Check for updates

Framework From a Multidisciplinary Approach for Transitioning Variants of Unknown Significance From Clinical Genetic Testing in Kidney Disease to a Definitive Classification

Uyenlinh L. Mirshahi[1], Ahana Bhan[2], Lotte E. Tholen[3], Brian Fang[4], Guoli Chen[5], Bryn Moore[1], Adam Cook[1], Prince Mohan Anand[6], Kashyap Patel[7], Mary E. Haas[8], Luca A. Lotta[8], Peter Igarashi[9], Jeroen H.F. de Baaij[3], Silvia Ferrè[10], Joost G.J. Hoenderop[3], David J. Carey[1] and Alexander R. Chang[2]

[1]Department of Molecular and Functional Genomics, Geisinger, Pennsylvania, USA; [2]Kidney Health Research Institute, Geisinger, Danville, Pennsylvania, USA; [3]Department of Physiology, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, the Netherlands; [4]Geisinger Commonwealth School of Medicine, Scranton, Pennsylvania, USA; [5]Department of Pathology, Penn State Health Hershey Medical Center, Hershey, Pennsylvania, USA; [6]Department of Nephrology, Medical University of South Carolina, Lancaster, South Carolina, USA; [7]Institute of Biomedical and Clinical Science, College of Medicine and Health, University of Exeter, Exeter, UK; [8]Regeneron Genetics Center, Sawmill Road, Tarrytown, New York, USA; [9]Department of Medicine, University of Minnesota, Minneapolis, Minnesota, USA; and [10]National Kidney Foundation, New York, New York, USA

Introduction: Monogenic causes in over 300 kidney-associated genes account for approximately 12% of end stage kidney disease (ESKD) cases. Advances in sequencing and large customized panels enable the noninvasive diagnosis of monogenic kidney disease at relatively low cost, thereby allowing for more precise management for patients and their families. A major challenge is interpreting rare variants, many of which are classified as variants of unknown significance (VUS). We present a framework in which we thoroughly evaluated and provided evidence of pathogenicity for *HNF1B*-p.Arg303His, a VUS returned from clinical diagnostic testing for a kidney transplant candidate.

Methods: A blueprint was designed by a multidisciplinary team of clinicians, molecular biologists, and diagnostic geneticists. The blueprint included using a health system-based cohort with genetic and clinical information to perform deep phenotyping of VUS heterozygotes to identify the candidate VUS and rule out other VUS, examination of existing genetic databases, as well as functional testing.

Results: Our approach demonstrated evidence for pathogenicity for *HNF1B*-p.Arg303His by showing similar burden of kidney manifestations in this variant to known *HNF1B* pathogenic variants, and greater burden compared to noncarriers.

Conclusion: Determination of a molecular diagnosis for the example family allows for proper surveillance and management of *HNF1B*-related manifestations such as kidney disease, diabetes, and hypomagnesemia with important implications for safe living-related kidney donation. The candidate gene-variant pair also allows for clinical biomarker testing for aberrations of linked pathways. This working model may be applicable to other diseases of genetic etiology.

Correspondence: Uyenlinh L. Mirshahi, Department of Molecular and Functional Genomics Geisinger Medical Center, 100 North Academy Ave, Danville, Pennsylvania 17822, USA. E-mail: umirshahi1@geisinger.edu; or Alexander R. Chang, Kidney Health Research Institute, Geisinger Medical Center, 100 North Academy Ave, Danville, Pennsylvania 17822, USA. E-mail: achang@geisinger.edu

Monogenic causes account for up to 40% of patients in cohorts enriched with ESKD or familial kidney disease.[1–4] Genetic testing has become an invaluable tool in the diagnosis of various types of chronic kidney disease (CKD), including but not limited to autosomal dominant tubulointerstitial kidney disease, autosomal dominant polycystic kidney disease, congenital anomalies of the

kidney and urinary tract, focal segmental glomerulosclerosis, and CKD of unknown cause.[2,5] Genetic testing allows for more precise molecular diagnosis, in some cases diagnostic reclassification. Benefits of establishing a molecular diagnosis include earlier treatment with disease-modifying therapies, screening for extrarenal manifestations, avoidance of invasive procedures such as kidney biopsy, and important implications for family planning and living kidney donation.[3,5,6]

HNF1B encodes the hepatocyte nuclear factor 1β (HNF1B) and is a member of the homeodomain-containing superfamily of transcription factors involved in the development of kidney, urogenital tract, pancreas, liver, brain, and parathyroid gland.[2] Pathogenic *HNF1B* variants lead to a wide spectrum of phenotypic expressions ranging from noninsulin dependent, maturity onset diabetes of the young, pancreatic hypoplasia, liver cholestasis, and several renal phenotypes. Renal phenotypes include an autosomal dominant polycystic kidney disease phenocopy spectrum (renal cysts and diabetes syndrome), an autosomal dominant tubulointerstitial kidney disease phenocopy spectrum (autosomal dominant tubulointerstitial kidney disease-HNF1B), congenital anomalies of the kidney and urinary tract, and biochemical anomalies (e.g., hypomagnesemia, hyperuricemia, hyperparathyroidism).[7–12] The phenotypic expression of *HNF1B* heterozygotes varies even between individuals with the same mutation within families, possibly as a result of temporal stochastic variations in *HNF1B* expression during nephrogenesis.[9] Because there is a large number of genes associated with *HNF1B* renal phenotypes, the use of exome sequencing or massively parallel sequencing (MPS) can be very helpful.[1,2,4,5,13–15]

MPS has become increasingly available and more affordable in clinical practice.[16,17] MPS allows the evaluation of multiple genes and thus can be particularly useful for monogenic kidney disorders with broad differential genetic causes. A consequence of testing dozens if not hundreds of genes linked to kidney disease is that many VUS are often detected. In various cohorts, from 10% to 100% of genetic results may be VUS, classified according to the American College of Medical Genetics-Association for Molecular Pathology (ACMG-AMP) criteria.[3,18–20] VUS present a diagnostic and ethical challenge in genetic testing and lack of resolution may result in delays in treatment and management.[21] Variant reclassification from VUS to (likely) benign, or (likely) pathogenic improves as data sharing and variant curation efforts from expert panels (e.g., ClinGen and Genomics England) expand.[5] Apart from case studies, implementation and strategies to efficiently transition kidney gene VUS to a more definitive classification are lacking.

Clinically unselected research population databases present a unique resource that can be used to triage VUS. The MyCode DiscovEHR database, currently comprised of approximately 173,000 individuals who have exome sequencing and linked-electronic health records,[22] includes rich and longitudinal in-patient and out-patient data on multiple generations spanning over 20 years that could be leveraged for studying clinical features of heterozygotes of rare VUS that are returned from clinical genetic testing.

In this exemplar study, we present a framework of efforts from a multidisciplinary team with expertise in nephrology, endocrinology, molecular biology, and diagnostic genetics to gather evidence for a diagnostic reclassification of *HNF1B* c.907C>T p.Arg303His from a VUS to a likely pathogenic variant, identified through a clinical MPS panel for a patient with CKD of unknown cause.

METHODS

Study Populations, Genetic Testing, and Clinical Data Abstraction

Clinical Family

The index patient (proband) is a female who was referred to Geisinger Medical Center for kidney transplant evaluation. Because she had CKD of unknown cause and family history of CKD, she underwent genetic testing with Natera Renasight for exonic and copy number variations in 385 genes associated with kidney disease (Natera, TX). To further investigate the *HNF1B* Arg303His VUS, family testing was performed on affected family members and unaffected family members to evaluate for cosegregation. Pertinent laboratory testing (serum magnesium, fractional excretion of magnesium (FEMg), uric acid, renal, and liver function tests) were offered as part of routine care in affected individuals. Renal and extrarenal involvement was recorded using patient interview and review of electronic health records. Permission to publish the case series was obtained from the proband and her family members.

