Clinical Genetic Screening in Adult Patients with Kidney Disease

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Abstract
Expanded accessibility of genetic sequencing technologies, such as chromosomal microarray and massively parallel sequencing approaches, is changing the management of hereditary kidney diseases. Genetic causes account for a substantial proportion of pediatric kidney disease cases, and with increased utilization of diagnostic genetic testing in nephrology, they are now also detected at appreciable frequencies in adult populations. Establishing a molecular diagnosis can have many potential benefits for patient care, such as guiding treatment, familial testing, and providing deeper insights on the molecular pathogenesis of kidney diseases. Today, with wider clinical use of genetic testing as part of the diagnostic evaluation, nephrologists have the challenging task of selecting the most suitable genetic test for each patient, and then applying the results into the appropriate clinical contexts. This review is intended to familiarize nephrologists with the various technical, logistical, and ethical considerations accompanying the increasing utilization of genetic testing in nephrology care.

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Introduction
Kidney disease is associated with significant morbidity and mortality and affects over 20 million patients in the United States (1). A family history of nephropathy is reported in approximately 30% of cases, revealing the strong role of genetics in kidney disease (2–4). Genetic testing is increasingly used in clinical nephrology due to expanded utilization and accessibility of genetic sequencing technologies (5,6). As a result, Mendelian forms of kidney disease are increasingly detected in adult and pediatric patients. In fact, although historically more clinically apparent in pediatric populations, it is now clear that genetic forms of kidney disease are also highly prevalent in adults, with some studies reporting a Mendelian cause of kidney disease in up to 37% of adult cases (7,8). Establishing a genetic diagnosis has significant implications for nephrology care because it may inform prognosis (9–11) and selection of therapy (12,13), spare patients from undergoing invasive diagnostic procedures such as a kidney biopsy (14,15), and guide family planning (2).

Many genetic testing modalities are currently available (e.g., targeted sequencing, microarrays, gene panels, genome-wide approaches, etc.), and selection of the most appropriate diagnostic sequencing approach is made on the basis of various factors. These include diagnostic yield of the different sequencing modalities, the patients’ clinical picture, their preferences for the types of results that may emerge with broader sequencing approaches, out-of-pocket costs, and third-party payer coverage. This review is intended to familiarize clinical nephrologists with concepts relating to clinical genetic testing.

Mendelian Nephropathies, Genetic Testing Modalities, and Diagnostic Yields
The human genome is divided into protein-coding (approximately 1%, known as the “Exome,” composed of approximately 408,659 exons) and noncoding sequences (approximately 99%) (16,17). Overall, it harbors approximately 20,000 genes, of which approximately 4100 are currently associated with Mendelian disorders (18). Human genomes differ greatly between individuals, and variations in genetic sequence are summarized in Figure 1 and include:

- Single nucleotide variants (SNVs): substitution of a single base.
- Small insertion-deletion (INDEL): insertion or deletion of approximately 2–1000 bases.
- Copy number variation: duplication/deletion that affects ≥1 kb in one or more loci. Copy number variants encompass 5%–10% of the human genome (19,20). The differentiation between small INDELS and copy number variants is specifically on the basis of the length of the affected DNA, reflecting a higher number of genes possibly involved in the latter.
- Chromosomal imbalance and rearrangements: deletions and duplications of entire chromosomes or segments of chromosomes. Inversions and translocations can also occur as a result of genome breakage followed by a rejoining of the broken ends in a different order than the original one.

These variations can potentially lead to a Mendelian disease. A recent survey identified 625 Mendelian disorders associated with kidney and urological traits (21), whereas the number of gene–disease associations continues to grow with the expanded use of massively parallel sequencing, a technological advancement that...
has increased the throughput of genomic sequencing (21). Importantly, diagnostic yield varies according to the categories of variants and choice of test. In Figure 1 and Table 1, we summarize technical and clinical aspects of different sequencing approaches, along with their respective benefits and drawbacks. Next, we discuss the major genomic diagnostic modalities.

