

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

Nine Knoers¹, Corinne Antignac², Carsten Bergmann^{3,4}, Karin Dahan^{5,6}, Sabrina Giglio^{7,8}, Laurence Heidet⁹, Beata S. Lipska-Ziętkiewicz^{10,11}, Marina Noris¹², Giuseppe Remuzzi¹², Rosa Vargas-Poussou¹³, Franz Schaefer¹⁴ for the ERA-EDTA Working Group on Inherited Kidney Disorders (WGIKD) and the Molecular Diagnostics Taskforce of the European Rare Kidney Disease Reference Network (ERKNet)

¹ Department of Genetics, University Medical Centre Groningen, The Netherlands

² Institut Imagine (Inserm U1163) et Département de Génétique, 24 bd du Montparnasse, 75015 Paris, France

³ Medizinische Genetik Mainz, Limbach Genetics, Mainz, Germany

⁴ Department of Medicine, Nephrology, University Hospital Freiburg, Germany

⁵ Division of Nephrology, Cliniques Universitaires Saint-Luc, Avenue Hippocrate, 10, B-1200 Brussels, Belgium

⁶ Center of Human Genetics, Institut de Pathologie et de Génétique, Avenue Lemaître, 25, B-6041 Gosselies, Belgium

⁷ Unit of Medical Genetics, Department of Medical Sciences and Public Health, University of Cagliari, Cagliari, Italy

⁸ Department of Clinical and Experimental Biomedical Sciences "Mario Serio", University of Florence, Florence, Italy

⁹ Service de Néphrologie Pédiatrique, Hôpital Universitaire Necker-Enfants Malades, 149 rue de Sèvres, 75743 Paris Cedex 15, France

¹⁰ BSL-Z – ORCID 0000-0002-4169-9685, Centre for Rare Diseases, Medical University of Gdansk, Gdansk, Poland

¹¹ Clinical Genetics Unit, Department of Biology and Medical Genetics, Medical University of Gdansk, Gdansk, Poland

¹² Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Aldo & Cele Daccò Clinical Research Center for Rare Diseases, Bergamo, Italy

¹³ Département de Génétique, Hôpital Européen Georges Pompidou, 20 rue Leblanc 75908 Paris Cedex 15, France

¹⁴ Division of Pediatric Nephrology, Center for Pediatrics and Adolescent Medicine, University of Heidelberg, Germany

Correspondence to: Nine Knoers; E-mail: v.v.a.m.knoers@umcg.nl



Working Group on Inherited
Kidney Disorders

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ABSTRACT

The overall diagnostic yield of massively parallel sequencing (MPS) based-tests in patients with chronic kidney disease (CKD) is around 30% for pediatric cases and 6-30% for adult cases. These figures should encourage nephrologists to frequently use genetic testing as a diagnostic means for their patients, but in reality several barriers appear to hinder the implementation of NGS diagnostics in routine clinical practice. In this paper, we aim to support the nephrologist to overcome these barriers. After a detailed discussion on the general items that are important to genetic testing in nephrology, namely (1) genetic testing modalities and their indications, (2) clinical information needed for high-quality interpretation of the genetic test, (3) clinical benefit of genetic testing and (4) genetic counselling, we will 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

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 (ERKReg)¹, and on published data on genetic testing of monogenic causes of ESKD in different cohorts.²⁻⁵ Mutations in over 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 enormously increased. 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 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 a 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, 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 paper, 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: (1) different genetic testing modalities and their indications, (2) the clinical information that is needed to allow high-quality interpretation of the genetic test, (3) the predicted clinical benefit of genetic testing, and (4) the importance of pre- and post-test counselling, including ethical and psychosocial implications of genetic testing. In the second part of this paper, we will more specifically discuss each of these items for the different groups of monogenic kidney disorders (glomerulopathies, renal tubulopathies, complement disorders, congenital abnormalities (CAKUT) and cystic kidney diseases/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 tests 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 massive parallel sequencing (MPS) techniques are preferably being applied in diagnostics (**table 1**). MPS techniques enable the