Geisinger DiscovEHR Cohort

The Geisinger cohort consisted of 172,589 individuals who received health care at Geisinger, a health system in central and northeastern Pennsylvania, USA (last date of query June 18, 2021). Individuals were consented, on an ongoing basis, to the MyCode Community Initiative to create a biorepository of blood, serum, and DNA samples for broad research use, including genomic analysis,[22] with linkage to the electronic health record. Renal and extrarenal involvement in the cases, including clinical diagnosis, procedures, imaging

results, and laboratory results were reviewed. Genetic analysis (for research purposes) was carried out as part of the DiscovEHR collaboration between Geisinger and the Regeneron Genetics Center by exome sequencing.[23] This study was reviewed by the Geisinger Institutional Review Board and determined as not including human subject research as defined in 45CFR46.102(f) (Study # 2021-0177); the family consented to publication of their cases.

ClinGen Monogenic Diabetes Expert Panel and Exeter Cohort

We contacted the ClinGen Monogenic Diabetes Expert Panel (inclusive of >100 laboratories) to identify other individuals with the *HNF1B* Arg303His variant. Among these laboratories, the Exeter cohort comprised of more than 5000 individuals with clinical suspicion of monogenic diabetes referred from routine clinical practice across the UK for genetic testing at the Molecular Genomics Laboratory at the Royal Devon and Exeter Hospital, Exeter, UK. Informed consent was obtained from these patients or their parents or guardians. The study was approved by the North Wales ethics committee.

Variant Annotation and Classification

The variants from clinical genetic testing and from Geisinger DiscovEHR research studies were annotated using clinically relevant RefSeq transcripts and classified by the clinical genetic laboratories (family A) or at Geisinger (MyCode participants) using ACMG-AMP guidelines.[24,25] *HNF1B* copy number loss were called from a referenced-based CLAMMS algorithm using exome sequencing,[26] and confirmed by Illumina chip array. Samples underwent quality assurance, including removal of samples of sex mismatch, other large chromosomal abnormalities, and outliers of derivative log ratio spread and genomic wave factors.[27] Outliers were defined as 1.5 times the interquartile range from the third quartile of the distribution of derivative log ratio spread. Patients referred as having a *HNF1B* whole gene deletion in this study included 25 clean samples with 17q12 microdeletion, a 1.26Mb deletion ranging from *AATF* to *HNF1B* which results in complete loss of the entire *HNF1B*. Noncarriers in this study refers to samples in the MyCode cohort without 17q12 microdeletion, known likely pathogenic or pathogenic *HNF1B* variants as defined by ClinVar,[28] or protein truncating variants (all except for the last exon).

Luciferase Reporter Assay

Human embryonic kidney 293 (HEK293) cells were seeded in 24 well plates and transfected with 350 ng of the promoter firefly luciferase constructs (pGL3) previously generated containing the kidney-specific promoters of Na$^+$/K$^+$-ATPase subunit gamma (*FXYD2*), or (*PKHD1*), or empty vector.[29] All 3 constructs were cotransfected with 25 ng of pCINEO-empty, pCINEO-*hHNF1B*, pCINEO-*hHNF1B*-Arg303His or pCINEO-*hHNF1B*-Lys156Glu cDNAs. Additionally, for controlling the transfection efficiency in each reaction, 10 ng of Renilla luciferase construct (pRL) under a CMV promoter was co-transfected and used to normalize luciferase counts in all conditions. Cotransfections were performed using polyethylenimine cationic polymer (Thermo FisherScientific, Waltham, MA) in 1:6 DNA to polyethylenimine cationic polymer ratio. For dose-response experiments, HEK293 cells were cotransfected with human *FXYD2 or PKHD1* promoter construct and 50 (PKHD1 only), 25, 12.5, 6.25, or 3.125 ng pCINEO-*hHNF1B* or pCINEO-*hHNF1B*-Arg303His cDNAs. Firefly and Renilla luciferase luminescence were measured 48 hours after transfection with the dual-luciferase reporter assay (Promega) using a plate reader (VICTOR, PerkinElmer, Waltham, MA).

Biomarkers

We compared presence of clinical laboratory measures (estimated glomeruler filtration rate [eGFR], magnesium, amylase, lipase, uric acid) related to *HNF1B*-related disease among *HNF1B*-Arg303His heterozygotes to individuals carrying the 17q12 microdeletion encompassing *HNF1B,* and noncarriers. We calculated eGFR for individuals aged less than 18 years using the Schwartz equation,[30] and for those aged 18 years or more using the CKD-EPI equation.[31] Longitudinal outpatient serum biomarkers were restricted to the age range of the *HNF1B*-Arg303His cases (15–60 years); measures following kidney transplantation and dialysis were excluded. Measures from each patient were pooled per group, regressed using a linear model against age at measurement, and plotted with 95% confidence intervals using Matplotlib seaborn library. Regression statistics were generated using R lm package (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Kidney Disease is Observed in 3 Generations of Family A Seen in Geisinger Nephrology Clinic in Heterozygotes of HNF1B-p.Arg303His

Family A Proband case AIII-1 is a 25-year-old female with a history of chronic pancreatitis, CKD stage 4, and asthma presented for kidney transplant evaluation (Note: neither this patient nor her family members had previously enrolled in the MyCode Community research cohort). At the age of 13, she had been evaluated for elevated amylase and lipase, abdominal pain, and CKD with no blood or urine on urinalysis. A kidney biopsy showed chronic tubulointerstitial
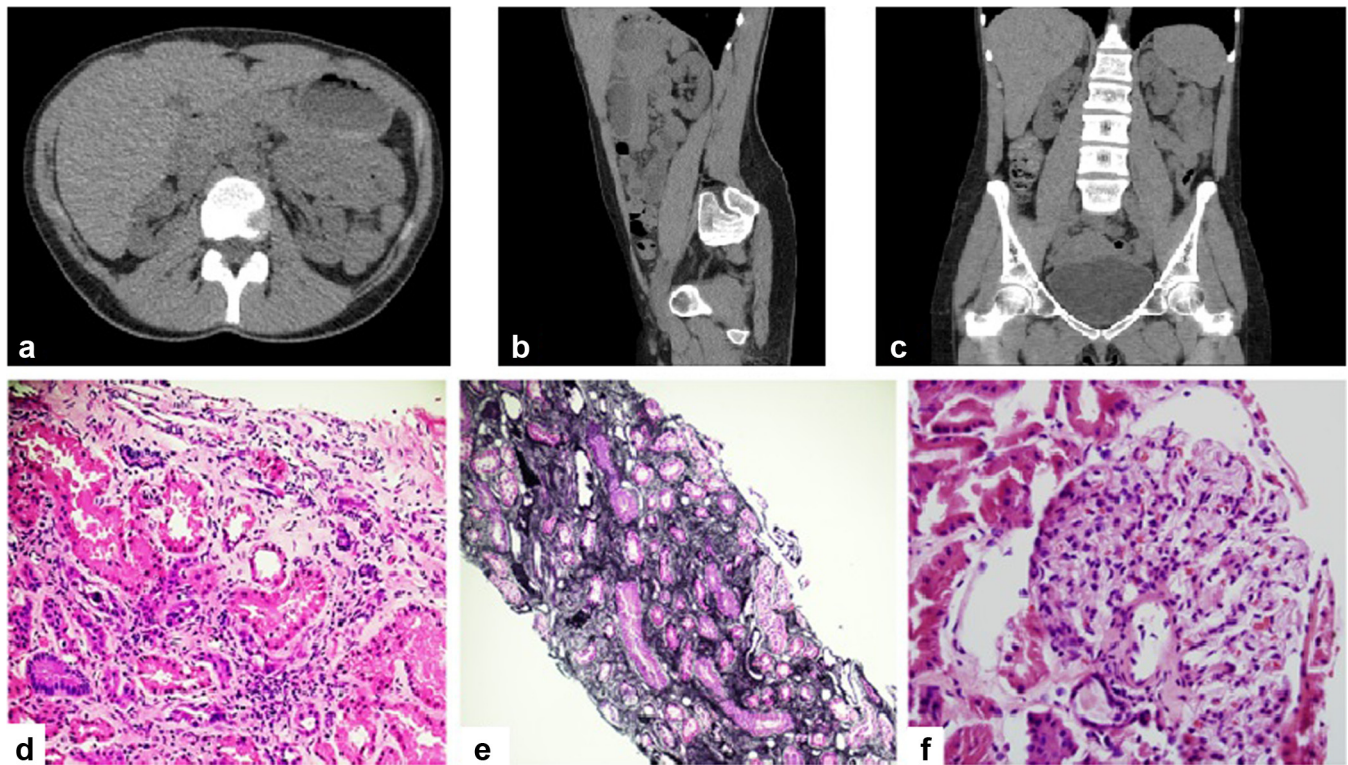
Figure 1. Imaging and histopathology of Family A proband (case AIII-1) *HNF1B*-p.Arg303His heterozygote showed bilateral small kidneys and oligomeganephronia. (A) Axial, (B) sagittal, and (C) coronal images from noncontrast CT scan taken at age 24 show small kidneys without cysts, and no overt abnormality of the pancreas and the liver. (D) Hematoxylin and eosin staining of renal biopsy specimen showed focal interstitial inflammation and mild tubular injury (10×10 magnification). Focal interstitial fibrosis is also present, which is highlighted by (E) Jones silver staining (4×10 magnification). (F) In the cortical areas, the number of glomeruli is reduced. Mild tubular hypertrophy associated with mildly enlarged glomeruli is observed as shown in a representative image (20×10 magnification).