**Targeted Dideoxy Terminator (Sanger) Sequencing**

This test aims to identify SNVs and INDELs in a specific gene or gene region (22). The execution is simple and results are highly reliable (error rate: 0.001%–1%) (23). Moreover, this method achieves a long reading length (approximately 800 bp) resulting in significant advantages for de novo variants confirmation and sequencing of...
Table 1. Genetic testing options

<table>
<thead>
<tr>
<th>Analysis Modality</th>
<th>Primary Scope</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Uses</th>
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<tr>
<td>Sanger sequencing</td>
<td>Identification of small variants (SNVs/INDELs) in a specific DNA region</td>
<td>Simple technical execution</td>
<td>Limited resolution (&lt;1 kb) unsuitable for large structural variants</td>
<td>Confirmation of a specific suspected mutation in a gene</td>
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<td>High analytical accuracy (error rate 0.001%–1%)</td>
<td>Time- and cost-inefficient for analysis of large DNA segments</td>
<td>Confirmation of MPS-identified variants</td>
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<td>Fast and simple interpretation</td>
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<td>Analysis of regions refractory to MPS analysis, such as repetitive regions</td>
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<td>No risk of secondary results</td>
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<td>Long reading lengths (approximately 800 bp)</td>
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<tr>
<td>Chromosomal microarrays</td>
<td>Identification of small chromosomal rearrangements/CNVs (≥200–400 kb)</td>
<td>Higher resolution than standard karyotyping (50–100 kb)</td>
<td>Cannot detect small mutations/CNVs</td>
<td>Patients with phenotype strongly suggestive of large rearrangements, such as multiple congenital anomalies and developmental diseases</td>
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<td>Genome-wide analysis</td>
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<td>Targeted MPS panels</td>
<td>Identification of small variants (SNVs/INDELs) within genes of interest for the clinical phenotype</td>
<td>Analysis of all genes possibly related to specific phenotypes</td>
<td>Limited ability to detect balanced chromosomal rearrangements, low-grade somatic mosaicism, and CNVs in pseudogenes and repetitive regions</td>
<td>Patients with phenotypes pointing to specific disorders and with low genetic heterogeneity</td>
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<td>Diagnostic yield up to 50% (depending on the patient phenotype and genes selection method)</td>
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<td>Restricted number of genes that minimizes risk of secondary findings and reduces analysis time</td>
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<td>Exome sequencing</td>
<td>Identification of small variants (SNVs/INDELs) within coding regions of the genome</td>
<td>Analysis of all coding regions in the genome</td>
<td>Coverage per base is generally lower than with targeted panels</td>
<td>Patients undiagnosed with more specific methodologies</td>
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<td>Unbiased approach increases diagnostic sensitivity</td>
<td>Challenging and time-consuming interpretation (high number of candidate variants)</td>
<td>Screening of patients with undefined phenotype</td>
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<td>Cover almost all sites related to Mendelian diseases (approximately 85%)</td>
<td>Potential for detection of secondary findings</td>
<td>Patients with heterogeneous/unspecific phenotype</td>
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<tr>
<td>Genome sequencing</td>
<td>Identification of small variants (SNVs/INDELs) within coding and noncoding regions of the genome</td>
<td>Identification of deep splicing and intrinsic variants unidentifiable with other techniques</td>
<td>Limited coverage in repetitive regions</td>
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<td>Better analytic performance than exome sequencing</td>
<td>Limited reliability for INDELs</td>
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<td>Efficient CNV identification</td>
<td>Maximizes results interpretation difficulty and time</td>
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<td>Extremely useful for reanalysis</td>
<td>Maximizes potential detection of secondary findings</td>
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SNV, single nucleotide variant; INDEL, insertion/deletion; MPS, massively parallel sequencing; CNV, copy number variation.
repetitive regions (24). *De novo* variants are those that are absent in parents and found only in the proband (*i.e.*, the individual undergoing genetic sequencing) as a result of a mutation in a parental germ cell. Repetitive regions are patterns of DNA fragments that occur in multiple copies, and can represent up to two thirds of the human genome (25). Sanger sequencing is generally used as a first-line test when there is strong clinical suspicion of a mutation in a specific gene, for screening at-risk family members for a known mutation, or as a confirmatory test for variants identified by massively parallel sequencing. It is also used for sequencing of specific genes or regions that are not attainable with massively parallel sequencing approaches (*e.g.*, guanine-cytosine-rich, highly repetitive segments, etc.).

