists to frequently use genetic testing as a diagnostic means for their patients. However, in reality, several barriers appear to hinder the implementation of massively parallel sequencing-based diagnostics in routine clinical practice. In this article we aim to support the nephrologist to overcome these barriers. After a detailed discussion of the general items that are important to genetic testing in nephrology, namely genetic testing modalities and their indications, clinical information needed for high-quality interpretation of genetic tests, the clinical benefit of genetic testing and genetic counselling, we describe each of these items more specifically for the different groups of genetic kidney diseases and for CKD of unknown origin.

**Keywords:** chronic kidney disease, clinical benefit, genetic counselling, genetic testing, massively parallel sequencing, monogenic diseases

## ADDITIONAL CONTENT

An author video to accompany this article is available at: [https://academic.oup.com/ndt/pages/author\\_videos](https://academic.oup.com/ndt/pages/author_videos).

## INTRODUCTION

Monogenic diseases are an underestimated yet very important cause of chronic kidney disease (CKD). They are estimated to account for 70% and 10–15% of the overall prevalence of end-stage kidney disease (ESKD) in children and adults, respectively. These prevalence estimates are based on large registries, such as the European Rare Kidney Disease Registry [1], and on

## KEY LEARNING POINTS

### What is already known about this subject?

- genetic testing using modern massively parallel sequencing techniques is becoming more and more important in nephrology but is not yet used routinely in daily clinical practice; and
- barriers to incorporating genetic testing in routine nephrology practice include limited genetic literacy, a lack of perceived benefit, difficulties in interpreting the identified genetic variants, and the need for genetic counselling.

### What this study adds?

- in this article, we aim to support the nephrologist to overcome these barriers;
- we discuss general items that are important to genetic testing in nephrology, including testing modalities and indications, clinical information needed, clinical benefit of genetic testing and genetic counselling; and
- we then discuss each of these items for different groups of inherited kidney diseases and for chronic kidney disease of unknown origin.

### What impact this may have on practice or policy?

- with these general and specific recommendations on genetic testing, we hope to encourage and support nephrologists to frequently use genetic testing as a diagnostic means for their patients and increase diagnostic yield and clinical benefit.

published data on genetic testing of monogenic causes of ESKD in different cohorts [2–5]. Mutations in >400 genes are related to inherited kidney diseases. Early detection of a monogenic cause for CKD can have important implications for patients and their family members, for instance in terms of management, prognosis, genetic counselling and screening of at-risk family members [6]. With the advent of massively parallel sequencing (MPS) techniques [previously referred to as next-generation sequencing (NGS)], the possibilities of accurately diagnosing inherited kidney diseases have increased enormously. In recent studies, the overall diagnostic yield of genetic testing using MPS technology in patients with CKD was 30% in paediatric cohorts and 6–30% in adult cohorts [4, 5, 7–9]. In these studies, not only patients with presumably monogenic causes of CKD based on clinical and/or histological phenotype and/or family history were included, but also patients with CKD of unknown origin in whom the clinical/histological phenotype nor family history pointed towards a monogenic cause of the disease. In studies examining the diagnostic yield of MPS-based testing in patients with specific diseases (or disease groups), even higher yields were reported, such as 55–80% in Alport syndrome and 64% in patients with renal tubulopathies [10, 11]. Despite the evidence for the diagnostic utility of MPS in CKD, genetic testing is not always used as a diagnostic means in routine clinical practice, especially not in adult nephrology. Barriers to incorporating MPS diagnostics in routine nephrology practice include limited genetic literacy, a lack of perceived benefit, the challenge of identifying the best diagnostic test for an individual patient, the difficulties in interpreting the identified genetic variants, concerns about costs and reimbursement and the need of pre- and post-test counselling.

In this article we aim to support the nephrologist to overcome these barriers and to encourage them in (further) implementation of genetic testing as a diagnostic means in their daily clinical practice. We will first discuss, in more general terms,

important items related to genetic testing in nephrology, including different genetic testing modalities and their indications, the clinical information that is needed to allow high-quality interpretation of the genetic test, the predicted clinical benefit of genetic testing and the importance of pre- and post-test counselling, including ethical and psychosocial implications of genetic testing. In the second part of this article we will more specifically discuss each of these items for the different groups of monogenic kidney disorders [glomerulopathies, renal tubulopathies, complement disorders, congenital anomalies of the kidney and urinary tract (CAKUT) and renal ciliopathies] and for CKD of unknown origin.

## DIFFERENT GENETIC TESTING MODALITIES AND THEIR INDICATIONS

Most genome diagnostic laboratories offer a wide range of genetic analysis for diagnostic testing (Box 1). Previously, Sanger sequencing of one or only a few selected genes sequentially was the major genetic test for diagnosing inherited kidney diseases. Nowadays, Sanger sequencing is rarely used, and only for disorders with minimal locus heterogeneity (only one gene involved), and MPS techniques are preferably being applied in diagnostics (Table 1). MPS techniques enable the simultaneous sequencing of the exons of a subset of genes associated with a particular phenotype (targeted phenotype-associated gene panels), of the exons of all 21 000 protein-coding human genes [exome sequencing (ES), previously referred to as whole ES] or of the complete genome [genome sequencing (GS), previously referred to as whole GS].

The primary scope of targeted MPS-based phenotype-associated gene panels and ES is the identification of small variants [single-nucleotide variants (SNVs) or small insertions/deletions (INDELs)] within the genes of interest for the clinical phenotype or within the coding region of the genome, respectively.

## Box 1. Genetic analysis techniques used in diagnostic laboratories

### Sanger sequencing

Sanger sequencing is a 'first-generation' sequencing technique and is based on the incorporation of labelled chain-terminating dideoxynucleotides during polymerase chain reaction (PCR), followed by electrophoretic size separation and subsequent visualization of the label signals.

### Copy number variation (CNV) assays:

#### Comparative genomic hybridization (CGH)/Single Nucleotide polymorphism (SNP) arrays

- Microarray-based techniques to detect large CNVs (deletions and duplications).

#### Multiplex ligation-dependent probe amplification (MLPA)

- Targeted PCR-based technique to detect both large and small CNVs (can detect CNVs in anything from complete chromosomes to single exons).

### Massively Parallel Sequencing (MPS)

- MPS encompasses several high-throughput sequencing approaches.

#### Targeted sequencing

- Targeted sequencing is the sequencing of specific areas of interest of the genome for in-depth analysis. Before sequencing, the genome is enriched for these areas of interest (either genes associated with a specific phenotype or the whole exome).

#### Targeted gene panel sequencing

- Simultaneous sequencing of a specific pre-selected set of genes relevant to a disease phenotype.

#### Exome Sequencing (ES)

- ES is targeted sequencing of the exome (the coding part of the genome), which constitutes 1–2% of the genome.

#### Genome Sequencing (GS)

- GS is sequencing of the entire genome, including the non-coding, regulatory DNA.

The targeted phenotype-associated gene panels are used for the diagnosis of disorders with locus heterogeneity, disorders with overlapping phenotypes or disorders with common pathways. Until recently these gene panels were mostly targeted gene enrichment-based panels, only allowing the sequencing of a set of preselected genes. This approach has the advantage that it will not yield incidental findings (IFs) in genes unrelated to the primary indication for testing, but the disadvantage is that updating of these enrichment-based panels with newly discovered relevant genes requires redesign and validation of the assay [12]. Nowadays, many diagnostic labs prefer to use phenotype-associated gene panels that are exome-based (targeted ES,

virtual gene panels; Table 1). This means that the exome is sequenced but only indication-relevant genes are analysed and interpreted by using *in silico* bioinformatics tools. This exome-based approach is more attractive to diagnostic laboratories because it allows dynamic gene content update with minimal design and validation. So when new disease-causing genes are discovered, a bioinformatics reanalysis of the already available data is sufficient. At present, in most diagnostic labs, such a bioinformatic reanalysis of the exome only takes place after a request of the physician treating the patient. In addition, when no (likely) pathogenic variants are identified in the indication-relevant genes, it is relatively easy to 'open up' the exome backbone data and look beyond the known genes, maximizing the opportunity for finding the causal variant and new candidate variants (second-tier test) [13]. A disadvantage of using exome-based virtual gene panels is that exome data tend to have less uniform sequence coverage than the targeted phenotype-associated gene panels, so genes of specific interest might need to be gap-filled using Sanger sequencing. It is important to emphasize that opening up the exome backbone data may reveal variants predictive for other diseases not related to the initial reason for testing (IFs; for further elaboration, see genetic counselling) and therefore specific consent related to the reporting of these findings is necessary.

In cases of unexplained kidney failure, targeted ES, with a second-tier option of analysis of the full exome, is the preferred first-tier test (Table 1).

Importantly, larger copy number variants (CNVs), some of which are important causes of inherited kidney diseases (Table 2), are not easily picked up by MPS-based gene panels or ES. Sophisticated bioinformatic tools are necessary to detect these large CNVs from gene panel or ES data and these are not yet routinely used in all diagnostic laboratories. Instead, the still preferred methodology for routine diagnostics of large CNVs in many labs is the microarray-based technique [comparative genomic hybridization (CGH) or single-nucleotide polymorphism (SNP) arrays; Table 1]. Multiplex ligation-dependent probe amplification (MLPA) is another technique to pick up CNVs. In addition, several important disease-causing variants, such as pathogenic variants in *PKD1*, can be missed using MPS-based panels or ES, due to the complexity of the involved genomic region [14, 15] (Table 2).

Some of the limitations of MPS-based gene panels and/or ES can be addressed by GS. GS can not only identify SNVs and INDELS in both coding and non-coding regions, but it can also pick up large CNVs and detect pathogenic variants in complex genomic regions, such as the *PKD1* mutations (Table 1) [16]. However, GS is not yet commonly used in clinical practice. This is due to the costs and time associated with GS and the complex interpretation of variants, especially intronic and other non-coding variants. However, this may change in the near future with the anticipated decline in sequencing costs and the expectation that the capability to interpret non-coding regions in the genome will improve over time [17].

