Review Article

Kidney360

A Practical Guide to Genetic Testing for Kidney Disorders of Unknown Etiology

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Abstract

Genetic testing is increasingly used in the workup and diagnosis of kidney disease and kidney-related disorders of undetermined cause. Out-of-pocket costs for clinical genetic testing have become affordable, and logistical hurdles overcome. The interest in genetic testing may stem from the need to make or confirm a diagnosis, guide management, or the patient's desire to have a more informed explanation or prognosis. This poses a challenge for providers who do not have formal training in the selection, interpretation, and limitations of genetic tests. In this manuscript, we provide detailed discussion of relevant cases in which clinical genetic testing using a kidney gene panel was applied. The cases demonstrate identification of pathogenic variants for monogenic diseases—contrasting them from genetic risk alleles—and bring up diagnostic limitations and diagnostic utility of these tests in nephrology. This review aims to guide clinicians in formulating pretest conversations with their patients, interpreting genetic variant nomenclature, and considering follow-up investigations. Although providers are gaining experience, there is still risk of testing causing more anxiety than benefit. However, with provider education and support, clinical genetic testing applied to otherwise unexplained kidney-related disorders will increasingly serve as a valuable diagnostic tool with the potential to reshape how we consider and treat many kidney-related diagnoses.

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Introduction

Despite standard workup, some patients with kidney disorders may not have a clear diagnosis. Depending on the year, 0.3%–4% of incident ESKD patients have "unknown" listed as their underlying etiology (1), with 20%–27% of patients with CKD/ESKD reporting a positive family history (2–5). Depending on the patient cohort, 10%–65% of patients with a family history may have a genetic cause identified (6–8), whereas up to up to 24% of a more general CKD cohort may have a genetic cause identified (9–11). Implementation of genetic testing has been fruitful, demonstrating notable yield in patients with nephrotic syndrome (12,13), focal segmental glomerulonephritis (14,15), nephrolithiasis (16,17), congenital anomalies of the kidney and urinary tract (18), and cystic kidney disease (19). Genetic sequencing of panels of hundreds of kidney-related genes are now available from multiple commercial or institutional genetic testing services for less than $500 out-of-pocket cost, with sample collection and professional genetic counseling services included. This is affordable to many patients and thus can be more readily offered in a variety of clinical settings (20). Groopman *et al.* (21) suggest that genetic findings can positively impact clinical care by making a diagnosis or further characterizing an existing clinical diagnosis, and guiding subspecialty referrals. They show that in some—particularly pediatric—cases, a genetic diagnosis may have an important impact on treatment decisions. A genetic diagnosis can also affect screening of living related donors for transplantation (22,23). For some patients, a significant benefit may come simply from providing an explanation for their condition.

Many nephrology providers do not feel comfortable counseling, ordering, or interpretating genetic testing (24). Although a one-time patient referral to a center with genetic kidney disease expertise to make a diagnosis is an option, we expect that the increasing role of genetics in nephrology will push most clinicians to want to over time develop a comfort level with this themselves. This review provides a practical framework of important concepts and nomenclature to utilize in genetic testing of patients with kidney disorders of unclear etiology. For excellent reviews of genetic diagnoses found most often, patient characteristics that predict highest likelihood of genetic diagnosis, and the types of genetic testing useful in kidney disease, we direct the reader to additional references (9,25–27). In this review, we detail the basics of genetic testing in clinical care.

Pretest Preparation and Counseling

Ordering clinicians should provide basic pretest communication and—to the extent of their comfort level—counseling, recognizing that patient education by a certified genetics counselor will be necessary in some circumstances (28). In preparation for pretest discussion, the ordering clinician should know (*1*) the

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type of test ordered (2), where to find the list of genes tested and available counseling services, and (3) whether the test will return findings regarding "actionable genes" such as cancer risk or cardiovascular disease that may prompt further testing in addition to genes relevant to kidney-related disease (29). For many testing services, the clinician will be asked to decide in advance whether he or she wishes for variants of unknown significance (VUS) to be included in the results report or withheld. This is of relevance for certain kidney phenotypes and is discussed below.

With the above in mind, we recommend the following be conveyed to the patient as part of pretest counseling (30,31):

- The clinician's expectation of what may or may not be found, and how that would affect management. In other words, define a gestalt pretest probability of making a possible or definitive genetic diagnosis. Also, convey whether cancer risk might be found and reported or will not be considered as part of the test.
- Genetic testing may not always provide a definitive result. Sometimes, the results are inconclusive due to either a VUS or the lack of a variant that could be due to the sequencing modality missing it. Genetic testing of additional family members may be a helpful aid in the interpretation of a variant.
- A positive test result may have implications for the health of other family members. See discussion of "Is the variant(s) present in all affected family members?" and case 4 below.
- A federal law, Genetic Information Nondiscrimination Act of 2008, prohibits health insurance and most employment discrimination based on genetic data, but it does not protect against denial of coverage for other types of coverage such as life and disability insurance (32).