**Comparative Genomic Hybridization/Chromosomal Microarray Analysis**

This represents an important improvement over classical karyotyping. Due to its high resolution (50–100 kb), this technique is able to identify smaller rearrangements than standard karyotyping (limited to a resolution of 1–2 Mb) in both coding and noncoding regions. Thus, this is the test of choice when a structural variant is suspected, such as in individuals with developmental disorders of the kidney. In fact, recent studies have detected a high frequency of copy number variation in children and young adults with congenital urinary tract malformations (26), implicating over 45 different genomic disorders, with six loci accounting for 65% of cases with pathogenic copy number variations (1q21, 4p16.1-p16.3, 16p11.2) (27,28). Overall diagnostic yield in children with congenital anomalies of kidney and urinary tract ranges between 10% and 17% (29,30) in nonsyndromic and syndromic cases (31,32). The drawbacks of chromosomal microarray include its limitations in detecting SNVs and INDELs, balanced chromosomal rearrangements, and deletion/duplications of <50,000 bp.

**Massively Parallel Sequencing**

This technique, which is sometimes called next-generation sequencing, was developed in the last decade. It allows for simultaneous sequencing of millions of DNA fragments, at a relatively low cost. It is best for identifying small variants, although large copy number variants can also be detected. Compared with Sanger sequencing, this is characterized by lower precision (error rate approximately 0.5%–2%) and shorter read length (generally 100–250 bp) (33). Possible applications differ in the size of genome sequenced, from specific regions to the entire genome. These include the approaches discussed below.

**Targeted Gene Panels.** If there is a limited number of genes that can cause a specific phenotype, a gene panel can be a cost-effective first-line test. This technique targets coding regions in a selected set of genes and is the best modality for conditions with a clearly distinct phenotype. Gene lists for different kidney phenotypes have been published (3,34,35). This approach leads to a diagnostic yield that is dependent on disease and patient selection procedures. Recent reports indicate diagnostic yields of 13% in children with congenital anomalies of kidney and urinary tract patients (3), approximately 20% in adult and children nephrotic syndrome cases (34), and up to 62% in glomerular disease and 78% in cystic diseases, in both adult and pediatric cases (35). Due to its targeted approach, gene panels may also simplify the interpretation of the results. In addition, panels are usually optimized for a specific set of genes, minimizing potential false negative findings due to incomplete sequence coverage. However, genes included in a particular panel vary between commercial services, and sometimes these panels fail to account for overlapping phenotypes. For example, nephrotic syndrome panels may not contain some congenital anomalies of kidney and urinary tract genes, such as *PAX2*, whose mutation may also manifest with proteinuria (36). The genes included in a specific panel must also be periodically updated as new genes are discovered.

**Exome Sequencing.** This sequencing approach examines nearly all coding regions of the genome (37). Exome sequencing proved its efficacy as a first-line test in both adult and pediatric patients, with a diagnostic yield extremely dependent on patient phenotype, age, and selection criteria. Recent studies demonstrate a diagnostic yield ranging from 11% in adult patients with FSGS (38), to 24% in primarily adult patients with congenital or cystic kidney disease (2). The yield is superior in pediatric cohorts: 32% in FSGS and 61% in cystic kidney disease (39). In general, the diagnostic yield outperforms targeted panels across different phenotypes in both adults and children (2,39,40). Importantly, exome sequencing allows for future reanalysis as new genes are discovered and analytic algorithms improve, without needing to resample the patient (41). As drawbacks, the coverage per base is generally lower than with targeted panels and can result in suboptimal coverage of some relevant genes (*e.g.*, *PKD1, GREB1L*) (42). Copy number variation detection with exome sequencing is possible but not always optimal, and the large amount of data generated can make interpretation challenging and time consuming (43). Several genomic regions relevant to nephrology are not optimally covered by exome sequencing and constitute blind spots that need to be recognized by clinicians ordering the test, such as duplicated region of *PKD1* or the *MLIC1* variable number tandem repeat (a tandem repeat is a short repetitive nucleotide sequence that is generally difficult to sequence with short read MPS technology) (44). Importantly, a very promising application of exome sequencing is in the evaluation of nephropathies of unknown etiology, where, depending on the populations studied, it can have a diagnostic yield ranging from 17% (2) to 38% (8) in adult patients.