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3 simultaneous sequencing of the exons of a subset of genes associated with a particular
4 phenotype (targeted phenotype-associated gene panels), of the exons of all 21,000 protein-
5 coding human genes (Exome sequencing, ES, previously referred to as whole exome
6 sequencing, WES) or of the complete genome (Genome sequencing, GS, previously referred
7 to as whole genome sequencing, WES).
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9 The primary scope of targeted MPS-based phenotype-associated gene panels and exome
10 sequencing is the identification of small variants (single nucleotide variants [SNVs] or small
11 insertions/deletions [INDELs]) within the genes of interest for the clinical phenotype or
12 within the coding region of the genome, respectively. The targeted phenotype-associated
13 gene panels are used for the diagnosis of disorders with locus heterogeneity, disorders with
14 overlapping phenotypes, or disorders with common pathways. Until recently, these gene
15 panels were mostly targeted gene enrichment-based panels, only allowing the sequencing
16 of a set of preselected genes. This approach has the advantage that it will not yield
17 incidental findings in genes unrelated to the primary indication for testing, but the
18 disadvantage that updating of these enrichment-based panels with newly discovered
19 relevant genes requires redesign and validation of the assay.¹² Nowadays, many diagnostic
20 labs prefer to use phenotype-associated gene panels that are exome-based (targeted ES,
21 virtual gene panels, **table 1**). This means that the exome is sequenced, but only indication-
22 relevant genes are analysed and interpreted by using in silico bioinformatics tools. This
23 exome-based approach is more attractive to diagnostic laboratories, because it allows
24 dynamic gene content update with minimal design and validation. So, when new disease-
25 causing genes are discovered, a bioinformatics re-analysis of the already available data is
26 sufficient. At present, in most diagnostic labs such a bioinformatic reanalysis of the exome
27 only takes place after request of the physician treating the patient. In addition, when no
28 (likely) pathogenic variants are identified in the indication-relevant genes, it is relatively
29 easy to 'open up' the exome backbone data and look beyond the known genes, maximizing
30 the opportunity for finding the causal variant and new candidate variants (second tier
31 test).¹³ A disadvantage of using exome-based virtual gene panels is that exome data tend to
32 have less uniform sequence coverage than the targeted phenotype-associated gene panels,
33 so genes of specific interest might need to be gap-filled using Sanger sequencing. It is
34 important to emphasize that opening up the exome backbone data may reveal variants
35 predictive for other diseases not related to the initial reason for testing (incidental findings,
36 for further elaboration, see genetic counseling) and therefore specific consent related to the
37 reporting of these findings is necessary.
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39 In cases of unexplained kidney failure, targeted ES, with a second-tier option of analysis of
40 the full exome, is the preferred first-tier test (**table 1**).

41 Importantly, larger copy number variants (CNVs), some of which are important causes of
42 inherited kidney diseases (**table 2**), are not easily picked up by MPS-based gene panels or
43 ES. Sophisticated bioinformatic tools are necessary to detect those large CNVs from gene
44 panel or ES data and these are not yet routinely used in all diagnostic laboratories. Instead,
45 the still preferred methodology for routine diagnostics of large CNVs in many labs is the
46 microarray-based technique (CGH- or SNP-arrays, **table 1**). Multiplex ligation-dependent
47 probe amplification (MLPA) is another technique to pick up CNVs. In addition, several
48 important disease-causing variants, such as pathogenic variants in *PKD1*, can be missed
49 using MPS-based panels or ES, due to the complexity of the involved genomic region.^{14,15}
50 (**table 2**).
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Some of the limitations of MPS-based gene panels and/or ES can be addressed by Genome Sequencing. GS can not only identify SNVs and INDEls in both coding and non-coding regions, 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 the variant's effect on, amongst 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 variant(s) is (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 datasharing.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 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), LOVD (<https://www.lovd.nl/>) (open access) or HGMD professional (<https://digitalinsights.qiagen.com/products-overview/clinical-insights-portfolio/human-gene-mutation-database/>) (licensed). In recent years, the efforts of organisations like ClinGen (<https://clinicalgenome.org/>) and Genomics England with PanelApp (<https://panelapp.genomicsengland.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 center to center, largely depending on the type of test, the local infrastructure/laboratory facilities, the bioinformatic capacity, and on 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, neonatal) should not exceed 2 weeks.

Box 1 Genetic analysis techniques used in diagnostic laboratories

Sanger Sequencing

Sanger sequencing is the “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 copy number variations (CNVs) (deletions and duplications).
- **Multiplex ligation-dependent probe amplification (MLPA)**
Targeted PCR-based technique to detect both large and small copy number variation (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 preselected 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
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Clinical information needed to allow high-quality interpretation of the genetic test

To help interpreting 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 and 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 test(s), 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 sibilings 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 over 13.000 terms describing phenotypic abnormalities and over 150.000 annotations to hereditary diseases and has become the *de facto* standard for deep phenotyping of rare diseases. Therefore, we recommend referring clinicians to 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 an Electronic Health Record (EHR) 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 diarrhea, 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-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

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3 diagnosis is crucial for precise genetic counselling, provides information about recurrence
4 risks, facilitates reproductive options, and in some cases offers presymptomatic testing
5 opportunities for family members at risk of having the same disorder. Finally, a genetic
6 diagnosis may be of pivotal importance in the setting of kidney transplantation, especially
7 when living-related donation is involved.³¹ Ascertaining the genetic origin of ESKD in a
8 transplant candidate is essential to evaluate the risk of transmission of kidney disease for
9 the biologically related donor, especially in hereditary nephropathies with age-dependent
10 manifestation (ADTKD-UMOD) or diseases with variable expressivity and reduced
11 penetrance such as HNF1B-associated disease (ADTKD-HNF1B). In families affected by these
12 diseases, gene-specific variant detection has considerable potential for accurate risk and
13 donor suitability assessment among relatives who are candidates for kidney donation.³²
14 Even a negative result of genetic screening may have clinical relevance. For instance, in
15 atypical hemolytic uremic syndrome, a multicenter prospective study has recently
16 documented that discontinuation of anti-C5 therapy based on negative genetic testing may
17 be reasonable and safe. Indeed the relapse after eculizumab discontinuation was predicted
18 by the presence of a complement gene abnormality.³³
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26 **Genetic counselling**