We will discuss the nomenclature used to describe variants (cases 1 and 2) and how the pretest probability of finding a variant will aid in variant interpretation (case 2). Case 3 illustrates interpreting a risk allele in contrast to a pathogenic variant for a monogenic disease. Case 4 illustrates a genetic finding that could have been missed by targeted next-generation sequencing (NGS) and prompts our outlining clinical scenarios where alternative or follow-up genetic testing many be necessary to confirm or detect a relevant variant. Overall, these cases highlight real-life findings and a patient-centered approach. We continue to refine our experience in genetic testing and reach out to certified genetics counselors or genetic specialists to help interpret and manage results when needed.

The Type of Genetic Test

Given the low cost, high sensitivity, and widespread availability of NGS to sequence multiple genes in parallel (also called massively parallel sequencing), this will be the first choice for most situations (21). A kidney gene panel refers to the assessment of multiple genes sequenced by NGS-based methods. Some testing services tailor the list of genes to the specified phenotype (*i.e.*, nephrotic syndrome) (12,33), whereas others have a fixed panel evaluated for any kidney-related indication (8). These lists are often quite thorough; however, we encourage providers to review the panel to ensure the genes of interest are being evaluated. There are many commercially available genetic testing products, and many institutions may also have clinical genetic testing available.

For a NGS panel, DNA obtained from cells in a patient's blood sample, cheek swab, or saliva is fragmented and barcoded for sequencing. Unless performing the more complete and costly whole genome sequencing, the next step is to capture and amplify the DNA fragments containing exons—the approximately 1% of the genome that encodes proteins. If the exons of all genes are collected and sequenced, this is known as whole exome sequencing (WES), whereas if the exons of only a subset of genes are

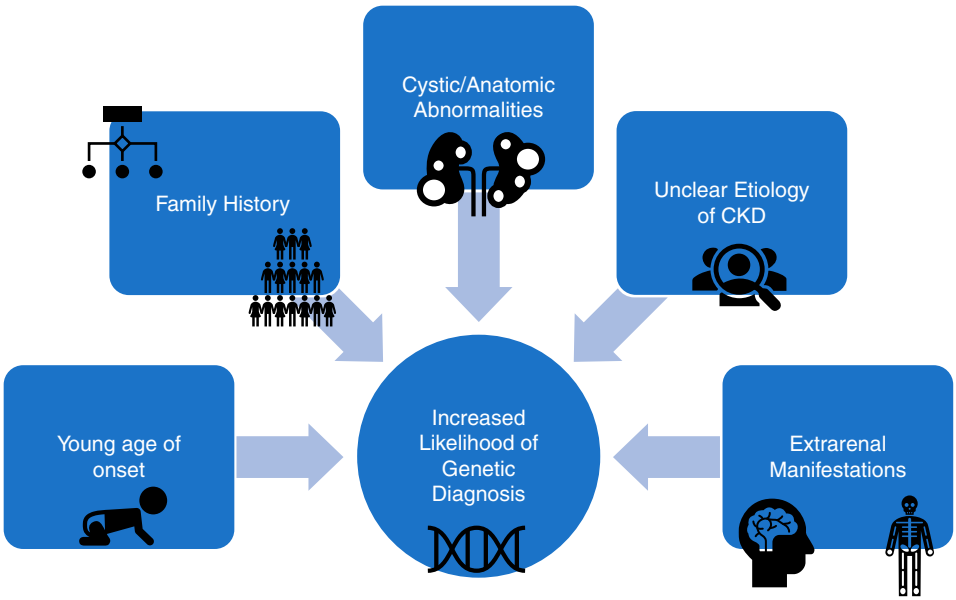

Figure 1. | **Patient factors that correlate with an increased likelihood of a genetic diagnosis.**

used, this is known as "targeted" NGS (20,21,34,35). Either way, analysis is limited to the genes listed in the panel. At present, there are more than 3900 genes associated with monogenic disorders (36), with more than 625 of these associated with kidney or urologic disease (37).

How to Know What to Expect

Certain clinical presentations convey a near certainty that a causative genetic variant exists, whereas in others, genetic testing is being used as one of many tools in the workup. In the latter case, a conclusive positive finding is meaningful, but an inconclusive or negative finding may not be. Figure 1 illustrates factors that correlate with increased likelihood of a genetic diagnosis (6,25). Having an estimated gestalt pretest probability of the likelihood of a positive result is useful both for setting patient expectations and preparing to interpret the genetic test results.

Case 1

A 20-year-old male with normal renal function has a renal ultrasound as part of an initial evaluation for hypertension. Multiple large cysts are noted in each kidney, resulting in mild symmetric enlargement. A follow-up abdominal magnetic resonance imaging scan confirms the kidney cysts and notes absence of liver cysts. He has no known family history of autosomal dominant polycystic kidney disease (ADPKD), kidney failure, or kidney transplant.