MPS creates a bulk of genomic data, thus the sequencing data need to be adequately annotated and filtered for variant calling. Estimating the pathogenicity of variants is performed based on the population frequency of a variant, the *in silico* prediction of

**Table 1. Different testing modalities and their current indications in nephrology**

Test	Indications	Examples
Sanger sequencing	Disorders with minimal locus heterogeneity	Fabry disease ( <i>GLA</i> ), Denys–Drash ( <i>WT1</i> ), cystinosis ( <i>CTNS</i> )
CGH/SNP array, MLPA	Large CNVs suspected	CAKUT, aHUS ( <i>CFH</i> , <i>CFHR</i> ), nephronophthisis ( <i>NPHP1</i> )
Targeted phenotype-associated gene panel	Disorders with locus heterogeneity	SRNS
Targeted ES (virtual gene panel)	Disorders with overlapping phenotypes	Hereditary tubulopathies,
	Disorders associated with genes from common pathway	Complement-related disorders
ES	Phenotype indistinct and underlying cause unknown	Unexplained kidney failure
	Second-tier test after gene panel testing	
GS	Due to high costs, interpretation challenges and long analytical period, currently only used in research for cases still unsolved after ES	ADPKD ( <i>PKD1</i> )
	Emerging clinical use	

**Table 2. Possible reasons for negative results after gene testing using MPS gene panels or ES**

Reason	Examples
Mutations in genes that represent phenocopies of a disease may be missed when using phenotype-associated gene panels	<i>HNF1B</i> -mediated cystic kidney disease can mimic ADPKD/ADTKD Mutations in <i>GLA</i> (Fabry) can mimic SRNS
Not all genes associated with given phenotype are tested in phenotype-associated gene panels	Currently unknown or very recently identified genes for heterogeneous diseases such as renal ciliopathies and steroid-resistant nephrotic syndrome
Detection of large CNVs from MPS gene panels/ES data is challenging; specific CNV detection algorithms are not automatically performed in diagnostic setting and therefore CNVs might be missed	<i>HNF1B</i> and <i>NPHP1</i> full gene deletions (CAKUT and nephronophthisis, respectively)
Variants in some genomic regions are poorly discovered with MPS gene panels or ES, such as mutations in regions with high GC content and/or with high sequence homology	High GC content in first exon of <i>COL4A3</i> (Alport syndrome) <i>PKD1</i> (ADPKD) has high GC content and sequence homology with six pseudogenes located nearby
Some pathogenic variants are not discovered by any of the MPS-based techniques	Cytosine insertion in variable number tandem repeat sequences in <i>MUC1</i> ( <i>MUC1</i> -ADTKD)
Variants in non-coding (intronic or regulatory) regions or imprinting defects are not detected with disease-specific gene panels or ES	Deep intronic mutations in <i>DGKE</i> (aHUS) Imprinting defect in Beckwith–Wiedemann syndrome

the variant's effect on, among others, the protein structure, if the variant could explain the phenotype and if the variant segregates with the disease in the family. A multidisciplinary approach involving clinical geneticists, genetic laboratory specialists and medical specialists (nephrologists) to adequately consider all these aspects is usually necessary for a correct interpretation of the MPS results. If variants are then deemed (likely) pathogenic, results can be translated back to the individual patient [13].

One of the most challenging questions in genetic diagnostics using MPS is how to deal with variants of uncertain significance (VUS), variants for which available evidence, if any, fails to significantly support either a pathogenic or a neutral significance. Local hospital policies differ on whether or not to disclose these VUS to patients. In the classification guidelines of the American College of Medical Genetics and Genomics (ACMG), it is recommended not to use VUS for clinical decision making and to undertake major efforts to resolve the classification of VUS to either benign or pathogenic, for instance, by

segregation analysis in the family, functional studies and data sharing [18]. It is important to realize that the pathogenicity of some of the previously reported variants has been called into question upon reanalysis because they appeared to have a relatively high frequency in control exomes/genomes [19, 20]. We therefore recommend, when using older literature describing 'pathogenic' variants, to carefully determine what is the strength of the supporting evidence for pathogenicity and also consult updated clinical variant databases, such as ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar/> open access), Leiden Open Variant Database (<https://www.lovd.nl/> open access) or the Human Gene Mutation Database (<https://digitalinsights.qia.gen.com/products-overview/clinical-insights-portfolio/human-gene-mutation-database/>; licensed). In recent years, the efforts of organizations like ClinGen (<https://clinicalgenome.org/>) and Genomics England with PanelApp (<https://panelapp.genomic.sengland.co.uk>), comprising specific clinical domain groups and/or expert panels have become very helpful in



defining the clinical relevance of the identified genes and variants (gene and variant curation) for various forms of genetic kidney diseases.

The reporting times of diagnostic genetic testing vary from centre to centre, largely depending on the type of test, the local infrastructure/laboratory facilities, the bioinformatic capacity and clinical urgency. However, for all clinical genetic testing indications we recommend that the reporting time should not exceed 8–12 weeks and for urgent indications (i.e. prenatal and neonatal) it should not exceed 2 weeks.

### CLINICAL INFORMATION NEEDED TO ALLOW HIGH-QUALITY INTERPRETATION OF THE GENETIC TEST

To help interpret MPS data for diagnostic purposes, a complete and precise clinical phenotype, a family history and clinical test results are important. In general, a medical history, including (presenting) renal symptoms, the age of onset, the course of the disorder and the findings of physical exams, including the presence/absence of extrarenal features, should be provided. The results of laboratory tests (e.g. kidney function), renal imaging and/or a renal biopsy and earlier genetic tests are also helpful.

A family history with a detailed three-generation pedigree, including both affected and unaffected individuals, can give essential information on the most likely pattern of inheritance of the disorder. For example, a pedigree with affected siblings in one family only and/or parental consanguinity suggests a recessive inheritance pattern, while a pedigree with more affected individuals in successive generations, points towards dominant inheritance.

Recently numerous computational gene/variant ranking tools have been developed that incorporate a rare disease patient's phenotype into the interpretation of his/her sequencing data [e.g. Exomiser (<http://www.sanger.ac.uk/science/tools/exomiser>)] [21]. These tools all require that the patients' phenotypic information is encoded in terms from the Human Phenotype Ontology (HPO), a vocabulary of phenotypic abnormalities encountered in human diseases (<https://hpo.jax.org>) [22]. HPO contains >13 000 terms describing phenotypic abnormalities and >150 000 annotations to hereditary diseases and has become the *de facto* standard for deep phenotyping of rare diseases. Therefore we recommend referring clinicians provide the clinical abnormalities to the diagnostic lab using HPO terms. In order to spare clinicians hours of work to manually find and encode the matching phenotypic HPO terms for a specific patient, tools are being developed that automatically convert clinical notes from electronic health records (EHRs) into a prioritized list of a patient's phenotype in HPO terms [e.g. ClinPhen (<http://bejerano.stanford.edu/clinphen>)] [23].

### PREDICTED CLINICAL BENEFIT OF GENETIC TESTING

The potential benefits of molecular genetic testing for inherited kidney diseases are numerous. First, genetic testing has the potential to provide an accurate diagnosis of the underlying cause of

the disease through a minimally invasive and increasingly time- and cost-effective test. An early genetic diagnosis may avoid the 'diagnostic odyssey' that many rare disease patients face, with unnecessary and potentially harmful diagnostic procedures, multiple misdiagnoses and incorrect treatments. An early genetic diagnosis might even obviate the need for a diagnostic kidney biopsy, although some investigators consider genetic testing as a complementary diagnostic technique to biopsy in the evaluation of patients with kidney disease [24]. A genetic diagnosis can lead to a reclassification of the original clinical or histological diagnosis. For instance, patients with Alport syndrome, caused by *COL4A3-5* mutations, have been misdiagnosed as having membranoproliferative glomerulonephritis (MPGN) and patients with congenital chloride diarrhoea, caused by *SLC26A3* mutations, have been misdiagnosed as having Bartter syndrome [25, 26]. In large cohorts of CKD patients it has been shown that genetic testing leads to a reclassification of the original diagnosis in 10–22% of cases [4, 5, 8]. Second, a genetic diagnosis can give clues towards early detection of potential extrarenal features. For instance, in CAKUT patients with *PAX2* mutations, screening for eye abnormalities is recommended [27]. In patients with *HNF1B* mutations, clinicians should be aware of potential extrarenal complications such as diabetes, liver abnormalities and pancreatic abnormalities [28]. Patients with *WT1* nephropathy, depending on their genotype, are at high risk of developing either nephroblastoma or gonadoblastoma, necessitating close monitoring [29]. Third, a genetic diagnosis can guide prognostic and therapeutic decision making in patients with nephrogenetic diseases. For instance, the type of *COL4A* mutation in a patient with Alport syndrome can provide information regarding renal and extrarenal (e.g. hearing loss) phenotypes and the risk of post-transplantation anti-glomerular basement membrane (GBM) glomerulonephritis [30]. A genetic diagnosis can also prevent the prescription of ineffective therapies, such as immunosuppressive drugs in genetic forms of nephrotic syndrome. Fourth, a genetic diagnosis is crucial for precise genetic counselling, provides information about recurrence risks, facilitates reproductive options and in some cases offers presymptomatic testing opportunities for family members at risk of having the same disorder. Finally, a genetic diagnosis may be of pivotal importance in the setting of kidney transplantation, especially when living-related donation is involved [31]. Ascertaining the genetic origin of ESKD in a transplant candidate is essential to evaluate the risk of transmission of kidney disease for the biologically related donor, especially in hereditary nephropathies with age-dependent manifestation [autosomal dominant tubulointerstitial kidney disease (ADTKD)-*UMOD*] or diseases with variable expressivity and reduced penetrance such as hepatocyte nuclear factor-1-beta (*HNF1B*)-associated disease (ADTKD-*HNF1B*). In families affected by these diseases, gene-specific variant detection has considerable potential for accurate risk and donor suitability assessment among relatives who are candidates for kidney donation [32].

Even a negative result of genetic screening may have clinical relevance. For instance, in atypical haemolytic uraemic syndrome (aHUS), a multicentre prospective study recently documented that discontinuation of anti-C5 therapy based on negative genetic testing may be reasonable and safe. Indeed, the

## Box 2. Recommendations for using MPS-based tests in nephrology

### Requesting genetic tests by clinicians

Nephrologists can order MPS-based genetic testing for symptomatic patients for diagnostic purposes, after appropriate counselling. When gene panels contain a large number of genes (or the exome) with a potential of IFs, consider consulting a clinical geneticist or refer the patient to a clinical geneticist for pre-test counselling.

Orders should be accompanied by detailed phenotypic information, including a family history/pedigree.

Ordering of pre-symptomatic tests for asymptomatic, at-risk family members is as yet reserved for clinical geneticists and only after adequate genetic counselling.

### Pre-test genetic counselling

Inform patients about the possible outcomes of the genetic test, including the possibility that the test may not give any positive results.

Discuss the possibility of VUS and IFs, and their potential implications, including those for family members, explain the hospital's policy with regard to these findings and emphasize the patient's right to not receive these results.

Emphasize that a genetic diagnosis may, but not always, will lead to a change in management and/or to prognostic information.

Mention the possible psychosocial and insurance consequences of receiving a definite diagnosis, including consequences for prognosis and the chances of developing extrarenal symptoms in some disorders.

### Post-test genetic counselling

In case of (likely) pathogenic variants, provide more information on the associated disease and, if possible, on the prognosis and possibilities for (a change of) management. This also means that the patient is eligible for (future) clinical trials for the associated disease. Also mention the patient advocacy groups for the specific disease(s).