27 Diagnostic genetic testing should be accompanied by careful pre- and post-test genetic
28 counselling, especially when MPS-based techniques are used (**Box 2**). Appropriate pre-test
29 counselling on the opportunities, limitations and possible results of genetic testing allows
30 patients or parents of an affected child to make an informed decision on whether or not to
31 undergo genetic testing and to understand the potential outcomes of the test. When the
32 local policy is to return VUS to patients, the chance of finding these variants should be
33 emphasized, including the possibility that additional investigations and data sharing might
34 be necessary to interpret these VUS, and also their potential to become meaningful over
35 time. The counselling should also include the chance of incidental findings, unanticipated
36 findings not related to the initial reason for genetic testing, but which could be predictive of
37 risk for other diseases, which may or may not be medically actionable. The reporting of
38 these unexpected findings is subject of an ongoing international ethical debate. In recent
39 years, several policy documents have been published regarding the return of incidental
40 findings in the US, Europe and Canada. The ACMG has established a list of 59 genes, for
41 which (likely) pathogenic mutations are believed to be strongly predictive of potentially life-
42 threatening diseases, such as cancer and cardiovascular diseases.³⁴ Because an early
43 detection of these diseases may be beneficial in terms of surveillance or treatment, the
44 ACMG advocates routine analysis of these 59 genes (recently updated to 73 genes), and
45 reporting all the (likely) pathogenic variants when performing clinical ES, unless patients opt
46 out.^{34,35} They also changed the terminology to “secondary” findings, following their advice
47 to intentionally analyse these 59 genes. By contrast, the European Society of Human
48 Genetics (ESHG) and the Canadian College of Medical Geneticists (CCMG) advocate a strict
49 and proportionate application of clinical sequencing and preferable perform targeted exome
50 (or genome)-based gene panel testing, analysing only the known disease-associated genes
51 and thereby minimizing discovery of secondary findings.^{36,37} In practice, none of these
52 policy documents have been accepted as the general standard and the policies and tools
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(e.g. consent) regarding incidental findings 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 mutation(s) or VUS) or the meaning of negative results (**table 2 and Box 2**). In case of pathogenic variant(s), 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 variant(s) 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 presymptomatic genetic testing; this offers them the opportunity to receive effective early treatment to slow CKD progression and prevent secondary morbidity.

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 large number of genes (or the exome) with a potential of incidental findings, consider consulting a clinical geneticist, or refer the patient to a clinical geneticist for pretest counselling.
- Orders should be accompanied by detailed phenotypic information, including a family history/pedigree.
- Ordering of presymptomatic 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 on 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 incidental findings (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 (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 presymptomatic 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 incidental findings, small in panels and larger in ES/GS. However, this is also very dependent on local policies, whether or not centers/laboratories offer patients opt-out or opt-in protocols on incidental findings. The local policy about dissemination of these incidental findings 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.

Glomerulopathies

The term glomerulopathies refers to abnormalities affecting the establishment and maintenance of the glomerular filtration barrier comprised of the podocyte, the glomerular basement membrane and the fenestrated endothelial cells. Hereditary entities account for at least one quarter of pediatric proteinuric glomerulopathies and are also identified in a growing fraction of adult-onset cases.^{4,38-40} 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, more than 60

genes (**table 3; see supplementary material**) 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), or *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*, *INF2*), the membrane protein complex linking these structures (e.g. *NPHS2*, *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*, *mt-tRNAs*) or tRNA modification (e.g. *KEOPS* complex genes, *WDR4*), nuclear transcription factors (e.g. *WT1*, *LMX1B*, *SMARCA11*), nuclear pore complex proteins (e.g. *NUP93*, *NUP107*) and membrane proteins involved in the anchoring of podocyte foot processes in the extracellular matrix (*LAMB2*, *ITGB4*, *ITGA3*). Alterations in the genes encoding the triple helix proteins that form collagen type IV collagen (*COL4A1,3,4,5*), the main constituent of the glomerular basement membrane, cause glomerulopathies with initially predominant hematuria 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 as "type IV collagen disorders".

Genetic testing: Indications and preferred testing strategies

In patients with **congenital nephrotic syndrome** (CNS) genetic testing is recommended as a first-line diagnostic procedure, which 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, hematuria or CKD of unknown origin, cases with extra-renal 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 (**table 3; see supplementary material**) is recommended with either a targeted phenotype-associated gene panel or targeted ES.

