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Reclassification of Variants Following Renal Genetics Testing: Uncommon Yet Impactful for Diagnosis and Management

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Introduction: Genetic testing is increasingly utilized in nephrology practice, but limited real-world data exist on variant reclassification following renal genetics testing.

Methods: A cohort of patients at the Cleveland Clinic Renal Genetics Clinic who underwent genetic testing through clinical laboratories was assessed with their clinical and laboratory data analyzed.

Results: Between January 2019 and June 2023, 425 new patients with variable kidney disorders from 413 pedigrees completed genetic testing through 10 clinical laboratories, including 255 (60%) females with median (25th, 75th percentiles) age of 36 (22–54) years. Multigene panel was the most frequently used modality followed by single-gene testing, exome sequencing (ES), chromosomal microarray (CMA), and genome sequencing (GS). At initial report, 52% of patients had ≥1 variants of uncertain significance (VUS) with or without concurrent pathogenic variant(s). Twenty amendments were issued across 19 pedigrees involving 19 variants in 17 genes. The overall variant reclassification rate was 5%, with 63% being upgrades and 32% downgrades. Of the reclassified variants, 79% were initially reported as VUS. The median time-to-amendments from initial reports was 8.4 (4–27) months. Following the variant reclassifications, 60% of the patients received a new diagnosis or a change in diagnosis. Among these, 67% of patients received significant changes in clinical management.

Conclusion: Variant reclassification following genetic testing is infrequent but important for diagnosis and management of patients with suspected genetic kidney disease. The majority of variant reclassifications involve VUS and are upgrades in clinically issued amended reports. Further studies are needed to investigate the predictors of such events.

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Chronic kidney disease is a leading cause of global morbidity and mortality with high economic burden, affecting >10% of the population worldwide.[1–3] Studies have suggested that genetic kidney diseases constitute approximately 10% of all chronic kidney disease cases[4]; although individually, these diseases may be considered rare. In recent years, genetic assessment has played an increasingly important role in the diagnosis and management of chronic kidney disease.[5–8] For example, a cohort study demonstrated that more than two-thirds of patients with positive genetic testing results received a new diagnosis or a change in diagnosis.[5] Over one-third of these patients experienced a substantial alteration in disease management.[5] As genetic testing becomes more accessible to patients with suspected genetic kidney disease, an increasing number of nephrologists are integrating genetic diagnostic tools into their practice.[5,9] However, interpretation of genetic variants identified in these tests, particularly those classified as VUS, remains challenging.[8,9]

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The American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) recommend a 5-tier classification system that classifies the variant as pathogenic, likely pathogenic, a VUS, likely benign, or benign.[10] They have provided 2 sets of criteria: 1 for the classification of pathogenic or likely pathogenic variants and 1 for the classification of benign or likely benign variants.[10] For a given variant, the interpretation is informed by several factors, including variant type, frequency at which the variant is found in the general population, previous observation of the variant in other individuals with similar presentations, the computer-predicted impact of the variant, functional assay studies, and segregation analysis.[10,11] A genetic variant with insufficient or conflicting evidence supporting its involvement in disease, such that it cannot be classified as pathogenic/likely pathogenic, or as benign/likely benign, is a VUS.[11]

A distinctive feature of VUS, in comparison to other forms of ambiguous test results, is that although the results itself may remain static, its significance is often resolved over time as more data become available.[12] For this reason, the ACMG encourages laboratories to consider proactive amendment of variant reports when a variant with a near-definitive classification (pathogenic or benign) must be reclassified.[10] Understanding variant reclassification is important for appropriate management of genetic disorders. Recent studies have examined the rates of gene variant reclassification in epilepsy syndromes,[13] inherited arrhythmia syndromes,[14] and hereditary cancers.[15,16] To our knowledge, no such data have been reported on variants detected in association with genetic kidney diseases.

In this study, we aimed to bridge this knowledge gap by analyzing a cohort of patients who were evaluated for suspected genetic kidney disease and underwent genetic testing across 10 Clinical Laboratory Improvement Amendments-certified laboratories in the United States over a 4.5-year period. We assess the prevalence, genotypic and phenotypic features, and potential predictors of variant reclassification, as well as its impact on diagnosis and management in patients presenting with a wide spectrum of kidney disease phenotypes.

METHODS

Study Cohort

This study was approved by the Cleveland Clinic Foundation institutional review board (IRB 18-705). A cohort of 425 patients who were evaluated at the Cleveland Clinic Renal Genetics Clinic and underwent renal genetic testing between January 2019 and June 2023 consented to this study. Review of medical records was performed independently by 2 researchers (EL and CB). Data collected include demographic and clinical characteristics at the time of index visit, including personal and family history of kidney disease, as well as laboratory data, including genetic testing modalities and results. All initial reports of genetic testing and amended reports, if received, were reviewed. Estimated glomerular filtration rates were calculated using the 2021 Chronic Kidney Disease Epidemiology Collaboration creatinine equation for adult patients and the 2009 creatinine-based "Bedside Schwartz" equation for pediatric patients.