nephritis and oligomeganephronia, and imaging showed diffusely hyperechogenic, small kidneys (8.8 cm-left, 9.2 cm-right) (Figure 1). Clinical genetic testing for hereditary pancreatitis (Ambry Genetics panel for *CFTR, SPINK1, PRSS1*) showed that she was heterozygous for *CFTR* p.Leu1065Pro, a pathogenic variant. Evaluation of sweat test, and pulmonary and genetics consultation eliminated the variant as causal for her pancreatic issues. Although her body mass index was low normal (21.5 kg/m^2), no testing for pancreatic insufficiency had been performed at that time. At age 14, allopurinol therapy was initiated because she was found to have an elevated uric acid level (7.7 mg/dl). At the age of 20, her CKD had progressed along with proteinuria (511 mg/g protein-to-creatinine ratio). A repeat kidney biopsy done at age 20 showed progressive chronic tubulointerstitial injury, moderate or patchy interstitial fibrosis and tubular atrophy, mild fibro-intimal thickening of arteries, and oligomeganephronia (Figure 1). Further testing revealed chronically elevated amylase or lipase, with normal liver function tests (Table 1). Over several years, serum magnesium levels ranged from 1.4 to 1.8 mg/dl, and at age 25 her serum magnesium was 1.9 mg/dl with FEMg indicating magnesium wasting (15%). Repeat abdomen

or pelvis noncontrast computed tomography at age 25 showed small kidneys and an unremarkable pancreas.

Additional Family Testing Reveals Cosegregation of the HNF1B-p.Arg303His Variant With Disease in Family A

Clinical genetic testing performed using the 385-gene Natera Renasight panel revealed that the proband, her mother, and her sister harbored *HNF1B-p*.Arg303His (chr17:37731732:C:T on GRCh38, Supplementary Table S1, Figure 2). Family A proband's 48-year-old mother, case AII-1, had a history of pancreatitis, chronically elevated amylase and lipase, and ureteral stenosis for which she underwent dilation in her late 20s (Table 1, Figure 2). Her body mass index was low normal at 19.8 kg/m^2, and she had never undergone testing for pancreatic insufficiency. No history of diabetes or liver problems were noted. She developed CKD in her late 30s, (eGFR 43 ml/min per 1.73 m^2 at 46 years of age) with no proteinuria or hematuria. Magnesium at the age 38 was low at 1.4 mg/dl; FEMg at age 48 was 9%. Kidney biopsy revealed somewhat dilated tubules and mild arterial fibro-intimal thickening without active interstitial inflammation. No renal or pancreatic imaging was available for this patient. Family A proband's 21-year-old sister, case AIII-

Table 1. Clinical characteristics of heterozygotes of *HNF1B* c.908G>A p.Arg303His at first presentation

Characteristics	Family A			Family B	Family C	
	Case AIII-1 (Index case)	Case AII-1	Case AIII-2	Case BI-1	Case CII-1 proband	Case CI-1 proband's mother
Study	This study	This study	This study	This study	Snoek et al.[32]	Snoek et al.[32]
Age, yrs	25	48	21	57	33	50
Sex	Female	Female	Female	Male	Male	Female
BMI, kg/m^2	21.5	19.8	NA	25.9	NA	NA
Hypertension	No	No	No	No	NA	NA
Diabetes (age)	No	No	No	Yes (51)	NA	NA
ESRD (age)	Yes (25)	No	No	No	NA	NA
Genitourinary abnormalities	No	Ureteral stenosis requiring dilatation in her 20s	No	No	NA	NA
Pancreas abnormalities	Pancreatitis	Pancreatitis	No	Mild atrophy pancreatitis	NA	NA
Creatinine, mg/dl	3.2	1.6	1.2	1.7 (51)	NA	NA
eGFR ml/min/1.73 m^2	20.7	39.0	62.7	45.6 (51)	39 (33)	NA
Albuminuria or proteinuria	ACR: 331 mg/g	ACR: 37.3 mg/g	Dipstick: trace	Dipstick: negative	24-h urine protein: 0.6 g/d	NA
Microscopic hematuria	Negative	Negative	Negative	Negative		NA
Albumin g/dl	4.5	4.4	NA	NA	Normal	NA
AST U/l	15	32	30	23	Normal	NA
ALT U/l	10	44	9	33	Normal	NA
Alkaline phosphatase U/l	51	59	220	73	Normal	NA
Serum magnesium mg/dl	1.9	1.7	1.8	1.6 (43)	NA	NA
FEMg %	15	9	3	NA	NA	NA
Uric Acid, mg/dl	7.7 (14)	5.8 (48)	4.9 (20)	NA	NA	NA
Kidney biopsy findings	Chronic tubulointerstitial nephritis, oligomeganephronia at 13 yrs	Zonal interstitial fibrosis thought to be due to ureteral stenosis at 43 yrs	NA	Chronic kidney disease, unknown etiology	FSGS unknown etiology with 45% glomerulosclerosis at 33 yrs	Hypodysplasia
Abdominal imaging	Small diffusely hyperechoic kidneys, normal pancreas on MRI at 13 yrs Small kidneys, unremarkable pancreas by CT at 24 yrs	NA	NA	Kidney unremarkable, mild atrophy pancreas	Kidney unremarkable in appearance and size	NA

ACR, albumin/creatinine ratio; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CT, computed tomography; eGFR, estimated glomerular filtration rate; ESRD, end stage renal disease; FEMg, fractional excretion of magnesium; FSGS, focal segmental glomerulosclerosis; MRI, magnetic resonance imaging; NA, not available.
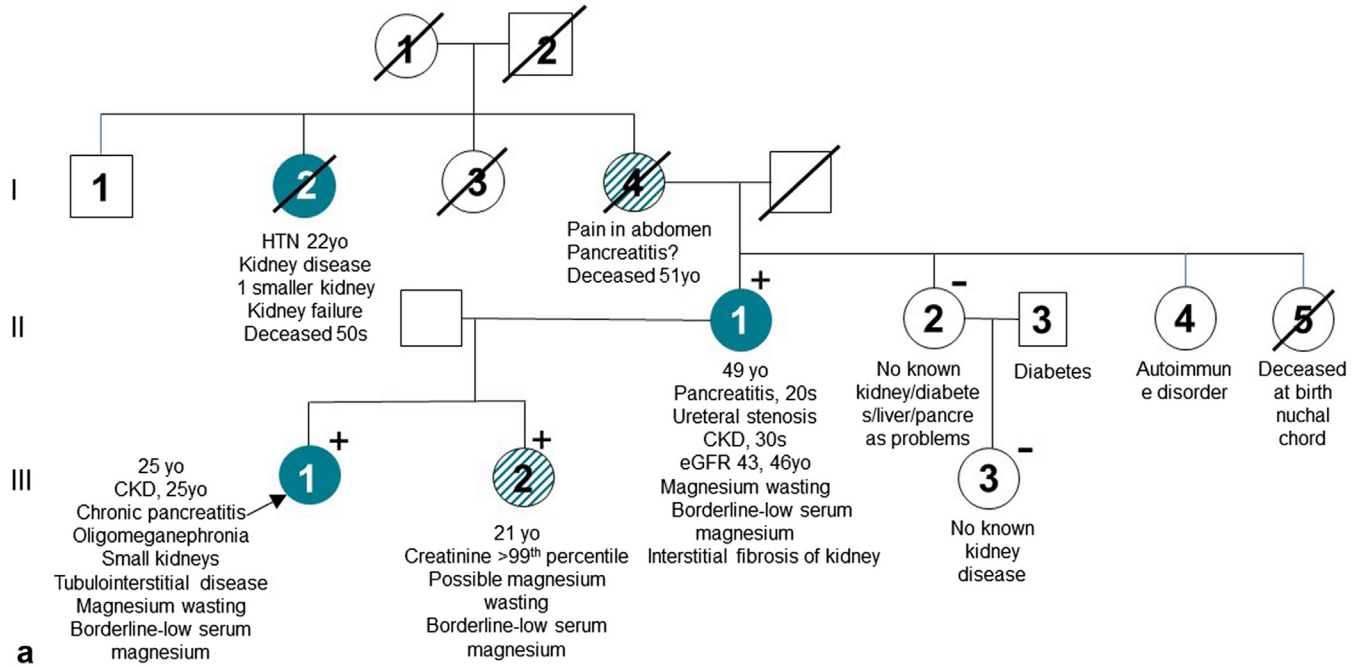If laboratory values were obtained at an earlier age, the age appears in parentheses.