**Genome Sequencing.** This technique has the best analytic performance when compared with the aforementioned approaches, owing to its uniform coverage of the genome. Genome sequencing has superior coverage at a minimum depth of 10 reads compared to exome sequencing, and limits PCR artifacts, guanine-cytosine bias, and a high variance in allele fraction (42,45). It is also more efficient for copy number variant detection, even outperforming microarrays approaches (46). Genome sequencing can characterize noncoding regions, enabling detection of splicing or regulatory variants with large phenotypic effect, although this is currently limited by our incomplete understanding of the function of most noncoding regions (47,48). Thus, genome sequencing represents the most promising future of genetic
testing because of its technical superiority, analytic performance, and mapping capacity, along with its progressively decreasing cost. Nonetheless, it still has blind spots shared with exome sequencing (e.g., \( PKD1 \) and \( MUC1 \)). There is still a limited amount of research on the use of genome sequencing in the context of nephrology, but data support its superiority to traditional techniques in several other contexts (49,50). For example, genome sequencing had a significant improvement in diagnostic yield (124%) when compared with targeted panels as a first-tier genetic test for pediatric disorders (51). Similar to exome sequencing, genome sequencing allows for future reanalysis and technologies application. Its potential drawbacks include the relatively high cost of the test (although this is progressively decreasing) and the possibility of detecting genetic findings not related to the primary indication for testing (i.e., secondary findings).

**Clinical Predictors of Diagnostic Yield**

Several clinical factors influence the diagnostic potential of each test. To date, some studies support the use of exome sequencing as a first-line diagnostic approach for noncystic forms of genetic nephropathies. However, there is a paucity of data comparing the yield of targeted panel and exome/genome sequencing for different kidney phenotypes. In Figure 2, we summarize the clinical predictors of diagnostic yield and highlight optimal sequencing approaches on the basis of the broad kidney disease category. Then, in Figure 3, we compare the diagnostic yields achieved in genetic studies of diverse phenotypes using different sequencing techniques.

The main factors that contribute to variation in the diagnostic yield of the specific sequencing approach are discussed below.

**Clinical Diagnosis**

**Cystic Kidney Disease.** These disorders are predominantly attributable to mutations in \( PKD1/PKD2 \) associated with adult-onset autosomal dominant polycystic kidney disease (the majority of cystic kidney diseases) and mutations in \( PKHD1 \) in pediatric-onset autosomal recessive polycystic kidney disease. This clinical distinction is not absolute: 2% of patients with \( PKD1/PKD2 \) mutations show a pediatric-onset severe phenotype clinically indistinguishable from autosomal recessive polycystic kidney disease (52), and other genes have been implicated as causal for cystic disease in both adults and pediatric patients (such as \( GANAB \) or \( DNAJB11 \) in adults and \( HNF1B \) in both) (53,54). For cystic phenotypes, when there is a strong clinical suspicion of mutation in a specific gene, Sanger sequencing is the test of choice; otherwise, targeted panels are often an appropriate initial test because of the limited numbers of identified genes implicated to date. Furthermore, the assays can be optimized to cover the duplicated region of \( PKD1 \) (45), which are otherwise not well covered by most other sequencing approaches. As such, the reported
diagnostic yield of targeted panels for cystic kidney disease range in adult patients from 24% (2) to 88% (35), and 23%–80% with exome sequencing (2,8,55), based on the clinical characteristics of the cohort.

Congenital Anomalies of Kidney and Urinary Tract and Nephronophthisis. To date, these disorders are the most common cause of kidney disease in children, frequently involving congenital defects in other organ systems (56). With hundreds of genes implicated, they are highly heterogeneous in both phenotype and genotype. In addition, these phenotypes are often caused by structural genomic variants (57), detectable by chromosomal microarray (58), whose diagnostic yield is greatest in patients with kidney parenchymal malformations and those with extrarenal manifestations (27,31,59). The remaining cases are caused by SNVs in a single gene, identifiable with exome sequencing or targeted panels, which can provide a diagnostic yield of around 14% in recent reports (3) and is slightly superior in syndromic cases (25% versus 9% [32], 23% versus 15% [31,60]) in pediatric and young adult cohorts.