Genetic Testing

Genetic testing was pursued at the discretion of the ordering physician and with the consent of the patient. All participants received pretest counseling, including a review of the Genetic Information Nondiscrimination Act.[17,18] DNA was collected from a buccal swab or blood specimen and analyzed at a Clinical Laboratory Improvement Amendments-certified laboratory. These laboratories were GeneDx (Gaithersburg, MD), PreventionGenetics (Marshfield, WI), Natera (Austin, TX), Blueprint Genetics (Seattle, WA), Invitae (San Francisco, CA), Otolaryngology and Renal Research Laboratories of the University of Iowa (Iowa City, IA), Genetics and Genomics Diagnostic Laboratory at the Cincinnati Children's Hospital Medical Center (Cincinnati, OH), Cleveland Clinic Molecular Genetics Laboratory (Cleveland, OH), Variantyx (Framingham, MA), and Mayo Clinic Molecular Genetics Laboratory (Rochester, MN). Laboratory selection for each patient was determined by available testing options and the patient's insurance coverage.

The genetic testing types ordered include single-gene test, multigene panel, CMA, ES, and GS. The laboratory methods to identify sequence variants included the use of next-generation sequencing, Sanger sequencing, or both. Large rearrangements were detected with next-generation sequencing dosage analysis, microarray-based comparative genomic hybridization, or multiplex ligation-dependent probe amplification analysis.

Variant Classification, Reclassification, and Reporting

Genetic testing results were issued by the testing laboratories. Following the ACMG-AMP Standards and Guidelines, variants were classified as pathogenic, likely pathogenic, of uncertain significance, likely benign, or benign.[10] As described before,[19] initial reports were received by the Renal Genetics Clinic and categorized into 5 groups as follows: (i) positive,

defined as a pathogenic or likely pathogenic variant in an autosomal dominant, mitochondrial, or X-linked disorder or as homozygous or compound heterozygous pathogenic or likely pathogenic variants in an autosomal recessive condition; (ii) carrier, defined as a heterozygous pathogenic or likely pathogenic variant in an autosomal recessive disorder; (iii) 2 *APOL1* kidney disease risk alleles, G1 (rs73885319, p.S342G) and G2 (rs71785313, p.N388_Y389del), in the homozygous or compound heterozygous state[20]; (iv) VUS; and (v) negative, if only benign or likely benign variants were identified. When multiple variants were identified in an individual, the test report interpretation was based on the most clinically severe classification.

Amended reports were issued independently by the testing laboratory upon receipt of additional evidence according to the ACMG-AMP Standards and Guidelines. The testing laboratory sent amended reports to the ordering clinicians at the Cleveland Clinic Renal Genetics Clinic, who then shared them with the affected patients along with appropriate counseling.

Statistical Analysis

Demographic, clinical, and laboratory data were summarized using descriptive statistics. Categorical variables were summarized using frequency with proportion, and continuous variables were summarized using medians with interquartile ranges. Chi-square test, with the Monte Carlo simulation method when needed,[21] was used to compare categorical variables. Unpaired 2-sample Wilcoxon test was used to compare continuous variables. Reported variant classification, reclassification, and amended reports were analyzed. Reclassifications were considered upgrades if the variant was reclassified to a more severe category and downgrades if the variant was reclassified to a less severe category.[15] Time-to-amendment was calculated using the issue dates for the initial and amended reports. Binomial logistic regression model was used to assess characteristics associated with variant reclassification.[22] All statistical analyses were performed using R version 4.3.0 (The R Foundation for Statistical Computing, Vienna, Austria). *P*-values <0.05 were considered significant.

RESULTS

Study Sample

As shown in Table 1, a total of 425 patients from 413 pedigrees were evaluated for suspected genetic kidney disease at the Renal Genetics Clinic of the Cleveland Clinic and completed genetic testing through 10 Clinical Laboratory Improvement Amendments-certified laboratories between January 2019 and June 2023. There were 253 female (59.5%), 170 male (40.0%), and 2 (0.5%) transgender patients in this cohort. The median age (25th, 75th percentiles) at index visit was 36.0 (22.0–54.0) years. At index visit, 39 patients (9.2%) had end-stage kidney disease on dialysis or with history of kidney transplantation. Among the rest, median estimated glomerular filtration rate (interquartile range) for the entire cohort was 80.0 (56.6–110.0) ml/min per 1.73 m^2. Eighty-nine patients (20.9%) had undergone kidney biopsy. Of these, 64.3% had an indication for glomerular disease. Further, 264 patients (62.1%) in the entire cohort reported family history of kidney disease. For 19 patients (4.5%), family history was limited or uncertain due to adoption, estrangement, or other social factors.

