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► **To cite this version:**

Ilias Bensouna, Alice Doreille, Marine Dancer, Anne-Sophie Lebre, Thomas Robert, et al.. Nephrogenomics, precision medicine and the role of genetic testing in adult kidney disease management. *Nature Reviews Nephrology*, 2025, <10.1038/s41581-025-00970-1>. <hal-05199260>

HAL Id: hal-05199260

<https://hal.science/hal-05199260v1>

Submitted on 4 Aug 2025

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Nephrogenomics, precision medicine and the role of genetic testing in adult kidney disease management

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Abstract

Genetic investigations in nephrology have long been viewed as the prerogative of paediatricians or restricted to archetypal genetic nephropathies with highly penetrant variants affecting young adults. However, genetic testing has emerged as a pivotal tool in the field of adult nephrology, with the ability to revolutionize the understanding and management of adult kidney diseases. Here, we explore the multifaceted role of genomic testing (such as exome or genome sequencing) in chronic kidney disease, shedding light on current genetic findings for reframing diagnostic paradigms and tailoring treatment strategies. Genomic testing has enhanced our comprehension of kidney diseases of unknown origin by showing that ~20% are attributable to kidney Mendelian genetic disorders with yet unsuspected phenocopies. Beyond genetic counseling, genetic integration can optimize therapeutic interventions, kidney transplantation and kidney disease prevention, both in index cases and at-risk family members. Furthermore, the emerging field of rapid nephrogenomics promises streamlined diagnosis and management, with potential impact on early therapeutic strategy. Importantly, although costs continue to decrease, the integration of genomic technologies in nephrology practice raises several ethical concerns, especially regarding variants of uncertain significance, and incidental or secondary findings. Establishing multidisciplinary frameworks should maximize the potential of nephrogenomics to improve patient outcomes.

[H1] Introduction

Several advances have positioned genetic testing as a pivotal tool in the management of adult kidney disease. Genomic testing — whole-exome sequencing (WES) and whole genome sequencing (WGS) — has fundamentally transformed our understanding of adult kidney diseases by enabling precise disease reclassification, particularly in cases of kidney disease of unknown origin, where ~20% of cases are genetic Mendelian diseases.¹⁻³

Additionally, WES and WGS have greatly expanded our knowledge of genetic kidney diseases in adults, where genotype–phenotype correlations are often poor.^{4,5} The use of these tools has unveiled numerous **phenocopies [G]** previously obscured by panel-based analysis approaches. The integration of genetic information can enhance therapeutic efficacy and minimize adverse effects by tailoring the treatment to the specific cause of the disease; in some cases, two or more genetic diseases are concurrent.^{6,7} Genetic information can help clinicians predict disease progression and identify individuals at risk of disease development, paving the way for proactive and personalized interventions, and heralding a new era of precision medicine in nephrology.⁸ Moreover, genetic testing has an important role in preventive medicine and in prompting genetic counseling, empowering individuals to make informed choices to mitigate disease risk.

However, genomic testing also involves important ethical considerations, especially in managing incidental or secondary findings, which are genetic variants or result discovered during genomic testing that are unrelated to the primary purpose of the test; these findings require careful attention and organized multidisciplinary interactions for optimal patient benefit.^{9,10} **Variants of uncertain significance [G]** (VUS) are challenging to interpret owing to limited evidence. However, the growing understanding of genotype–phenotype associations enables the refinement of molecular diagnoses during reevaluation over time. Finally, the introduction of rapid genomic technologies has potential to improve workflow efficiency and accelerate diagnoses in nephrology, with paradigmatic examples in the management of thrombotic microangiopathy (TMA).^{11,12} Therefore, genetic testing is emerging as a cornerstone of contemporary kidney disease management in adults, offering unparalleled insights and enabling precision medicine.

In this Review, we explore the technical aspects of pangenomic analysis in adult nephrology, detailing sequencing methods available to nephrologists and geneticists, in particular for the management of unknown nephropathy. We highlight the specificity of pangenomic analysis, including the potential of long-read sequencing to improve molecular diagnosis in nephrology,

especially for previously challenging genomic regions. However, challenges remain, including the management and sharing of VUS, and of incidental and secondary findings, which raise ethical issues that most nephrologists have not been trained to handle. Multidisciplinary collaboration can help to address gaps in knowledge and maximize the benefits of genomic analyses in clinical nephrology.

[H1] Overview of sequencing methods and variant sharing frameworks

[H2] Summary of available sequencing methods

Sanger sequencing, developed by Frederick Sanger in 1977 as the first-generation sequencing technology, is highly accurate for detecting single-nucleotide variants (SNVs) and small insertions or deletions (<50 base pairs) in a targeted region. Despite the advent of next-generation sequencing (NGS), Sanger sequencing remains the gold-standard for confirming some variants identified by NGS, particularly single-nucleotide polymorphisms (SNPs) owing to its lower error rates and higher base-calling accuracy compared with NGS, and its capacity to validate specific regions of interest with precision. This method is not suitable for large-scale genomic studies but is still useful for segregation studies used to determine whether a trait follows the distribution of a suspected variant.

Since 2012, NGS has revolutionized genomics by enabling the simultaneous sequencing of millions of DNA fragments. This advance marked a substantial leap from Sanger sequencing, which is limited to processing a single DNA fragment at a time. NGS facilitates large-scale genetic studies and enables the analysis of entire genomes or targeted gene panels efficiently. NGS encompasses WES, which targets exons (these sections of the genome largely coincide with the coding regions of genes, covering 1–2% of the genome), as well as WGS, which goes beyond coding regions to include introns, intergenic regions, regulatory elements and non-coding sequences. Of note, WES captures exon–intron boundaries, usually ranging from 100–200 base pairs, which enables the bioinformatic detection of the most common splice junctions as nearby intronic variants. This expansive coverage contrasts sharply with the ability of Sanger sequencing to examine only small, specific regions at a time, and renders NGS indispensable for detecting a broader spectrum of genetic variants, including those in non-coding regions that have a crucial role in gene regulation and disease.¹³ (TABLE 1) Custom panel sequencing commonly uses an exome sequencing backbone, focusing on a specific set of genes associated with specific groups of

diseases. This approach requires accurate clinical suspicion before testing to ensure that the appropriate panel is selected.

In clinical nephrology, NGS technologies, although transformative, present several limitations that must be considered to ensure accurate diagnoses. First, short-read sequencing methods (SRS), such as WES and WGS, struggle to detect complex structural variations such as hybrid or fusion genes. For example, *CFH* hybrid genes, implicated in complement-mediated TMA (CM-TMA), are difficult to identify owing to their structural complexity and sequence similarity within the regulators of complement activation (RCA) locus, but could account for 5% of CM-TMA diagnoses.^{14,15} [Au: OK?] These genes are typically analyzed using **multiplex ligation-dependent probe amplification [G]** (MLPA), a technique that is currently recommended for use in combination with NGS to avoid missing these rare but clinically significant events.¹⁶ Similarly, short-read NGS methods are inadequate for resolving highly repetitive regions, such as those in *MUC1*, which is associated with autosomal dominant tubulointerstitial kidney disease (ADTKD).^{17,18} ^{19,20} The most common *MUC1* pathogenic variant, a cytosine insertion (27dupC) in a homopolymer stretch, is difficult to detect with traditional sequencing and is usually identified through PCR-based methods or mass spectrometry, or indirectly by specific staining of the truncated MUCIN1 protein on kidney biopsy tissue.^{21–23} Advances in bioinformatics pipelines and long-read sequencing technologies are promising solutions for overcoming these challenges, offering greater resolution in complex genomic regions.^{24,25} A bioinformatic pipeline reported in 2023 accurately calls variants within the *MUC1* coding variable number tandem repeats (VNTR) region using SRS data from targeted panels, including the key pathogenic variant 27dupC, and has also been adapted for WES data in routine clinical practice, enabling reliable detection of the 27dupC variant.^{24,25} This adaptation is currently limited to the 27dupC variant and further research is required to extend its application to other *MUC1* variants. Continued efforts in bioinformatics development and sequencing technologies are needed to fully overcome the diagnostic limitations posed by NGS in these complex regions²⁴ (TABLE 2).