2, had eGFR levels less than first percentile for her age or sex ranging 63 and 67 ml/min per 1.73 m^2 in the past year (Table 1, Figure 2). She had no history of diabetes or known abnormalities of the liver or pancreas although testing for pancreatic insufficiency or abdominal imaging was not performed. FEMg measured at age 21 was 3%. Family A proband's maternal great aunt (case AI-2, Figure 2) had a history of hypertension and kidney disease at age 22 and was born with one of her kidneys being smaller than the other. Per the proband's mother, case AI-2 later had kidney failure and died in her 50s. No additional clinical information is available. Family A proband's grandmother (case AI-4, Figure 2) was reported to have a possible history of pancreatitis.

Clinical genetic testing of unaffected family members (proband's maternal aunt (case AII-2, Figure 2) and maternal cousin (case AIII-3) as potential kidney donors using the same MPS panel test found that neither unaffected individual carried the *HNF1B* Arg303His.

Incidentally, case AIII-3 was found to have a likely pathogenic frameshift in *CFI* (c.1311dup p.Asp438Argfs*8).

Review of Genetic Databases and Literature

The *HNF1B*-p.Arg303His variant is absent from the broad gnomAD dataset. In reviewing the literature, a case was submitted to ClinVar (https://www.ncbi.nlm. nih.gov/clinvar/RCV001253232/) in which an individual was reported with renal cysts and diabetes; no other information was noted. A case study looking at genetic causes of focal segmental glomerulosclerosis reported a mother-son pair with ESKD at a young age who were heterozygous for *HNF1B*-p.Arg303His.[32] The proband had ESKD at age 33 (family C, case CII-1, Table 1) and focal segmental glomerulosclerosis on kidney biopsy. No renal structural abnormalities, electrolyte, glucose, or liver enzyme abnormalities were observed. His mother (CI-1, Table 1) was reported to be heterozygous

I

1 | **2** HTN 22yo Kidney disease 1 smaller kidney Kidney failure Deceased 50s | **3** | **4** Pain in abdomen Pancreatitis? Deceased 51yo

II

1 49 yo Pancreatitis, 20s Ureteral stenosis CKD, 30s eGFR 43, 46yo Magnesium wasting Borderline-low serum magnesium Interstitial fibrosis of kidney | **2** No known kidney/diabetes/liver/pancreas problems | **3** Diabetes | **4** Autoimmune disorder | **5** Deceased at birth nuchal chord

3 No known kidney disease

III

25 yo CKD, 25yo Chronic pancreatitis Oligomeganephronia Small kidneys Tubulointerstitial disease Magnesium wasting Borderline-low serum magnesium | **1** | **2** 21 yo Creatinine >99th percentile Possible magnesium wasting Borderline-low serum magnesium

a

Rules met	Explanation	Evidence	Before this Study	After this Study
PM1	Within critical functional DNA binding domain (POU$_H$ domain)	Amino acids 232-305 locate in POU$_H$ domain of *HNF1B*	met	met
PM2	Highly conserved region (pyloP100way = 7.32)		met	met
PP3	REVEL score 0.944, DANN 0.9995, fathmm_XF 0.950, fathmm_XF_pred Damaging, CADD_phred 27.7		met	met
PS4	The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls	No cases in gnomAD MAF = 2.9e-6 in MyCode DiscovEHR	met	met
PP1 moderate	Co-segregation evidence	Family A: Affected AI-2, AII-1 N = (½)^2 Family C: Affected CI-1 N = (½)^1 N total = (½)^2 * (½) = 1/8	not met (Family A co-segregation data was deemed not convincing)	met
Classification			**VUS**	**LP**

b

Figure 2. Family A pedigree and pathogenic classification of *HNF1B*-p.Arg303His. (A) Male members are represented in squares, females in circles. Filled-in symbols indicate affected members with clinical features previously observed in *HNF1B* loss of function patients are shown, open symbols show unaffected members, and hashed symbols are members highly suspicious for the kidney disease. Positive sign indicates that the individual is heterozygous for *HNF1B*-p.Arg303His, and a negative sign indicates that the individual was genotyped and does not harbor *HNF1B*-p.Arg303His. The proband is identified with an arrow. Each generation with informative health records are labeled with Roman numerals on the left. (B) Pathogenic reclassification of *HNF1B*-p.Arg303His per ACMG-AMP guidelines. The rules and the explanation for meeting the rules before this study and after this study are indicated. For cosegregation evidence, see text. CKD, chronic kidney disease; HTN, hypertension, eGFR, estimated glomerular filtration rate.

of the same variant and deceased at age 50 with ESKD, presumably from hypodysplastic kidneys. Of note, these cases were reported from investigators in the Netherlands and are not related to our proband's family. Likewise, these individuals were most likely not related to the individual reported in ClinVar because there were no citations for ClinVar in the publication.

Based on the initial evidence, the *HNF1B*-p.Arg303His variant was classified as a VUS (PM2, PP3, PP4) by the clinical diagnostic laboratory. The diagnostic laboratory noted the lack of strong evidence for variant segregation because only 2 meioses (AIII-2 and AIII-3) were observed in family A and 2 in family C.

The MyCode Population Cohort Provides Evidence of Variant Pathogenicity and Penetrance in an Unselected Population

We interrogated the MyCode research cohort of 172,589 participants and identified only 1 individual with *HNF1B* p.Arg303; this patient had no other known ClinVar pathogenic or likely pathogenic variants or PTVs in kidney genes.[3] This 58-year-old male (family B, case BI-1), who harbored a p.Arg303His (minor allele frequency 2.9e-6), has clinical features of *HNF1B* mutations including a history of gout, elevated amylase and/or lipase, pancreatitis, diabetes with insulin treatment for at least 10 years, and stage 3 CKD (Table 1). Last computed tomography taken at age 51 indicated mild atrophy of the pancreas, no kidney or liver cysts, or other genitourinary defects.