Involve a clinical geneticist or genetic counsellor to discuss recurrence risks, screening advice for family members and possibilities for family planning, including reproduction options, such as sperm cell donation, prenatal diagnosis and pre-implantation genetic testing.

Discuss the potential implications of the genetic results for family members and encourage patients to share this information with at-risk family members. Mention the possibility of pre-symptomatic testing for these family members via a clinical geneticist.

In case of VUS, discuss the need for additional investigations.

### General considerations

For phenotype-associated gene panels or ES/GS, specific consent is necessary, especially related to the increasing chance of IFs, which is small in panels and larger in ES/GS. However, this is also very dependent on local policies, whether centres/laboratories offer patients opt-out or opt-in protocols on IFs. The local policy about dissemination of these IFs should be clear to the patient.

Genetic testing can confirm but does not rule out a genetic cause of the disease, owing to factors such as technical limitations, phenocopies and the presence of unknown causative genes.

VUS should not be used for clinical decision making.

relapse after eculizumab discontinuation was predicted by the presence of a complement gene abnormality [33].

## GENETIC COUNSELLING

Diagnostic genetic testing should be accompanied by careful pre- and post-test genetic counselling, especially when MPS-based techniques are used (Box 2). Appropriate pre-test counselling on the opportunities, limitations and possible results of genetic testing allows patients or parents of an affected child to make an informed decision on whether to undergo genetic testing and to understand the potential outcomes of the test. When the local policy is to return VUS to patients, the chance of finding these variants should be emphasized, including the possibility that additional investigations and data sharing might be necessary to interpret these VUS, and also their potential to

become meaningful over time. The counselling should also include the chance of IFs, unanticipated findings not related to the initial reason for genetic testing but which could be predictive of risk for other diseases, which may or may not be medically actionable. The reporting of these unexpected findings is the subject of an ongoing international ethical debate.

In recent years, several policy documents have been published regarding the return of IFs in the USA, Europe and Canada. The ACMG has established a list of 59 genes for which (likely) pathogenic mutations are believed to be strongly predictive of potentially life-threatening diseases, such as cancer and cardiovascular diseases [34]. Because early detection of these diseases may be beneficial in terms of surveillance or treatment, the ACMG advocates routine analysis of these 59 genes (recently updated to 73 genes) and reporting all the (likely) pathogenic variants when performing clinical ES, unless patients opt

out [34, 35]. They also changed the terminology to ‘secondary’ findings, following their advice to intentionally analyse these 59 genes. In contrast, the European Society of Human Genetics and the Canadian College of Medical Geneticists advocate a strict and proportionate application of clinical sequencing and prefer performing targeted exome (or genome)-based gene panel testing, analysing only the known disease-associated genes and thereby minimizing discovery of secondary findings [36, 37]. In practice, none of these policy documents have been accepted as the general standard and the policies and tools (e.g. consent) regarding IFs revealed by MPS still differ enormously between and even within countries.

Finally, patients should be informed about the possible psychosocial implications of having a definite diagnosis and related prognosis and about potential consequences for insurability.

Post-test counselling should include detailed information on the results of the genetic test (pathogenic mutations or VUS) or the meaning of negative results (Table 2 and Box 2). In case of pathogenic variants, more information, when available, should be given on the related disease, its prognosis and treatment options. Patients or parents of an affected child should be informed about the recurrence risks and the implications of the results of the genetic test for family planning and reproductive options. They should also be informed about the potential implications of the pathogenic variants for family members. Patients and parents should be encouraged to share that information with their family members at risk. These family members can then be counselled and, when applicable, undergo pre-symptomatic genetic testing. This offers them the opportunity to receive effective early treatment to slow CKD progression and prevent secondary morbidity.

## GLOMERULOPATHIES

The term glomerulopathies refers to abnormalities affecting the establishment and maintenance of the glomerular filtration barrier composed of the podocyte, the GBM and the fenestrated endothelial cells. Hereditary entities account for at least one-quarter of paediatric proteinuric glomerulopathies and are also identified in a growing fraction of adult-onset cases [38–40]. The likelihood of identifying a causative genetic abnormality is inversely related to the age at disease onset. In congenital nephrotic syndrome (CNS), screening of *NPHS1*, *NPHS2*, *WT1* and *LAMB2* identifies the underlying genetic defect in ~80% of cases, while several other less commonly mutated genes account for an additional ~5% of diagnoses. *NPHS2*, *WT1* and *NPHS1* are also the most common causes of childhood-onset hereditary steroid-resistant nephrotic syndrome in European patients, while in Asians defects in *COQ8B* are also common [38, 39, 41]. To date, >60 genes (Supplementary data, Table S1) have been linked to glomerulopathies. Abnormalities in several other kidney disease genes may present as clinical phenocopies of a proteinuric glomerulopathy, e.g. *CUBN* (a tubular gene), *CLCN5* (Dent disease, a proximal tubular defect) and *PAX2* (a *CAKUT* gene).

Most glomerulopathy genes are selectively or preferentially expressed in the podocyte, justifying the term ‘podocytopathies’. These include components of the slit diaphragm (e.g. *NPHS1*),

the podocyte cytoskeleton (e.g. *MYO1E*, *ACTN4* and *INF2*) and the membrane protein complex linking these structures (e.g. *NPHS2* and *TRPC6*). Proteinuric glomerulopathies can also be caused by genetic abnormalities in genes encoding regulatory elements involved in podocyte differentiation and maintenance, including nuclear and mitochondrial genes involved in mitochondrial energy provision (e.g. *COQ2*, *COQ6*, *COQ8B* and *mt-tRNAs*) or transfer RNA modification (e.g. *KEOPS* complex genes, *WDR4*), nuclear transcription factors (e.g. *WT1*, *LMX1B* and *SMARCAL1*), nuclear pore complex proteins (e.g. *NUP93* and *NUP107*) and membrane proteins involved in the anchoring of podocyte foot processes in the extracellular matrix (*LAMB2*, *ITGB4* and *ITGA3*). Alterations in the genes encoding the triple helix proteins that form type IV collagen (*COL4A1*, 3, 4 and 5), the main constituent of the GBM, cause glomerulopathies with initially predominant haematuria that may progress to proteinuria and renal failure in the disease course, but may also present with a nephrotic syndrome phenotype. These disorders are collectively termed type IV collagen disorders.

### Genetic testing: indications and preferred testing strategies

In patients with CNS, genetic testing is recommended as a first-line diagnostic procedure that should be performed as part of the initial patient evaluation [42, 43]. For nephrotic syndrome manifesting in later childhood, genetic testing should be considered in all cases that do not respond to standard steroid therapy [steroid-resistant nephrotic syndrome (SRNS)] [44]. Priority should be given to family history of proteinuria, haematuria or CKD of unknown origin, cases with extrarenal features and those undergoing preparation for renal transplantation. Conversely, genetic testing is not recommended in initially steroid-responsive patients who develop steroid resistance later in their disease course.

Comprehensive genetic screening comprising all CNS/SRNS-related genes (Supplementary data, Table S1) is recommended with either a targeted phenotype-associated gene panel or targeted ES.

For patients with a multiorgan phenotype suggestive of syndromic SRNS (Supplementary data, Table S1), direct testing for defects in the related genes can be performed as the first step, followed by comprehensive genetic testing if no pathogenic variant is detected in the expected genes. This may require extended additional clinical and diagnostic evaluation with the aim of identifying subtle extrarenal signs and symptoms [42–44].

### Clinical benefit of genetic testing

In patients with proteinuric glomerulopathies, genetic screening is of high clinical relevance for clinical management and prognosis. Patients with hereditary disorders do not respond to immunosuppressive therapies and can therefore be spared the potential toxicity of these ineffective medications. Instead, renin–angiotensin system (RAS) inhibitors effectively lower proteinuria and are likely to extend the survival of kidney function in hereditary glomerulopathies [45]. Moreover, a specific pharmacotherapy is available for patients with biallelic

pathogenic variants in genes that lead to coenzyme Q10 synthase deficiency [46]. Another important benefit of genetic testing in this disease group is the fact that, unlike immune-mediated glomerulopathies, genetic kidney diseases are not recurring in the kidney after kidney transplantation. Finally, the confirmation of a genetic disease cause can be particularly useful in oligosymptomatic syndromic disorders where relevant extrarenal features can escape clinical detection or may arise later in the course of disease, such as hearing loss with the collagen IV genes and *COQ6*, sex reversal and Wilms tumours with *WT1* or adrenal insufficiency with *SPGL1* mutations.

### Genetic counselling

For families with hereditary glomerulopathies, genetic counselling can support their decision making regarding prenatal diagnosis, including prenatal and pre-implantation diagnostics in subsequent pregnancies. For certain syndromic entities (e.g. Galloway–Mowat syndrome, *SGPL1* glomerulopathy), palliative care may be considered depending on the severity of the disease. Genetic counselling is also relevant concerning potential organ donorship of first-degree relatives. Donor screening is obligatory for diseases with dominant transmission and in certain entities with significant intra- and interfamilial variability and incomplete or age-dependent penetrance (e.g. *WT1*; *COL4A3*, 4 or 5; and *NPHS2*).

## TUBULOPATHIES

The term tubulopathies refers to abnormalities of proteins involved directly or indirectly in epithelial transport along the renal tubules. They have an important role in body homeostasis, adjusting the reabsorption and secretion of water and solutes. Inherited tubulopathies cover a group of abnormalities with several modes of inheritance, a variable presentation (in terms of age and severity) and often substantial clinical and biological overlap [47, 48]. They are more frequently diagnosed in children than in adults, particularly those with autosomal recessive transmission. In a recent European collaborative study, hereditary disorders accounted for 64% and 29% of cases of paediatric and adult patients with a clinical diagnosis of tubulopathy, respectively [11, 49]. To date, >60 genes have been linked to tubulopathies (Supplementary data, Table S2). Most of the encoded proteins are directly responsible for the reabsorption or secretion of solutes and water (transporters, pumps or channels), which could be transepithelial or paracellular [e.g. renal hypophosphataemia, Bartter syndrome types 1–3, familial hypomagnesaemia with hypercalciuria and nephrocalcinosis (FHHNC), distal renal tubular acidosis and nephrogenic diabetes insipidus]. Other proteins regulate the expression or activity of transporters, pumps or channels (e.g. Bartter syndrome types 4a and 5, pseudohypoaldosteronism type 2 and nephrogenic diabetes insipidus) and others take part in intracellular processes such as endocytosis (Dent disease).

Some metabolic diseases have been included in this group because they have a tubulopathy as the first manifestation (e.g. De Toni–Debré–Fanconi syndrome in cystinosis or nephrocalcinosis in hyperoxaluria).