Some regions of interest in nephrogenomics that are not well-sequenced by SRS can be effectively explored using long-read sequencing, which offers several advantages, including the study of structural variants, better evaluation of repeated regions and accurate analysis of pseudogenes. The **Telomere-to-telomere [G]** (T2T) Consortium and the **Human Pangenome Reference [G]**

Consortium (HPRC) have used long-read technologies^{26,27}, which excel in capturing guanine–cytosine (GC)-rich regions [G], often found in gene regulatory areas. These techniques have contributed to the reduction of gaps in genome assemblies, providing a more accurate assessment of gene duplications and their orthologues, and enabling the study of repetitive and complex genomic regions, such as centromeric regions, long repeats and complex structural variants. In nephrogenomics, the choice between these methods depends on the specific questions, the need for depth versus breadth of genomic coverage and budget.

[H2] Potential impact of long-read sequencing and methylation profiling

Long-read sequencing is particularly effective in resolving complex genomic variations in nephrogenomics that are challenging for SRS, such as *CFH* hybrid genes or *MUC1* variants.^{12,28} This approach also handles disease-associated genes located near pseudogenes, which can interfere with the detection of pathogenic variants, as seen in autosomal-dominant polycystic kidney disease (ADPKD).²⁹ With long reads, variants can be phased to determine whether they are inherited together from the same parent (on the same chromosome) or from different parents (on different chromosomes); this information is important for understanding the functional impact of genetic variants, their contribution to disease and their transmission (recessive or dominant).³⁰ Long-read RNA sequencing can also be employed to describe RNA abundance, uncover RNA structures, and assess RNA modifications. Beyond DNA and RNA sequences, long-read sequencing also enables the study of epigenetic modifications, such as 5-methylcytosine on native DNA.³⁰

Over time, long-read sequencing has become more high-throughput and user-friendly, with costs gradually decreasing. However, price and speed remain important considerations and, given the resources required for data analysis, sequencing a large number of samples simultaneously is challenging. Two platforms currently dominate long-read sequencing. The cost per base of sequencing with PacBio's High Fidelity (HiFi) platform is still high, creating an access barrier for individual labs without substantial funding. ONT sequencers represent an alternative platform that has continually improved in accuracy as the technology matures. Both PacBio and ONT technologies have been adopted for large-scale projects.²⁷ ONT sequencers have empowered genomics projects in smaller labs, leading to the emergence of application-focused customers. The use of long-read sequencing holds promises for simplifying and shortening the diagnostic process

for patients with nephropathies in clinical settings in the near future. Compared with SRS, the cost remains higher, although more information can be analyzed within the same assay. Further studies are needed to establish the role of long-read sequencing in nephrology.

[H2] In-silico approaches for gene panel analysis, ClinGen curation and variant sharing

The field of nephrogenomics has identified hundreds of genes linked to nephropathies. As per American College of Medical Genetics and Genomics (ACMG) recommendations, these genes are clinically relevant because molecular diagnosis can guide specific treatments (for example, in ADPKD, primary oxaluria, Fabry disease, cystinosis, cobalamin C deficiency and CoQ10 deficiency), enables nephroprotective care, prevents unnecessary prolonged immunosuppression (for example, in Alport syndrome), enables assessment of recurrence risk post-kidney transplantation (for example, in atypical hemolytic uremic syndrome and monogenic lithiasis), screens for extra-renal manifestations (variants in *HNFB1B*, *PKDI*, *PKD2* and mitochondrial DNA), or facilitates genetic counseling, including prenatal and preimplantation diagnosis.⁸ Based on these recommendations, in-silico panels are designed to focus molecular analysis on relevant genes identified using pangenomic data.

Collaborative efforts can significantly improve understanding of genetic variants through sharing of pathogenicity information, as exemplified by the [Clinical Genome Resource](#) (ClinGen) database, founded by the US National Human Genome Research Institute. [PanelApp](#) is another valuable resource that enables the creation of virtual gene panels for molecular analysis.³¹ PanelApp is an online, open-source tool developed by Genomics England, where users can access all gene panels related to genomic tests listed in the UK National Health Service (NHS) National Genomic Test Directory, as well as virtual gene panels used in the 100,000 Genomes Project.³² [PanelApp Australia](#) follows a similar approach.³² As previously mentioned, virtual bioinformatic panels can be applied to genomic data and are constantly updated as new genes related to the pathology of interest are discovered and confirmed.

PanelApp employs a standardized gene curation framework and sets clear evidence thresholds for diagnostics. This tool records case-level and functional evidence for each gene–disease association, offers detailed panel descriptions, promotes the use of multiple panels, employs version-controlled panels and fosters the development of specific gene curation skills. PanelApp

also integrates data from various sources, including expert opinions, literature and genomic databases, to provide comprehensive and accurate gene panels for clinical application. The regular updates provided by PanelApp are a considerable advantage in a field where new genetic discoveries can quickly impact genetic diagnostics. In nephrogenomics, several panels are available for use, such as the CAKUT panel, the cystic kidney disease panel, the unexplained kidney failure in young people panel for chronic kidney disease of unknown aetiology (CKDu), and the tubulointerstitial kidney disease panel.³²

ClinGen is a nonprofit initiative funded by the US National Institutes of Health (NIH) aimed at collecting and disseminating genetic data, advancing knowledge, defining the clinical significance of genes, and enhancing patient care and research. Experts propose recommendations and classifications based on publicly available human and experimental data, contributing to gene curation.³³ ClinGen supports the development of clinical guidelines and regulatory policies that are applicable in nephrogenomics. [ClinVar](#), another NIH-supported endeavor, is a publicly accessible database that compiles information on genomic variation and its association with human health. ClinGen and ClinVar collaborate closely, fostering a partnership to enhance our understanding of clinically relevant genomic variations through shared data, archival efforts and collaborative curation. This partnership facilitates the characterization and dissemination of the clinical significance of genomic variations. Submissions to ClinVar are accepted from clinical testing laboratories, researchers, locus-specific databases, expert panels and professional societies. These submissions adhere to standardized formats for variant descriptions by the Human genome variation society (HGVS), medical conditions and terms indicating clinical significance.

The availability of these open-source data aids and streamlines the interpretation of gene variants. Additionally, international data accessibility promotes harmonization in genetic diagnoses. Databases that compile comprehensive information on gene physiopathology, types of variations associated with phenotypes, occurrence rates and related phenotypes represent substantial progress and time-saving resources in diagnostics, including the diagnosis of rare genetic diseases. Sharing data enables collaboration and advances in knowledge. [GeneMatcher](#), which is a free website supported by the Baylor-Hopkins Center for Mendelian Genomics, has emerged as a tool to enhance collaboration as it aims to connect patients, families, clinicians and researchers interested

in the same genes. The associated platform [Matchmaker Exchange](#) connects GeneMatcher with other related databases, some of which are curated by patients.³⁴ The primary goal of these platforms is to assist in establishing novel gene disease associations from both research and clinical cases. Users can post genes of interest and are automatically matched with others who have posted on the same gene, receiving email notifications in case of a match. These platforms enable the submission of gene symbols, variants, diagnoses and clinical features, adhering to strict safety and privacy protocols. Together, these initiatives enhance the accuracy, reliability and clinical relevance of genetic information, driving progress in precision medicine and nephrogenomics.