For patients with a multiorgan phenotype suggestive of syndromic SRNS (**table 3; see supplementary material**), direct testing for defects in the related gene(s) can be performed as the first step, followed by comprehensive genetic testing if no pathogenic variant is detected in the expected gene(s). This may require extended additional clinical and diagnostic evaluation with the aim of identifying subtle extra-renal 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, 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

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3 that lead to CoQ10 synthase deficiency.⁴⁶ Another important benefit of genetic testing in
4 this disease group is the fact that unlike immune-mediated glomerulopathies, genetic
5 kidney diseases are not recurring on the kidney after kidney transplantation. Finally, the
6 confirmation of a genetic disease cause can be particularly useful in oligosymptomatic
7 syndromic disorders where relevant extrarenal features can escape clinical detection or may
8 arise later in the course of disease, such as hearing loss with the collagen IV genes and
9 *COQ6*, sex reversal and Wilms tumours with *WT1*, or adrenal insufficiency with *SPGL1*
10 mutations.
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14 Genetic counselling

15 For families with hereditary glomerulopathies, genetic counselling can support their
16 decision making regarding prenatal diagnosis including prenatal and pre-implantation
17 diagnostics in subsequent pregnancies. For certain syndromic entities (e.g. Galloway-Mowat
18 syndrome, *SGPL1* glomerulopathy), palliative care may be considered depending on the
19 severity of the disease. Genetic counseling is also relevant concerning potential organ
20 donorship of first-degree relatives. Donor screening is obligatory for diseases with dominant
21 transmission and in certain entities with significant intra- and inter-family variability and
22 incomplete or age-dependent penetrance (e.g. *WT1*, *COL4A-3-4-5*, *NPHS2*).
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26 **Tubulopathies**

27 The term tubulopathies refers to abnormalities of proteins involved directly or indirectly in
28 epithelial transport along the renal tubules. They have an important role in body
29 homeostasis adjusting the reabsorption and secretion of water and solutes. Inherited
30 tubulopathies cover a group of abnormalities with several modes of inheritance, a variable
31 presentation (in terms of age and severity) and often substantial clinical and biological
32 overlap.^{47,48} They are more frequently diagnosed in children than in adults, particularly
33 those with autosomal recessive transmission. In a recent European collaborative study
34 hereditary disorders accounted for 64 and 29 % of cases of paediatric and adult patients
35 with a clinical diagnosis of tubulopathy, respectively.^{49,50} To date more than 60 genes have
36 been linked to tubulopathies (**table 4; see supplementary material**). Most of the encoded
37 proteins are directly responsible for the reabsorption or secretion of solutes and water
38 (transporters, pumps or channels), which could be transepithelial or paracellular (e.g. renal
39 hypophosphatemia, Bartter syndrome types 1 to 3, familial hypomagnesemia with
40 hypercalciuria and nephrocalcinosis (FHHNC), distal renal tubular acidosis, nephrogenic
41 diabetes insipidus). Other proteins regulate the expression or activity of transporters,
42 pumps or channels (e.g. Bartter syndrome types 4a and 5, pseudohypoaldosteronism type 2,
43 nephrogenic diabetes insipidus) and others take part in intracellular processes such as
44 endocytosis (Dent disease).
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46 Some metabolic diseases have been included in this group because they have a tubulopathy
47 as first manifestation (e.g. De Toni-Debré-Fanconi syndrome in cystinosis or
48 nephrocalcinosis in hyperoxaluria).
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50 Genetic testing: Indications and preferred testing strategies

51 For the majority of tubulopathies the confirmation of a genetic diagnosis is recommended.
52 As several diseases are genetically heterogeneous (e.g. Fanconi syndrome, Bartter
53 syndrome, distal renal tubular acidosis, hypercalcemia) and because symptoms can overlap
54 during disease evolution (e.g. patients with severe acidosis or hypokalemia could have a
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transient Fanconi syndrome⁵¹), 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 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 (**table 4; see supplementary material**). Extrarenal manifestations, can also be a helpful guide; they are described together with the associated diseases in table 4. 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 (**table 4; see supplementary material**); important examples are sensorineural hearing loss (antenatal bartter type 4, renal tubular acidosis, East syndrome⁵²⁻⁵⁴); ocular abnormalities (FHHNC type 2, Lowe syndrome, proximal tubular acidosis⁵⁵⁻⁵⁷); dental abnormalities (FHHNC, enamel renal syndrome⁵⁸⁻⁶⁰); neurological manifestations (Lowe syndrome, proximal tubular acidosis, mixed tubular acidosis, East syndrome^{55-57,61}); or cutaneous and exocrine gland abnormalities (HELIX syndrome⁶²).

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 chronic kidney disease (e.g. Dent disease, FHHNC), the Introduction of nephroprotective therapy is crucial. Even for diseases with a relative 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 preimplantation testing when indicated. For X-linked diseases, genetic counselling and screening allows the identification of female carriers, which can provide information for reproductive decision-making and neonatal management (e.g. nephrogenic diabetes insipidus, Dent disease).

In some genetic diseases, as FHHNC and infantile hypercalcemia or hypophosphatemic rickets with hypercalciuria, heterozygous relatives could develop hypercalciuria and

nephrolithiasis.⁶³ Finally, a reliable diagnosis is crucial to evaluate the eligibility of living kidney donors.⁶⁴

Complement disorders

Atypical hemolytic uremic syndrome (aHUS), immune-complex mediated membranoproliferative glomerulonephritis (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.^{65,66}

Pathogenic or likely pathogenic variants in complement genes are identified in 50-60% of aHUS and 15-20% of IC-MPGN/C3G patients.^{67,68}

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.^{67,68} DGKE variants are exceptions and cause a recessive form of aHUS with infantile onset.