### Genetic testing: indications and preferred testing strategies

For the majority of tubulopathies the confirmation of a genetic diagnosis is recommended. As several diseases are genetically heterogeneous (e.g. Fanconi syndrome, Bartter syndrome, distal renal tubular acidosis and hypercalcaemia) and because symptoms can overlap during disease evolution (e.g. patients with severe acidosis or hypokalaemia could have a transient Fanconi syndrome) [50], we strongly recommend using targeted phenotype-associated gene panels or exome-based panels (targeted ES) whenever available. This strategy also allows the identification of variants in genes presenting as clinical phenocopies; a frequent example is the identification of *CLCNKB* or *HNF1B* variants in patients with a Gitelman syndrome phenotype. In this group of diseases, the phenotypic criteria are very important and for each disease we have established a minimum data set for genetic diagnosis to facilitate the choice of regions of interest in MPS-based gene panels (Supplementary data, Table S2). Extrarenal manifestations can also be a helpful guide; they are described together with the associated diseases in Supplementary data, Table S2. A PanelApp panel is in place and a ClinGen expert panel in tubulopathies is being set up, which will be very helpful in guiding genetic data interpretation in the coming years.

### Clinical benefit of genetic testing

In patients with a clinical diagnosis of tubulopathy, genetic testing establishes a precise diagnosis, which has high relevance for clinical management and prognosis and in some cases can end diagnostic odysseys. The importance in clinical management is particularly true for diseases with a perinatal presentation with life-threatening situations (antenatal Bartter syndrome, type 1 pseudohypoaldosteronism, nephrogenic diabetes insipidus and recessive distal renal tubular acidosis) in which an adapted treatment is necessary. Specific therapies can be proposed, such as thiazides in pseudohypoaldosteronism type 2 or amiloride in Liddle syndrome.

A precise diagnosis allows focused screening for extrarenal manifestations in some tubulopathies (Supplementary data, Table S2); important examples are sensorineural hearing loss (antenatal Bartter type 4, renal tubular acidosis, East syndrome [51–53]); ocular abnormalities (FHHNC type 2, Lowe syndrome, proximal tubular acidosis [54–56]); dental abnormalities (FHHNC, enamel renal syndrome [57–59]); neurological manifestations (Lowe syndrome, proximal tubular acidosis, mixed tubular acidosis and East syndrome [53, 55, 56, 60]); or cutaneous and exocrine gland abnormalities (HELIX syndrome [61]).

There are also some long-term benefits in having a definite genetic diagnosis: most of the tubulopathies affect the quality of life (e.g. failure to thrive and severe polyuria in nephrogenic diabetes insipidus) and close follow-up is important, particularly in children. For diseases with an evolution towards CKD (e.g. Dent disease and FHHNC), the introduction of nephroprotective therapy is crucial. Even for diseases with a relatively good prognosis, such as Gitelman syndrome, genetic confirmation could be helpful to guide care and to survey and prevent specific chronic complications such as chondrocalcinosis.



### Genetic counselling

For families with hereditary tubulopathies, genetic counselling is important to evaluate the risk for future pregnancies and support decision making concerning prenatal or pre-implantation testing when indicated. For X-linked diseases, genetic counselling and screening allow the identification of female carriers, which can provide information for reproductive decision making and neonatal management (e.g. nephrogenic diabetes insipidus and Dent disease).

In some genetic diseases, such as FHHNC and infantile hypercalcaemia or hypophosphataemic rickets with hypercalciuria, heterozygous relatives could develop hypercalciuria and nephrolithiasis [62]. Finally, a reliable diagnosis is crucial to evaluate the eligibility of living kidney donors [63].

## COMPLEMENT DISORDERS

aHUS, immune complex-mediated MPGN (IC-MPGN) and C3 glomerulopathy (C3G) are prototypical complement-related rare diseases and are associated with genetic and acquired abnormalities in regulatory proteins and in the two components of the C3 convertase of the alternative pathway of complement [64, 65]. Pathogenic or likely pathogenic variants in complement genes are identified in 50–60% of aHUS and 15–20% of IC-MPGN/C3G patients [66, 67].

An autosomal dominant mode of transmission with incomplete penetrance has been reported for the large majority of aHUS-associated complement gene abnormalities. Penetrance is influenced by the presence of other rare variants and common risk haplotypes and by environmental triggers [66, 67]. Diacylglycerol kinase epsilon (DGKE) variants are exceptions and cause a recessive form of aHUS with infantile onset.

The diagnosis of aHUS is based on clinical parameters (haematologic abnormalities and acute renal failure), after ruling out Shiga toxin-producing *E. coli* (STEC)-HUS, thrombotic thrombocytopenic purpura (TTP) (severe ADAMTS13 deficiency with <10% protease activity) and secondary forms (autoimmune diseases, drugs, cancer and human immunodeficiency syndrome). HUS is an acute, devastating disease; specific complement inhibitor therapy is lifesaving and should be started without delay to prevent irreversible injury to the kidney and other organs [64].

In IC-MPGN/C3G, diagnosis is based mainly on biopsy (light microscopy, immunofluorescence and electron microscopy) and urinary abnormalities [64, 68].

For high-quality diagnostic interpretation of the results, accurate clinical information should be provided to the diagnostic laboratories (Supplementary data, Table S3).

### Genetic testing: Indications and preferred testing strategies

For patients with aHUS and IC-MPGN/C3G, we recommend comprehensive genetic screening comprising a minimum set of genes: *CFH*, *CD46*, *CFI*, *C3*, *CFB*, *THBD* and *DGKE* (Supplementary data, Table S3) [64, 69]. All genes should be screened simultaneously using validated MPS-based multigene panels because in aHUS, and more rarely in IC-MPGN/C3G, the concurrence of two or more rare complement gene variants

with additive impact on disease risk and phenotype has been reported [70]. Genetic analysis should include genotyping for risk SNPs and haplotype blocks in *CFH* and *MCP* (Supplementary data, Table S3). We also suggest genetic analysis of the five *CFHR* genes, since variants and haplotypes of these genes have been found in association with aHUS and/or IC-MPGN/C3G [69, 71, 72].

For both aHUS and IC-MPGN/C3G, CNV assays are strongly recommended to ensure the identification of genomic rearrangements in the *CFH/CFHR* genomic region that result in deletions, duplications and hybrid genes (Supplementary data, Table S3) [73–75].

Genetic variants identified in aHUS, C3G and related complement disorders are published in the complement genetic variant database ([www.complement-db.org/home.php](http://www.complement-db.org/home.php)), with displays of the variant sequence, reported phenotype and structural, functional and allele frequency data. This database represents a valuable tool in guiding genetic data interpretation [76].

ES and even GS could be indicated in familial recessive forms with infantile onset to identify rare intronic pathogenic *DGKE* variants [77] or other underlying genetic conditions [78, 79].

In addition to genetic testing, screening for acquired complement abnormalities is strongly recommended, namely anti-FH autoantibodies in aHUS and IC-MPGN/C3G and anti-C3b, anti-FB and anti-CR1 antibodies and C3 nephritic factors in IC-MPGN/C3G [80–82].

In both aHUS and IC-MPGN/C3G, we recommend parallel screening for genetic and biochemical abnormalities as soon as the clinical diagnosis has been established. The analyses should be performed in experienced laboratories. Since the screening is complex and takes time, therapy should be initiated while analyses are being performed.

### Clinical benefit of genetic testing

The identification of genetic and/or acquired complement abnormalities is of clinical relevance, both to confirm the diagnosis and to optimize patient management. The nature of the underlying complement defect influences disease progression, the risk of relapses and responses to therapies [83]. Terminal complement blockade at the level of C5 is effective in the vast majority of aHUS patients [84] but apparently not in patients with *DGKE* mutations [85, 86]. Patients with anti-FH autoantibodies may benefit from plasma exchange and immunosuppressive therapy that limits antibody titre and production. The risk of disease recurrences after discontinuation of C5 blockade, as well as after kidney transplantation in aHUS patients who developed ESRD, is strongly influenced by the genetic background [87].

In IC-MPGN/C3G, identification of the specific genetic and acquired complement defects may be helpful for identifying the underlying pathogenesis [88] and will have an impact on clinical management. Patients with acquired or genetic defects resulting in intense activation of the complement terminal pathway could benefit from C5 blockade, whereas those with abnormalities mainly affecting the initial steps of the complement cascade might benefit from new molecules that target the C3 convertase of the alternative complement pathway. Genetic

and biochemical screening and stratification will be particularly relevant in clinical trials of new complement inhibitors to ensure that each patient receives the treatment best targeted to the specific complement defect and to provide the best path to success.

### Genetic counselling

In addition to the general reasons for genetic counselling mentioned in the introduction, counselling in HUS is specifically recommended before deciding on living-related kidney donation, which carries the risk of recurrence in the recipient and of *de novo* disease in the donor should the donor carry an at-risk genetic variant.

## CAKUT

The term CAKUT refers to abnormalities affecting kidney development, a complex process that involves reciprocal interaction between the ureteric bud and the metanephric mesenchymal tissue. CAKUT occurs in 3–6 of 1000 live births, represents ~20% of the prenatally detected anomalies [89] and constitutes the main cause of CKD in children [90] and a likely underestimated proportion of CKD of unknown origin in adults. The phenotypic spectrum is very large and can include variable degrees of renal parenchymal defects of the kidney (such as agenesis, hypoplasia, dysplasia or multicystic dysplastic kidney), upper urinary tract defects (such as uretero-pelvic junction obstruction, obstructive and/or refluxing megaureter or low-grade vesicoureteral reflux) and lower urinary tract obstruction (such as posterior urethral valve and urethral atresia). Severity also greatly varies from benign conditions such as ectopic kidney to lethal diseases such as bilateral renal agenesis or bilateral multicystic renal dysplasia [91]. CAKUT can present as isolated or syndromic, associated with various extrarenal phenotypes. The familial clustering suggests a major genetic contribution to the aetiology of CAKUT [92]. Pathogenic variations in >50 genes have been reported in isolated or syndromic CAKUT (Supplementary data, Table S4), with an autosomal dominant or, more rarely, autosomal recessive mode of inheritance. Mutations in genes involved in syndromic CAKUT can also lead to isolated kidney disease. CNVs have also been shown to be frequently associated with isolated or syndromic CAKUT. Up to 16% of individuals with renal hypodysplasia have been shown to have a molecular diagnosis attributable to a CNV disorder [93]. More recently, 45 distinct known genomic disorders at 37 loci were identified in 4.1% of a series of 2824 CAKUT cases (however, 6 loci accounted for 65% of these cases) and novel CNVs were associated with an additional 2% cases [94]. Interestingly, the genomic architecture seems to be different according to CAKUT subcategories in that series, with an enrichment for novel large or intermediate-size CNVs in vesicoureteral reflux or obstructive uropathy and an excess of duplications for lower urinary tract obstruction and duplicated collecting system.