[H2] WES in clinical practice and diagnosis yield in adult nephrology

One of the challenges in adult chronic kidney disease, is that phenotypes are often poorly described and atypical. These characteristics increase the risk of prescribing the wrong gene panel and potentially missing a diagnosis with targeted molecular analysis. This problem is overcome by WES, which enables molecular diagnoses that are not limited to genes that are initially suspected. In previous adult nephropathy cohorts, indications for WES were broader than those for targeted analysis, including nephropathy of unknown origin lacking the phenotype of a specific genetic disease.^{1,3,35-37} In all of these cohorts, <50% of patients had a family history of nephropathy. Diagnosis yield with WES ranged from 10% in American studies to 25% in European studies.^{1,3,36} Of note, the diagnosis does not always match the initial presentation. For example, in a French cohort of patients with CKDu, 20% of people with ADTKD according to a molecular diagnosis were initially diagnosed as having glomerular nephropathy.³ In other cohorts, incidental findings were found in 5% of patients, meaning that molecular diagnostics identified additional causes of disease, risk factors or other relevant genetic findings.³

[H1] Regional variability in genetic reporting and CKD management

[H2] Current recommendations from the ACMG and ESHG

The ACMG has released guidelines for interpreting sequence variants, featuring a standardized classification system that includes categories such as pathogenic, likely pathogenic, uncertain significance, likely benign and benign³⁸⁻⁴⁰ (FIG. 1).] These guidelines emphasize the integration of various types of evidence, including population data, computational predictions and functional studies. Additionally, specific Clinical Practice Guidelines have been developed by ClinGen,

offering recommendations for genetic testing across various conditions such as neurodevelopmental disabilities, hereditary cancer syndromes and cardiomyopathies⁴¹, and Kidney Disease: Improving Global Outcomes (KDIGO) recommendation also exist for screening for kidney disease risk alleles, such as *APOLI*.⁸ Moreover, Dutch, NHS and Australian guidelines have been published or updated in the past 10 years.^{42–44}

The European Society of Human Genetics (ESHG) is dedicated to advancing research, education, and clinical practice in human genetics. The Society also supports professional development, establishes quality standards in genetic testing and advocates for ethical practices in genetics. The ESHG has issued several key recommendations and guidelines, particularly focusing on genetic testing and clinical genomics, such as guidelines on the use of NGS in clinical practice. These guidelines stress the importance of confirming reported variants to ensure accuracy and prevent diagnostic errors resulting from sample mix-ups or issues within bioinformatics pipelines.^{43,45} These risks have been addressed in many regions by certifying or accrediting reference laboratories for genetic testing.

Both the ACMG and the ESHG address the ethical aspects of genetic testing, including informed consent, privacy concerns and the implications of disclosing secondary findings. These associations advocate for clear communication strategies to help patients understand the potential outcomes of genetic testing and the significance of any findings. These guidelines have a crucial role in standardizing practices in clinical genetics, ensuring that genetic testing and reporting maintain high standards of accuracy, ethics and patient benefit (FIGs. 2 and 3). To help manage secondary findings, the latest ACMG guidelines (ACMG SF v3.2) specify that particular genes that should be reported if secondary findings are detected during clinical exome and genome sequencing.⁴⁶

[H2] Lack of standardization in genetic reports

Standardizing genetic reporting is crucial in medical genetics as it impacts the quality of care, result interpretation and clinical decision-making directly. However, achieving this standardization poses significant challenges owing to various substantial and complex factors. Different laboratories and institutions use various formats for reporting genetic results, leading to inconsistencies in presentation and comprehension. Moreover, the absence of unified standards for

interpreting genetic variants results in discrepancies, highlighting the importance of open-source tools to aid in interpretation.^{38,41,47} Additionally, diverse terminologies and coding systems across platforms complicate the integration and comparison of genetic data. Collectively, these challenges impede the establishment of a consistent and reliable genetic reporting system.

Variations in evidence weighting and interpretation can lead to differing classifications of the same genetic variant. Several initiatives and recommendations have emerged to address these challenges. The ClinVar database supports variant interpretation by archiving observations of the relationships between genotypes and phenotypes.⁴⁸ Moreover, national platforms as Shariant in Australia enable genetic-testing laboratories to share variant interpretation and associated evidence.⁴⁹

Gain-of-function (GOF) variants in kidney disease-related genes such as *UMOD*, *PAX2* and *HNF1B* present substantial challenges for variant effect predictors, including widely used tools such as SIFT, UMD predictor and PolyPhen-2.^{50,51,52} These tools primarily assess loss-of-function (LOF) variants by predicting whether a variant disrupts protein structure or function. However, these tools often struggle with GOF mutations, which are likely to have more heterogeneous molecular effects, such as excessive protein stabilization, toxic aggregation, modulation of protein activity or changes in protein interaction selectivity. Computational predictors might systematically underperform regarding dominant-negative (DN) and GOF variants in genes related to kidney disease (for example, *UMOD*, *PAX2*, *INF2*, *C3*, *TRPC6*, *CFB*, *CFHR1:CFH*).^{53–55} This underperformance can lead to large numbers of true pathogenic variants being missed in current sequencing studies, which is particularly concerning given the widespread use of these predictors to prioritize potentially causal variants in precision medicine and genomic research. Accurate interpretation of GOF variants requires more specialized tools and functional assays to ensure that pathogenic variants are not overlooked (FIG. 2).

Several challenges of interpreting genetic variations and implementing guidelines in computational tools, particularly in nephrogenomics, have been highlighted, including issues such as disparities between guidelines and real-world applications, ambiguity in variant classification, and the need for improved standards and tools.⁵⁶ For example, a consensus statement on molecular diagnostics in Alport syndrome, refined the criteria set by ACMG, outlining standardized

guidelines for genetic testing, aiming to enhance accuracy and consistency in diagnosis.⁴⁴ Such examples underscore the importance of consistent guideline application across different contexts and the efforts that need to be made by the nephrogenomic community (FIG. 3).

Standardization of genetic reporting can ensure care quality and consistency in clinical interpretation. Despite considerable advances, global adoption of these standards is needed to overcome current challenges. Efforts to standardize genetic reporting should involve education and training of healthcare providers to enable effective understanding and use of genetic information. Continuous professional development is vital to keeping abreast of genetic advances. Continued collaboration, technological advancements and comprehensive guidelines are also essential for achieving greater consistency in genetic reporting.

[H1] Clinician education and collaboration with geneticists and genetic counselors

For many nephrologists, integrating genomics into clinical practice represents a substantial new skill set.^{57,58} This integration involves acquiring technical competencies in genetic technologies to understand newly developed genetic tests, their limitations and the nuances of result interpretation. It also requires practical skills such as facilitating informed consent and effectively communicating genetic results within the context of genetic counseling. Moreover, this integration requires a shift towards expanding patient care to include consideration of family members and addressing the ethical challenges associated with genetic testing. Consequently, genomics education is essential to cultivate a competent nephrogenomic workforce.^{59,60} Comprehensive education in nephrogenomics could also help mitigate disparities in access to genetic testing for patients and alleviate barriers during the pre-referral period.^{57,61}

In line with this need, both practicing nephrologists and the next generation of medical professionals are increasingly advocating for enhanced educational resources in the field of genetics.^{57,62-64} Given the rapidly increasing volume and complexity of genetic data, as well as the development of new genetic testing strategies, nephrogenomics requires a multidisciplinary team approach that emphasizes close collaboration among clinical, laboratory and research experts. This approach aligns with KDIGO recommendations and is exemplified by successful multidisciplinary nephrogenetic clinics developed in Australia.^{8,61,65,66} Such collaborative interactions among specialists ensure comprehensive patient care, provide educational opportunities for all specialists

involved to learn from diverse perspectives, foster innovation and contribute to advances in nephrogenomics.