Table 1. Characteristics of patients at index visit at the Cleveland Clinic Renal Genetics Clinic who underwent renal genetics testing from January 2019 through June 2023

Characteristics	Total ($N = 425$)
Age, median (25th, 75th percentiles), yr	36.0 (22.0–54.0)
Male, *n* (%)	170 (40.0)
Race, *n* (%)	
Asian	9 (2.1)
Black	53 (12.5)
Hispanic	10 (2.4)
White	323 (76.0)
Other	30 (7.1)
eGFR, ml/min per 1.73 m^2, median (IQR)	88.0 (56.5–110.0)
ESKD, *n* (%)	39 (9.2)
Kidney biopsy, *n* (%)	89 (20.9)
Family history of kidney disease, *n* (%)	264 (62.1)
Phenotype, *n* (%)	
Electrolytes disturbance and or kidney stones/nephrocalcinosis	119 (28.0)
Glomerular disease	117 (27.5)
Cystic kidney disease	79 (18.6)
CAKUT	21 (4.9)
aHUS/TMA	14 (3.3)
Tubulointerstitial disease	5(1.2)

aHUS, atypical hemolytic uremic syndrome; CAKUT, congenital anomalies of kidneys and urinary tract; eGFR, estimated glomerular filtration rate; ESKD, end-stage kidney disease; IQR, interquartile range; TMA, thrombotic microangiopathies.

Electrolyte disturbance and/or kidney stones and nephrocalcinosis (28.0%) were the leading phenotypes, followed by glomerular disease (27.5%), cystic kidney disease (18.6%), congenital anomalies of the kidney and urinary tract (4.9%), atypical hemolytic uremia syndrome and thrombotic microangiopathy (3.3%), tubulointerstitial disease (1.2%), and other conditions (13.4%). Thirteen (3.1%) individuals were asymptomatic but underwent genetic testing due to family history of kidney disease or as part of living donor evaluation.

Genetic Testing Modalities and Initial Variant Reports

As shown in Figure 1a, the most frequently utilized testing modality in this cohort was multigene panel (324 cases, 76.2%), followed by single-gene testing (81

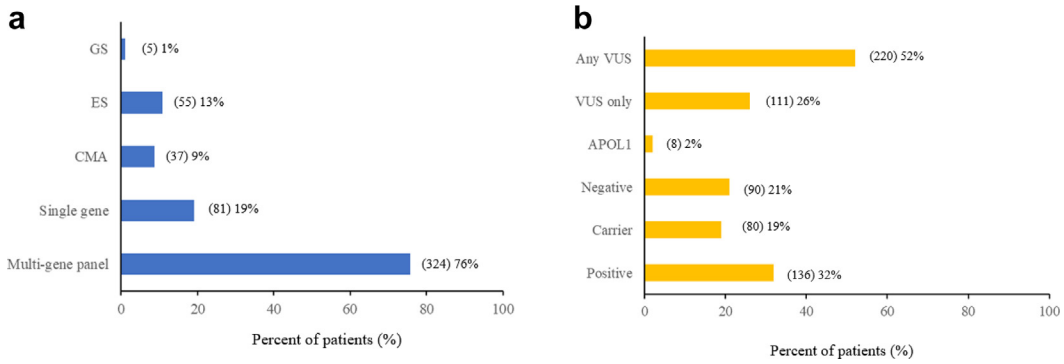

Figure 1. Genetic testing modalities and results at initial reports among 425 patients. (a) Genetic testing modalities. (b) Genetic testing results at initial reports. APOL1, positive for 2 *APOL1* risk alleles; CMA, chromosomal microarray; ES, exome sequencing; GS, genome sequencing; VUS, variant of uncertain significance.Any VUS, presence of at least 1 variant of uncertain significance with or without concurrent pathogenic variant(s); VUS only, only variant of uncertain significance detected without any concurrent pathogenic variant.

cases, 19.1%), ES (55 cases, 12.9%), CMA (37 cases, 8.7%), and GS (5 cases, 1.2%). Multiple testing modalities were applied in 73 (17.2%) patients.

As shown in Figure 1b, among the 425 patients who underwent genetic testing, 136 (32.0%) had positive results with pathogenic or likely pathogenic variants in an autosomal dominant, mitochondrial, or X-linked disorder or with homozygous or compound heterozygous pathogenic or likely pathogenic variants in an autosomal recessive condition; 80 patients (18.8%) were carriers of autosomal recessive disorders with a heterozygous pathogenic or likely pathogenic variant; and 8 patients (1.9%) had the *APOL1* kidney disease risk alleles G1 or G2 in the homozygous or compound heterozygous state (G1/G1, G2/G2, or G1/G2). Two hundred twenty patients (51.8%) had at least 1 VUS identified, with or without concurrent pathogenic variant(s). Of these, 111 patients had VUS(s) as their only findings at the initial reports. Ninety patients (21.2%) had negative results, with only benign or likely benign variants identified.