However, a monogenic cause of CAKUT is currently found in only 10–15% of cases, even by MPS, suggesting that inheritance may frequently be more complex. *PAX2*, *HNF1B* and *EYA1* are the three genes most frequently involved in

monogenic CAKUT (representing 23% of the cases of CAKUT with a known CNV in Verbitsky *et al.* [94]); more than half of the *HNF1B* defects correspond to the recurrent 17q12 microdeletion. The prognosis of CAKUT is mainly related to the extent of reduction in nephron number and associated risk of renal failure and extrarenal anomalies in syndromic forms of CAKUT. In the majority of CAKUT with an identified monogenic cause there is a variable expressivity, and identical pathogenic variation can result in different CAKUT subphenotypes and in extremely variable severity, even within the same family.

### Genetic testing: indications and preferred testing strategies

A molecular diagnosis for patients affected with CAKUT is in most cases useful but not urgent. It is mainly useful for genetic counselling when pathogenic variants responsible for the phenotype are identified. Whether to start with a small MPS-based gene panel including the most often mutated genes, before proceeding to a larger panel, or a virtual (exome-based) panel is mainly dependent on local/national organization. Testing frequent genes in a first tier is not necessarily easier/cheaper than testing all known genes at once. CNVs can be screened by CGH or SNP analysis (Table 1). Large series of patients tested with targeted ES including coding exons of known genes, with or without candidate genes, led to the identification of pathogenic or likely pathogenic variants (including CNVs) in 3–18% of cases [19, 20, 95]. These differences are due, at least in part, to differences in inclusion criteria used for testing. Some phenotypes such as posterior urethral valve are essentially sporadic. Unilateral/benign CAKUT such as unilateral multicystic kidney dysplasia with normal contralateral kidney, low-grade vesico-ureteral reflux and unilateral pelvi-ureteric obstruction, which are frequent, also seem to be very rarely associated with a known monogenic cause [19]. A higher rate of mutations will be obtained when testing CAKUT affecting both renal parenchymas, with or without urinary tract defect, with or without a familial history of CAKUT and with or without extrarenal defects. Mutations in genes other than *HNF1B*, *PAX2* and *EYA1* represent, for each gene, only a small percentage of cases.

Interpretation of variants in CAKUT genes can be even more difficult because the pathogenicity of some variants previously reported as pathogenic mutations is today, with the available knowledge of large databases such as gnomAD, questionable. For instance, this is the case for gene variants that would currently be classified as VUS, as they have been reported in only a few reports without data regarding segregation (or sometimes with the variant inherited from a healthy parent), and with a minor allele frequency too high in population and/or in-house databases. However, as expressivity and penetrance can vary greatly in monogenic CAKUT with autosomal dominant inheritance, it is sometimes difficult to definitely rule out the causality of some variants. This is the case for *CDC5L* [96], *CRIM1* [19], *FOXF1* [97], *ROBO2* [98], *SOX17* [99], *SRGAP1* [98], *TRAP1* [100], *UPK3A* [101] and even for *DSTYK* [102].

Notably, the large majority of CAKUT cases are currently not explained by pathogenic variation in known or novel genes even when tested by ES (Jeanpierre *et al.* unpublished data) [95]. This might be due to mutations difficult to detect by ES (Table 2), to the involvement of somatic events, environmental factors or epigenetic mechanisms and to oligogenicity, which may explain both the known familial aggregation of CAKUT and the low rate of mutations identified in genes involved in monogenic forms of the disease. As for any other molecular testing, it is necessary to collect a detailed family history and precise phenotypic information regarding the index case and family (including renal ultrasound of the parents when possible). Because inheritance is frequently autosomal dominant with variable expressivity, because of the frequency of *de novo* mutations in these genes and because of the large number of rare variants of unknown significance identified by MPS screening, results will be much easier to interpret when cases are tested as trios (proband and parents). Future studies aimed at understanding the complex inheritance of CAKUT will require collaborative efforts in order to share data of a very large number of cases with deep phenotyping.

### Clinical benefit of genetic testing

The molecular diagnosis is helpful in order to orientate complementary extrarenal explorations and for specific follow-up of the patient, such as the monitoring for diabetes in cases of *HNF1B* mutations, ocular defect (risk of retinal detachment) in cases of *PAX2* mutations, hearing testing in cases of *EYA1* mutation and close follow-up of developmental milestones in children with 17q12 deletion. The identification of CNVs will be especially important for the evaluation of potential extrarenal defects, particularly for neurodevelopment. In the prenatal setting, except for the cases with recurrent pathogenic CNVs, the molecular diagnosis would not help in predicting the severity of extrarenal defects. Identification of a pathogenic variant in a CAKUT gene will also improve management of patients presenting with heavy proteinuria associated with FSGS secondary to reduced nephron number (in particular in association with *PAX2* mutations), which can phenocopy SRNS [103]. The same holds true for phenotypes in isolated small hyperechogenic kidneys associated with renal failure (without proteinuria or abnormalities of the urinary sediment) in adults, a frequent presentation usually considered as CKD of unknown origin, which can be secondary to tubulo-interstitial kidney disease, ciliopathy or CAKUT.

A molecular diagnosis is also useful when living-related kidney transplantation is planned. In view of variable disease expressivity, genetic testing ensures that the donor does not carry the pathogenic variation identified in the index case.

### Genetic counselling

Many cases with an identified monogenic cause of CAKUT are associated with a *de novo* mutation in one gene involved in autosomal dominant disease, thus molecular testing of the case and parents will reassure parents for future pregnancies that the risk of recurrence is limited to the risk of germline mosaicism. Because the severity of the renal disease varies greatly, even

within a given family, in most autosomal dominant monogenic CAKUT, knowledge of the molecular defect is not very helpful to predict the prognosis, which is much better correlated with the renal morphology. This is particularly true when CAKUT is diagnosed in the prenatal period. In families in which a pathogenic variant has been identified in a gene involved in autosomal dominant CAKUT with variable expressivity, even if the risk of transmission of the variant is 50%, parents will frequently opt for prenatal monitoring based on ultrasound screening targeted on foetal kidney morphology rather than for early prenatal molecular testing after chorionic villus sampling. However, this must be discussed on a case-by-case basis.

Extrarenal anomalies, which can be associated with pathogenic variations/CNV in many of the genes involved in monogenic CAKUT, also frequently have variable expressivity, making the molecular diagnosis a poor predictor of the severity of extrarenal symptoms. This is the case for instance for the ocular anomalies associated with *PAX2* mutations [104] and the hearing/ear/branchial defects associated with *EYA1* mutations [105].

The recurrent 17q12 deletion including *HNF1B*, which is a frequent cause of foetal hyperechogenic normal-sized kidneys, is considered by some authors as conferring a high risk of neurodevelopmental disorders [106]; however, the penetrance and severity of neuropsychological disorders associated with that CNV seem less important in the cohort of patients diagnosed secondary to kidney anomalies [107]. In families affected with autosomal recessive CAKUT such as bilateral renal agenesis associated with *ITGA8* mutations, Fraser syndrome, megacystis microcolon intestinal hypoperistalsis or in cases of renal tubular dysgenesis, genetic counselling can support their decision making regarding prenatal or pre-implantation testing.

## RENAL CILIOPATHIES

Renal ciliopathies are a clinically and genetically heterogeneous group of inherited disorders. Extrarenal manifestations are frequently associated. Because of this huge heterogeneity, we will focus here on the group of cystic kidney diseases that includes polycystic kidney diseases [PKDs; autosomal dominant PKD (ADPKD) and autosomal recessive PKD (ARPKD)] and on nephronophthisis (NPHP). We will only briefly touch upon less frequent cilia-related disorders (Supplementary data, Table S5) and other genetic syndromes that may phenocopy renal ciliopathies (e.g. metabolic and mitochondrial). PKD is characterized by enlarged kidneys [108]. In children with early severe disease manifestations, variants in genes for ARPKD (mainly *PKHD1*) and ADPKD (mainly *PKD1* and *PKD2*) are most common [109]. Remarkably, ADPKD mutations often occur *de novo* without a family history. In those severe cases, pathogenic *PKD1* variants can affect both disease alleles in a recessive mode of inheritance. Furthermore, variants in *DZIP1L* and many other genes can lead to an ARPKD-like phenotype [110]. The majority of patients with PKD are adults and explained by heterozygous variants in *PKD1* or *PKD2*. However, there is a growing list of genes that when mutated either mimic ADPKD or give rise to more atypical ADPKD phenotypes (*GANAB*, *DNAJB11*, *HNF1B*, *PKHD1*, *DZIP1L*, *TSC1/2*, *VHL*, *OFD1* in women etc.).



PKD can usually be easily distinguished on clinical grounds from NPHP, a tubulointerstitial disease characterized by tubulointerstitial cysts and small or normal-sized kidneys. More than 20 *NPHP* genes (mostly autosomal recessive) are known. A large deletion of *NPHP1* accounts for 20–40% of all juvenile cases. Variants in all *NPHP* genes are largely pleiotropic and can lead to several extrarenal manifestations [111–113].

ADPKD can sometimes be mistaken for ADTKD, although ADTKD is usually associated with renal impairment without kidney enlargement and with only a few or no cysts. To simplify matters, ADTKD could be described as the autosomal dominant equivalent of NPHP. It is most commonly caused by mutations in *MUC1* (primarily a specific 1-bp insertion not detectable by conventional assays, including ES) or *UMOD*. In ADTKD, ESKD is typically reached later in life than in NPHP [114].

Many other ciliopathies may present with kidney cysts mimicking PKD or NPHP (Supplementary data, Table S5), especially when extrarenal features are mild or not yet detectable (e.g. in the prenatal environment and early childhood).

### Genetic testing: indications and preferred testing strategies

In all patients with cystic kidney disease manifesting prenatally or in early childhood, genetic testing is highly recommended as a first-line diagnostic procedure as part of the initial patient evaluation. Testing might not be required in children with a single cyst, absent extrarenal abnormalities and a negative family history of ADPKD, but is indicated in children with progressive disease indicated by kidney cysts increasing in size or number. When one of the parents is a confirmed ADPKD patient and the child has cysts and a course that may well fit typical ADPKD, testing may be postponed.

In patients with adult disease onset, such as in most cases of ADPKD, genetic testing is increasingly recommended due to the reasons discussed in greater detail below.

Given the large clinical and genetic heterogeneity and vast pleiotropy, a comprehensive gene testing approach is recommended for renal ciliopathies. A stepwise approach might only be indicated in a minority of patients in which there is clear phenotypic evidence for a specific disease, such as von Hippel–Lindau syndrome, for which there is only a single (small) gene known with a high mutation detection rate. However, in most other cases we would clearly recommend a broader testing approach at the very beginning, due to massive heterogeneity and a large number of phenocopies.