Genetic testing is pursued because his cyst number and hypertension at this age point to a genetic cause, and diagnosis and prognosis are in question due to lack of family history.

His genetic testing reveals a heterozygous variant in the *ALG8* gene and no other pathogenic or likely pathogenic variant in a panel of genes that includes *PKD1*, *PKD2*, *PRKCSH*, *SEC63*, *GANAB*, *ALG8*, *ALG9*, *PKHD1*, *SEC61B*, *DNAJB11*, *IFT140*, *UMOD*, *REN*, *HNF1B* (38,39) (Figure 2).

Genetic Variant Nomenclature

The Human Genome Variation Society establishes the standards for annotation of genetic variants (40,41). Figure 3

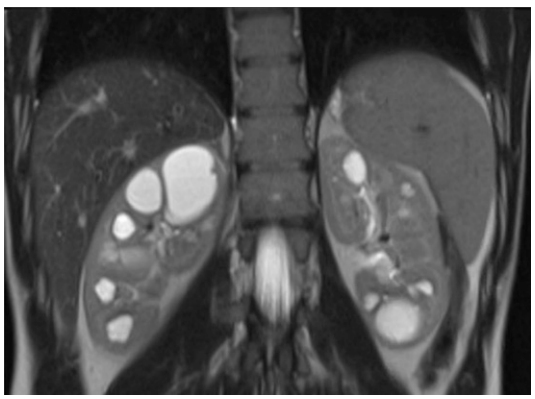

Figure 2. | **Abdominal MRI of patient described in Case 1.**

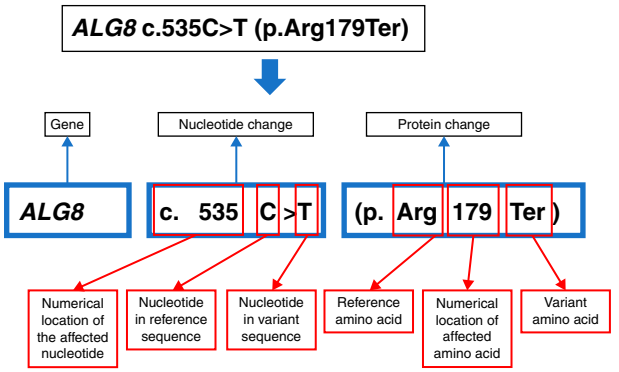

Figure 3. | **Illustration of variant nomenclature for Case 1.** Human gene names are capitalized and italicized.

provides a guide to this interpretation. A variant is annotated with reference to the gene and the numerical position(s) in that gene's coding DNA sequence and protein (chain of amino acids [AA]) sequence. The numbering for the coding DNA starts with nucleotides 1–3, which encode AA number 1, and continues for the length of the protein. Because some genes may have more than one way of splicing its exons together ("alternative transcripts"), which would affect the numbering for the coding DNA and protein sequence, a transcript name such as "NM_024079" may also be provided. Each of the 64 possible three-nucleotide combinations encodes 1 of 20 different AAs or a stop codon. AAs are abbreviated as three letters or a single letter (*e.g.*, "Arg" or "R" for arginine), and the stop codon is abbreviated as "Ter" for termination or "X." Table 1 illustrates the potential consequences that may result from the substitution, insertion, or deletion of one or a small number of nucleotides.

Genetic Variant Interpretation

Identified variants affecting a gene of interest are compared with large databases to determine whether they are common or rare in the general population and ethnic subgroups, and whether they have previously been associated with disease. The largest resource for assessing a variant's frequency in the general population and ethnic subgroups is known as the Genome Aggregation Database (gnomAD) (42). GnomAD provides data from WES and whole genome sequencing for more than 120,000 individuals (more than 240,000 alleles). A minor allele frequency of >1% is considered common. Although a patient's gene sequence may differ from the reference human genome with common variants, a clinical genetics report will only list variants that are sufficiently rare to be of possible relevance to the rare monogenic disease phenotype in question or that are specific established risk alleles. The American College of Medical Genetics and Genomics and the Association for Molecular Pathology provide criteria for the characterization of a variants as "pathogenic," "likely pathogenic," "uncertain significance," "likely benign," and "benign," with "likely" being used when there is a >90% certainty that the variant may be either pathogenic or benign (40).