Importantly, this collaboration is essential for achieving accurate diagnoses. Although genomic testing is a powerful tool, it is not a diagnostic test in itself; it identifies genetic variations, but diagnosis is achieved by interpreting these variants within the context of the clinical features of the patient. Therefore, as discussed earlier, clinico-biological correlation is crucial to reach a definitive diagnosis.⁶⁷ The initial phenotyping might be limited or incomplete before testing, but can be enriched through reverse phenotyping after identifying highly suspicious variants, as well as screening for paucisymptomatic or asymptomatic manifestations of the disease that might have been previously overlooked (see later discussion). The diagnostic yield of genetic testing depends heavily on the quality and quantity of phenotypic information available, and the ability to engage in discussions with clinicians regarding variant interpretations.^{68,69} A multidisciplinary team approach to genomics data is being implemented in various centres, with studies consistently showing an increased diagnostic yield and improved accuracy following WES.^{70,71}

Moreover, genomic multidisciplinary teams have demonstrated high proficiency in interpreting variants of uncertain significance (VUS).^{71,72} These teams efficiently resolve VUS by maximizing clinical–molecular information for more accurate variant interpretation^{72,73}, thereby preventing false positives⁷⁴ and reducing the need for additional time and analyses by up to 80% in cases involving VUS, as exemplified for epilepsy.⁷⁵ In addition to enhancing the diagnostic yield of genetic testing, multidisciplinary teams help mitigate the risk of generating reports laden with irrelevant VUS, which can be challenging and time-consuming for clinicians to interpret and difficult to communicate effectively to patients (Supplementary TABLE 1).

[H2] Family and variant cosegregation analysis in practice

Variant cosegregation analysis is an important step in the interpretation of genetic variants, particularly for those classified as VUS. In real-world settings, however, logistical constraints often limit its feasibility. This section outlines a pragmatic framework for implementing segregation analysis in clinical nephrogenomics. The selection of family members for testing is performed collaboratively during a multidisciplinary case interpretation meeting that includes

nephrologists and biologist geneticists. This step is critical for prioritizing individuals with informative genetic relationships and accessible clinical data. Importantly, segregation analysis can only begin once the index individual has informed their relatives and received their consent to participate. Once key relatives are identified, the nephrologist initiates the process, ideally supported by a genomics care coordinator or genetic counsellor. Family members involved in segregation studies do not always undergo a full nephrology consultation, which can accelerate the process and reduce unnecessary medicalization. The coordinator has a central role in contacting the relatives directly, based on the contact information provided by the index individual. They can also distribute explanatory materials, answer questions and ensure that informed consent and logistics are coordinated smoothly across geographic distances. To accommodate relatives who live elsewhere, home-based saliva collection kits are mailed with instructions and consent documents. The genetic testing typically focuses on Sanger or qPCR sequencing, depending on the specific variant(s) of interest. Each participating relative is also asked to complete a standardized renal work-up, including: blood tests (serum creatinine, urea, electrolytes and albumin), urine analysis (proteinuria, haematuria, albumin-to-creatinine ratio) and kidney imaging (kidney ultrasound).

This coordinated and decentralized approach helps to generate useful phenotype–genotype correlation data without the need for in-person specialist visits. Moreover, this strategy can reduce the need for trio exome sequencing (that is, the genomic analysis of the index individual and their biological parents), thus offering a cost-saving alternative, while still yielding valuable segregation data. Even when segregation analysis does not lead to immediate variant reclassification, the generated data might support future reinterpretation efforts, especially when shared with national or international variant databases. It might also clarify inheritance patterns and inform clinical surveillance or preventive strategies for relatives. Clinically, segregation results might inform follow-up recommendations for asymptomatic variant carriers, refine recurrence risk estimates in families and avoid unnecessary surveillance in unaffected relatives. Finally, ethical and privacy safeguards are essential. Family members are contacted only after consent from the index individual, and results are communicated only if explicitly agreed. The process follows all applicable data protection regulations and respects the autonomy of each participant. By embedding segregation into a structured and scalable process, clinicians can

enhance the interpretability of genetic results and contribute to a more accurate delineation of monogenic disease liability within families.

[H2] Variability in reporting secondary or incidental findings in high-income countries

Incidental and secondary findings refer to pathogenic or likely pathogenic variants that are unrelated to the primary diagnosis and phenotype for which the genetic test was initiated. The key distinction between incidental and secondary findings lies in the intentionality behind their discovery. Incidental findings are discovered unintentionally and are not specifically sought after; they represent genetic variations in genes unrelated to the initial indication for exome sequencing in the patient. By contrast, secondary findings are actively searched for according to a predefined list of actionable genes or conditions.

In France and several other European countries, the bioethics law of 2021 mandates that incidental findings should be disclosed if they are actionable, meaning that they carry substantial health implications that can be addressed through preventive or therapeutic measures. French law emphasizes patient consent, requiring that individuals are informed about the possibility of discovering such findings and given the option to decide whether they want to receive this information. This approach represents an ‘opt-in’ consent model for incidental findings. Additionally, patients can modify their consent at any time before or after sequencing. The active search for secondary findings is currently prohibited in France.^{76,77}

By contrast, in the USA, the ACMG has established guidelines recommending management of certain secondary findings in clinical exome and genome sequencing. These guidelines list specific genes that are considered medically actionable. Consequently, the reporting of both incidental and secondary findings is generally mandated in the USA, although patients can opt out of receiving this information. The approach in the USA prioritizes maximizing patient benefit and preventive care using sequencing data.^{46,78,79}

In Canada, the Canadian College of Medical Geneticists (CCMG) recommends that patients be informed about the possibility of incidental findings and that consent should be obtained regarding which types of incidental findings they wish to receive. The CCMG does not endorse the intentional analysis of disease-associated genes beyond those linked to the primary indication (that

is, secondary findings). Each province might have slightly different regulations and practices, but the emphasis is generally on informed consent with an ‘opt-in’ approach to incidental findings. The intentional search for secondary findings is not recommended, although it is not prohibited as in France.⁸⁰

These differences highlight the diverse approaches to managing incidental and secondary findings in genetic testing, reflecting the legal, ethical and cultural context of each region — an important consideration when interpreting nephrogenomics research. Although the USA and Canada emphasize guidelines from professional organizations, France and several European countries have codified specific requirements into their national law, illustrating a more regulated approach to bioethics in genetic testing. These variations underscore the ongoing ethical debate and the differing national priorities in the management of genetic information, reflecting broader healthcare policies and cultural values regarding patient autonomy and public health.