FSGS and Steroid-Resistant Nephrotic Syndrome. This is a genetically heterogeneous phenotype with over 30 known causal genes (61), affecting primarily children and young adults (62,63). The diagnostic yield can be as high as 32% (39) in recently analyzed cohorts with exome sequencing, and is higher in pediatric cases than in adult ones. Steroid-resistant nephrotic syndrome reached diagnostic yield of 25%–30% (39,64–66) in pediatric patients versus 12% in adults (67), whereas FSGS proved around 22% in pediatric patients (68) and 11% in adults (38). Importantly, a genetic diagnosis can have strong implications for patient management and should always be considered in early-onset disease, particularly in the presence of a clear family history. For example, most genetic forms of steroid-resistant nephrotic syndrome may not respond to immunosuppression therapy (69). Therefore, a positive finding may spare a patient from unwanted complications from this therapy (70). Similarly, a negative test may also guide management as it may influence clinical decision-making toward a longer and/or more aggressive immunosuppression course, and may provide prognostic insights too, as hereditary forms are associated with lower recurrence rate after transplantation (9). Finally, genetic testing can identify cases that may respond to therapy to glucocorticoids, such as cases owing to PLCE1 (71), or cases that may benefit from other therapies that can be effectively treated with coenzyme Q10 (CoQ10) supplementation, such as steroid-resistant nephrotic syndromes owing to mutations in genes of the CoQ10 biosynthesis pathway (ADCK4, COQ2, COQ6, or PDSS2) (72,73). There are many genetic panels available as a first-line diagnostics, but exome sequencing can also be justifiable as a test of choice because of the rapid pace of discovery of new genes for nephrotic syndrome.

Tubulopathies (e.g., Autosomal Dominant Tubulointerstitial Kidney Disease [ADTKD]). Recent studies show targeted panels to have the highest diagnostic yield in this disease category. In fact, diagnostic yield ranges from 62% (38,74) to 83% (75) in pediatric cohorts and 75% (75) to 100% (8) in adult cohorts. This is probably because of the highly characteristic clinical presentation and subsequent selection of cases. Importantly, recent findings suggest that trio analysis with exome sequencing, whereby both the
proband and their parents are sequenced for comparison, can significantly increase the targeted panels diagnostic performance because the availability of parental data enables segregation analysis and detection of de novo mutations (63). Several genes have been implicated in ADTKD to date, such as UMOD, HNF1B, REN, and MUC1 in autosomal dominant tubulointerstitial kidney disease (76). Importantly, though commercial-targeted panels may include MUC1, the ADTKD is associated with variable-number tandem repeat mutations, which makes massively parallel sequencing approaches challenging because of the low coverage in these genomic regions (77).

Kidney Disease of Unknown Etiology. This is an important clinical category representing approximately 5%–15% of patients with kidney failure (1). If a thorough nephrology workup is nondiagnostic, genetic testing should be considered, particularly in the setting of early age of disease onset and a positive family history. The diagnostic yield in both adults and children ranges from 7% to 40% depending on patient characteristics and clinical history, with over two dozen different disorders described across developmental, glomerular, and tubulointerstitial categories (2,72,75). Given the heterogeneous disorders contributing to this disease category, exome sequencing had superior diagnostic performance as compared with targeted gene panels (2).

Family History and Extrarenal Manifestations

Studies indicate a three- to four-fold greater chance of a positive genetic finding in the setting of a positive family history of kidney disease (2,72). For example, a positive family history finding was much more likely in adults (53%) than in pediatric patients (12%) in a cohort of patients with monogenic nephrotic syndrome (34). It is worth noting that the clinical presentation can be different in different family members with the same mutation. For example, the same mutation in PAX2, responsible for papillorenal syndrome, may present as hearing or visual defect in some family members, but present as kidney diseases in other relatives (78). This variable expression motivates obtaining a thorough family history of disease in patients suspected of genetic disease.

In addition, multiorgan involvement is a compelling clinical finding suggestive of a genetic disorder. In a recent study of exome sequencing in adult nephrology patients, the presence of extrarenal features increased the diagnostic yield up to 69% (8). Although obtaining a detailed family history, or being able to decipher extrarenal features associated with a genetic diagnosis, may seem challenging for nongenetic experts, it is important to appreciate that these are key features suggestive of a genetic diagnosis and must be emphasized in medical education going forward. Moreover, collaborating with genetic counselors and/or clinical geneticists can ensure better collection of pertinent family and clinical data. Furthermore, the introduction of decision-support tools and electronic algorithms in medical health records, able to flag potential Mendelian disorders, will help clinicians in detecting genetic disorders and extrarenal features they might not have recognized (79).