In the subset of 220 patients with at least 1 VUS identified with or without concurrent pathogenic variant(s), multigene panels (189 cases, 85.9%) were the most frequent modality, followed by ES (23 cases, 10.5%), CMA (3 cases, 1.4%), single-gene testing (2 cases 1.0%), and GS (1 case, 0.5%).

Among those who underwent genetic testing, male patients with kidney disease were more likely to have a VUS identified compared to female patients (58.2% [99/170] vs. 47.4% [120/253], $P = 0.03$). No statistical difference in the identification of VUS was observed between White and non-White patients (50.2% [162/323] vs. 56.9% [58/102], $P = 0.24$).

Variant Reclassification at Amended Reports

As shown in Table 2, in the 4.5-year analysis, a total of 20 amendments across 19 pedigrees involving 19 unique variants in 17 genes were issued by 5 laboratories. Of these, 1 *CASR* variant (c.398A>T; p.Glu133Val) was reclassified when the proband's child tested positive for the same variant in familial variant testing while the proband's report had not been updated. Another *CYP24A1* variant (c.1186C>T; p.Arg396Trp) was reclassified in twin siblings simultaneously. The remaining amendments were issued exclusively to probands. Overall, the reclassification rate among all pedigrees was 4.6% (19/413 pedigrees). A majority of reclassified variants were initially reported as VUS (78.9%, 15/19 pedigrees) representing a VUS-specific reclassification rate of 7.0% (15/213 pedigrees).

As shown in Table 2 and Figure 2a, among the 19 reclassified variants, 63.2% (12/19) were upgrades, 31.6% (6/19) were downgrades, and 5.3% (1/19) were reclassification of inheritance pattern of gene-related condition. As shown in Table 2 and Figure 2b, among the 15 reclassified VUS, 10 (66.7%) were upgrades, and 5 (33.3%) were downgrades. As shown in Table 2 and Figure 3, electrolyte disturbance and/or kidney stones or nephrocalcinosis (35.3%, 6/17) were the leading kidney-related phenotype of genes in which variants were reclassified, including *ALPL*, *ATP7A*, *CASR*, *CYP24A1*, *MOCOS*, and *SLC12A3*; followed by glomerular disease (23.5%, 4/17), including *COL4A3*, *NPHS2*, *NUP107*, and *WT*; cystic kidney disease (17.6%, 3/17), including *IFT140*, *PKD1*, and *PKD2*; and congenital anomalies of kidneys and urinary tract (11.8%, 2/17), including *SALL1* and *WFS1*. One variant in the *AIP* gene and 1 variant in the *SCN1A* gene were incidentally identified by ES, which were related to pituitary adenoma and Dravet syndrome, respectively.

Stratified by testing modality, 65% (13/20) of the amended reports were issued following reanalysis of multigene panel results, followed by ES results

Table 2. List of variants and genes which were reclassified

Gene	OMIM phenotype	Variant	Initial classification	Amended classification
ALPL	Hypophosphatasia	c.318G>C p.Gln106His	VUS	Pathogenic
		c.1375G>A p.Val459Met	Likely pathogenic	Pathogenic
AIP	Pituitary adenoma 1, multiple types	c.799C>T	Likely pathogenic	Risk variant
	Pituitary adenoma predisposition	p.Gln267*		
ATP7A	Menkes disease	c.1019C>T	VUS	Likely benign
	Neuronopathy, distal hereditary motor, X-linked	p.Pro340Leu		
	Occipital horn syndrome			
CASR	Hyperparathyroidism, neonatal	c.398A>T	VUS	Likely pathogenic
	Hypocalcemia, autosomal dominant	p.Glu133Val		
	Hypocalcemia autosomal dominant, with Bartter syndrome			
	Hypocalciuric hypercalcemia, type I			
COL4A3	Alport syndrome, autosomal dominant or autosomal recessive	c.8083G>A p.Gly695Arg	VUS	Pathogenic
CYP24A1[a]	Hypercalcemia, infantile	c.1186C>T p.Arg396Trp	VUS	Pathogenic
IFT140	Retinitis pigmentosa	c.217_218del	Heterozygous pathogenic variant in autosomal recessive condition	Heterozygous pathogenic variant in autosomal dominant condition
	Short-rib thoracic dysplasia 9 with or without polydactyly	p.Arg73Alafs*16		
MOCOS	Xanthinuria, type II	c.300-1G>C	VUS	Likely pathogenic
NPHS2	Nephrotic syndrome	c.686G>A p.Arg229Gln	Benign	VUS
NUP107	Galloway-Mowat syndrome Nephrotic syndrome	c.503G>A p.Ser168Asn	VUS	Likely benign
PKD1	Polycystic kidney disease 1	c.7546C>T p.Arg2516Cys	VUS	Likely pathogenic
		c.7271C>T p.Thr2424Met	VUS	Likely pathogenic
PKD2	Polycystic kidney disease 2	c.1057G>A p.Glu353Lys	VUS	Likely pathogenic
SALL1	Townes-Brocks branchiootorenal-like syndrome	c.478G>A p.Gly160Ser	VUS	Likely benign
SCN1A	Developmental and epileptic encephalopathy 6B, non-Dravet Dravet syndrome	c.1170+4A>G	VUS	Likely pathogenic
SLC12A3	Gitelman syndrome	c.1400C>T p.Ser467Phe	VUS	Likely pathogenic
WFS1	Deafness, autosomal dominant Wolfram syndrome 1 Wolfram-like syndrome, autosomal dominant	c.683G>A p.Arg228His	VUS	Likely benign
WT1	Denys-Drash syndrome Frasier syndrome Meacham syndrome Mesothelioma, somatic Nephrotic syndrome, type 4 Wilms tumor, type 1	c.208G>A p.Gly70Ser	VUS	Benign