[H2] Genetic testing landscape in the Global South and lack of representation in large databases

Large genomic databases such as the Genome Aggregation Database (GnomAD) or the Regeneron genomic database (RGD) have a crucial role in advancing our understanding of genetic architecture and the impact of genetic variations on patient phenotypes, given that allele frequency is one of the parameters used in determining variant classification.⁴⁴ These databases are built on the principle that they should ideally be devoid of rare diseases and rare disease-causing variants. By comparing the genotype of a patient with those in genomic databases, clinicians and researchers can assess whether an uncommon variant is likely to be pathogenic. However, a considerable imbalance exists in the representation of different populations within these databases, with most of the data coming from individuals of European ancestry.⁸¹⁻⁸³ This imbalance is important because substantial genotype differences in allele frequency exist across geographical populations,⁸⁴ which can be taken into account during analysis by calculating maximum population frequency and combined frequency. Having a genomic database with >1 million alleles from individuals without rare diseases can limit false positive findings if sample diversity is sufficient, and samples from minoritized groups of individuals are included, although the risk of assigning pathogenic or likely pathogenic status owing to an underestimated variant frequency in the control

database remains.^{85–87} Moreover, using the allele frequency filter during molecular analysis, in regard of available data on current incidence or frequency of the kidney disease in the general population, can mitigate the risk of falsely assign pathogenic variants to patients with rare alleles not represented in common databases.^{41,88}

The Human Hereditary and Health in Africa (H3Africa) program aims to address current disparities in genomic database representation by promoting the development of specific infrastructure and advancing intra-continental genomic research across Africa.⁸⁹ This initiative is vital for improving our understanding of genome specificity and the distribution of rare alleles among individuals from the 28 participating African countries underrepresented in the reference genome, especially as African populations have the highest levels of human genetic variation.^{90,27,91,92} In particular, alleles from East African populations are underrepresented in variant databases, which can lead to important knowledge gaps. For example, the presence of homozygous *APOL1* G1 or G2 risk alleles, which are most frequent among individuals of East African ancestry, is associated with accelerated progression of chronic kidney disease, with an odds ratio greater than 5 for CKD progression to kidney failure, especially in individuals with HIV-associated nephropathy.^{93–95}

Beyond this specific example, rare variants are now more comprehensively cataloged than they were 10 years ago, thanks to global efforts to sequence minoritized populations. Currently, ~20% of patients in genomic databases are of African ancestry. This increased representation enables the calculation of population maximum allele frequencies, which helps to refine the list of potential causal variants for rare diseases and reduces the likelihood of false positive diagnoses in people of African ancestry.

[H1] Reverse phenotyping and clinical–biological discussion of variants

The traditional ‘phenotype-first’ approach, guided by clinical features, can lead to biases and underdiagnosis. By contrast, WES and WGS can expand the scope of variant detection, requiring clinical–biological correlation. Reverse phenotyping involves re-examining the clinical presentation of the patient based on molecular findings. This approach might require specialized genomic expertise and can be resource-intensive, requiring detailed medical record reviews and additional tests compared with the ‘phenotype-first’ approach.^{60,69}

Reverse phenotyping is particularly valuable when a pathogenic variant is linked to an atypical phenotype or when a highly suspicious variant has not been documented in databases. For example, a LOF variant in a gene not associated with a known disease in the Online Mendelian Inheritance in Man (OMIM) database (see below) might require further investigation. Evaluating different kidney phenotypes requires a thorough review of clinical history and a comprehensive examination of the patient to gather data on metrics such as proteinuria type, urinary sediment activity and kidney morphology, which can be time-consuming and resource intensive. Certain key assessments, such as glomerular basement membrane width might not be routinely included in standard medical records, further complicating this process. Phenotype descriptions are often imprecise but can be substantially improved and guided by genetic sequencing. Deep phenotyping, which refines clinical observations based on genetic insights, is essential for improving often imprecise phenotype descriptions and represents the future of precision medicine, helping to connect disease subtypes with specific genetic variations.⁹⁶

A genomics-driven phenotypic approach might clarify how individual genomic differences influence the associated phenotypic spectrum. For now, clinicians must use the available tools to formulate accurate diagnostic hypotheses, stratifying patients according to shared biological mechanisms of disease (**FIG. 4**).

Furthermore, phenotype characterization can vary considerably among clinicians owing to differences in education and clinical experience. Clinical phenotyping involves translating disease symptoms into standardized concepts within a disease ontology. Some disease ontologies are highly detailed, including more concepts than necessary to phenotype a typical patient. Three major phenotype repositories are freely available online: the Human Phenotype Ontology⁹⁷ (HPO) which includes 10,000 disease phenotype profiles for rare and common diseases; OMIM, which covers 9,500 primarily heritable diseases⁹⁸ and Orphanet, containing genotypic and phenotypic data on 9,346 rare diseases.⁹⁹

Reverse phenotyping relies heavily on the specificity of gene-related impairments. The phenotype encompasses measurable elements such as clinical observations (including signs, symptoms, mode of onset, course of disease progression and response to treatment), biomarkers or radiological findings. Although combinations of these elements might be specific to certain gene-associated Mendelian diseases, the absence of some of these elements does not lead to the elimination of a

candidate variant (**FIG. 4**). Histopathology can be suggestive of collagenopathies with basement membrane abnormalities despite the findings not being specifically indicative of Alport syndrome. Moreover, certain genetic diseases, including those with syndromic or pathognomonic features, might be non-penetrant in the patient or family, or might only manifest later in life. As a growing number of genetic kidney diseases fall into this category, reverse phenotyping should always be part of the diagnostic process.^{100,101} Close collaboration between geneticists, nephrologists and nephropathologists is essential to avoid misdiagnoses.

Thus, after sequencing results are obtained, the granularity of the phenotype of the patient is retrospectively enhanced using the co-interpretation model.^{3-5,102} Clinical cases that undergo genomic testing are typically reviewed in regular co-interpretation meetings between nephrologists and geneticists, enabling the discussion of suspicious variants using existing literature and genetic databases. Following this discussion, the nephrologist might recommend additional clinical exams, specialist consultations, imaging and enzyme assays to detect any overlooked signs or symptoms related to syndromic genetic disorders. In parallel, the nephropathologist determines whether and how to re-evaluate the kidney biopsy tissue, looking for specific signs of the suspected genetic nephropathy.¹⁰³ For example, a patient labeled with indeterminate nephropathy (**FIG. 3**) might undergo specific electron microscopy analysis of kidney biopsy tissue to investigate potential glomerular basement membrane abnormalities. Such a detailed examination might help to confirm the pathogenicity of a variant or classify it as likely pathogenic. This reverse phenotyping approach directs further diagnostic explorations based on genetic findings.

The *de novo* nature of a variant in the context of a dominant pathology strengthens its pathogenicity but is not part of reverse phenotyping. Diagnoses misattributed to another pathology can occur. In one example, a patient receiving dialysis, with a family history of steroid-resistant IgA nephropathy, was found to carry a *TRPC6* pathological variant, rather than having IgA nephropathy (**FIG. 2**).

In the case of a pathogenic variant, reverse phenotyping primarily focuses on extra-renal impairments that might have been missed owing to asymptomatic presentation or **variable expressivity [G]** of a gene. *PAX2* represents a classic example, whereby LOF in this gene results in a renal-coloboma phenotype.¹⁰⁴ However, ocular anomalies might be limited to asymptomatic

papillary abnormalities in some patients, requiring specific ocular reverse phenotyping for detection. Specialists performing these examinations must be knowledgeable about the gene-related pathology to ensure accurate detection and interpretation of subtle manifestations.

Beyond the presence of a *de novo* variant that can be confirmed in the index case, several possible explanations exist for a lack of disease symptoms. For example, the variants might be non-pathogenic at the clinical level, have reduced **penetrance [G]** or variable expressivity, or late-onset of disease might account for the lack of genotype–phenotype associations. Incomplete family or medical history might preclude the clinical diagnosis of a genetic disease. Finally, co-expression of unidentified protective alleles¹⁰⁵ might mask the effect of the pathogenic variant.⁴¹

With deep phenotyping, heterozygous carriers of variants in genes classically associated with autosomal recessive diseases might be found to have detectable phenotypic changes not initially suspected. Typically, these carriers are assumed to be healthy and without symptoms related to the recessive disease, although they might present subtle or milder symptoms, such as those observed in individuals with heterozygous *IFT140* frameshift variants.¹⁰⁶ Such investigations can also reveal a dominant transmission of genes associated with different clinical features than those that follow a recessive pattern of inheritance.