The diagnosis of aHUS is based on clinical parameters (hematologic abnormalities and acute renal failure), after ruling out STEC-HUS (infections by shiga-toxin producing bacteria), TTP (severe ADAMTS13 deficiency with <10% protease activity) and secondary forms (autoimmune diseases, drugs, cancer, HIV). HUS is an acute, devastating disease; specific complement inhibitor therapy is life-saving and should be started without delay to prevent irreversible injury to the kidney and other organs.⁶⁵

In IC-MPGN/C3G, diagnosis is based mainly on biopsy (light microscopy, immunofluorescence and electron microscopy) and on urinary abnormalities.^{65,69}

For high-quality diagnostic interpretation of the results accurate clinical information should be provided to the diagnostic laboratories (**table 5; see supplementary material**).

Genetic testing: Indications and preferred testing strategies

For patients with aHUS and IC-MPGN/C3G we recommend a comprehensive genetic screening comprising a minimum set of genes: CFH, CD46, CFI, C3, CFB, THBD, and DGKE (**table 5; see supplementary material**).^{65,70} All genes should be screened simultaneously using validated MPS-based multi-gene 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.⁷¹ Genetic analysis should include genotyping for risk SNPs and haplotype blocks in CFH and MCP (**table 5; see supplementary material**).

We also suggest genetic analysis of the five CFHR genes, since variants and haplotypes in these genes have been found in association with aHUS and/or IC-MPGN/C3G.^{70,72,73}

For both aHUS and IC-MPGN/C3G, copy number variation assays are strongly recommended to ensure the identification of genomic rearrangements in the CFH/CFHRs genomic region that result in deletions, duplications and hybrid genes (**table 5; see supplementary material**).⁷⁴⁻⁷⁶

Genetic variants identified in aHUS, C3G, and related complement disorders are published in the complement genetic variant database (www.complement-db.org/home.php), with displays of the variant sequence, reported phenotype, and structural, functional and allele

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3 frequency data. This database represents a valuable tool in guiding genetic data
4 interpretation.⁷⁷

5 ES and even GS could be indicated in familial recessive forms with infantile onset to identify
6 rare intronic pathogenetic DGKE variants⁷⁸ or other underlying genetic conditions.^{79,80}

7 In addition to genetic testing, screening for acquired complement abnormalities is strongly
8 recommended, namely anti-FH autoantibodies in aHUS and IC-MPGN/C3G, and anti-C3b,
9 anti-FB, and anti-CR1 antibodies and C3 nephritic factors (C3NeFs) in IC-MPGN/C3G.⁸¹⁻⁸³

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

14 Clinical benefit of genetic testing

15 The identification of genetic and/or acquired complement abnormalities is of clinical
16 relevance, both to confirm the diagnosis and to optimize patient management. The nature of
17 the underlying complement defect influences disease progression, the risk of relapses and
18 responses to therapies.⁸⁴ Terminal complement blockade at the level of C5 is effective in the
19 vast majority of aHUS patients⁸⁵ but apparently not in patients with DGKE mutations.^{86,87}

20 Patients with anti-FH autoantibodies may benefit from plasma-exchange and
21 immunosuppressive therapy that limits antibody titer and production. The risk of disease
22 recurrences after discontinuation of C5 blockade, as well as after kidney transplantation in
23 aHUS patients who developed ESRD is strongly influenced by the genetic background.⁸⁸

24 In IC-MPGN/C3G, identification of the specific genetic and acquired complement defects may
25 be helpful for identifying the underlying pathogenesis⁸⁹, and will have an impact on clinical
26 management. Patients with acquired or genetic defects resulting in intense activation of the
27 complement terminal pathway could benefit from C5 blockade, whereas those with
28 abnormalities mainly affecting the initial steps of the complement cascade might benefit from
29 new molecules that target the C3 convertase of the alternative complement pathway. Genetic
30 and biochemical screening and stratification will be particularly relevant in clinical trials of
31 new complement inhibitors, to ensure that each patient receives the treatment best targeted
32 to the specific complement defect and to provide the best path to success.