OMIM, Online Mendelian Inheritance in Man. VUS, variants of uncertain significance.
[a]This variant reclassification was issued in twin siblings.

(30%, 6/20), and a single-gene test result (5%, 1/20). Within each testing modality, these amendments represent reclassification rates of 9.1% (5/55) for ES, 4.0% (13/324) for multigene panels, and 1.2% (1/81) for single-gene testing, respectively. No amended reports were issued for CMA or GS tests.

Time From Initial Report to Amended Report

The median time-to-amendment (interquartile range) from the date of initial report was 8.4 (4.4–27.1) months. As shown in Figure 4, the time-to-amendment followed a descending trend across the years of initial report. For variants reported in 2019 to 2022, the median time-to-amendment declined from 27.1 months in 2019 to 5.3 months in 2022. There were 2 amendments for initial reports received by outside institutions in 2013 and 2015 that were transferred to this institution. These reports were amended in 88.4 months and 59.2 months, respectively. The time-to-amendment did not differ significantly between variants that were upgraded and those that were downgraded (14.7 months vs. 5.45 months, $P = 0.52$).

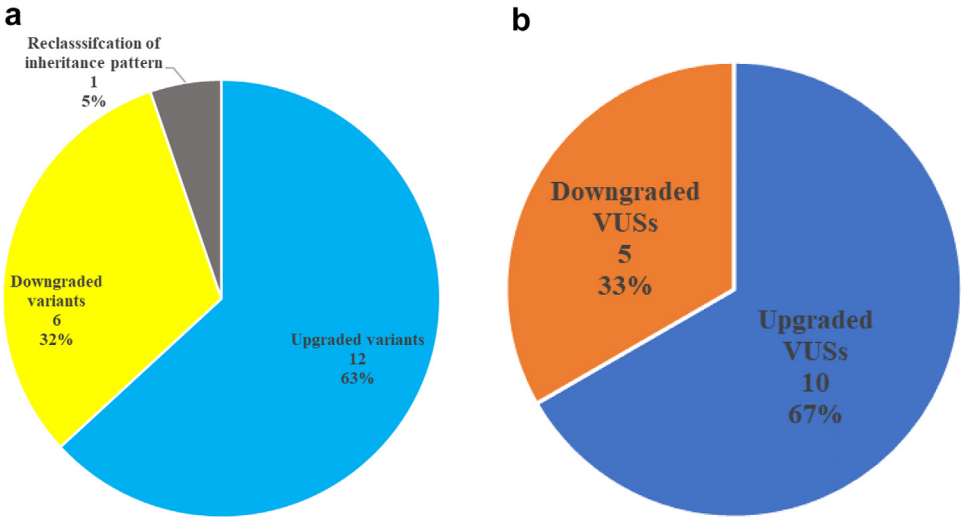

Figure 2. Upgraded vs downgraded reclassification of all variants (a) and VUSs (b). VUS, variant of uncertain significance.

Factors Associated With Reclassification

As shown in Table 3, self reported female gender on medical records was identified as a factor associated with variant reclassification in the univariate analysis (odds ratio 4.79 (1.59–18.8), $P = 0.01$), and this association persisted after adjusting for age and family history of kidney disease (odds ratio 4.36 (1.42–17.65), $P = 0.02$). Of the 20 patients with amended reports, 90% (18/20) were female. Segregation analyses were completed for 38.8% (7/18) of these female patients, whereas none (0.0%) of the 2 male patients with amended reports underwent variant reclassification.