[H1] Integrating rapid genomic technologies into clinical practice

Molecular diagnoses are becoming increasingly rapid and could thus be potentially incorporated into the initial management of rare diseases and genetic nephropathies.^{11,12,107} Preparation, sequencing and analysis times have become progressively shorter, counted in hours, thanks to technological advances such as third-generation sequencing (Oxford Nanopore) that can avoid delays caused by the need to batch samples.^{107,108} Thus far, in the field of nephrogenomics, nanopore sequencing has been successfully implemented in the context of TMA, achieving a molecular diagnosis in less than 3 days, with potential major impact on patient therapeutic management (that is, initiation of anti-C5 therapy).^{11,12,12} Rapid genomic sequencing (RPS) could avoid the need for a kidney biopsy by providing early molecular diagnosis, potentially guiding treatment without the need for histological analysis. Although the impact of this approach in nephropathies other than TMAs has not yet been extensively studied, early molecular diagnosis might reduce diagnostic delays and avoid unnecessary procedures with potential overall cost

reduction of 20%.^{12,109} RPS enabled large cost reductions in a US paediatric healthcare system.^{108,110} In children, the clinical utility of genetic diagnosis during admission to intensive care units (ICUs) was reported to be inversely proportional to the time to result, despite the ideal cost-to-speed ratio still needing to be clarified.^{111,112} Further studies are needed in nephrogenomics, especially in the management of adults with non-TMA related CKD.

[H1] Health economics studies and the cost of genetic testing for CKD

Advances in genetic testing have revealed that monogenic kidney diseases in adults are often underdiagnosed. A substantial proportion of cases of ‘nephropathy of unknown origin’ has a genetic cause. Moreover, certain nonspecific patterns (such as focal segmental glomerulosclerosis (FSGS) lesions or nephroangiosclerosis on kidney biopsy tissue) can lead to the mislabeling of nephropathies, which might also have a genetic cause in some patients.^{1,3,4,4,5,36,102}

Recognizing genetic nephropathies and making accurate diagnoses is crucial, with considerable implications for healthcare costs.^{3,9} In certain cases, kidney biopsy and its associated complications could be avoided (**FIG. 5**). Accurate diagnoses also enable tailored treatment, avoiding the unnecessary use of immunosuppressive therapy and its associated complications (for example, individuals with *COL4A*-related variants do not benefit from treatment with corticosteroids or rituximab; discontinuation of eculizumab might also be warranted in cases of TMA without an identified complement variant). Additionally, a genetic diagnosis might prompt the initiation of specific treatments (for example, hydroxycobalamin for cobalamin C deficiency or enzyme replacement therapy for Fabry disease). In 20% of cases, a genetic diagnosis led to adjustments in the treatment plan.⁶⁵ Finally, cascade screening through genetic testing can identify at-risk relatives early, facilitating nephroprotection and the management of disease progression risk factors to delay or prevent the need for kidney replacement therapy.⁹

The cost of genetic sequencing has decreased dramatically in the past 25 years.¹¹³ However, genetic tests are often still perceived as rare and costly resources. The cost of a genetic test varies depending on the type of test (panel, exome or genome sequencing). For example, as of 2025, prices from a recognized private US-based company for panel sequencing, which are representative of typical costs in high-income countries, range from USD 990 (Alport panel, 4 genes) to USD1,790 (comprehensive inherited kidney disease panel, 329 genes), whereas exome sequencing costs USD1,890.¹¹⁴ Of note, the cost of genetic testing is often lower than of the overall

cost of a kidney biopsy¹¹⁵, which is not only a financially costly procedure but also carries a 5% risk of major bleeding complications.¹¹⁶ However, discussions about the cost-effectiveness of kidney biopsies are relatively scarce. Moreover, these numbers must be considered within the global cost of chronic kidney disease, which can reach nearly USD60,000 per year for patients requiring haemodialysis.¹¹⁷

Cost-effectiveness studies are scarce, and economic evaluation studies from a wide variety of jurisdictions and health systems are needed, along with long-term global evaluations of cost-effectiveness. A 2021 Australian study evaluated the cost-effectiveness and optimal timing of WES in adults and children with suspected monogenic glomerular diseases compared with non-genomic investigations (biochemical, imaging and/or kidney biopsy tests).¹¹⁸ In children, early WES was cost-saving, providing an incremental cost saving of USD2,120 per additional diagnosis compared with non-genomic investigations. Conversely, in adults, early WES resulted in an incremental cost of USD3,585 per additional diagnosis. This study focused on the cost to achieve a diagnosis but did not consider the repercussions of this diagnosis and the potential savings (for example, guiding therapy, avoiding side-effects of unnecessary treatments and nephroprotection of relatives diagnosed through cascade screening).

A reevaluation of this cohort in 2023, using a lifetime horizon, showed that genomic testing was associated with an additional cost of USD290 and a gain of 0.04 quality-adjusted life-years (QALYs) per patient, translating to an incremental cost-effectiveness ratio of USD7,100 per QALY. According to this report, WES is likely to be cost-effective, with a 75% probability of cost-effectiveness across the whole cohort, particularly in people with glomerular disease.¹¹⁹

Accordingly, a modeled cost analysis in a population selected through a diagnostic workflow for genetic kidney disorders indicated that early genetic testing reduced costs by 20% per patient, whereas a real cost analysis of 66 patients demonstrated a 41% actual cost reduction.¹⁰⁹

The classical approach to genetic testing has traditionally involved phenotype-driven gene panels. This 'phenotype-to-genotype' strategy often requires a thorough identification of renal and extrarenal manifestations, histopathological evaluation and multiple biochemical tests before genetic tests can be ordered. The success of phenotype-driven genetic panel testing thus relies heavily on the clinical hypothesis of the nephrologist, which can introduce indication bias by favoring patients with well-defined, typical phenotypes and excluding those with less obvious or

variable phenotypic presentations. In adult nephrology, the initial presentation might not align often with the expected clinical features of the genetic disorder.^{1,3,120} Consequently, a negative result from one panel might necessitate additional panels or exome sequencing, increasing both the cost and time to diagnosis.

As of 2024, when a genetic kidney disease is suspected, the strategy is shifting towards broad-spectrum genetic analyses, such as genomic testing or comprehensive nephropathy panels, adopting a ‘one test fits all’ approach, despite WES alone not being diagnostic in some cases. The approach to diagnosis is ‘phenotype-to-genotype-to-phenotype’, with clinical and broad-spectrum genetic evaluations being carried out simultaneously, followed by a clinical re-evaluation considering the genetic sequencing results to confirm or refute the diagnosis suggested by the genetic test. In the Australian cohort previously mentioned, panel testing was costlier and less effective than early WES.¹¹⁸ A 2020 American study also demonstrated that a panel-based approach misses one molecular diagnosis out of four, compared with exome sequencing.¹²¹

Advances in bioinformatics continue to reduce the limitations of WES, enabling the implementation of a ‘one test fits all’ approach. The choice of a genotype-first approach is increasingly supported by the expanding capabilities of WES, which can now detect both SNPs and structural variants within the exons of 22,000 genes.¹²² In addition, mitochondrial variants responsible for nephropathy, such as the *MT-TL1* m.3243A>G variant, which could be responsible for MELAS (mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes) or MIDD (maternally inherited diabetes and deafness) syndromes can also now be detected.^{123,124} This variant is also associated with non-specific glomerular nephropathy, which rarely motivates a specific search for this variant. Specific bioinformatics pipelines also enable the detection of the 27dupC variant in the *MUC1* gene.²⁴

[H1] Conclusion

Genetic testing has emerged as a crucial tool in adult nephrology, transforming understanding and management of CKD. Genomic testing, including WES and WGS, revealed that a substantial proportion of kidney diseases of unknown origin are attributable to Mendelian genetic disorders, challenging traditional diagnostic paradigms based on phenotype-first approaches, especially in adult nephropathies. The integration of genetic data into clinical practice has not only redefined

diagnostic strategies but also optimized treatment approaches and preventive measures, particularly in cases with a genetic aetiology. However, the widespread adoption of these technologies also raises several ethical concerns, particularly regarding the management of VUS and incidental findings, necessitating a multidisciplinary approach, such as a specialized nephrogenomic clinic, to ensure optimal patient care.