Table 1. Types of variants resulting from single or small oligonucleotide variants

Variant Type	Definition	Nucleotide Change	Coding DNA Nomenclature "c. prefix"	Protein Nomenclature "p. prefix"	Comments	Implication
Substitution (synonymous)	Single nucleotide substitution resulting in unchanged AA	ATG AGA (ref) c. 1 2 3 4 5 6 Met Arg p. 1 2 ATG AGG (var)	c.6**A>G**	p.Arg2= or p.Arg2Arg	• 6th nucleotide adenine changed to guanine • Variant occurs at 2nd codon; both AGA and AGG encode arginine to the protein sequence is not affected	No change in AA; likely benign unless near splice junction
Substitution (missense)	Single nucleotide substitution changing AA	ATG AGA ↓ ATG ATA	c.5**G>T**	p.Arg2Ile	• 5th nucleotide guanine changed to thymine • 2nd codon encoded arginine, but variant encodes isoleucine (Ile)	AA changed; potentially pathogenic if significant change in important AA
Substitution (nonsense)	Single nucleotide substitution creating a stop codon	ATG AGA ↓ ATG TGA	c.4**A>T**	p.Arg2**Ter** or p.Arg2**X**	• 4th nucleotide adenine changed to thymine • 2nd codon encoded arginine, but variant encodes a premature stop codon	Truncated protein; pathogenic in majority of disease mechanisms
Insertion/deletion (frame shift)	Insertion or deletion of # nucleotides (# ≠ multiple of 3)	ATG AGA CAG T Met Arg Gln ↓ ATG GAC AGT Met Asp Ser	c.4**delA**	p.Arg2**fs**	• 4th nucleotide adenine is deleted • A resultant shift in the reading frame no longer encodes the intended protein and by chance will reach a stop codon	Truncated/altered protein; all AAs distal to frame shift are changed; pathogenic in majority of disease mechanisms
Insertion/deletion (nonframeshift)	Insertion or deletion of # nucleotides (# = multiple of 3)	ATG AGA CAG Met Arg Gln ↓ ATG CAG Met Gln	c.4_6delAGA	p.Arg2del	• 4th through 6th nucleotides are deleted, causing loss of the Arg but no frameshift	AA deleted; potentially pathogenic if critical AA
Substitution (splice variant)	Single nucleotide substitution	AAGgtaatt... Lys intron ↓ AAGttaatt... Lys intron	c.21 + 1G>T		• 1st intronic nucleotide (guanine) is changed to a thymine; no protein consequence is defined given intronic variant	Truncated protein expected; splice site abolished if first or second intronic base is modified; pathogenic in majority of disease mechanisms

AA, amino acid.

Truncating versus Nontruncating Variants

An important distinction in variant interpretation is the difference between a variant that results in a shortened ("truncated") protein versus a variant that still encodes the full-length protein but with a focal modification. The "truncating variant" can be expected to encode a nonfunctional protein fragment that is typically degraded. Examples of truncating variants are (*1*) nonsense variant (premature termination ["stop"] codon), (*2*) frameshift "fs" variant (insertion or deletion of nucleotides not a multiple of three), (*3*) splice variant affecting either of the essential two intronic nucleotides adjacent to a splice junction, or (*4*) a large deletion of multiple exons (Table 1). In contrast, a "nontruncating" variant encodes a full-length protein that may be fully functional, fully dysfunctional, or partially functional, or alternatively take on a different function—a so-called gain of function. Examples of nontruncating variants include (*1*) a missense variant (single AA substitution), or (*2*) small nonframeshifting insertion/deletion of three or six or nine *etc*. nucleotides (Table 1).

Truncating variants can typically be characterized as likely pathogenic or pathogenic for disorders caused by loss-of-function mechanism even if not previously reported with disease and without family data. In contrast, nontruncating mutations will need to be previously associated with the disorder, found in multiple affected family members, or assessed by biologic assay if they are to be characterized as more than just a VUS.

The *ALG8* variant in case 1 is a truncating variant, also known as loss of function variant, because the 179th AA, arginine, is replaced by a stop codon. The *ALG8*: c.535C>T: p.Arg179Ter variant has been previously reported (43) and can, in the opinion of the authors, be interpreted as pathogenic. The variant classification of "pathogenic" refers to the effect of the variant on the encoded protein but not necessarily whether a single "pathogenic" heterozygous variant can be expected to cause disease as discussed next.

Inheritance and Zygosity

In autosomal dominant (AD) conditions, a single pathogenic variant on one (maternal or paternal) allele—a "heterozygous" variant—results in disease. In autosomal recessive (AR) conditions, both (maternal and paternal) alleles must have a disease-causing variant (*i.e.*, there are no normal copies of the gene). For recessive genotypes, if the two disease-causing alleles are identical, the variant is referred to as "homozygous," whereas if the two disease-causing alleles are different, the patient's genotype is referred to as "compound heterozygous." Patients with a single pathogenic copy of a gene associated with an AR disease are called carriers. They may have either no expression of disease characteristics or, with some diseases, mild or unique attributes (44,45). X-linked variants are on the X chromosome. Males will have greater disease expression of X-linked traits because they only have one X chromosome and thus no normal copy (46,47). *De novo* mutations spontaneously arose in the gamete or early embryo and are not inherited from either parent.