Impact of Variant Reclassification on Diagnosis and Management

As shown in Table 4 and Supplementary Table S1, following variant reclassification, 60% (12/20) patients received a new diagnosis (11/20) or a change in diagnosis (1/20). *A priori* diagnoses were confirmed in 5% (1/20) of the patients. The new or changed diagnosis among patients led to a significant change in management in 67% (8/12) of patients. These changes included avoidance of steroids and immunosuppressive treatment in 1 case with autosomal dominant Alport syndrome; initiation of enzyme replacement therapy in 1

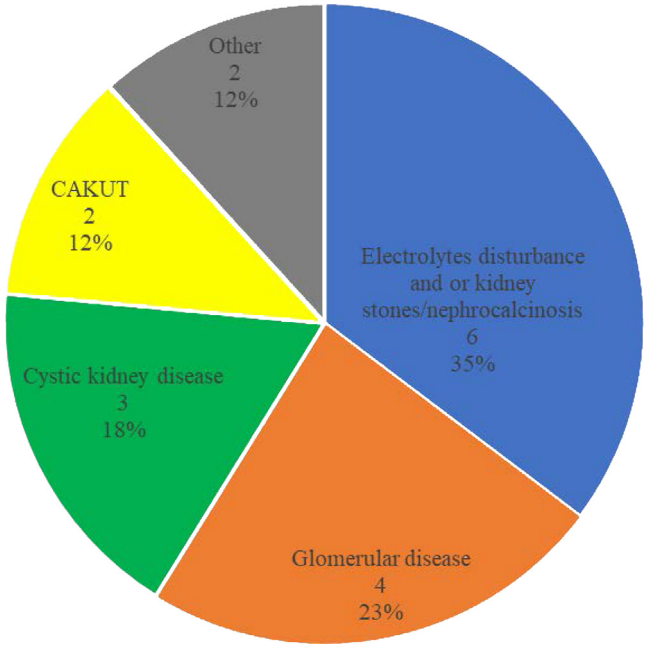

Figure 3. Renal phenotype of 17 genes in which variants were reclassified (*n*, %). CAKUT, congenital anomalies of kidneys and urinary tract.

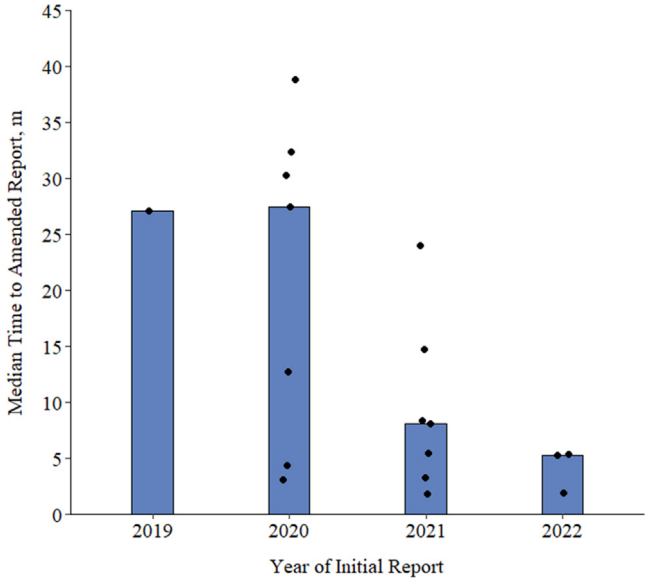

Figure 4. Year-specific median time from initial report to amended report with variant reclassification. Each dot represents a single reclassification event.

Table 3. Univariate analysis for factors associated with variant reclassification

Factor	Variant Reclassification	
	OR (95% CI)	*P*-value
Age <18 yr	0.21 (0.01–1.04)	0.13
Female	4.79 (1.59–18.8)	0.01
White	0.94 (0.56–1.36)	0.78
Non-White	1.83 (0.60–7.97)	0.34
ESKD	1.11 (0.17–4.04)	0.90
eGFR <60 ml/min per 1.73 m^2	1.00 (0.99–1.02)	0.45
Family history of kidney disease	0.52 (0.18–1.23)	0.18
Phenotype	1.01 (0.87–1.15)	0.83
Glomerular disease		
Electrolytes and/or stones/nephrocalcinosis		
Cystic kidney disease		
CAKUT		
Tubulointerstitial disease		
aHUS/TMA		
Had kidney biopsy	1.27 (0.41–3.39)	0.65
Testing modality	1.04 (0.92–1.14)	0.45
Single-gene		
Multi-gene		
CMA		
ES		
GS		

aHUS, atypical hemolytic uremic syndrome; CAKUT, congenital anomalies of kidneys and urinary tract; CI, confidence interval; CMA, chromosomal microarray; eGFR, estimated glomerular filtration rate; ES, exome sequencing; ESKD, end-stage kidney disease; GS, genome sequencing; OR, odds ratio; TMA, thrombotic microangiopathies.