33 Genetic counselling

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

38 **CAKUT**

39 The term Congenital anomalies of the kidney and urinary tract (CAKUT) refers to
40 abnormalities affecting kidney development, a complex process that involves reciprocal
41 interaction between the ureteric bud and the metanephric mesenchymal tissue. CAKUT occur
42 in three to six of 1000 live births, represent about 20% of the prenatally detected anomalies⁹⁰,
43 and constitute the main cause of chronic kidney disease (CKD) in children⁹¹, and a likely
44 underestimated proportion of CKD of unknown origin in adults. The phenotypic spectrum is
45 very large and can include variable degrees of renal parenchymal defects of the kidney (such
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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.⁹² CAKUT can present as isolated or syndromic, associated with various extra-renal phenotypes. The familial clustering suggests a major genetic contribution to the etiology of CAKUT.⁹³ Pathogenic variations in more than 50 genes have been reported in isolated or syndromic CAKUT (**table 6; see supplementary material**), 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. Copy number variations (CNVs) have also been shown to be frequently associated with isolated or syndromic CAKUT. Up to 16% of individuals with renal hypo-dysplasia have been shown to have a molecular diagnosis attributable to a CNV disorder.⁹⁴ More recently 45 distinct known genomic disorders at 37 loci were identified in 4.1% of a series of 2824 CAKUT cases (however six loci were accounting 65% of these cases), and novel CNVs were associated with additional 2% cases.⁹⁵ 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 vesico-ureteral 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 next generation sequencing, suggesting that inheritance may frequently be more complex. *PAX2*, *HNF1B* and *EYA1* are the three genes most frequently involved in monogenic in CAKUT (representing 23% of the cases of CAKUT with a known CNV in ⁹⁵); more than half of the *HNF1B* defects correspond to the recurrent 17q12 microdeletion. The prognosis of CAKUT is mainly related to (1) the extent of reduction in nephron number and associated risk of renal failure, and (2) extra-renal 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 counseling when pathogenic variant(s) responsible for the phenotype is (are) identified. Whether to start with a small MPS-based gene panel including most often mutated genes, before proceeding to a larger panel, or a virtual (exome-based) panel is mainly depending 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 exome sequencing 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 to 18% of cases.^{19,20,96} 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, unilateral pelviureteric 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

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3 parenchymas, with or without urinary tract defect, with or without familial history of CAKUT
4 and with or without extra-renal defects. Mutations in genes other than *HNF1B*, *PAX2* and
5 *EYA1* represent, for each gene, only a small percentage of cases.

6 Interpretation of variants in CAKUT genes can be even more difficult because the
7 pathogenicity of some variants previously reported as pathogenic mutations, is nowadays,
8 with the available knowledge of large databases such as gnomAD, questionable. This is for
9 instance the case for gene variants that would currently be classified as VUS, as they have
10 been reported in only a single (or in few) report(s) without data regarding segregation (or
11 sometimes with the variant inherited from a healthy parent), and with a minor allele
12 frequency too high in population and/or inhouse databases. However, as expressivity and
13 penetrance can vary greatly in monogenic CAKUT with autosomal dominant inheritance, it is
14 sometimes difficult to definitely rule out the causality of some variants. This is the case for,
15 *CDC5L*⁹⁷, *CRIM1*¹⁹, *FOXF1*⁹⁸, *ROBO2*⁹⁹, *SOX17*¹⁰⁰, *SRGAP1*⁹⁹, *TRAP1*¹⁰¹, *UPK3A*¹⁰², and even for
16 *DSTYK*.¹⁰³

17 Notably, the large majority of CAKUT cases are currently not explained by pathogenic
18 variation in known or in novel genes even when tested by exome sequencing (Jeanpierre et
19 al. unpublished data)⁹⁶. This might be due to mutations difficult to detect by ES (**table 2**), to
20 the involvement of somatic events, environmental factors or epigenetic mechanisms, and to
21 oligogenicity, which may explain both the known familial aggregation of CAKUT and the low
22 rate of mutations identified in genes involved in monogenic forms of the disease. As for any
23 other molecular testing, it is necessary to collect detailed family history and precise
24 phenotypic information regarding the index case and family (including renal ultrasound of the
25 parents when possible). Because inheritance is frequently autosomal dominant with variable
26 expressivity, because of the frequency of *de novo* mutations in these genes, and because of
27 the large number of rare variants of unknown significance identified by MPS screening, results
28 will be much easier to interpret when cases are tested as trios (proband and parents). Future
29 studies aiming at understanding the complex inheritance of CAKUT will require collaborative
30 efforts in order to share data of a very large number of cases with deep phenotyping.

31 Clinical benefit of genetic testing

32 The molecular diagnosis is helpful in order to orientate complementary extra-renal
33 explorations and for specific follow up of the patient, such as the monitoring for diabetes in
34 cases of *HNF1B* mutations, ocular defect (risk of retinal detachment) in case of *PAX2*
35 mutations, hearing testing in case of *EYA1* mutation, and close follow up of developmental
36 milestones in children with 17q12 deletion. The identification of CNV(s) will be especially
37 important for the evaluation of potential extra-renal defects, particularly for
38 neurodevelopment. In the prenatal setting, except for the cases with recurrent pathogenic
39 CNV(s), the molecular diagnosis would not help in predicting the severity of extra-renal
40 defects. Identification of a pathogenic variant in a CAKUT gene also will improve
41 management of patients presenting with heavy proteinuria associated with FSGS secondary
42 to reduced nephron number (in particular in association with *PAX2* mutations), which can
43 phenocopy steroid resistant nephrotic syndrome.¹⁰⁴ The same holds true for phenotypes
44 consisting in isolated small hyperechogenic kidneys associated with renal failure (without
45 proteinuria or abnormalities of the urinary sediment) in adults, a frequent presentation
46 usually considered as CKD of unknown origin, which can be secondary to tubulo-interstitial
47 kidney disease, ciliopathy, or CAKUT.