Considering the Data Linking the Gene to a Phenotype

ALG8 encodes a protein required for the synthesis of sugar molecules known as N-glycans that help protein maturation in the endoplasmic reticulum. *ALG8* is essential for appropriate maturation of the ADPKD protein polycystin-1 (43). *ALG8* is curated in some but not yet all public databases as an official disease gene for autosomal dominant polycystic kidney and liver phenotypes. The authors and others having growing experience with this genetic diagnosis (38,48). Given what is known about this gene, the lack of variants in the disease genes for typical ADPKD in the case 1 patient, and the phenotype that fits reported cases, we were comfortable providing the patient with reassurance that the *ALG8* variant is likely to be the cause of his cystic kidney disease. Based on previously described cases and the mechanism of cyst formation being an incomplete loss of polycystin levels, his course will hopefully be relatively indolent, without renal failure. We expect therefore, based on the non-*PKD1* or -*PKD2* genetic etiology, that he is at low-risk of progression and thus not a candidate for tolvaptan therapy. In general, genotype alone should not be used to determine those at risk for rapid progression because sometimes even patients with truncating *PKD1* mutations may have slowly progressive course (49). For patients with normal renal function and ADPKD, we rely on Mayo imaging classification and/or the finding of average kidney length >16.5 cm in a young patient to determine eligibility for tolvaptan therapy (50).

In other cases, if a gene variant is called pathogenic but the implicated gene does not fit the patient's phenotype, it is always reasonable to consider the level of evidence and experience (*i.e.*, case numbers, pedigrees, biologic mechanism, animal models) that support the implication of a gene with a phenotype because the literature is still a work in progress.

Case 2: A VUS

A 38-year-old male with no past medical history has an elevated creatinine of 1.7 mg/dl. Seven years prior, he had normal renal function. He has no hearing or vision loss and no family history of kidney disease, and he takes no medication. Physical exam and history are unremarkable. Urinalysis consistently demonstrates 2+ blood but no protein. Urine microscopy demonstrates isomorphic red blood cells without casts.

Due to his young age, elevated creatinine, and hematuria of unclear etiology, a renal biopsy is performed. This shows unremarkable light microscopy and immunofluorescence, but diffuse thinning of the glomerular basement membrane on electron microscopy.

This did not explain his renal impairment but suggested thin basement membrane disease which has been associated with mutations in *COL4A3, COL4A4,* or *COL4A5* (51) (Figure 4).

To explore this possibility further and to address patient interest in whether there was a genetic cause, he underwent

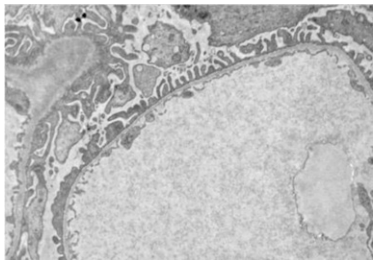

Figure 4. | **Electron microscopy of renal biopsy in Case 2.**

clinical genetic testing. No positive findings were reported; however, his list of VUSs are shown in Table 2.

VUS

VUSs, as the name implies, are of unknown significance. It is typically advised that the clinician will not base clinical decisions on VUSs. Nonetheless, there are characteristics about a case/gene/VUS that can increase the likelihood that a VUS is indeed pathogenic such that the finding may still provide clinically relevant information that can be interpreted with caution. The following considerations are assessed when determining the potential significance of a VUS (52):

Patient/Family/Phenotype Factors

- Does the patient's phenotype fit with reported phenotype for the disease gene/genotype? Keep in mind that heterozygous VUS will typically not be of relevance for recessive disease unless two variants are found in the same gene.
- Is the variant(s) present in all affected family members? This is a very important way to support or exclude the pathogenicity of a variant. Given this, it may be wise to include in pretest counseling that some results may lead to the suggestions that other family members get tested. In general, affected family members will provide more information than unaffected family members—because for some diseases, not all carriers of the pathogenic variant get disease. In contrast, lack of the variant in a family member affected with the same phenotype has the power to rule out that variant as the causative variant in that family.

Gene/Protein/Variant Factors

- Does the variant cause a significant alteration in AA characteristics in an important region of the encoded protein? If the specific AA at that position in the protein is conserved (maintained) through evolution of species, this supports its importance. If the affected AA is in a functional domain of the protein, it is more likely to have a consequential effect. If the AA change results in change of size, polarity, or charge of the AA, it is more likely to have an effect than a more subtle change.
- Is the variant sufficiently rare in the general population? Rarer is more likely pathogenic in rare monogenic disorders. Often a threshold of <1% in the general population is set to define a variant as being "rare." This may be a logical threshold for many AR phenotypes because only when two such rare alleles occur in the same person does the phenotype present. However, for AD diseases, the general population frequency for any one pathogenic variant should be expected to be significantly less than the prevalence of the disease, thus certainly <0.1% but typically much rarer than that. For example, the authors consider any variant with frequency in the general cohort or ethnic subpopulations >0.01% as too common to be pathogenic for ADPKD where the 1:1000 disease prevalence is made up of patients who each have any one of many hundreds to thousands of potential unique rare pathogenic alleles. A variant's frequency in the general population or ethnic subpopulations can be found on the GnomAD resource listed below. Many pathogenic variants will be so rare that they are "novel" or "absent" in GnomAD; the variant was not found in any of the volunteers represented in the GnomAD cohort and thus would not be listed on the site.
- Is the variant near a splice junction and predicted by algorithms to effect splicing efficiency?
- Is the implicated disease known to be caused by very rare deleterious variants (as opposed to specific gain-of-function mutations)? To use the example of ADPKD again, many disease-causing nontruncating alleles are characterized as VUS because they haven't been previously reported in association with disease; thus, their consequence cannot be conclusively interpreted. Nonetheless, in a patient with clinically diagnosed ADPKD, finding a VUS in *PKD1* that is convincing by the parameters described above is often helpful. For this reason, we suggest requesting VUSs to be reported at least when testing for ADPKD variants.