Age of Onset

Mendelian disorders are more common in children (75), and age of onset has been shown to significantly influence the probability that the cause is an underlying genetic diagnosis for certain clinical subtypes. For example, genetic cystic kidney disease is almost three-fold more probable in pediatric cases than in adult ones (80). Hence, early onset of disease should increase suspicion for a genetic disease.

Genetic Data Analysis

Interpretation of genetic test results requires close collaboration between the clinician and the molecular pathologist, akin to the collaboration between the nephrologist and kidney pathologist in the evaluation of a biopsy. The interpretation of sequence data depends on the genomic tests used and requires gene- and variant-level analyses, phenotypic-level comparison, and determination of actionability. It also requires domain-specific expertise about the molecular and clinical features of disease. In an effort to standardize this process, the American College of Medical Genetics and Genomics (ACMG) has established guidelines for interpretation of sequence variants (81). Variants are classified as “pathogenic,” “likely pathogenic,” “variants of unknown significance (VUS),” “likely benign,” or “benign.” These guidelines recommend using terms such as “pathogenic” and “benign” for variants that are almost certainly disease causing or known to be benign, respectively; “likely pathogenic” and “likely benign” is used for variants with >90% certainty of either being disease causing or benign. VUS is used for variants for which there is insufficient evidence to classify in any of the other categories. As a general rule, clinicians should only act on variants classified as “pathogenic” and “likely pathogenic.” These categories of variants are generally very rare in the population (typically with allele frequency <1:10,000), and have robust evidence of disease association on the basis of multiple prior reports, predicted effect on protein function, and potentially in vitro functional studies. Once a “pathogenic” or “likely pathogenic” variant has been identified, a second-level comparison is warranted to determine concordance of the patient’s characteristics with the phenotype reported for the disease. Any discrepancy should be reviewed by both the molecular pathologist and clinician, and prior reports should be reviewed. Importantly, recent data suggest that many VUS will eventually be classified as benign (82–86) and can usually be resolved with subsequent clinical correlations, in discussions between the molecular diagnostician, clinician, and the patient.

Although a number of gene lists for nephropathy traits have been generated (2,3,21,34,35,87), nephrology lacks standardized, well-curated gene and variant lists. This will change as kidney disease is increasingly represented in initiatives such as ClinGen, which aims to define the clinical relevance of genes and variants for clinical and research use (88). The development of expertly-curated gene and variant lists is critical because of the many nuances in sequence interpretation. For example, although gene-disrupting mutations are often pathogenic, some disorders are almost exclusively caused by missense variants affecting specific domains of the protein
(e.g., FSGS owing to mutations in the diaphanous inhibitory domains of INF2 [89], or autosomal dominant tubulo-interstitial kidney disease owing to mutations in exons 3 or 4 of UMOD [90], etc.). Therefore, loss-of-function (e.g., nonsense, frameshift, splice, etc.) and missense variants outside of functional domains, even if exceedingly rare, are unlikely to be causal for these diseases (91). Conversely, a relatively common NPHS2 variant (R229Q) can be disease causing if in trans with specific 3’ NPHS2 mutations that exert a dominant-negative effect on the R229Q protein (92).

Currently, we still have an incomplete catalog of rare variants of the genome and limited understanding of their functional effect, hence many rare variants do not clearly fit into “pathogenic” or “benign” categories and are designated as VUS. A VUS classification frequently arises when a variant is novel and in silico algorithms predict it to be damaging, but there is insufficient data on its in vivo functional consequences and inheritance pattern. Classification as a VUS can also occur when a variant has been previously reported as disease causal, but there is contradictory data about its pathogenicity. This is the case of some population-specific polymorphisms or modestly rare variants that were erroneously thought to be extremely rare and reported as pathogenic (91). With the availability of larger and more diverse reference data sets, many previously reported pathogenic variants are now shown to have an allele frequency that exceeds the disease prevalence and can now be reclassified as benign (82,93). For example, a recent study showed that the top genes potentially contributing to most false positive results for kidney diagnostics were all identified before the availability of large publicly available databases (21). It is expected that the increasing number of genomes sequenced across populations of different ancestries will provide better estimates of variant allele frequencies, reducing the numbers of VUS and false positives. In addition, the availability of saturation mutagenesis of human genes (94) raises the possibility of obtaining functional information about every potential genetic variant in the future (in saturation mutagenesis, a single amino acid can be substituted to any of the other 19 possible substituents, which allows a comprehensive analysis of the function of amino acids at each position in the protein).