Serum Biomarkers of *HNF1B*-p.Arg303His Cases Indicate Decline in Kidney and Pancreatic Function, Similar to *HNF1B* Whole Gene Deletion

Because no differences in renal and extrarenal clinical characteristics between patients with *HNF1B* coding and splicing pathogenic variants and those with *HNF1B* whole gene deletion (17q12 microdeletion) have been reported,[9] we compared eGFR, magnesium, and pancreatic enzyme levels of *HNF1B*-p.Arg303His cases to individuals with 17q12 microdeletion and noncarriers in the MyCode cohort. Figure 3 shows outpatient laboratory measures of cases in family A and family B compared to the lifetime profile of individuals with 17q12 microdeletion and noncarriers of pathogenic *HNF1B* variants. As expected, eGFR decline over time for the 17q12 microdeletion cases were greater
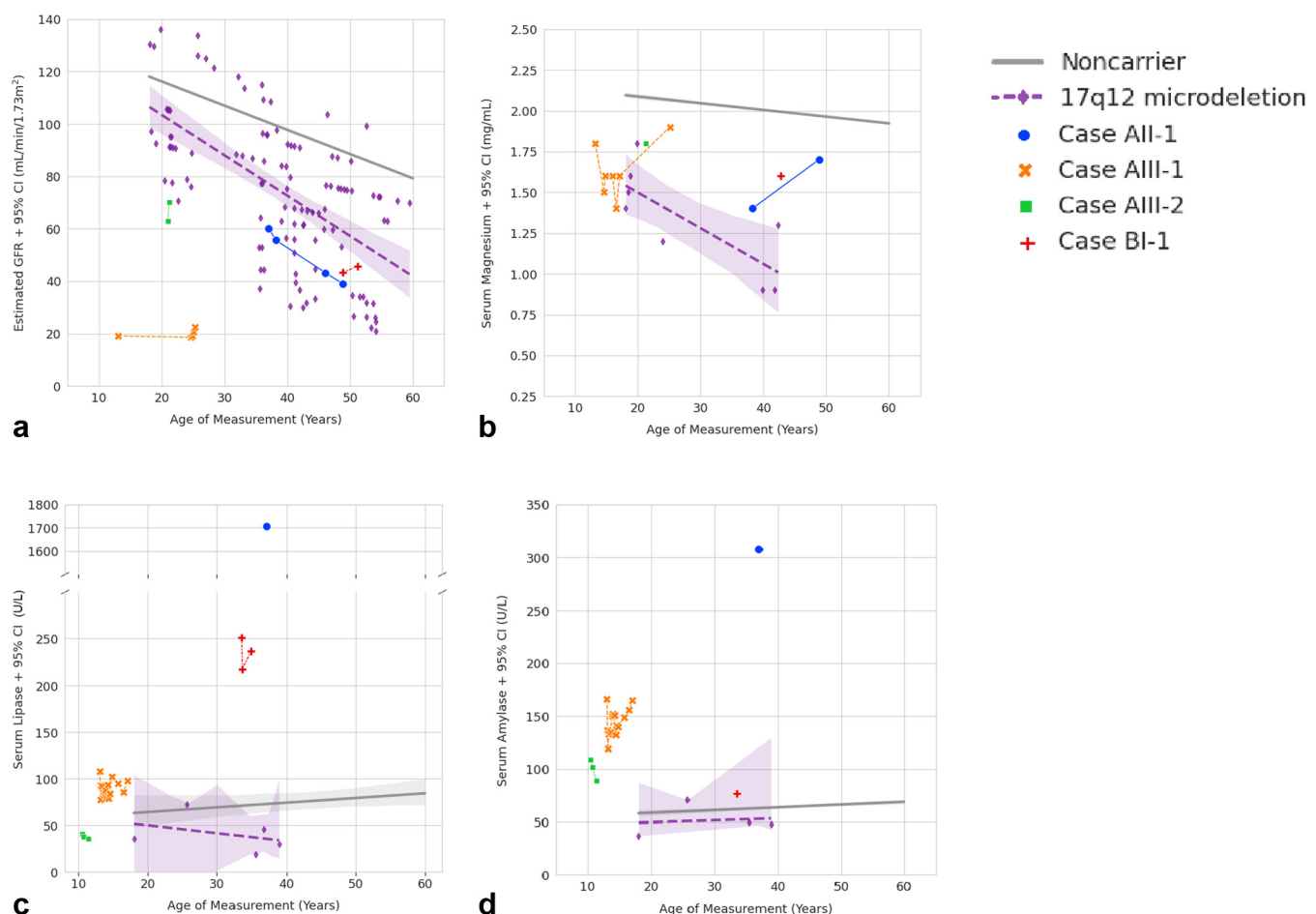

Figure 3. Serum biomarkers of *HNF1B*-p.Arg303His cases indicate decline in kidney and pancreatic function similar to *HNF1B* whole gene deletion. Lifetime laboratory measures from age 18 to 60 years for individuals with *HNF1B* whole gene deletion (17q12 microdeletion), *HNF1B*-p.Arg303His (case AII-1, case AIII-1, case AIII-2, and case BI-1), and noncarriers of HNF1B are plotted against the age of measurement. Linear regression was performed for 17q12 microdeletion and noncarrier groups. For clarity, only regression lines for noncarriers and 95% confidence intervals are shown. Data for *HNF1B*-p.Arg303His are shown as individual measurement per case due to a smaller number of tests. Biochemical measures and number of individuals with measures are as follow: (A) Estimated GFR (n for noncarriers = 115,298; n for 17q12 microdeletion = 12), (B) Serum magnesium (n noncarriers = 21,411; n 17q12 microdeletion = 4), (C) Serum lipase (n noncarriers = 24,323, n 17q12 microdeletion = 4), (D) Serum amylase (n noncarriers = 18,116; n 17q12 microdeletion = 4). See materials and methods for details.

(-1.7 ml/min per 1.73 m^2/year [95% confidence interval -1.9, -1.4]) vs. noncarriers (-0.928 ml/min per 1.73 m^2/year [-0.932, -0.924]; $P < 0.0001$). All HNF1B-p.Arg303His heterozygotes had eGFR levels lower than noncarriers and 17q12 microdeletion cases at the same age (Figure 3). Serum magnesium in all HNF1B-p.Arg303His cases were similar to levels from 17q12 microdeletion cases and lower than noncarriers (Figure 3). Three of the 4 HNF1B-p.Arg303His cases had elevations in lipase whereas 17q12 microdeletion cases had no differences in mean lipase or amylase levels compared to noncarriers.

Individuals Heterozygous for Other Variants at the Arg303 Locus Also Show Renal Abnormalities Consistent With Pathogenic HNF1B Variants

To our knowledge, other HNF1B-p.Arg303 variations have been observed in only 3 other individuals to date. Lalièvre et al.[33] reported a school-aged heterozygote of HNF1B c.907C>T p.Arg303Cys with multicystic dysplasia (LOVD Patient ID 00231109, http://www.lovd.nl/HNF1B). In addition, from the Molecular Genomics Laboratory at the Royal Devon and Exeter Hospital, we report a mother-daughter pair with diabetes, both of whom harbor c.907C>A p.Arg303Ser. The daughter was referred for genetic testing for HNF1B due to early-onset diabetes (age 26) and solitary kidney. Her mother had diabetes at age 39 and also had the same variant. Diabetes was also present in 5 maternal uncles and aunts but genetic analyses on these family members are not available.

HNF1B Arg303His Mutation Mildly Affects FXYD2 and PKHD1 Promoter Activation In Vitro

HNF1B acts as a transcriptional activator or repressor by binding to the promoter of target genes. We tested the binding properties of Arg303His on 2 kidney-specific promoters, PKHD1 or FXYD2, in a dual-luciferase reporter assay system. HNF1B-p.Arg303His transcriptional activity on FXYD2 promoter significantly increased by 13% compared to wildtype HNF1B (Supplementary Figure S1A). Nevertheless, saturation curves with decreasing concentrations of promoter construct for FXYD2 showed comparable transcriptional activity between the mutant Arg303His construct and wildtype HNF1B (Supplementary Figure S1B). In contrast, HNF1B-p.Arg303His showed a reduced transcriptional activity to wildtype HNF1B for PKHD1 promoter (11%, $P < 0.05$, Supplementary Figure S1A). Nevertheless, the difference was not observed at saturating concentrations of 25 ng and 50 ng constructs (Supplementary Figure S1C). These studies suggest that compared to the wildtype, the His

at position 303 mildly alters transactivation potential of HNF1B in the tested HNF1B target promoters.