Table 2. Case 2 Variant Report: VUS

Gene	Associated Disease(s)	Inheritance	Variant	Zygosity	Classification
COL4A4	*COL4A4*-related Alport syndrome	AD and AR	c.816G>A (p. Lys272=)	Heterozygous	Unknown significance

Provided Interpretation: This synonymous variant is located near an intron-exon boundary located in exon 13 and is predicted by multiple in silico splice predictor algorithms to reduce the splice site activity significantly. This variant has not been reported as associated with a clinical condition in the Human Gene Mutation Database (HGMD) and is absent from the Broad Institute gnomAD dataset.
VUS, variant of uncertain significance; AD, autosomal dominant; AR, autosomal recessive.

Clinical geneticists have access to algorithms and prediction scoring systems that incorporate some of this assessment and may innumerate relevant data to the variant interpretation in the report. In addition, assessment will consider whether the specific variant has been reported previously for the provided phenotype. If this information is not provided in the genetic testing report, some details can be found on websites such as ClinGen (53), ClinVar (54), gnomAD (42), and other broad or disease-specific publicly available databases.

The VUS listed in case 2 was one of six reported VUSs from a panel testing 385 "kidney genes" in this patient. Although it is reasonable to pass over most VUSs, in cases such as this where the pretest probability for finding a *COL4A3* or *COL4A4* heterozygous variant was considered high, the reported VUS in *COL4A4* can be considered with added interest. *COL4A4* encodes the α4 collagen protein integral to the α3α4α5 helix that forms the adult glomerular basement membrane. Patients with heterozygous mutations negatively impacting *COL4A4* may have AD Alport syndrome or thin basement membrane disease (51,55). Although this VUS is a synonymous variant—no change in the 272nd AA lysine—algorithms identified that this novel (never before reported) variant occurs near an exon-intron boundary and is predicted based on the surrounding sequence to interfere with splicing. Unlike variants in the first or second base of the intron from the exon/intron junction, which will undoubtedly abolish splicing, exonic nucleotides near the splice site may also alter splicing—potentially resulting in exclusion of exon 13 in the case above—but their consequence is less predictable. This case certainly raises the likelihood that indeed this variant has consequence; however, without biologic assessment or more familial data, this suspicion remains unproven.

Case 3: *APOL1* Risk Alleles

A 38-year-old male with hypertension and prediabetes presents to clinic for evaluation of proteinuria. His mother had developed ESKD in her late 30s, but the details of her diagnosis are not well known by the family. His physical exam, aside from mild hypertension, is unremarkable. His laboratory data show a serum creatinine of 1.2 mg/dl, a urine protein-to-creatinine ratio of 0.97 mg/mg Cr, and no other abnormalities. His renal biopsy shows focal segmental glomerulosclerosis (FSGS). Due to his family history and young age of disease onset, the patient underwent genetic testing. There were no variants reported for monogenic causes of FSGS; however, positive variants were found, as detailed in Table 3.

This case highlights the discovery of a risk allele—an allele that on its own is not pathogenic but can increase the risk of developing a disease in the setting of other clinical or environmental "second hit" factors. *APOL1* risk alleles (termed G1 or G2) were discovered among people with recent West African ancestry who had higher rates of ESKD, even after accounting for traditional risk factors. Further studies demonstrated that individuals homozygous for either allele (G1/G1 or G2/G2) or heterozygous for both alleles (G1/G2) as seen in this case are at significant risk for developing hypertensive ESKD, biopsy-proven FSGS, HIV-associated nephropathy, and possibly coronavirus disease 2019–related glomerular disease (56–60). *APOL1* G1 or G2 heterozygosity will be encountered frequently by a clinician performing genetic testing because more than one third of Black individuals carry one high-risk allele—thought to confer no significant added risk—whereas 13% have a high-risk genotype (60,61). The discovery of the high-risk genotype may lead the clinician to consider its associated disease(s) and/or aggressively treat known risk factors that may contribute to the "second hit" such as HIV or other viral infections, hypertension, and obesity.