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3 A molecular diagnosis is also useful when a living related kidney transplantation is planned.
4 In view of variable disease expressivity, genetic testing allows to ensure that the donor does
5 not carry the pathogenic variation identified in the index case.
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8 Genetic counseling

9 Many cases with an identified monogenic cause of CAKUT are associated with a *de novo*
10 mutation in one gene involved in autosomal dominant disease, thus the molecular testing of
11 the case and parents will allow to reassure parents for future pregnancies, the risk of
12 recurrence being limited to the risk of germline mosaicism. Because the severity of the renal
13 disease varies greatly, even within a given family, in most autosomal dominant monogenic
14 CAKUT, knowledge of the molecular defect is not very helpful to predict the prognosis, which
15 is much better correlated with the renal morphology. This is particularly true when CAKUT is
16 diagnosed in the prenatal period. In families in which a pathogenic variant has been identified
17 in a gene involved in autosomal dominant CAKUT with variable expressivity, even if the risk
18 of transmission of the variant is 50%, parents will frequently opt for prenatal monitoring
19 based on ultrasound screening targeted on fetal kidney morphology rather than for early
20 prenatal molecular testing after chorionic villus sampling. However, this must be discussed
21 on a case-by-case basis.
22

23 Extra-renal anomalies, which can be associated with pathogenic variations/CNV in many of
24 the genes involved in monogenic CAKUT, also frequently have a variable expressivity, making
25 the molecular diagnosis not a good predictor of the severity of the extra-renal symptoms. This
26 is the case for instance for the ocular anomalies associated with *PAX2* mutations¹⁰⁵, and the
27 hearing/ear/branchial defects associated with *EYA1* mutations¹⁰⁶.

28 The recurrent 17q12 deletion including *HNF1B*, which is a frequent cause of fetal
29 hyperechogenic normal sized kidneys, is considered by some authors as conferring a high risk
30 of neurodevelopmental disorders¹⁰⁷; however, the penetrance and severity of
31 neuropsychological disorders associated with that CNV seems less important in cohort of
32 patients diagnosed secondary to kidney anomalies.¹⁰⁸ In families affected with autosomal
33 recessive CAKUT such as bilateral renal agenesis associated with *ITGA8* mutations, Fraser
34 syndrome, Megacystis microcolon intestinal hypoperistalsis, or in cases of renal tubular
35 dysgenesis, genetic counseling can support their decision making regarding prenatal or
36 preimplantation testing.
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46 **Renal ciliopathies**

47 Renal ciliopathies are a clinically and genetically heterogeneous group of inherited disorders.
48 Extrarenal manifestations are frequently associated. Due to this huge heterogeneity, we
49 would here like to focus on the group of cystic kidney diseases, which includes Polycystic
50 kidney diseases (ADPKD and ARPKD), and on nephronophthisis (NPHP). We will only shortly
51 touch upon less frequent cilia-related disorders (**Table 7; see supplementary material**) and
52 other genetic syndromes that may phenocopy renal ciliopathies (e.g., metabolic,
53 mitochondrial). Polycystic kidney disease (PKD) is characterized by enlarged kidneys.¹⁰⁹ In
54 children with early severe disease manifestations, variants in genes for ARPKD (mainly *PKHD1*)
55 and ADPKD (mainly *PKD1* and *PKD2*) are most common.¹¹⁰ Remarkably, ADPKD mutations
56 often occur *de novo* without a family history. In those severe cases, pathogenic *PKD1* variants
57 can affect both disease alleles in a recessive mode of inheritance. Furthermore, variants in
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DZIP1L and many other genes can lead to an ARPKD-like phenotype.¹¹¹ 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*, *HNF1 β* , *PKHD1*, *DZIP1L*, *TSC1/2*, *VHL*, *OFD1* in women, etc.).

PKD can usually be easily distinguished on clinical grounds from nephronophthisis (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.¹¹²⁻¹¹⁴

ADPKD can sometimes be mistaken for Autosomal Dominant Tubulointerstitial Kidney Disease (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 either 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.¹¹⁵

Many other ciliopathies may present with kidney cysts mimicking PKD or NPHP (**Table 7; see supplementary material**), 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 already 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 be not 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 one single (small) gene known with a high mutation detection rate. However, in most other cases we would clearly recommend a broader testing approach already at the very beginning due to massive heterogeneity and the large number of phenocopies.

Whatever primary strategy is chosen, an expanded gene panel or ES, the testing approach should be able to detect copy-number variations (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 extra-renal phenotypic features.