HNF1B-p.Arg303His Classification per ACMG-AMP Guideline

We combined all the evidence of HNF1B-p.Arg303His family members to classify HNF1B-p.Arg303His per ACMG-AMP criteria. Per Jarvick and Browning, we assumed that the variant has full penetrance and that it is inherited from 1 ancestor given its absence in gnomAD exomes and genomes and rare frequency in the MyCode population (minor allele frequency = 2.9e-6).[25] By this assumption, for family A, members AI-2 and AI-4 are untyped heterozygotes (Figure 2a). Using a conservative approach, we considered the proband, AI-2, and AII-1 as affected heterozygotes for family A, and the proband and CI-1 as affected heterozygotes in family C. We calculated the total N, the probability that the observed variant-kidney phenotype cosegregation is not by chance. The total N is the product of all Ns in each family. By definition, the probability of each proband in the 3 families is 1. We assigned $N = \frac{1}{2}$ for each affected heterozygote, resulting in $N_{total} = 1/8$ (Figure 2b). A probability of 1/8 in 2 families provides a moderate level of pathogenicity support that the observed kidney phenotype did not occur by chance. Of note, the probability for cosegregation would have been higher had we included the contributions from the likely affected heterozygotes (AI-4 and AIII-2) and the unaffected noncarrier members (AII-2 and AIII-3) in family A. Per ACMG-AMP guidelines, additional supportive evidence confirming pathogenicity of this variant includes the following (Figure 2b): rarity of this variant in MyCode and its nonexistence in control populations like gnomAD; prevalence of the variant in affected individuals is significantly higher than in unaffected individuals; variant is in a hotspot of the gene that is highly conserved; and in silico evidence suggests a deleterious effect on the gene product.

DISCUSSION

In an effort to streamline the genetic diagnosis for a kidney transplant candidate, we present a framework to prioritize rare VUS results from clinical genetic testing for kidney disease developed through efforts from a multidisciplinary team (Figure 4). The standard workflow following receipt of a rare VUS result consistent with the clinical phenotype should include literature review, examination of reference databases (e.g., ClinVar, LOVD, gnomAD), and if other affected family members exist, additional deep phenotyping (e.g., FEMg),[11,12] and family testing to test for cosegregation. Consultation with a ClinGen curation panel or experts in the gene of interest should be considered. If resources allow, additional research from other sources may be
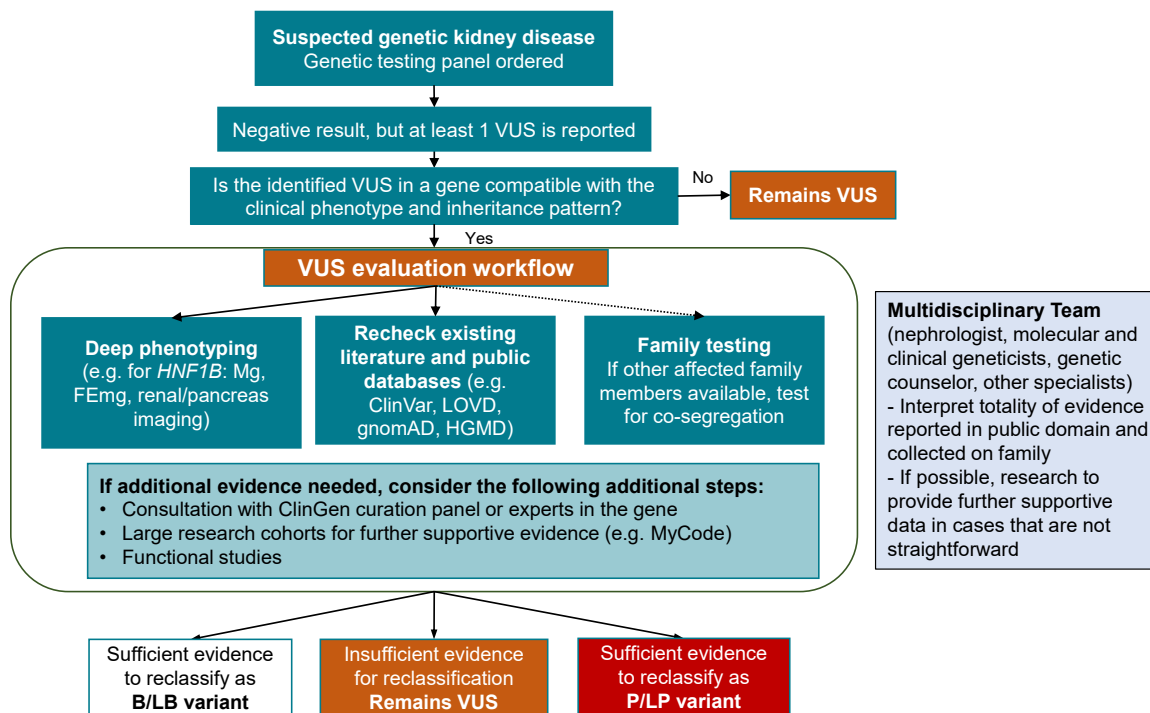
Figure 4. Proposed framework for transitioning a VUS from clinical genetic testing to a more definitive classification. The case presented in this study provided evidence for a framework that can be utilized to prioritize rare VUS results from clinical genetic testing for other kidney diseases. In the study, clinical genetic testing returned multiple VUS for a kidney transplant candidate and her family members. Identifying the VUS and other known pathogenic variants of that gene in the MyCode database and deep phenotyping of individuals with the VUS compared to noncarriers narrowed the VUS pool to *HNF1B*-p.Arg303His. Studies of variant effect on protein function, additional clinical workup (Mg wasting), and studies of variant cosegregation with disease traits provide further supporting evidence of pathogenicity. gnomAD, genome aggregation database; HGMD, human gene database; LOVD, Leiden Open Variation Database; P/LP, pathogenic/likely pathogenic, VUS, variant of unknown significance.

helpful to provide supportive evidence. In this case, we collaborated with colleagues who performed *in vitro* functional testing, albeit with inconclusive results. We also interrogated a large research cohort (MyCode) to compare phenotypic traits of *HNF1B* Arg303His heterozygotes with individuals with 17q12 microdeletion and noncarriers to demonstrate consistency in supporting pathogenicity. It should be noted that these types of associative analyses do not explicitly fulfill ACMG criteria. Regardless, demonstration of lower magnesium levels and lower eGFR in the heterozygotes compared to noncarriers provided additional supportive evidence to fulfill the ACMG level of pathogenicity support criteria, hence, reclassification of the variant from VUS to likely pathologic.

Using data from our research population cohort, we show that *HNF1B*-p.Arg303His heterozygotes had eGFR and serum magnesium levels that were comparable or lower than those observed in individuals with 17q12 microdeletion. Even though the proband's sister does not currently have CKD as defined as eGFR < 60 ml/min per 1.73 m^2, her eGFR measured twice at age 21 (less than the first percentile for her age and sex), and her serum magnesium level at age 21 were significantly lower than noncarriers of pathogenic *HNF1B* variants (mean [95%

confidence interval] 1.8 mg/dl vs. 2.3 [2.1, 2.5], $n = 174$ individuals measured at age 21 years old in noncarriers). Notably, we observed that 3 of the 4 *HNF1B*-p.Arg303His cases had elevated serum lipase compared to individuals with 17q12 microdeletion and noncarriers of pathogenic *HNF1B* variants. With *HNF1B* as the candidate gene, we further confirmed that the proband and her mother had hypermagnesuria (FEMg) as observed with *HNF1B* extrarenal abnormalities.[11,34]

Our index case and 4 other family members had multiple VUS returned from clinical genetic testing. There were only 2 VUS that were shared among the family members, namely *PTH1R*-p.Ala72Val and *HNF1B*-p.Arg303His, but the phenotype of tubulointerstitial kidney disease, hypomagnesemia, and pancreas dysfunction was consistent only with *HNF1B*. Typical signs of *PTH1R* mutations such as Murk Jansen type of metaphyseal chondrodysplasia, characterized by abnormal height, hypercalcemia, bone deformities, and renal calcification, were absent,[35] and the *PTH1R* p.Ala72Val was also harbored by the proband's asymptomatic cousin (case AIII-3).

Our study exemplifies the important role of large, unselected cohorts with robust electronic health records data to provide corroborating evidence of clinical

traits for the gene-disease pair associated with rare VUS. It is important to note that variable penetrance and clinical phenotype of monogenic disorders (e.g., *HNF1B*) can make determination of pathogenicity more challenging, and large cohorts can be very useful to provide confidence in pathogenicity (Mirshahi2022-medrxiv). In a disease with high heterogeneity even within the same family, the presence of similar clinical spectrum between the proband, her family members, the MyCode participant with *HNF1B*-p.Arg303His, and an individual in ClinVar is highly supportive of this variant being causal for her CKD. Indeed, we observed renal and extrarenal features in all 7 individuals from clinical data (family A and family C, ClinVar individual with scant data) as well as the participant from the MyCode research study (family B) who all had *HNF1B*-p.Arg303His in common. Reports of other renal abnormalities in individuals with *HNF1B*-p.Arg303Ser and p.Arg303Cys lend support that the arginine residue at this locus is important in *HNF1B* function. The Grantham's distance which predicts the dissimilarity of amino acid substitutions by composition, polarity, and molecular volume for arginine to histidine (29) < arginine to serine (110) < arginine to cysteine (180) suggesting that the cysteine and serine substitutions create a greater physicochemical difference than the histidine.[36] Further, the arginine 303 is in the POU_H domain and is conserved in multiple species including human, mouse, rat, frog, and zebrafish (Supplementary Figure S2).