Despite the clear advantages of genomic testing, limitations still exist, particularly with SRS technologies, which might fail to detect complex genomic variations such as fusion genes or variants in highly repetitive regions. These challenges highlight the need for ever evolving complementary technologies, such as long-read sequencing, which offers improved accuracy in detecting structural variants, repetitive sequences and mitochondrial genome variants, as well as phasing of variation. Long-read sequencing is particularly promising for its potential to streamline the diagnostic process, although its higher cost and slower adoption rate require further studies to clarify its role in nephrology.

The importance of standardized genetic reporting cannot be overstated, as inconsistencies in variant interpretation and reporting can lead to diagnostic errors and hinder clinical decision-making. The ACMG developed guidelines to address these challenges, promoting standardized practices and ethical considerations in genetic testing in nephrology. However, disparities in the representation of different populations within genomic databases, particularly those of African ancestry, underscore the need for more inclusive research efforts to improve the accuracy and relevance of genetic data across diverse populations and given that information about population maximum allele frequency can help in the analysis of variant pathogenicity.

The ‘one test fits all’ approach, facilitated by advances in bioinformatics, is increasingly becoming the standard in nephrogenomics. This approach, which integrates both clinical and genetic evaluations, promises to enhance diagnostic accuracy and reduce costs associated with traditional, phenotype-driven genetic testing. However, its successful implementation requires extensive education and training for nephrologists, as well as close collaboration with geneticists and genetic counselors to ensure accurate interpretation and application of genetic data.

In conclusion, genetic testing is poised to become a cornerstone of nephrology, offering unparalleled insights into the genetic underpinnings of kidney diseases and paving the way for precision medicine. As genomic technologies become more broadly adopted, addressing the

associated ethical, educational and logistical challenges will be crucial to fully realize the potential of nephrogenomics in improving patient outcomes.

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Author contributions

All authors made substantial contributions to discussions of the content. I.B., A.D., M.D., T.R. and L.M. researched data for the article. I.B., A.D., T.R. and L.M. wrote the article. A.L., T.R. and L.M. reviewed or edited the manuscript before submission.

Competing interests

M.D. is employed by Eurofins Biomnis. The other authors declare no competing interests.

Peer review information

TBC

Supplementary information

Supplementary information is available for this paper at <https://doi.org/10.1038/s41581-XXX-XXXX-X>.

Related links

Clinical Genome resource: <https://clinicalgenome.org/>

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ClinVar: <https://www.ncbi.nlm.nih.gov/clinvar/>

GeneMatcher: <https://genematcher.org/>

Matchmaker Exchange : <https://www.matchmakerexchange.org/>

Key points

- Next-generation sequencing enables rapid, comprehensive and extensive genomic analysis. To improve the detection and interpretation of complex genetic variants, long-read sequencing and collaborative databases are valuable tools in nephrogenomics.
- The differences in genetic reporting standards and management of incidental and secondary findings across different regions reflects a need for more uniform guidelines and ethical frameworks in genetic testing.
- Increasing the representation of minoritized populations in genomic databases is crucial for reducing diagnostic disparities and improving the accuracy of genetic testing worldwide.
- A multidisciplinary approach and reverse phenotyping enhance diagnostic accuracy by re-evaluating clinical presentations in light of genetic findings, facilitating in-depth discussions

between clinicians and geneticists to uncover atypical or overlooked manifestations, and identifying new phenocopies.

- Rapid genomic technologies hold considerable promise in nephrology, with potential major impact on early therapeutic strategies.
- Broad-spectrum genetic testing approaches enhance diagnostic accuracy and cost-effectiveness for chronic kidney disease by reducing diagnostic delays and avoiding unnecessary procedures, compared with traditional phenotype-driven methods.

Table 1. Sequencing technique applications and limitations

Technique	Technological features	Applications	Limitations
Sanger sequencing	Targeted DNA sequencing based on primed synthesis with DNA polymerase	Confirmation test (good accuracy for variants within tested loci) Segregation analysis (no incidental findings)	Restricted region of analysis Intermediate reads (80–1000 bp)
Snapshot PCR	Primer extension-based method for single-base extension and termination	Detection of recurrent pathogenic variant (highly sensitive)	Analysis restricted to a single position Allele amplification dependent on conservation of hybridization region
MPLA	Analysis of PCR products using 5' and 3' MPLA probes	Detection of copy number variant Identification of hybrid genes	Challenging probe design Limited applications
NGS gene panel	Massive parallel sequencing of multiple targeted gene regions	Analysis of genes associated with suspected diagnosis (no incidental findings, accessible clinical interpretation)	Coverage restricted to targeted genes Short read lengths (100–300 bp) Limited yield in patients with atypical or unspecific presentation
NGS exome sequencing	Massive parallel sequencing of exonic regions and surrounding intronic regions of the whole genome	Systematic investigation for patients with unspecific phenotypes Detection of copy number variants possible	Specialized bioinformatics required Challenging clinical interpretation Management of incidental findings required Short-read lengths (100–250 bp) Missing information about intronic and intergenic regions
NGS genome sequencing	Massive parallel sequencing of the coding and non-coding DNA of the whole genome	Systematic investigation for patients with unspecific phenotypes Research applications	Specialized bioinformatics required Highly challenging clinical interpretation Management of incidental findings required Short read lengths (100–250 bp) Low sequencing depth Massive data storage required
Third-generation sequencing	Direct interrogation of single molecules with no prior PCR amplification (either targeted gene panels, exome sequencing or genome sequencing)	Analysis of targeted regions, whole exome or genome (region enrichment using adaptive sampling) Analysis of highly repeated regions and some hybrid genes (long reads: 5,000–100,000 bp)	Short insertion-deletion detection still have to be confirmed by another method Complex and specialized bioinformatics (novel method)

		Ultrafast genomics in research settings (simplified workflow)	Massive data storage required
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MPLA, multiplex ligation-dependent probe amplification; NGS, next-generation sequencing; SNV, single-nucleotide variants.