With the accelerating pace of new discoveries, an emerging challenge is the continuous review of data. Thus, a reanalysis of exomes may reveal a new diagnostic finding and revise a negative genetic test result. A recent report from the National Institutes of Health–sponsored Centers for Mendelian Genomics indicated that a new genetic discovery is made for every 28 exomes sequenced (95). This rapid pace of genetic discovery necessitates systematic updating of variant databases and gene panels, and provides clinicians and patients with the future prospect of achieving a diagnosis when the initial genetic findings are unrevealing. For example, a recent re-evaluation of exome sequence data from two cohorts initially analyzed in 2012–2013 revealed that the diagnostic yield increased from 25% to 37% over a 5-year period, attributable mainly to newly discovered disease-causal genes (96). There are additional strategies for addressing unsolved cases, as shown by the Undiagnosed Disease Network of the National Institutes of Health (97):

transcriptome analysis (also called RNA sequencing, which uses massive parallel sequencing approaches to reveal the sequence and quantity of RNA in a biologic sample) can reveal the possible effect of noncoding DNA sequence variants on splicing or allele-specific expression; or proteomic analysis can detect translation defects or point to metabolic defects. Although these approaches create opportunities for improving clinical care, they are resource intensive for the diagnostic laboratories and clinicians. Furthermore, sequence interpretation can be a burdensome process, particularly when each step is performed manually (98). Hence, semiautomated algorithms can rapidly characterize variant allele frequencies, identify previously reported pathogenic variants, incorporate results of in silico prediction tools, and expedite some of the more labor-intensive steps. There are also currently no guidelines and standards for reanalysis of genetic test results, and this challenge will need to be addressed in the next few years.

Return of Genetic Results and Clinical Implementation

The diagnosis of a Mendelian disorder, whether in the context of a diagnostic finding explicative of the patient’s kidney disease or a medically actionable secondary finding, has the potential to meaningfully inform nephrology care (see Box 1; 99–101). The clinical value of actionable genetic findings underscores the importance of returning genetic findings to patients. Today, most experts agree that clinically valid, medically actionable genetic findings should be returned to adult patients who opted to receive them at the time of consent (102), including for individuals who underwent genetic sequencing through their participation in research (103). Importantly, however, genetic testing results can affect insurability and confidentiality, which many patients and providers may not fully realize. Although legislation exists through the 2009 Genetic Information Nondiscrimination Act to protect employers and health insurance carriers from discriminating against individuals on the basis of their genetic results (104,105), it is not fully comprehensive. Furthermore, clinicians should also be aware of the potential psychosocial implications of a genetic diagnosis for a patient, which may arise from multiple factors, including marginalization and stigmatization from members of their family and community. Therefore, it is essential that all patients thoroughly understand the implication of genetic testing and that genetic counseling services are available for them and their families.

On the clinical side, implementing genetic results into clinical care is an important and challenging task that can lead to effective targeted treatment; for example, patients with steroid-resistant nephrotic syndrome owing to mutations in genes of the CoQ10 biosynthesis pathway can be effectively treated with CoQ10 supplementation (72), and RNA interference therapies have been developed for primary hyperoxaluria type 1 caused by mutations in AGXT (105,106). In the same manner, secondary results affect patient health and need to be reported. The role of clinical nephrologists in this regard is generally limited to identifying and activating the right specialist for patient screening/follow-up. Moreover, ACMG guidelines
currently limit the list to 59 actionable genes in which mutations strongly affect patient prognosis and life expectancy (104), but larger sets are used in different laboratories (107). These challenging tasks require support to ensure the nephrologists’ correct use of diagnostic genetic testing. This will also require collaborations with genetic counselors and clinical geneticists, development of clinical decision-support tools in electronic health records that address physician knowledge gaps in genomics, and the availability of remote consultation via telemedicine for providers with limited access to genetic services.