case with hypophosphatasia; evaluation of candidacy for tolvaptan in 1 case with ADPKD1; brain MRI in 1 case with ADPKD2, which was previously denied by insurance; optimization of antiepileptic drugs in 1 case with Dravet syndrome; personalized calcium and vitamin D management in 1 patient with autosomal dominant hypocalcemia; discontinuation of imaging surveillance in 1 case after removal of diagnosis of pituitary adenoma multiple types; and personalized management and monitoring of hypercalcemia in 1 case during pregnancy with 24-hydroxylase deficiency.

DISCUSSION

To the best of our knowledge, this is the first real-world study to assess variant reclassification following variable genetics testing in a cohort of renal genetics patients through multiple laboratories. Over the 4.5-year analysis period, we evaluated the initial and amended genetic testing reports among 425 patients through 10 different Clinical Laboratory Improvement Amendments laboratories and found several novel findings. First, amended reports with variant reclassification following renal genetics testing are uncommon, with only 4.6% of patients receiving such reports. Second, VUS are frequently reported in renal genetics testing across various testing modalities, and the majority of variant reclassifications involve VUS. Third, upgrades in variant reclassification account for most of the reclassification reports in clinical practice. Fourth, female patients appear to be more likely to experience variant reclassification, and segregation analysis plays a role in the variant reclassification process. Finally, variant reclassification often leads to a new diagnosis or a change in diagnosis, impacting the clinical management of renal genetics patients.

Table 4. Impact of variant reclassification on diagnosis and management among patients who received amended reports following genetics testing

Impact of Variant Reclassification	Total ($N = 20$)
New diagnosis	11 (55%)
Change of diagnosis	1 (5%)
Confirmation or no change of a prior diagnosis	8 (40%)
Change in management[a]	7 (58%)

[a]Indicates analysis was performed only among those with new diagnosis or change of diagnosis.

Different from studies in cancer genetics or cardiogenetics assessing variant reclassification with a single testing modality such as multigene panel,[14–16] we analyzed data with multiple testing modalities including single-gene testing, multigene panel, ES, GS, and CMA. This provided data, which more closely reflect routine clinical practice, in which different laboratories are utilized depending on target genes, available modalities, and insurance coverage. We observed a reclassification rate of 4.6% for all pedigrees with reported variants and a reclassification rate of 7.0% for pedigrees with at least 1 variant initially reported as VUS across various testing modalities. These values encompass both upgrades and downgrades in pathogenicity, and the infrequent reclassification rate reflects the stability of definitive classifications. These results provide important references for clinicians in their daily practice when ordering and following up on genetic testing for patients with suspected genetic kidney disease.

Our data suggest that VUS are common in renal genetic testing reports, with more than half of our patients receiving reports containing at least 1 VUS with or without any concurrent pathogenic variants. These VUS were most frequently identified in multigene panels followed by ES. Variations in genetic sequence are classified as VUS when the association with disease risk and the significance to function are unclear.[23] These are usually missense, silent, and intronic variants or in-frame deletions and insertions,[23] as was also seen in this study. The popularity of multigene testing increases the probability of identifying VUS, and data from functional studies or clinical studies on these large numbers of VUS are urgently needed.[23–25] In this study, we observed that the vast majority of reclassification events involved VUS. Interestingly, electrolytes disorder and/or kidney

stones and nephrocalcinosis comprise the leading phenotype associated with genes whose variants were reclassified. This may potentially relate to research advances in the field of genetic kidney stones or nephrocalcinosis, and renal channelopathies.[26–28] Further, the declining median time from initial reports to amended reports with variant reclassification over the past few years in this cohort support the notion that active research in renal genetics is improving the understanding of VUSs.

VUS can be a source of frustration for clinicians and patients and are susceptible to misinterpretation.[29] The categorization of a VUS is typically based on various criteria and evidence.[10,29–31] Different laboratories may employ slightly different criteria, but common factors include typical types of variant evidence (e.g., population data, computational data, functional data, and segregation data). In our practice, we actively follow-up with patients who had VUS but a highly suspected genetic etiology, including facilitating segregation analysis and/or offering a follow-up visit. During these follow-ups, updated clinical information, if any significant changes occurred, will be shared with the testing laboratories to assist in any potential VUS reclassification. When a nephrologist orders genetic testing, it is helpful to provide pretest counseling about possible results, including VUS, to prepare patients. When a nephrologist receives a report with VUS, it is important to help patients understand that the VUS cannot confirm a diagnosis, but they may be reclassified as pathogenic or benign in the future when additional evidence becomes available. Segregation analysis for VUS of interest, continued follow-up, and communication with the testing laboratory if any significant changes in the patient's clinical condition, such as kidney biopsy results, will be helpful to assist in the further clarification of the VUSs. Indeed, phenotype data can be instrumental in the reclassification of variants. The 2015 guidelines by the ACMG and the AMP clearly state that classifying pathogenic variants (i.e., PP4) involves phenotype evidence: "Patient's phenotype or family history is highly specific for a disease with a single genetic etiology."[30] In renal genetics practice, kidney biopsy data are valuable for the testing laboratory in variant interpretation, and nephrologists should be encouraged to share this data whenever available.