Whatever primary strategy is chosen—an expanded gene panel or ES—the testing approach should be able to detect CNVs (e.g. deletions account for 50% of abnormalities in *HNF1B*) and to cover complex genomic regions such as in *PKD1* [15].

Clinical information helpful for diagnostics and classification includes kidney morphology, cyst location, family history and renal and extrarenal phenotypic features.

### Clinical benefits of genetic testing

The high gene detection rate in cystic kidney diseases allows one to rapidly establish a definite diagnosis and avoids a

‘diagnostic odyssey’ with unnecessary diagnostic measures such as renal or liver biopsy for the majority of patients. To establish a definite diagnosis is often of psychological benefit for patients and families. Knowledge of the genotype may point to renal and extrarenal comorbidities, which would otherwise have taken considerably longer to diagnose, and may allow early detection and disease monitoring (e.g. diabetes mellitus in *HNF1B* disease). Having said that, it may highlight possible future complications, allowing focused screening and better prevention. Since any mode of inheritance can be present in cystic kidney diseases and associated renal ciliopathies, valid information on the recurrence risk for future children or other family members and on the possibility to offer prenatal or pre-implantation genetic testing is only possible with knowledge of the genotype. The genotype can also be relevant to the inclusion of patients in clinical trials and the future choice of treatment options. With the vasopressin receptor 2 antagonist tolvaptan, the first treatment specifically for ADPKD has been approved, and other gene-specific treatments may become available soon.

### Genetic counselling

Genetic counselling is highly recommended due to variable expressivity and the variety of extrarenal features seen in patients with renal ciliopathies. It can also address the complex aspects of prenatal testing and pre-implantation testing in line with regional practices and regulations.

### CKD OF UNKNOWN ORIGIN

CKD of unknown origin is frequently seen among CKD cohorts, accounting for up to one-third of all cases with adult-onset CKD [115]. In several recent studies using MPS techniques, it was demonstrated that monogenic causes are responsible for a significant proportion of those ‘unknown’ cases. The diagnostic yield in these studies varied between 12% and 56%, depending on the inclusion criteria, number of patients in the study and the MPS approach [4, 5, 8, 9]. Indicators of a higher diagnostic yield were a positive family history, younger age of onset of CKD, the presence of extrarenal features and congenital/cystic disease phenotypes. Further research is necessary to explore the diagnostic yield of genetic testing in CKD of unknown origin in a clinical setting.

### Genetic testing: indications and preferred testing strategies

Since features attributable to an underlying genetic diagnosis were not recognized prior to the genetic analysis in many patients with CKD of unknown origin, it seems appropriate to perform a genetic test in patients with severe CKD/ESKD and onset before the age of 50 years in whom a clear-cut non-genetic diagnosis (e.g. acute nephrotoxicity, diabetic nephropathy and infectious nephropathy) has been excluded [116]. Although there are rare cases of patients >50 years of age with adult-onset CKD in whom a genetic diagnosis was identified, the *a priori* chance of a genetic diagnosis in these older CKD patients seems extremely low and does not yet warrant genetic testing unless there is a clear family history [117]. We recommend a tiered exome-based diagnostic approach in patients with CKD



### Box 3. Gaps in knowledge, unmet needs and helpful strategies for implementation of genetics and MPS approaches in clinical nephrology practice

#### Genetics literacy among clinicians needs improvement

Educational resources for professionals: <https://www.genomicseducation.hee.nhs.uk>), review paper with helpful online resources to aid physicians [119].

Practice-based education for nephrologists: ERKNet webinar series (<https://www.erknet.org/>), WGIKD Continuing Medical Education courses and e-seminars (<https://www.era-online.org/en/wgikd/>).

#### Genetic variant interpretation needs improvement

Improvements in bioinformatic algorithms.

Effective sharing of genetic data while preserving patient privacy [i.e. GeneMatcher (<https://genematcher.org/>), development of federated systems).

Gene and variant curation by specific clinical domain groups and expert panels organized within ClinGen (<https://clinicalgenome.org/>).

Gene panels curated by experts through Genomics England (<https://panelapp.genomicsengland.co.uk/>).

#### Guidelines for MPS-based diagnostic testing

Validate diagnostic outcomes of MPS-based genetic testing in a real-life clinical setting rather than a research setting (i.e. variety study in 1000 CKD patients, University Medical Centre Groningen) [120].

#### Determine the costs versus benefits of genetic testing

Studies on the long-term effects of establishing a molecular diagnosis on healthcare utilization and clinical outcomes.

Cost-benefit analyses evaluating genetics-based care versus standard of care to determine health economic utility and facilitate coverage for diagnostic testing by healthcare insurance companies.

#### How to effectively organize genetic testing and counselling in daily clinical practice

Development of clinical decision support tools.

Genetic counsellors in nephrology practice.

Computing platforms that facilitate integration of genomic data with EHRs.

Development of centres of excellence (e.g. ERKNet).

Access to and organization of multidisciplinary case discussion platforms.

of unknown origin, starting with a large targeted multigene panel involving all known nephropathy genes and opening up the whole exome backbone to look beyond the known diseases genes in case no causative variants are identified.

#### Clinical benefit of genetic testing

For many patients, the answer to why they have developed CKD is very important. Knowing the exact aetiology of their disease generally has a positive impact on their lives [118]. In addition, knowing that a disease is heritable also means that family members that might be affected can be counselled on their likelihood of developing renal disease and, when applicable, be genetically screened for the identified mutation(s) in the patient. In addition, an unequivocal diagnosis in a patient with CKD of unknown origin may give important clues for management, screening of potential associated extrarenal problems, decisions about transplantation, eligibility of living-related kidney donors and family planning.

#### Genetic counselling

All patients in whom a genetic diagnosis has been established should receive genetic counselling, especially when there are questions on family planning, reproduction and kidney transplantation with living-related donation. In addition,

patients should be informed about the possible implications of the molecular diagnosis for their family members.

#### CONCLUSION

In this article we have described the enormous potential of using MPS-based testing as a diagnostic means in patients with known and unknown causes of CKD. We have shown that in many of these kidney disease patients, MPS-based gene panel testing or ES in the diagnostic process can provide an accurate diagnosis, thereby facilitating prognostication and personalized management, including nephroprotection and decisions around kidney transplantation. An accurate diagnosis is also crucial for genetic counselling and family planning and allows reproductive options, such as prenatal or pre-implantation genetic testing. It allows screening of at-risk family members, which may also be important in determining their eligibility as kidney transplant donors. At present, phenotype-associated multigene panels and ES are the preferred diagnostic MPS-based testing modalities, but it is expected that when GS becomes more feasible, both in terms of cost-effectiveness and complex data interpretation, GS-based diagnostic testing will replace most current testing modalities [17].

Here we have given recommendations for when and how to use MPS-based diagnostics in current clinical practice. For further implementation of genetic testing early in the diagnostic process of patients with kidney disorders, it is important that we increase both awareness and evidence on the benefits of genetic diagnostic testing, including health economic utility, and find answers to additional knowledge gaps and unmet needs. Helpful strategies (Box 3) to achieve these goals are education to increase genetic literacy; bioinformatic innovations and data-sharing strategies to improve variant interpretation; large-scale validation of research findings in the clinical setting, such as the diagnostic yield of genetic testing for kidney diseases; cost-benefit analyses of genetic testing and further development of organizational and counselling support in daily practice.

## SUPPLEMENTARY DATA

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

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## CONFLICT OF INTEREST STATEMENT

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## DATA AVAILABILITY STATEMENT

No new data were generated or analysed in support of this research.

## REFERENCES

- Bassanese G, Wlodkowski T, Servais A *et al*. The European Rare Kidney Disease Registry (ERKReg): objectives, design and initial results. *Orphanet J Rare Dis* 2021; 16: 251
- Devuyt O, Knoers NV, Remuzzi G *et al*. Rare inherited kidney diseases: challenges, opportunities, and perspectives. *Lancet* 2014; 383: 1844–1859

- Vivante A, Hildebrandt F. Exploring the genetic basis of early-onset chronic kidney disease. *Nat Rev Nephrol* 2016; 12: 133–146
- 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
- Connaughton DM, Kennedy C, Shril S *et al*. Monogenic causes of chronic kidney disease in adults. *Kidney Int* 2019; 95: 914–928
- Stokman MF, Renkema KY, Giles RH *et al*. The expanding phenotypic spectra of kidney diseases: insights from genetic studies. *Nat Rev Nephrol* 2016; 12: 472–483
- Connaughton DM, Hildebrandt F. Personalized medicine in chronic kidney disease by detection of monogenic mutations. *Nephrol Dial Transplant* 2020; 35: 390–397
- Lata S, Marasa M, Li Y *et al*. Whole-exome sequencing in adults with chronic kidney disease: a pilot study. *Ann Intern Med* 2018; 168: 100–109
- 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
- Morinière V, Dahan K, Hilbert P *et al*. Improving mutation screening in familial hematuric nephropathies through next generation sequencing. *J Am Soc Nephrol* 2014; 25: 2740–2751
- Ashton EJ, Legrand A, Benoit V *et al*. Simultaneous sequencing of 37 genes identified causative mutations in the majority of children with renal tubulopathies. *Kidney Int* 2018; 93: 961–967
- Groopman EE, Rasouly HM, Gharavi AG. Genomic medicine for kidney disease. *Nat Rev Nephrol* 2018; 14: 83–104
- Snoek R, van Eerde AM, Knoers NVAM. Importance of reliable variant calling and clear phenotyping when reporting on gene panel testing in renal disease. *Kidney Int* 2017; 92: 1325–1327
- Xue Y, Ankala A, Wilcox WR *et al*. Solving the molecular diagnostic testing conundrum for Mendelian disorders in the era of next-generation sequencing: single-gene, gene panel, or exome/genome sequencing. *Genet Med* 2015; 17: 444–451
- Ali H, Al-Mulla F, Hussain N *et al*. PKD1 duplicated regions limit clinical utility of whole exome sequencing for genetic diagnosis of autosomal dominant polycystic kidney disease. *Sci Rep* 2019; 9: 4141
- Mallawaarachchi AC, Hort Y, Cowley MJ *et al*. Whole-genome sequencing overcomes pseudogene homology to diagnose autosomal dominant polycystic kidney disease. *Eur J Hum Genet* 2016; 24: 1584–1590
- Lionel AC, Costain G, Monfared N *et al*. Improved diagnostic yield compared with targeted gene sequencing panels suggests a role for whole-genome sequencing as a first-tier genetic test. *Genet Med* 2018; 20: 435–443
- 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
- Nicolaou N, Pulit SL, Nijman IJ *et al*. Prioritization and burden analysis of rare variants in 208 candidate genes suggest they do not play a major role in CAKUT. *Kidney Int* 2016; 89: 476–486
- Heidet L, Morinière V, Henry C *et al*. Targeted exome sequencing identifies PBX1 as involved in monogenic congenital anomalies of the kidney and urinary tract. *J Am Soc Nephrol* 2017; 28: 2901–2914
- Smedley D, Jacobsen JO, Jäger M *et al*. Next-generation diagnostics and disease-gene discovery with the Exomiser. *Nat Protoc* 2015; 10: 2004–2015
- Köhler S, Carmody L, Vasilevsky N *et al*. Expansion of the human phenotype ontology (HPO) knowledge base and resources. *Nucleic Acids Res* 2019; 47: D1018–1027
- Deisseroth CA, Birgmeier J, Bodle EE *et al*. ClinPhen extracts and prioritizes patient phenotypes directly from medical records to expedite genetic disease diagnosis. *Genet Med* 2019; 21: 1585–1593
- Murray SL, Dorman A, Benson KA *et al*. Utility of genomic testing after renal biopsy. *Am J Nephrol* 2020; 51: 43–53
- Adam J, Connor TM, Wood K *et al*. Genetic testing can resolve diagnostic confusion in Alport syndrome. *Clin Kidney J* 2014; 7: 197–200
- Choi M, Scholl UI, Ji W *et al*. Genetic diagnosis by whole exome capture and massively parallel DNA sequencing. *Proc Natl Acad Sci USA* 2009; 106: 19096–19101
- Deng H, Zhang Y, Xiao H *et al*. Diverse phenotypes in children with PAX2-related disorder. *Mol Genet Genomic Med* 2019; 7: e701