Genetic Testing and Clinical Trials

Genetics can affect the development and design of clinical trials in multiple ways. First, discoveries in human genetics can uncover new targets for therapy. In fact, recent analyses suggest that therapies targeting pathways discovered through human genetic studies have a greater chance of successful validation in clinical trials (108,109). A well known success story involves the identification of PCSK9 mutations in rare forms of hyperlipidemia, which led to the development of treatment for common forms of this trait (110,111), and promising results are emerging in nephrology, such as chaperone-based therapies in Fabry disease (112). The incorporation of genetic analysis in clinical trials can also identify a subset of patients who may preferentially benefit from therapy, such as the discovery of clinical responsiveness to a tyrosine kinase inhibitor in patients with nonsmall cell lung cancer with specific somatic mutations in the EGFR gene (113). Similarly, JAK1/JAK2 mutations in solid tumors can predict response to anti-PD1/PDL1 therapies (114). It is thus not surprising that different initiatives have been taken in this sense; for example, the Precision Medicine Initiative is an investment of $70 million to the National Cancer Institute to “scale up efforts to identify genomic drivers in cancer and apply that knowledge in the development of more effective approaches to cancer treatment” (115). In addition, several trials are now being developed to target specific molecular defects rather than the clinical category of disease, aiming to treat cancer according to underlying genomic alterations, regardless of clinical cancer type (116). Even if the trial is not targeting specific mutations, genetic testing may enhance the study power by identifying subsets of patients who would likely not respond to the investigation therapy. For example, exclusion of patients with mutations affecting kidney structure or function (such as COL4A5 or NPHS2) may improve the power of studies targeting immunologic pathways for the treatment of steroid-resistant nephrotic syndrome and also minimize exposure to side effects in patients unlikely to benefit from such therapy. Moreover, identification of patients with genetic predisposition to outcomes such as cancer or cardiomyopathy may also help with adjudication of adverse events in clinical trials. Finally, pharmacogenomic analyses can help identify variants that affect drug absorption or metabolism, as exemplified by variants in CYP2C9/VKORC1 for warfarin toxicity (117) or TPMT for azathioprine toxicity, enabling better assessment of dosage, safety, and side effects.

Conclusions

Achieving a precise diagnosis is a fundamental goal of medical practice. Although historically focused on pediatric populations, genetic testing has emerged as a powerful diagnostic tool in adult nephrology, with important implications for diagnosis and management. As genetic testing is increasingly incorporated into clinical practice, many challenges are yet to be addressed, such as optimizing the accuracy of variant interpretation, deploying genetic testing affordable on a large scale, and improving physician and patient education. More research is also needed to investigate the long-term effect of establishing a molecular diagnosis on health care utilization and outcomes, to facilitate third-party payer coverage for diagnostic testing. In this regard, there are emerging data on the cost benefit of genetic testing in fields such as diabetes or cancer (118–120), and similar research would benefit the nephrology field. With the rapid pace of discovery, we also anticipate that the nephrology field will increasingly deploy clinical trials stratified on specific molecular defects rather than the clinical category of disease, increasing the power and safety profile of studies.

Box 1. | Establishing a genetic diagnosis can support personalized nephrology care: A case study.

Case Study 1

A male patient with Focal Segmental Glomerulosclerosis on kidney biopsy is found to have a pathogenic large truncating variant in the COL4A5 gene, diagnostic for Alport syndrome. In addition to establishing a molecular diagnosis for Alport syndrome, the type of variant may provide insights into the patient’s clinical course, wherein Loss-of-Function variants, such as the one in this example, are associated with early onset of kidney failure, hearing loss and ocular abnormalities (116) and large deletions in the COL4A5 gene are associated with increased risk for Anti-glomerular basement membrane disease post-transplantation (117). In addition, the genetic diagnosis may inform allograft donor selection among at-risk family members, as mothers of males with X-linked Alport syndrome are typically discouraged from serving as donors (118). Furthermore, conservative management of the patient’s proteinuria with an agent that inhibits the Renin-Angiotensin-Aldosterone-system (RAAS) (e.g., ACE-Inhibitor or ARB) is recommended (3). Finally, a genetic diagnosis can also help identify disease-specific support groups, which can provide psychosocial support for affected individuals and their families, guide patients to clinical drug trials, registries, and other relevant resources.
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Supplemental Material. References for Figure 3 studies.

References
6. Phillips KA, Deverka PA, Hooker GW, Douglas MP: Genetic test availability and spending: Where are we now? Where are we going? Health Aff (Millwood) 37: 710–716, 2018