Use of Genetic Testing in Living Related Kidney Donation

Living kidney donors are at increased risk of developing of CKD/ESKD (62). Although this may be attributed to reduced nephron mass following donation, an alternative explanation could be that some living related donors might carry the genetic cause or genetic risk that already destined them to kidney problems. As such, it may be valuable to attempt to identify a genetic cause of CKD/ESKD in a recipient such that if found, the living related donor can be screened for the same variant (single variant testing would be all that would be necessary). This is particularly of relevance when the donor is younger than the age at which clinical manifestations would become apparent (22,23,63–65).

The presence of risk alleles such as *APOL1* is a different situation because <15% of those with the high risk *APOL1* genotype will develop ESKD (66). Even though studies indeed report an increased risk of ESKD in donors with the *APOL1* high-risk genotype, that is a risk that some living related donors may still wish to take in order to give their loved one a kidney (67). Importantly, attitudes toward genetic testing in potential donors are mixed, with some demonstrating dissatisfaction knowing that genetic testing may preclude them from donating their kidney to a loved one and would be willing to undergo donation despite these potential risks of transplant (68).

Case 4: An Unexpected but Fitting Diagnosis

A 53-year-old female with hypertension and insulin-dependent diabetes mellitus is noted to have chronic

Table 3. Case 3 Variant Report: Two *APOL1* risk alleles

Gene	Condition(s)	Inheritance	Variant(s)	Zygosity	Classification
APOL1	Susceptibility to ESKD and focal segmental glomerulosclerosis	Complex	G1 allele	Heterozygous	Risk allele
APOL1			G2 allele	Heterozygous	Risk allele

Table 4. Case 4 Variant Report: Whole gene deletion

Gene	Associated Disease(s)	Inheritance	Variant	Zygosity	Classification
HNF1B	Renal cysts and diabetes syndrome	AD	Whole gene deletion	Heterozygous	Pathogenic

AD, autosomal dominant.

hypomagnesemia. Multiple family members have been diagnosed with type 2 diabetes and CKD attributed to diabetes. She does not take any diuretics, proton pump inhibitors, or alcohol. During a prior hospitalization, she rarely achieved normal magnesium levels despite significant intravenous and oral magnesium repletion. Renal ultrasound demonstrates symmetric, echogenic kidneys in addition to multiple right upper pole simple cysts measuring up to 1.7 cm in diameter.

Due to severe renal magnesium wasting, renal cysts, CKD, and positive family history for CKD, there was moderate suspicion for a genetic etiology, although it was unclear to what extent diabetes could explain the familial kidney disease.

Serum creatinine: 1.5 mg/dl (gradual upward trend from a creatinine of 1.0 six years ago).

Serum magnesium: 1.4 mg/dl (normal range 1.7–2.4 mg/dl) despite supplementation with magnesium lactate 336 mg twice daily.

Fractional excretion of magnesium: 25% (expected <3% in patients with hypomagnesemia).

Urinalysis is remarkable for 1+ protein, 2+ glucose.

Findings are detailed in Table 4.

Given the whole gene deletion, the patient underwent clinical chromosomal microarray analysis to confirm the variant and define the extent of the deletion. This confirmed the presence of a large (1.4 Mb) heterozygous deletion at 17q12, which includes the disease gene *HNF1B*.

When NGS May Not Be Enough

Large deletions such as seen in this case can be characterized as a type of structural variation. Since the heterozygous deletion results in only one remaining allele, these deletions are also referred to as copy number variation (CNV; *i.e.*, only one copy of the gene is present rather than the usual two copies provided by the maternal and paternal allele). CNVs, broadly defined as deletions or duplications of large segments of the genome, can occur *de novo* or be inherited (69). They are not optimally detected by the short reads of targeted NGS or WES. Although NGS-based sequencing will be sufficient as the genetic testing modality for many patients with kidney disorders and was sufficient to detect the large deletion in this case, Table 5 outlines specific indications where alternative genetic testing modalities are used as the preferred initial test, follow-up

Table 5. Specific indications for alternative sequencing modalities in nephrology

Clinical Scenario	Alternative Testing Options
Large deletions or chromosomal rearrangements suspected (typically pediatric: CAKUT, multi-organ manifestations) or needed as a follow-up assessment to a negative test with suboptimal assessment of copy number variation (*e.g.*, whole gene deletions of *NPHP1*) or to confirm large deletion found by WES or targeted NGS	SNP or chromosomal microarray *Other options:* • Comparative genomic hybridization • Whole genome sequencing • Multiplex ligation probe amplification
ADTKD-*MUC1* suspected (ADTKD phenotype but no mutations found in other ADTKD genes)	Variant-specific testing available without cost from the Broad Institute (contact ableyer@wakehealth.edu)
Single variant testing: An established familial pathogenic variant is known, and patient desires testing only for presence/absence of that variant	Sanger sequencing following PCR amplification of a small genomic region containing the variant location
Gold standard[a] for ADPKD	Sanger sequencing of long-range PCR amplicons designed to amplify only *PKD1*, *PKD2*, but not the duplicated regions (pseudogenes) homologous to *PKD1*
High suspicion for genetic etiology but no variant found	Consider testing for large deletions (above), and/or contacting testing facility to ask about sequencing quality for specific genes of interest