28. Nagano C, Morisada N, Nozu K *et al.* Clinical characteristics of HNF1B-related disorders in a Japanese population. *Clin Exp Nephrol* 2019; 23: 1119–1129
29. Lipska BS, Ranchin B, Iatropoulos P *et al.* Genotype-phenotype associations in WT1 glomerulopathy. *Kidney Int* 2014; 85: 1169–1178
30. Yao XD, Chen X, Huang GY *et al.* Challenge in pathologic diagnosis of Alport syndrome: evidence from correction of previous misdiagnosis. *Orphanet J Rare Dis* 2012; 7: 100
31. Aymé S, Bockenhauer D, Day S *et al.* Common elements in rare kidney diseases: conclusions from a Kidney Disease: Improving Global Outcomes (KDIGO) Controversies Conference. *Kidney Int* 2017; 92: 796–808
32. Kuppachi S, Smith RJH, Thomas CP. Evaluation of genetic renal diseases in potential living kidney donors. *Curr Transpl Rep* 2015; 2: 1–14
33. Fakhouri F, Fila M, Hummel A *et al.* Eculizumab discontinuation in children and adults with atypical hemolytic uremic syndrome: a prospective multicentric study. *Blood* 2021; 137: 2438–2449
34. Kalia SS, Adelman K, Bale SJ *et al.* Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. *Genet Med* 2017; 19: 249–255
35. Miller DT, Lee K, Chung WK *et al.* ACMG SF v3.0 list for reporting of secondary findings in clinical exome and genome sequencing: a policy statement of the American College of Medical Genetics and Genomics (ACMG). *Genet Med* 2021; doi: 10.1038/s41436-021-01172-3.
36. Matthijs G, Souche E, Alders M *et al.* Guidelines for diagnostic next generation sequencing. *Eur J Hum Genet* 2016; 24: 2–5
37. Boycott K, Hartley T, Adam S *et al.* The clinical application of genome-wide sequencing for monogenic diseases in Canada: position statement of the Canadian College of Medical Geneticists. *J Med Genet* 2015; 52: 431–437
38. Sadowski CE, Lovric S, Ashraf S *et al.* A single-gene cause in 29.5% of cases of steroid-resistant nephrotic syndrome. *J Am Soc Nephrol* 2015; 26: 1279–1289
39. Trautmann A, Bodria M, Ozaltin F *et al.* Spectrum of steroid-resistant and congenital nephrotic syndrome in children: the PodoNet registry cohort. *Clin J Am Soc Nephrol* 2015; 10: 592–600
40. Warejko JK, Tan W, Daga A *et al.* Whole exome sequencing of patients with steroid-resistant nephrotic syndrome. *Clin J Am Soc Nephrol* 2018; 13: 53–62
41. Song X, Fang X, Tang X *et al.* COQ8B nephropathy: early detection and optimal treatment. *Mol Genet Genomic Med* 2020; 8: e1360
42. Boyer O, Niaudet P. Nephrotic syndrome: rituximab in childhood steroid-dependent nephrotic syndrome. *Nat Rev Nephrol* 2013; 9: 562–563
43. Lipska-Ziętkiewicz BS, Ozaltin F, Hölttä T *et al.* Genetic aspects of congenital nephrotic syndrome: a consensus statement from the ERKNet-ESPN inherited glomerulopathy working group. *Eur J Hum Genet* 2020; 28: 1368–1378
44. Trautmann A, Vivarelli M, Samuel S *et al.* IPNA clinical practice recommendations for the diagnosis and management of children with steroid-resistant nephrotic syndrome. *Pediatr Nephrol* 2020; 35: 1529–1561
45. Gross O, Licht C, Anders HJ *et al.* Early angiotensin-converting enzyme inhibition in Alport syndrome delays renal failure and improves life expectancy. *Kidney Int* 2012; 81: 494–501
46. Atmaca M, Gulhan B, Korkmaz E *et al.* Follow-up results of patients with ADCK4 mutations and the efficacy of CoQ10 treatment. *Pediatr Nephrol* 2017; 32: 1369–1375
47. van der Wijst J, Belge H, Bindels RJM *et al.* Learning physiology from inherited kidney disorders. *Physiol Rev* 2019; 99: 1575–1653
48. Downie ML, L, Garcia SC, Kleta R *et al.* Inherited tubulopathies of the kidney: insights from genetics. *Clin J Am Soc Nephrol* 2020; 16: 620–630
49. Hureau M, Ashton E, Dahan K *et al.* High-throughput sequencing contributes to the diagnosis of tubulopathies and familial hypercalcaemia hypocalcaemia in adults. *Kidney Int* 2019; 96: 1408–1416
50. Watanabe T. Renal Fanconi syndrome in distal renal tubular acidosis. *Pediatr Nephrol* 2017; 32: 1093
51. Birkenhäger R, Otto E, Schürmann MJ *et al.* Mutation of BSND causes Bartter syndrome with sensorineural deafness and kidney failure. *Nat Genet* 2001; 29: 310–314
52. Karet FE, Finberg KE, Nelson RD *et al.* Mutations in the gene encoding B1 subunit of H<sup>+</sup>-ATPase cause renal tubular acidosis with sensorineural deafness. *Nat Genet* 1999; 21: 84–90
53. Bockenhauer D, Feather S, Stanescu HC *et al.* Epilepsy, ataxia, sensorineural deafness, tubulopathy, and KCNJ10 mutations. *N Engl J Med* 2009; 360: 1960–1970
54. Konrad M, Schaller A, Seelow D *et al.* Mutations in the tight-junction gene claudin 19 (CLDN19) are associated with renal magnesium wasting, renal failure, and severe ocular involvement. *Am J Hum Genet* 2006; 79: 949–957
55. Bökenkamp A, Ludwig M. The oculocerebrorenal syndrome of Lowe: an update. *Pediatr Nephrol* 2016; 31: 2201–2212
56. Igarashi T, Inatomi J, Sekine T *et al.* Mutations in *SLC4A4* cause permanent isolated proximal renal tubular acidosis with ocular abnormalities. *Nat Genet* 1999; 23: 264–266
57. Bardet C, Courson F, Wu Y *et al.* Claudin-16 deficiency impairs tight junction function in ameloblasts, leading to abnormal enamel formation. *J Bone Miner Res* 2016; 31: 498–513
58. Yamaguti PM, Neves FA, Hotton D *et al.* Amelogenesis imperfecta in familial hypomagnesaemia and hypercalcaemia with nephrocalcinosis caused by *CLDN19* gene mutations. *J Med Genet* 2017; 54: 26–37
59. Jaureguierry G, De la Dure-Molla M, Parry D *et al.* Nephrocalcinosis (enamel renal syndrome) caused by autosomal recessive *FAM20A* mutations. *Nephron Physiol* 2012; 122: 1–6
60. Roth DE, Venta PJ, Tashian RE *et al.* Molecular basis of human carbonic anhydrase II deficiency. *Proc Natl Acad Sci USA* 1992; 89: 1804–1808
61. Hadj-Rabia S, Brideau G, Al-Sarraj Y *et al.* Multiplex epithelium dysfunction due to *CLDN10* mutation: the HELIX syndrome. *Genet Med* 2018; 20: 190–201
62. Dasgupta D, Wee MJ, Reyes M *et al.* Mutations in *SLC34A3/NPT2c* are associated with kidney stones and nephrocalcinosis. *J Am Soc Nephrol* 2014; 25: 2366–2375
63. Gambaro G, Zaza G, Citterio F *et al.* Living kidney donation from people at risk of nephrolithiasis, with a focus on the genetic forms. *Urolithiasis* 2019; 47: 115–123
64. Goodship TH, Cook HT, Fakhouri F *et al.* Atypical hemolytic uremic syndrome and C3 glomerulopathy: conclusions from a “Kidney Disease: Improving Global Outcomes” (KDIGO) Controversies Conference. *Kidney Int* 2017; 91: 539–551
65. Smith RJH, Appel GB, Blom AM *et al.* C3 glomerulopathy - understanding a rare complement-driven renal disease. *Nat Rev Nephrol* 2019; 15: 129–143
66. Noris M, Remuzzi G. Glomerular diseases dependent on complement activation, including atypical hemolytic uremic syndrome, membranoproliferative glomerulonephritis, and C3 glomerulopathy: Core Curriculum 2015. *Am J Kidney Dis* 2015; 66: 359–375
67. Noris M, Remuzzi G. Atypical hemolytic-uremic syndrome. *N Engl J Med* 2009; 361: 1676–1687
68. Cook HT, Pickering MC. Histopathology of MPGN and C3 glomerulopathies. *Nat Rev Nephrol* 2015; 11: 14–22
69. Iatropoulos P, Noris M, Mele C *et al.* Complement gene variants determine the risk of immunoglobulin-associated MPGN and C3 glomerulopathy and predict long-term renal outcome. *Mol Immunol* 2016; 71: 131–142
70. Bresin E, Rurali E, Caprioli J *et al.* Combined complement gene mutations in atypical hemolytic uremic syndrome influence clinical phenotype. *J Am Soc Nephrol* 2013; 24: 475–486
71. Bu F, Borsa NG, Jones MB *et al.* High-throughput genetic testing for thrombotic microangiopathies and C3 glomerulopathies. *J Am Soc Nephrol* 2016; 27: 1245–1253
72. Bernabéu-Herrero ME, Jiménez-Alcázar M, Anter J *et al.* Complement factor H, FHR-3 and FHR-1 variants associate in an extended haplotype conferring increased risk of atypical hemolytic uremic syndrome. *Mol Immunol* 2015; 67: 276–286
73. Józsi M, Tortajada A, Uzonyi B *et al.* Factor H-related proteins determine complement-activating surfaces. *Trends Immunol* 2015; 36: 374–384
74. Valoti E, Alberti M, Tortajada A *et al.* A novel atypical hemolytic uremic syndrome-associated hybrid *CFHR1/CFH* gene encoding a fusion protein that antagonizes factor H-dependent complement regulation. *J Am Soc Nephrol* 2015; 26: 209–219
75. Gale DP, de Jorge EG, Cook HT *et al.* Identification of a mutation in complement factor H-related protein 5 in patients of Cypriot origin with glomerulonephritis. *Lancet* 2010; 376: 794–801
76. Osborne AJ, Breno M, Borsa NG *et al.* Statistical validation of rare complement variants provides insights into the molecular basis of atypical