Table 2. Pitfalls associated with short-read sequencing in nephrology

SRS pitfalls	Description	Examples	References
Complex gene structures	SRS struggles to detect complex gene structures such as fusion genes, especially in regions with high sequence similarity or gene duplications.	<i>CFH</i> hybrid genes in aHUS	16,125,126
Segmental duplications	SRS might miss gene duplications or deletions in regions with segmental duplications depending on the reference genome used, leading to incomplete genomic coverage (variant non-calling)	Loss of <i>CFHR3</i> and <i>CFHR1</i> (relevant in aHUS) in new reference genomes (for example, T2T)	125,126
Highly repetitive regions	SRS is limited in resolving highly repetitive sequences owing to alignment challenges, potentially missing important variants.	<i>MUC1</i> in autosomal dominant tubule-interstitial kidney disease (27dupC variant)	17,19,20,23–25
Fusion genes	SRS often fails to detect fusion genes, which can be crucial for the diagnosis of certain genetic disorders.	<i>CFH</i> hybrid genes, which represent <5% of deleterious molecular events in severe complement-mediated TMA	16
Mitochondrial genome detection by exome sequencing	Probes used for short-read targeted or exome sequencing might dismiss extra-nuclear mitochondrial genome	m.3243A>G variant in <i>MTTL1</i> associated with MELAS and MIDD syndromes	123
Alternative detection methods required	Given the limitations of SRS, alternative methods such as MLPA or long-read sequencing are recommended for accurate detection.	MLPA for <i>CFH</i> hybrid genes; long-read sequencing for <i>MUC1</i> VNTR region	16,24,25

SRS: short read sequencing; aHUS: atypical hemolytic and uremic syndrome; TMA: thrombotic microangiopathy; MELAS: mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; MIDD: maternally inherited diabetes and deafness; MLPA: multiplex ligation-dependent probe amplification; T2T, Telomere-to-telomere consortium; VNTR: variable number tandem repeats. The table summarizes pitfalls associated with SRS in key genetic driver of kidney disease, highlighting the importance of using complementary methods for accurate genetic analysis when SRS is used.

Figure 1: Overview of ACMG classification and impact of VUS management for clinicians

According to the initial American College of Medical Genetics and Genomics (ACMG) ranking, genetic variations can lead to reports identifying causal variants (Class 4/5), corresponding to a probability of association with the kidney phenotype >90%. For variants with a probability <90% (ranging from variants of unknown significance (VUS) to neutral/benign variants), reclassification can occur through post-hoc reanalysis. This reanalysis might consider additional family co-segregation data, reappraisal of previously unnoticed clinical arguments (that is, reverse phenotyping), additional functional studies, or reevaluation based on novel genetic variation data. Reporting practices for VUS vary across laboratories and countries, ranging from providing a list of VUS to omitting this information in the medical report. Consequently, VUS classification or reclassification can differ across laboratories despite efforts toward standardization. The [ClinGen](#) initiative aims to standardize variant interpretation according to disease groups and gene–phenotype associations.

Figure 2: Benefits of genetic diagnosis in adults with kidney dysfunction of unknown cause

Overview of deep phenotyping in clinical case of nephrotic-range proteinuria. A 33-year-old patient with isolated microhematuria and non-significant kidney biopsy tissue findings 8 years earlier had been lost to follow up until an incidental discovery of nephrotic-range proteinuria exceeding 4 g/day during an allergy workup. A subsequent kidney biopsy revealed focal and segmental glomerulosclerosis. Genetic consultation indicated isolated glomerular disease of unknown origin, with no family history or extrarenal involvement. Genetic testing identified two hypomorphic variants, Leu1474Pro and Gly631Val in *COL4A3* gene, in trans configuration, with class III and IV American College of Medical Genetics and Genomics (ACMG)-Association for Molecular Pathology (AMP) classification, respectively. Further genetic-based histological analysis using electron microscopy confirmed abnormalities consistent with Alport syndrome. ACMG-AMP reclassification of these variants updated the Leu1474Pro to Class IV, leading to a recessive Alport syndrome diagnosis. This case underscores the importance of genetic counseling, with a very limited risk of disease transmission dependent on partner genotype, and suggests the potential for inclusion in trials for specific treatment targeting Alport syndrome and deep screening for asymptomatic extrarenal involvement.

Figure 3: Multiple benefits of genetic analysis in families with a history of kidney disease

Overview of a case of misattributed nephropathy. Illustration of the variable penetrance and expressivity within a family affected by glomerular kidney disease attributed to IgA nephropathy. Dynamic nephrology consultation, prompted by the discovery of IgA nephropathy in the son of the index case, facilitated the diagnosis through exome analysis. This analysis revealed a known pathogenic heterozygous *TRPC6* variant, which segregated perfectly with the kidney disease within the family. The impact of the genetic diagnosis on histological reanalysis and patient management highlights the importance of genetic analysis in deepening the understanding of the phenotype in patients and families affected by kidney disease.

Figure 4: Illustration of locus heterogeneity and variable phenotypes

- A. Phenotype specificity is generally inversely proportional to the expression heterogeneity associated with a variant. Thus, we can contrast highly penetrant variants with a highly specific phenotype, such as those in *PKD1* associated with autosomal dominant polycystic kidney disease, with *TRPC6* variants associated with podocytopathies.
- B. In cases of complete penetrance, all individuals carrying the pathogenic variant develop clinical features, whereas some variant carriers remain asymptomatic in cases of incomplete penetrance. Expressivity is uniform when a phenotype is consistent across affected individuals. By contrast, variable expressivity results in clinical manifestations that differ in severity of organ involvement despite an identical genotype.

Figure 5: Reverse phenotyping in nephrogenomics

- (A) Description of arguments that support post sequencing data. Integration of genomic information and phenotypical data post sequencing induce post sequencing reverse phenotyping now driven by genetic variation found post sequencing. Different outcome are described ranging from confirmation of genetically related kidney disease, uncertain diagnosis remains that might spurred additional follow up and surveillance, or at the far end negative diagnosis without impact of genetic testing
- (B) Overview of reverse phenotyping tests that can be selected according to the identified variation and the clinical context. Given a specific variation, additional exams, beyond a kidney biopsy, could be performed. Some, at the forefront of reverse phenotyping capabilities. are not accessible in some clinical practices This might explain that in some institution, depending on tools available, diagnostics capability following and genomic test might vary for the same genetic variation. Exchanging detailed phenotype across the globe will fostered incrementally VUS interpretation, ranking them in actionable categories (e.g likely pathogenetic or pathogenic, see figure 1)

Glossary terms

Phenocopies: Clinical presentations that resemble the phenotype of a genetic disorder but are caused by a different genetic or non-genetic factor. Phenocopies can complicate diagnosis and genetic interpretation in clinical practice.

Variants of uncertain significance: (VUS) Genetic variant that lacks clear categorization of pathogenicity, typically with a probability of pathogenicity between 10% and 90%. Its association with disease risk or phenotype remains unclear due to insufficient or conflicting evidence. VUS requires further research, functional studies, or clinical data to determine its relevance. (See example in Figure 2A)

Multiplex ligation-dependent probe amplification: (MLPA) Molecular technique used to detect copy number variations (CNVs) in specific genomic regions. MLPA can identify deletions, duplications, and other alterations in multiple genes simultaneously by using probes that hybridize to target DNA sequences.

Telomere-to-telomere: (T2T) Term used to describe the complete sequencing of a chromosome from one telomere (chromosome end) to the other, without any gaps. T2T sequencing represents the highest level of genome assembly, providing a fully contiguous and uninterrupted sequence, which is critical for understanding genomic regions previously inaccessible, such as centromeres and repetitive elements.

Human pangenome reference: project funded by the US National Human Genome Research Institute to sequence and assemble genomes from 47 genetically diverse individuals with phased and diploid assemblies.

GC-rich regions: DNA sequences with a high G and C content commonly found near promoters and associated with CpG islands. GC-rich regions are more difficult to sequence because the hydrogen bonds are stronger, and these regions tend to create secondary structures (for example, zinc fingers) that can affect polymerases during sequencing.

Variable expressivity: Reflects differences in phenotypic characteristics, including variability in the severity and range of symptoms, influenced by factors such as genetic background, environment, and epigenetic modifications, between individuals with the same pathogenic variant.

Penetrance: Proportion of individuals with a specific genetic variant who exhibit the associated phenotype. Complete penetrance occurs when all individuals with the variant show the phenotype, whereas incomplete penetrance refers to situations where some individuals with the variant do not express or have lower expression of the associated traits.