PKHD1 and *FXYD2* are known transcriptional targets of HNF1B, and disturbed transcription of these genes may cause kidney malformation and hypomagnesemia, respectively.[37–39] Our luciferase reporter experiments using wildtype HNF1B and HNF1B-p.Arg303His showed similar transactivation of the *PKHD1* and *FXYD2* promoters. Nevertheless, absence of an effect by the mutant on transactivation effect does not exclude pathogenicity. For example, HNF1B-p.Val61Gly showed comparable transactivation potential to wildtype HNF1B in a luciferase reporter assay,[40] even though this variant was observed in 3 children with *HNF1B*-related disorders as follows: a child with a single ovary, a single kidney, and a hemi-uterus;[7] a child with prune belly syndrome and congenital genitourinary malformation;[40] and a child with multicystic dysplastic kidney.[41] Mild to no alterations in *in vitro* studies were also observed in another homeodomain variant, *HNF1B*-p.Arg295His; this variant was observed in a family of multiple kidney and pancreatic anomalies.[42,43] It was previously shown that the C-terminal domain of HNF1B and coactivators modify histone acetylase activity;[38] therefore, mutations of *HNF1B* may reduce histone acetylation on target promoters.[44] The absent or mild effect of mutants on luciferase activity may be due to

transient nature of the expression of *HNF1B* in the assay as well as differences in chromatin state compared to the more complex *in vivo* situation.[45]

Our study is not without limitations. Whereas clinical features of *HNF1B* were observed in the proband's grandmother and grand-aunt, we lacked genetic testing on these 2 individuals.[9] In addition, we cannot exclude the presence of unknown pathogenic structural variants in the intronic regions not covered on the gene panel. Regardless, the clinical spectrum observed in the 4 Arg303His families, the Arg303Ser family, and the Arg303Cys patient provides robust evidence that this locus does not tolerate these amino acid changes. Given the rarity of the variant (only 1 unrelated individual in MyCode), we could not conduct formal case-control analyses that are used in ACMG classifications. Future large scale collaborations are needed to improve the power to conduct formal case-control statistical comparisons for rare variants.

Despite the high utility of genetic testing in CKD, upwards of 10% to 100% of tests returned a VUS in part because of the painstaking work of gathering and adjudicating evidence to determine the pathogenicity.[3,18–20,46] Variant classification for CKD-associated variants is disproportionately affected because very few CKD genes are recommended as returnable in incidental findings by the ACMG-AMP guidelines compared to cardiac disease or cancer genes.[47] A VUS presents an ethical challenge to report to patients and their families due to insurance liability, genetic counseling availability, and the concern that the disease causality of these variants can be overstated leading to unnecessary stress.[48] In families with genetic kidney disease, resolution of VUS is particularly important because detection of genetic kidney disease in family members can not only allow for appropriate precision management but also help avoid a situation where an asymptomatic family member with a genetic pathogenic variant donate a kidney and then later develops ESKD.[49] Similarly, an unresolved VUS could delay family member carriers from serving as potential kidney donors if the variant is later unequivocally determined benign.

In conclusion, we present a VUS observed in *HNF1B* from clinical genetic testing of a kidney transplant candidate and 2 affected family members. Literature search alone showed that variations at this locus led to kidney disease phenotype consistent with the *HNF1B* disease spectrum; however, there was not enough evidence for pathogenicity. Using a multidisciplinary approach, we propose a multistage process in evaluating VUS. Altogether, the data support the pathogenicity of the *HNF1B*-p.Arg303His variant, providing a genetic diagnosis for the proband, her mother, and her sister. The utility of this model can be applied to other genetic diseases as genetic testing become more routine.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF).

Supplementary References.

Regeneron Genetics Center Banner Author List and Contribution Statements.

Figure S1. In vitro experiments showed mildly altered, differential transactivation potential of HNF1B Arg303His.

Figure S2. The Arg303 is conserved in the Pit-1Oct-1/2-UNC-86 homeodomain of HNF1B.

Table S1. Rare variants among members of family A.

REFERENCES

1. Westland R, Renkema KY, Knoers N. Clinical integration of genome diagnostics for congenital anomalies of the kidney and urinary tract. *Clin J Am Soc Nephrol*. 2020;16:128–137. https://doi.org/10.2215/CJN.14661119

2. Groopman EE, Marasa M, Cameron-Christie S, et al. Diagnostic utility of exome sequencing for kidney disease. *N Engl J Med*. 2019;380:142–151. https://doi.org/10.1056/NEJMoa1806891

3. Connaughton DM, Kennedy C, Shril S, et al. Monogenic causes of chronic kidney disease in adults. *Kidney Int*. 2019;95:914–928. https://doi.org/10.1016/j.kint.2018.10.031

4. Mann N, Braun DA, Amann K, et al. Whole-exome sequencing enables a precision medicine approach for kidney transplant recipients. *J Am Soc Nephrol*. 2019;30:201–215. https://doi.org/10.1681/ASN.2018060575

5. Knoers N, Antignac C, Bergmann C, et al. Genetic testing in the diagnosis of chronic kidney disease: recommendations for clinical practice. *Nephrol Dial Transplant*. 2022;37:239–254. https://doi.org/10.1093/ndt/gfab218

6. Jayasinghe K, Stark Z, Kerr PG, et al. Clinical impact of genomic testing in patients with suspected monogenic kidney disease. *Genet Med*. 2021;23:183–191. https://doi.org/10.1038/s41436-020-00963-4

7. Edghill EL, Bingham C, Ellard S, Hattersley AT. Mutations in hepatocyte nuclear factor-1beta and their related phenotypes. *J Med Genet*. 2006;43:84–90. https://doi.org/10.1136/jmg.2005.032854

8. Ulinski T, Lescure S, Beaufils S, et al. Renal phenotypes related to hepatocyte nuclear factor-1beta (TCF2) mutations in a pediatric cohort. *J Am Soc Nephrol*. 2006;17:497–503. https://doi.org/10.1681/ASN.2005101040

9. Clissold RL, Hamilton AJ, Hattersley AT, et al. HNF1B-associated renal and extra-renal disease-an expanding clinical spectrum. *Nat Rev Nephrol*. 2015;11:102–112. https://doi.org/10.1038/nrneph.2014.232

10. Woolf AS, Johnstone KA, Wirsing A, et al. HNF1B mutations associate with hypomagnesemia and renal magnesium wasting. *J Am Soc Nephrol*. 2009;20:1123–1131. https://doi.org/10.1681/ASN.2008060633

11. van der Made CI, Hoorn EJ, de la Faille R, et al. Hypomagnesemia as first clinical manifestation of ADTKD-HNF1B: a case series and literature review. *Am J Nephrol*. 2015;42:85–90. https://doi.org/10.1159/000439286

12. Raaijmakers A, Corveleyn A, Devriendt K, et al. Criteria for HNF1B analysis in patients with congenital abnormalities of kidney and urinary tract. *Nephrol Dial Transplant*. 2015;30:835–842. https://doi.org/10.1093/ndt/gfu370

13. Mallett AJ, McCarthy HJ, Ho G, et al. Massively parallel sequencing and targeted exomes in familial kidney disease can diagnose underlying genetic disorders. *Kidney Int*. 2017;92:1493–1506. https://doi.org/10.1016/j.kint.2017.06.013

14. Rao J, Liu X, Mao J, et al. Genetic spectrum of renal disease for 1001 Chinese children based on a multicenter registration system. *Clin Genet*. 2019;96:402–410. https://doi.org/10.1111/cge.13606