Clinical benefits of genetic testing

The high gene detection rate in cystic kidney diseases allows to rapidly establish a definite diagnosis and avoid a “diagnostic odyssey” with unnecessary diagnostic measures such as

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

20 Genetic counselling

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

27 **CKD of unknown origin**

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29 Chronic kidney disease (CKD) of unknown origin is frequently seen among CKD cohorts,
30 accounting up to one third of all cases with adult-onset CKD.¹¹⁶ In several recent studies using
31 NGS techniques, it was demonstrated that monogenic causes are responsible for a significant
32 proportion of those “unknown” cases. The diagnostic yield in these studies varied between
33 12 and 56%, depending on the inclusion criteria, number of patients in the study and the
34 NGS approach.^{4,5,8,9} Indicators of a higher diagnostic yield were a positive family history,
35 younger age of onset of CKD, the presence of extrarenal features, and congenital/cystic
36 disease phenotypes. Further research is necessary to explore the diagnostic yield of genetic
37 testing in CKD of unknown origin in a clinical setting.

40 Genetic testing: indications and preferred testing strategies

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42 Since in many patients with CKD of unknown origin, features attributable to an underlying
43 genetic diagnosis were not recognized prior to the genetic analysis, it seems appropriate to
44 perform a genetic test in patients with severe CKD/ESKD and onset before the age of 50, in
45 whom a clear-cut non-genetic diagnosis (e.g. acute nephrotoxicity, diabetic nephropathy,
46 infectious nephropathy) has been excluded.¹¹⁷ Although there are rare cases of patients with
47 adult-onset CKD above the age of 50 in whom a genetic diagnosis was identified, the *a priori*
48 chance of a genetic diagnosis in these older CKD patients seems extremely low and does not
49 yet warrant genetic testing unless there is a clear family history.¹¹⁸ We recommend a tiered
50 exome-based diagnostic approach in patients with CKD of unknown origin, starting with a
51 large targeted multi-gene panel, involving all known nephropathy genes, and open up the
52 whole exome backbone to look beyond the known diseases genes, in case no causative
53 variant(s) is (are) identified.

Clinical benefit of genetic testing

For many patients the answer to why they have developed CKD is very important. Knowing the exact etiology of their disease generally has a positive impact on their lives.¹¹⁹ In addition, knowing that a disease is heritable, also means that family members that might be affected can be counseled 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 for family planning.

Genetic counseling

All patients in whom a genetic diagnosis has been established, should receive genetic counseling, especially when there are questions on family planning, reproduction and in setting of kidney transplantation with living-related donation. In addition, patients should be informed on 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 presumably 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 counseling and family planning and allows reproductive options, such as prenatal or preimplantation 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 multi-gene 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 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 renal 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 (1) education to increase genetic literacy, (2) bioinformatic innovations and datasharing strategies to improve variant interpretation, (3) large scale validation of research findings in the clinical setting, such as the diagnostic yield of genetic testing for kidney diseases, (4) cost versus benefit analyses of genetic testing, and (5) further development of organizational and counseling support in daily practice.

Box 3. Gaps in knowledge/unmet needs and helpful strategies for implementation of genetics and massively sequencing (MPS) approaches into clinical nephrology practice

- **Genetics literacy among clinicians needs improvement**
 - Educational resources for professionals: www.genomicseducation.hee.nhs.uk), review paper with helpful online resources to aid physicians¹²⁰
 - Practice-based education for nephrologists: ERKnet webinar series (<https://www.erknet.org/>), WGIKD CME courses and e-seminars (<https://www.era-edta.org/en/wgikd/>)

 - **Genetic variant interpretation needs improvement**
 - Improvements in bioinformatic algorithms
 - Effective data-sharing genetic data whilst preserving patient privacy (i.e. Gene matcher <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/>)
 - Genepanels curated by experts through Genomics England (<https://panelapp.genomicsengland.co.uk/>)

 - **We need guidelines for MPS-based diagnostic testing**
 - Validate diagnostic outcomes of MPS-based genetic testing in a real-life clinical setting rather than research setting (i.e. Variety-study in 1000 CKD patients, University Medical Centre Groningen¹²¹)

 - **We need to determine the costs versus benefits of genetic testing**
 - Studies on long-term effect of establishing a molecular diagnosis on health care 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 health care insurance companies

 - **We need to know how to effectively organize genetic testing and counseling in daily clinical practice?**
 - Development clinical decision support tools
 - Genetic counselors in nephrology practice
 - Computing platforms that facilitate integration of genomic data with electronic health records
 - Development of centres of excellence (e.g. ERKNet)
 - Access to and organization of multidisciplinary case discussion platforms
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DATA AVAILABILITY STATEMENT

No new data are generated or analysed in support of this research

CONFLICT OF INTEREST STATEMENT

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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 copy number variants (CNVs) suspected	CAKUT aHUS (<i>CFH,CFHR</i>) Nephronophthisis (<i>NPHP1</i>)
Targeted phenotype-associated gene panel	Disorders with locus heterogeneity	Steroid resistant nephrotic syndrome (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 & 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 at research basis for cases still unsolved after ES Emerging clinical use	ADPKD (<i>PKD1</i>)

Table 2. Possible reasons for negative results after gene testing using massively parallel sequencing (MPS) gene panels or exome sequencing (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, steroid-resistant nephrotic syndrome
Detection of large copy number variants (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 (VNTR) sequences in <i>MUC1</i> (<i>MUC1-ADTKD</i>)
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