Notably, most reclassified variants, including VUSs, detected in this study were upgraded to pathogenic or likely pathogenic variants. This pattern differs from what has been observed in studies on variants associated with pathologies other than kidney diseases, which report higher frequencies of downgrades to benign or likely benign variants.[13,15,16] This difference likely reflects variations in study methods. In other studies involving cancer genetics testing or cardiogenetics testing, variant calls were carried out as part of standardized research protocols at single institutions.[13,16] In our study, variant classification and reclassification were based on independent interpretations of the joint ACMG-AMP guideline by multiple academic and commercial laboratories. Some laboratories only reported amendments of diagnostic significance, namely upgrades to pathogenic or likely pathogenic variants. Although the standardized approach more closely approximates the maximal reclassification rates for both upgrades and downgrades, our data more closely mirrors routine clinical practice in renal genetics clinics.

Racial and gender disparities have been noted in genetic testing and access.[19,32,33] Unlike studies in other clinical areas,[29,34] our data did not show statistical significance in the identification of VUS between White and non-White patients with kidney disease. This is likely related to the sample size, and further studies are needed. It is interesting that our data suggest that male patients with kidney disease were more likely to receive a report with a VUS identified. Further, we observed that patients who received amended reports with variant reclassification were predominantly female, even after adjusting for age, race, kidney function, and phenotypes. The underlying reason is unclear. One potential explanation is that female probands are more likely to communicate genetic risks to relatives and promote segregation analysis through familial variant testing.[35,36] Indeed, we noted that more than half of the female patients, and none of the male patients who had variant reclassification underwent additional segregation analysis. One study has identified definite clinical diagnosis of inherited arrhythmia syndromes and a family history of genetic disorder, in general, as factors that predict variant reclassification[14]; whereas our data did not confirm these factors as predictors for variant reclassification. One caveat is the limitation of published research on familial variant testing for kidney disease, highlighting the need for disease-specific investigations into this clinically important question. Nevertheless, further studies with larger sample sizes will be needed to assess predictors for variant reclassification.

Our data clearly support the impact of variant reclassification. Nearly 60% of patients with variant reclassification received a new diagnosis or a change in diagnosis, and among them, 58% experienced a change in clinical management. The impact of variant reclassifications on individual and familial medical management, risk counseling, and screening has been supported by studies in cancer genetics,[24] cardio-

genetics,[14] and neurogenetics.[37] This study fills a gap in knowledge regarding the impact of variant reclassification in renal genetics and underscores the importance of periodic follow-up with patients who have undergone renal genetics testing to ensure that any variant reclassification, if it occurs, is appropriately communicated to patients and their families. Furthermore, this study supports the need for renal genetics societies to educate clinicians in general nephrology practice about the potential for variant reclassification and the necessary skills for managing and counseling patients with such amended reports.

This study has a few limitations. It is a retrospective cohort study with a relatively small number of patients whose variants were reclassified, limiting the multivariate analysis for predictors of variant reclassification. Furthermore, this study was based on variant calls from 10 different academic or commercial testing laboratories. Although the diversity of the laboratories enhances the generalizability of this analysis, variant classification and reclassification may not have been strictly uniform in practice across all the laboratories involved. This limitation is somewhat mitigated by the fact that all laboratories followed the joint ACMG-AMP guidelines for variant classification.[10]

In summary, this study suggests that variant reclassification following renal genetics testing is uncommon yet impactful on the diagnosis and management of patients with suspected genetic kidney disease. It provides real world data of the renal genetics clinic and indicates that VUS are the most involved in variant reclassification, often being upgraded when laboratories issue amended reports. This study strongly supports the importance of variant reclassification, because it frequently results in a new diagnosis and a change in the clinical management of patients. Our data indicate that female patients are more likely to undergo variant reclassification, which may be related to their higher likelihood of undergoing segregation analysis. Further studies are needed to assess predictors for variant reclassification.

DISCLOSURE

XW is a scientific advisory board member of Natera. M-BR reports receiving consulting fees from Alexion. All the other authors declared no conflicting interests.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplementary Table S1. Phenotype of patients who had variant reclassification and its implications on diagnosis and management.

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