15. Eckardt KU, Alper SL, Antignac C, et al. Autosomal dominant tubulointerstitial kidney disease: diagnosis, classification, and management–a KDIGO consensus report. *Kidney Int*. 2015;88:676–683. https://doi.org/10.1038/ki.2015.28

16. Cocchi E, Nestor JG, Gharavi AG. Clinical genetic screening in adult patients with kidney disease. *Clin J Am Soc Nephrol*. 2020;15:1497–1510. https://doi.org/10.2215/CJN.15141219

17. Phillips KA, Deverka PA, Hooker GW, Douglas MP. Genetic test availability and spending: where are we now? Where are we going? *Health Aff (Millwood)*. 2018;37:710–716. https://doi.org/10.1377/hlthaff.2017.1427

18. Elhassan EAE, Murray SL, Connaughton DM, et al. The utility of a genetic kidney disease clinic employing a broad range of genomic testing platforms: experience of the Irish Kidney Gene Project. *J Nephrol*. 2022. https://doi.org/10.1007/s40620-021-01236-2

19. Thomas CP, Freese ME, Ounda A, et al. Initial experience from a renal genetics clinic demonstrates a distinct role in patient management. *Genet Med*. 2020;22:1025–1035. https://doi.org/10.1038/s41436-020-0772-y

20. Lieberman KV, Chang AR, Block GA, et al. The KIDNEYCODE program: diagnostic yield and clinical features of individuals with chronic kidney disease. *Kidney360*. Forthcoming 2022. https://doi.org/10.34067/KID.0004162021

21. Hay E, Cullup T, Barnicoat A. A practical approach to the genomics of kidney disorders. *Pediatr Nephrol*. 2022;37:21–35. https://doi.org/10.1007/s00467-021-04995-z

22. Carey DJ, Fetterolf SN, Davis FD, et al. The Geisinger MyCode community health initiative: an electronic health record-linked biobank for precision medicine research. *Genet Med*. 2016;18:906–913. https://doi.org/10.1038/gim.2015.187

23. Mirshahi UL, Luo JZ, Manickam K, et al. Trajectory of exonic variant discovery in a large clinical population: implications for variant curation. *Genet Med*. 2019;21:1417–1424. https://doi.org/10.1038/s41436-018-0353-5

24. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17:405–424. https://doi.org/10.1038/gim.2015.30

25. Jarvik GP, Browning BL. Consideration of cosegregation in the pathogenicity classification of genomic variants. *Am J Hum Genet*. 2016;98:1077–1081. https://doi.org/10.1016/j.ajhg.2016.04.003

26. Packer JS, Maxwell EK, O'Dushlaine C, et al. CLAMMS: a scalable algorithm for calling common and rare copy number variants from exome sequencing data. *Bioinformatics*. 2016;32:133–135. https://doi.org/10.1093/bioinformatics/btv547

27. Fortier N, Rudy G, Scherer A. Detection of CNVs in NGS data using VS-CNV. *Methods Mol Biol*. 2018;1833:115–127. https://doi.org/10.1007/978-1-4939-8666-8_9

28. Landrum MJ, Lee JM, Benson M, et al. ClinVar: improving access to variant interpretations and supporting evidence. *Nucleic Acids Res*. 2018;46:D1062–D1067. https://doi.org/10.1093/nar/gkx1153

29. Kompatscher A, de Baaij JHF, Aboudehen K, et al. Loss of transcriptional activation of the potassium channel Kir5.1 by HNF1β drives autosomal dominant tubulointerstitial kidney disease. *Kidney Int*. 2017;92:1145–1156. https://doi.org/10.1016/j.kint.2017.03.034

30. Schwartz GJ, Muñoz A, Schneider MF, et al. New equations to estimate GFR in children with CKD. *J Am Soc Nephrol*. 2009;20:629–637. https://doi.org/10.1681/ASN.2008030287

31. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med*. 2009;150:604–612. https://doi.org/10.7326/0003-4819-150-9-200905050-00006

32. Snoek R, Nguyen TQ, van der Zwaag B, et al. Importance of genetic diagnostics in adult-onset focal segmental glomerulosclerosis. *Nephron*. 2019;142:351–358. https://doi.org/10.1159/000499937

33. F. Lalève, S. Decramer, L. Heidet, et al., School level of children carrying a HNF1B variant or a deletion, *Eur J Hum Genet*, 28, 2020, 56–63.

34. Hayes WN, Bryant WA, Booth J, et al. HNF1B mutations are associated with a Gitelman-like tubulopathy that develops during childhood. *Kidney Int Rep*. 2019;4:1304–1311. https://doi.org/10.1016/j.ekir.2019.05.019

35. Saito H, Noda H, Gatault P, et al. Progression of mineral ion abnormalities in patients with Jansen metaphyseal chondrodysplasia. *J Clin Endocrinol Metab*. 2018;103:2660–2669. https://doi.org/10.1210/jc.2018-00332

36. Grantham R. Amino acid difference formula to help explain protein evolution. *Science*. 1974;185:862–864. https://doi.org/10.1126/science.185.4154.862

37. Ferrè S, de Baaij JH, Ferreira P, et al. Mutations in PCBD1 cause hypomagnesemia and renal magnesium wasting. *J Am Soc Nephrol*. 2014;25:574–586. https://doi.org/10.1681/ASN.2013040337

38. Hiesberger T, Shao X, Gourley E, et al. Role of the hepatocyte nuclear factor-1beta (HNF-1beta) C-terminal domain in Pkhd1 (ARPKD) gene transcription and renal cystogenesis. *J Biol Chem*. 2005;280:10578–10586. https://doi.org/10.1074/jbc.M414121200

39. Ferrè S, Veenstra GJ, Bouwmeester R, et al. HNF-1B specifically regulates the transcription of the γa-subunit of the Na+/K+-ATPase. *Biochem Biophys Res Commun*. 2011;404:284–290. https://doi.org/10.1016/j.bbrc.2010.11.108

40. Granberg CF, Harrison SM, Dajusta D, et al. Genetic basis of prune belly syndrome: screening for HNF1β gene. *J Urol*. 2012;187:272–278. https://doi.org/10.1016/j.juro.2011.09.036

41. Hoskins BE, Cramer CH 2nd, Tasic V, et al. Missense mutations in EYA1 and TCF2 are a rare cause of urinary tract malformations. *Nephrol Dial Transplant*. 2008;23:777–779. https://doi.org/10.1093/ndt/gfm685

42. Bohn S, Thomas H, Turan G, et al. Distinct molecular and morphogenetic properties of mutations in the human HNF1beta gene that lead to defective kidney development. *J Am Soc Nephrol*. 2003;14:2033–2041. https://doi.org/10.1097/01.asn.0000078808.70309.c4

43. Barbacci E, Chalkiadaki A, Masdeu C, et al. HNF1beta/TCF2 mutations impair transactivation potential through altered co-regulator recruitment. *Hum Mol Genet*. 2004;13:3139–3149. https://doi.org/10.1093/hmg/ddh338

44. Gong Y, Ma Z, Patel V, et al. HNF-1beta regulates transcription of the PKD modifier gene Kif12. *J Am Soc Nephrol*. 2009;20:41–47. https://doi.org/10.1681/ASN.2008020238

45. Inoue F, Kircher M, Martin B, et al. A systematic comparison reveals substantial differences in chromosomal versus episomal encoding of enhancer activity. *Genome Res*. 2017;27:38–52. https://doi.org/10.1101/gr.212092.116

46. Bleyer AJ, Westemeyer M, Xie J, et al. Genetic etiologies for chronic kidney disease revealed through next-generation renal gene panel. *Am J Nephrol*. 2022;53:297–306. https://doi.org/10.1159/000522226

47. Green RC, Berg JS, Grody WW, et al. ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. *Genet Med*. 2013;15:565–574. https://doi.org/10.1038/gim.2013.73

48. Savatt JM, Myers SM. Genetic testing in neurodevelopmental disorders. *Front Pediatr*. 2021;9:526779. https://doi.org/10.3389/fped.2021.526779

49. Singh G, Gohh R, Clark D, et al. Vignette-based reflections to inform genetic testing policies in living kidney donors. *Genes (Basel)*. 2022;13:592. https://doi.org/10.3390/genes13040592