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

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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.

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Monogenic causes account for up to 40% of patients in cohorts enriched with ESKD or familial kidney disease.¹⁴ 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. 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.

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. 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). 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. 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.

MPS has become increasingly available and more affordable in clinical practice. 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. VUS present a diagnostic and ethical challenge in genetic testing and lack of resolution may result in delays in treatment and management. 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. 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, 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, 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. HNF1B copy number loss were called from a referenced-based CLAMMS algorithm using exome sequencing, 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. 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, 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 glomeruliferation 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, and for those aged 18 years or more using the CKD-EPI equation. 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
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, PRSSI) 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/m²), 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/m², 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 m² 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-
2, had eGFR levels less than first percentile for her age or sex ranging 63 and 67 ml/min per 1.73 m² 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 p.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. 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.
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.

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.
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. 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, 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.
(−1.7 ml/min per 1.73 m²/year [95% confidence interval −1.9, −1.4]) vs. noncarriers (−0.928 ml/min per 1.73 m²/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 showed elevations in lipase whereas 17q12 microdeletion cases have been observed in only 3 other individuals to date. Laliève et al. 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.

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. Laliève et al. 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). 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 AI-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 = 1/2 for each affected heterozygote, resulting in Ntotal = 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), 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
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 m², 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.37,38

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 (Mirshahi 2022-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. Further, the arginine 303 is in the POUH 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. 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, 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; a child with prune belly syndrome and congenital genital-urinary malformation; and a child with multicystic dysplastic kidney. 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. It was previously shown that the C-terminal domain of HNF1B and coactivators modify histone acetylase activity; therefore, mutations of HNF1B may reduce histone acetylation on target promoters. 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.

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. 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. 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. 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. 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. Similarly, an unresolved VUS could delay family member carriers from serving as potential kidney donors if the variant is late 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.
DISCLOSURE

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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.

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