ADTKD, autosomal dominant tubulointerstitial kidney disease; ADPKD, autosomal dominant polycystic kidney disease; CAKUT, congenital anomalies of the kidney and urinary tract; NGS, next-generation sequencing; SNP, single nucleotide polymorphisms; WES, whole exome sequencing.

[a]As NGS-based methodologies improve, using paired-end sequencing, improved capture reagents, with or without preceding long-range PCR, the superiority of this approach is less apparent, and thus NGS-based panels are often used recently as first test for genetic diagnosis in ADPKD.

to a suspected missed variant, or confirmation of a detected variant, as in case 4 (35,70–73).

Case 4 Continued: Reframing the Patient

HNF1B encodes the essential transcription factor hepatocyte nuclear factor 1β, which is expressed in the pancreas, liver, and kidney (74,75). Recessive loss is embryonically lethal, whereas a range of phenotypes and severities—which may be termed "variable expressivity"—can result from heterozygous variants or loss of this gene. In the kidney, some patients with an *HNF1B* mutation manifest with congenital anomalies of the kidney and urinary tract, whereas others have a progressive tubulointerstitial kidney disease often with gout with or without kidney cysts. Other potential manifestations are maturity-onset diabetes of the young (formerly known as "renal cysts and diabetes syndrome"), hypomagnesemia, elevated liver function tests, and hyperparathyroidism out of proportion to CKD (76–79). *HNF1B* gene deletion can be part of a larger chromosome 17q2 deletion syndrome, in which case there may also be developmental, learning, and psychiatric problems (80,81).

In this case, the genetic finding prompted the reframing of the patient's diabetes diagnosis and her CKD. She had undergone a liver biopsy 1 year prior for workup of persistent mild liver function test elevation, which had shown no actionable findings and arguably could have been avoided if the genetic diagnosis was known at the time. Given the AD nature of this disease, the patient was counseled by the ordering provider about potential implications for her family members. Specifically, 50% of her offspring would be expected to inherit this variant, and she may wish to discuss screening with them.

Depending on the clinician's comfort level, such post-test counseling can be performed by the clinician or by a genetics counselor—either *via* the genetic testing service or *via* an independent clinical referral. Although establishing a definitive pathogenic variant can create the opportunity to establish a potentially presymptomatic diagnosis in affected family members, proactively offering this should not be taken lightly. First, in diseases such as this case with a disease gene (*HNF1B*) with variable expressivity, knowing that a family member has the variant does not entirely predict the characteristics or severity of disease to expect. Second, genetic diagnosis in pediatrics is typically discouraged other than for familial disease where pre-emptive therapies are available.

Take-Home Points and Looking Ahead

It is important to note that with increasing use of genetic testing, public databases that document variants and their spectrum of clinical consequences will be updated. With time, this will contribute to more informed interpretations of VUSs discovered in the future (82). A clinician can aid in these efforts by accurately providing the patient phenotype at time of ordering the test—allowing the submitted data (phenotype) to be associated with the variant—and encouraging the sequencing service to submit data to public resources. As resources for variant curation improve and additional disease genes are identified, clinicians may consider contacting the testing company to have a patient's genetic sequencing data reanalyzed. Sequencing services that use WES as opposed to just targeted sequencing of the genes in the panel may be able not only to reinterpret VUSs but also to analyze genes not previously considered.

This review aimed to provide a foundation for nephrology providers to start to develop experience with the affordable tool of clinical genetic testing to aid in the workup of a wide range of unexplained kidney-related abnormalities. Some take home points are the following:

- Ordering a clinical genetic test should be feasible and no longer require extensive logistical coordination by the provider.
- Pretest discussion (counseling) to establish expectations is recommended as outlined above.
- A clinician can choose whether to refer for genetic counseling, and even whether to refer to a center with genetic kidney disease expertise to make a genetic diagnosis, but will want to understand genetic terminology in kidney disease diagnoses.
- Everyone has many genetic variants. Avoid overinterpreting VUS. Many, possibly most, VUS are benign, but if the VUS is in a gene that can cause the phenotype, further consideration may help determine clinical relevance.
- Seek advice from a genetics counselor or a colleague with genetics experience when there is uncertainty about the consequence or relevance of a variant.

When used in the proper circumstances, genetic testing will allow us to enrich our understanding and care of patients with kidney disorders.

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Author Contributions

All authors were responsible for the conceptualization, wrote the original draft of the manuscript, and reviewed and edited the manuscript.

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