- hemolytic uremic syndrome and C3 glomerulopathy. *J Immunol* 2018; 200: 2464–2478
77. Mele C, Lemaire M, Iatropoulos P *et al.* Characterization of a new DGKE intronic mutation in genetically unsolved cases of familial atypical hemolytic uremic syndrome. *Clin J Am Soc Nephrol* 2015; 10: 1011–1019
  78. Walsh PR, Johnson S, Brocklebank V *et al.* Glucose-6-phosphate dehydrogenase deficiency mimicking atypical hemolytic uremic syndrome. *Am J Kidney Dis* 2018; 71: 287–290
  79. Valoti E, Alberti M, Carrara C *et al.* Hemolytic uremic syndrome in an infant with primary hyperoxaluria type II: an unreported clinical association. *Nephron* 2019; 142: 264–270
  80. Noris M, Donadelli R, Remuzzi G. Autoimmune abnormalities of the alternative complement pathway in membranoproliferative glomerulonephritis and C3 glomerulopathy. *Pediatr Nephrol* 2019; 34: 1311–1323
  81. Marinozzi MC, Chauvet S, Le Quintrec M *et al.* C5 nephritic factors drive the biological phenotype of C3 glomerulopathies. *Kidney Int* 2017; 92: 1232–1241
  82. Blanc C, Togarsimalemath SK, Chauvet S *et al.* Anti-factor H autoantibodies in C3 glomerulopathies and in atypical hemolytic uremic syndrome: one target, two diseases. *J Immunol* 2015; 194: 5129–5138
  83. Noris M, Caprioli J, Bresin E *et al.* Relative role of genetic complement abnormalities in sporadic and familial aHUS and their impact on clinical phenotype. *Clin J Am Soc Nephrol* 2010; 5: 1844–1859
  84. Legendre CM, Licht C, Muus P *et al.* Terminal complement inhibitor eculizumab in atypical hemolytic-uremic syndrome. *N Engl J Med* 2013; 368: 2169–2181
  85. Brocklebank V, Kumar G, Howie AJ *et al.* Long-term outcomes and response to treatment in diacylglycerol kinase epsilon nephropathy. *Kidney Int* 2020; 97: 1260–1274
  86. Azukaitis K, Simkova E, Majid MA *et al.* The phenotypic spectrum of nephropathies associated with mutations in diacylglycerol kinase. *J Am Soc Nephrol* 2017; 28: 3066–3075
  87. Noris M, Remuzzi G. Managing and preventing atypical hemolytic uremic syndrome recurrence after kidney transplantation. *Curr Opin Nephrol Hypertens* 2013; 22: 704–712
  88. Iatropoulos P, Daina E, Curreri M, *et al.* Cluster analysis identifies distinct pathogenetic patterns in C3 glomerulopathies/immune complex-mediated membranoproliferative GN. *J Am Soc Nephrol* 2018; 29: 283–294
  89. Levi S. Mass screening for fetal malformations: the Eurofetus study. *Ultrasound Obstet Gynecol* 2003; 22: 555–558
  90. Sanna-Cherchi S, Ravani P, Corbani V *et al.* Renal outcome in patients with congenital anomalies of the kidney and urinary tract. *Kidney Int* 2009; 76: 528–533
  91. Madariaga L, Morinière V, Jeanpierre C *et al.* Severe prenatal renal anomalies associated with mutations in *HNF1B* or *PAX2* genes. *Clin J Am Soc Nephrol* 2013; 8: 1179–1187
  92. Bulum B, Özçakar ZB, Ustüner E *et al.* High frequency of kidney and urinary tract anomalies in asymptomatic first-degree relatives of patients with CAKUT. *Pediatr Nephrol* 2013; 28: 2143–2147
  93. Sanna-Cherchi S, Kiryluk K, Burgess KE *et al.* Copy-number disorders are a common cause of congenital kidney malformations. *Am J Hum Genet* 2012; 91: 987–997
  94. Verbitsky M, Westland R, Perez A *et al.* The copy number variation landscape of congenital anomalies of the kidney and urinary tract. *Nat Genet* 2019; 51: 117–127
  95. van der Ven AT, Connaughton DM, Ityel H *et al.* Whole-exome sequencing identifies causative mutations in families with congenital anomalies of the kidney and urinary tract. *J Am Soc Nephrol* 2018; 29: 2348–2361
  96. Hwang DY, Dworschak GC, Kohl S *et al.* Mutations in 12 known dominant disease-causing genes clarify many congenital anomalies of the kidney and urinary tract. *Kidney Int* 2014; 85: 1429–1433
  97. Hilger AC, Halbritter J, Pennimpede T *et al.* Targeted resequencing of 29 candidate genes and mouse expression studies implicate *ZIC3* and *FOXF1* in human VATER/VACTERL association. *Hum Mutat* 2015; 36: 1150–1154
  98. Hwang DY, Kohl S, Fan X *et al.* Mutations of the *SLIT2*-*ROBO2* pathway genes *SLIT2* and *SRGAP1* confer risk for congenital anomalies of the kidney and urinary tract. *Hum Genet* 2015; 134: 905–916
  99. Gimelli S, Caridi G, Beri S *et al.* Mutations in *SOX17* are associated with congenital anomalies of the kidney and the urinary tract. *Hum Mutat* 2010; 31: 1352–1359
  100. Saisawat P, Kohl S, Hilger AC *et al.* Whole-exome resequencing reveals recessive mutations in *TRAP1* in individuals with CAKUT and VACTERL association. *Kidney Int* 2014; 85: 1310–1317
  101. Jenkins D, Bitner-Glindzicz M, Malcolm S *et al.* De novo Uroplakin IIIa heterozygous mutations cause human renal adysplasia leading to severe kidney failure. *J Am Soc Nephrol* 2005; 16: 2141–2149
  102. Sanna-Cherchi S, Sampogna RV, Papeta N *et al.* Mutations in *DSTYK* and dominant urinary tract malformations. *N Engl J Med* 2013; 369: 621–629
  103. Vivante A, Chacham OS, Shril S *et al.* Dominant *PAX2* mutations may cause steroid-resistant nephrotic syndrome and FSGS in children. *Pediatr Nephrol* 2019; 34: 1607–1613
  104. Bower M, Salomon R, Allanson J *et al.* Update of *PAX2* mutations in renal coloboma syndrome and establishment of a locus-specific database. *Hum Mutat* 2012; 33: 457–466
  105. Krug P, Morinière V, Marlin S *et al.* Mutation screening of the *EYA1*, *SIX1*, and *SIX5* genes in a large cohort of patients harboring branchio-otorenal syndrome calls into question the pathogenic role of *SIX5* mutations. *Hum Mutat* 2011; 32: 183–190
  106. Moreno-De-Luca D, Mulle JG, Kaminsky EB *et al.* Deletion 17q12 is a recurrent copy number variant that confers high risk of autism and schizophrenia. *Am J Hum Genet* 2010; 87: 618–630
  107. Laliève F, Decramer S, Heidet L *et al.* School level of children carrying a *HNF1B* variant or a deletion. *Eur J Hum Genet* 2020; 28: 56–63
  108. Bergmann C, Guay-Woodford LM, Harris PC *et al.* Polycystic kidney disease. *Nat Rev Dis Prim* 2018; 4: 50
  109. Gimpel C, Bergmann C, Bockenbauer D *et al.* International consensus statement on the diagnosis and management of autosomal dominant polycystic kidney disease in children and young people. *Nat Rev Nephrol* 2019; 15: 713–726
  110. Bergmann C. ARPKD and early manifestations of ADPKD: the original polycystic kidney disease and phenocopies. *Pediatr Nephrol* 2015; 30: 15–30
  111. Braun DA, Hildebrandt F. Ciliopathies. *Cold Spring Harb Perspect Biol* 2017; 9: a028191
  112. Bergmann C. Genetics of autosomal recessive polycystic kidney disease and its differential diagnoses. *Front Pediatr* 2018; 5: 221
  113. McConnachie DJ, Stow JL, Mallett AJ. Ciliopathies and the kidney: a review. *Am J Kidney Dis* 2021; 77: 410–419
  114. Živná M, Kidd K, Přistoupilová A *et al.* Noninvasive immunohistochemical diagnosis and novel *MUC1* mutations causing autosomal dominant tubulointerstitial kidney disease. *J Am Soc Nephrol* 2018; 29: 2418–2431
  115. Neild GH. Primary renal disease in young adults with renal failure. *Nephrol Dial Transplant* 2010; 25: 1025–1032
  116. Snoek R, van Jaarsveld RH, Nguyen TQ *et al.* Genetics-first approach improves diagnostics of ESKD patients younger than 50 years. *Nephrol Dial Transplant* 2020; gfaa363. doi: 10.1093/ndt/gfaa363
  117. Snoek R, van Setten J, Keating BJ *et al.* NPHP1 (nephrocystin-1) gene deletions cause adult-onset ESRD. *J Am Soc Nephrol* 2018; 29: 1772–1779
  118. Esquivel-Sada D, Nguyen MT. Diagnosis of rare diseases under focus: impacts for Canadian patients. *J Community Genet* 2018; 9: 37–50
  119. Milo Rasouly H, Aggarwal V, Bier L *et al.* Cases in precision medicine: genetic testing to predict future risk for disease in a healthy patient. *Ann Intern Med* 2021; 174: 540–547
  120. de Haan A, Eijgelsheim M, Vogt L *et al.* Diagnostic yield of next-generation sequencing in patients with chronic kidney disease of unknown etiology. *Front Genet* 2019; 10: 1264

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