The Value of Genetic Testing in Polycystic Kidney Diseases Illustrated by a Family With *PKD2* and *COL4A1* Mutations

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The diagnosis of autosomal dominant polycystic kidney disease (ADPKD) relies on imaging criteria in the setting of a positive familial history. Molecular analysis, seldom used in clinical practice, identifies a causative mutation in >90% of cases in the genes *PKD1*, *PKD2,* or rarely *GANAB*. We report the clinical and genetic dissection of a 7-generation pedigree, resulting in the diagnosis of 2 different cystic disorders. Using targeted next-generation sequencing of 65 candidate genes in a patient with an ADPKD-like phenotype who lacked the familial *PKD2* mutation, we identified a *COL4A1* mutation (p.Gln247*) and made the diagnosis of HANAC (hereditary angiopathy with nephropathy, aneurysms, and muscle cramps) syndrome. While 4 individuals had ADPKD-*PKD2*, various *COL4A1*-related phenotypes were identified in 5 patients, and 3 individuals with likely digenic *PKD2*/*COL4A1* disease reached end-stage renal disease at around 50 years of age, significantly earlier than observed for either monogenic disorder. Thus, using targeted next-generation sequencing as part of the diagnostic approach in patients with cystic diseases provides differential diagnoses and identifies factors underlying disease variability. As specific therapies are rapidly developing for ADPKD, a precise etiologic diagnosis should be paramount for inclusion in therapeutic trials and optimal patient management.

Complete author and article information (including a list of HALT Progression of Polycystic Kidney Disease Group Investigators) provided before references.

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Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is characterized by progressive bilateral cyst development with highly variable kidney disease.[1] *PKD1* and *PKD2* mutations are, respectively, identified in ~78% and ~15% of the pedigrees,[2,3] with mutation of a third gene, *GANAB*, occurring rarely (~0.3%). The genetic lesion in ~7% remains unresolved.[4,5] Genetic variability strongly influences the severity of ADPKD, with median age at end-stage kidney disease of 58 years in individuals with *PKD1* truncating mutations, about 67 years for those with *PKD1* nontruncating mutations, and approximately 79 years in those with mutations in *PKD2*.[6] An ADPKD diagnosis is presently based on the conjunction of age-dependent imaging criteria with a positive familial history; molecular testing is rarely used.[6-9] However, the phenotypes associated with mutations in several genes can occasionally mimic ADPKD: *PKHD1*, causing autosomal recessive polycystic kidney disease (ARPKD); *HNF1B*, autosomal dominant tubulointerstitial disease (ADTKD-*HNF1B*); the tuberous sclerosis genes *TSC1* and *TSC2*; and the autosomal dominant polycystic liver disease (ADPLD) genes (*SEC63*,

Figure 1. Molecular and imaging data for pedigree M625 with autosomal dominant polycystic kidney disease (ADPKD)-*PKD2* and *COL4A1*-associated diseases. (A) Disease segregation in pedigree M625. Black symbols denote ADPKD-*PKD2*–affected individuals; blue symbols, patients with *COL4A1*-associated diseases; and gray shading, unknown status of individuals. Blue and black symbols are used in individuals with suspected digenic *COL4A1*-*PKD2* disease. Slash over symbols denotes death. Genetic results were not available for III.2, IV.1, and IV.3, but their status could be inferred from their medical records (IV.1 and IV.3) and/or from the dominant inheritance pattern of both diseases (III.2, IV.1, and IV.3; inferred genotype within parentheses). II.1, who died at age 63 years, had a positive familial history of ADPKD (ADPKD-affected descendants of II.4 are represented on the pedigree and live in Europe, but no further information is available). Whether he also carried the *COL4A1* variant is uncertain. III.2 died at age 46 years from uremia; she was likely carrying both variants that she passed on to IV.1 and IV.3; for her husband (III.1), who died at age 72 years, no relevant medical history was mentioned. (B) IGV (integrative genomics viewer; Broad Institute) view of the next-generation sequencing of V.5 shows *COL4A1* variant c.739C>T (p.Gln247*; reverse strand). Read depth: 2,822, C=49%, T=51%. (C) Sanger sequencing confirmation of heterozygous *COL4A1* variant c.739C>T (p.Gln247*) in V.5 and *PKD2* variant c.715_718dupTACG in VI.3. Wild-type sequences are shown for comparison. Magnetic resonance (MR) image, T2 weighted, of (D) COL4A1 individual V.5, age 61 years, shows bilateral renal cysts without kidney enlargement (height-adjusted total kidney volume [HtTKV] = 200 mL/m); (E) PKD2 patient VI.1 at age 53 years, shows enlarged polycystic kidneys (HtTKV = 1,953 mL/m); (F) PKD2 patient VI.2, who has moderately enlarged polycystic kidneys at age 46 years (HtTKV = 470 mL/m); and (G) COL4A1 individual VI.4, who has nonenlarged kidneys at age 35 years, with 6 cysts in the left kidney (the largest shown here measures 1.6 cm) and 2-mm sized cysts in the right kidney. Cerebral MR image of (H) COL4A1 individual V.5, axial FLAIR (fluid-attenuated inversion recovery) sequence shows bilateral areas of signal hyperintensity in the central semiovale (arrows) and (I) COL4A1 individual VII.1 (4 years), sagittal T1-weighted sequence shows marked volume loss in the superior cerebellar vermis (arrows).

PRKCSH, *LRP5*, *ALG8*, and *SEC61B*).[6] Mutations to *COL4A1* can also cause bilateral renal cysts and decline in kidney function after 50 years, as part of HANAC (hereditary angiopathy with nephropathy, aneurysms, and muscle cramps) syndrome.[10,11] We report how genetic testing of a multigenerational "ADPKD" pedigree explains the marked intrafamilial variability due to finding 2 distinct genetic disorders.

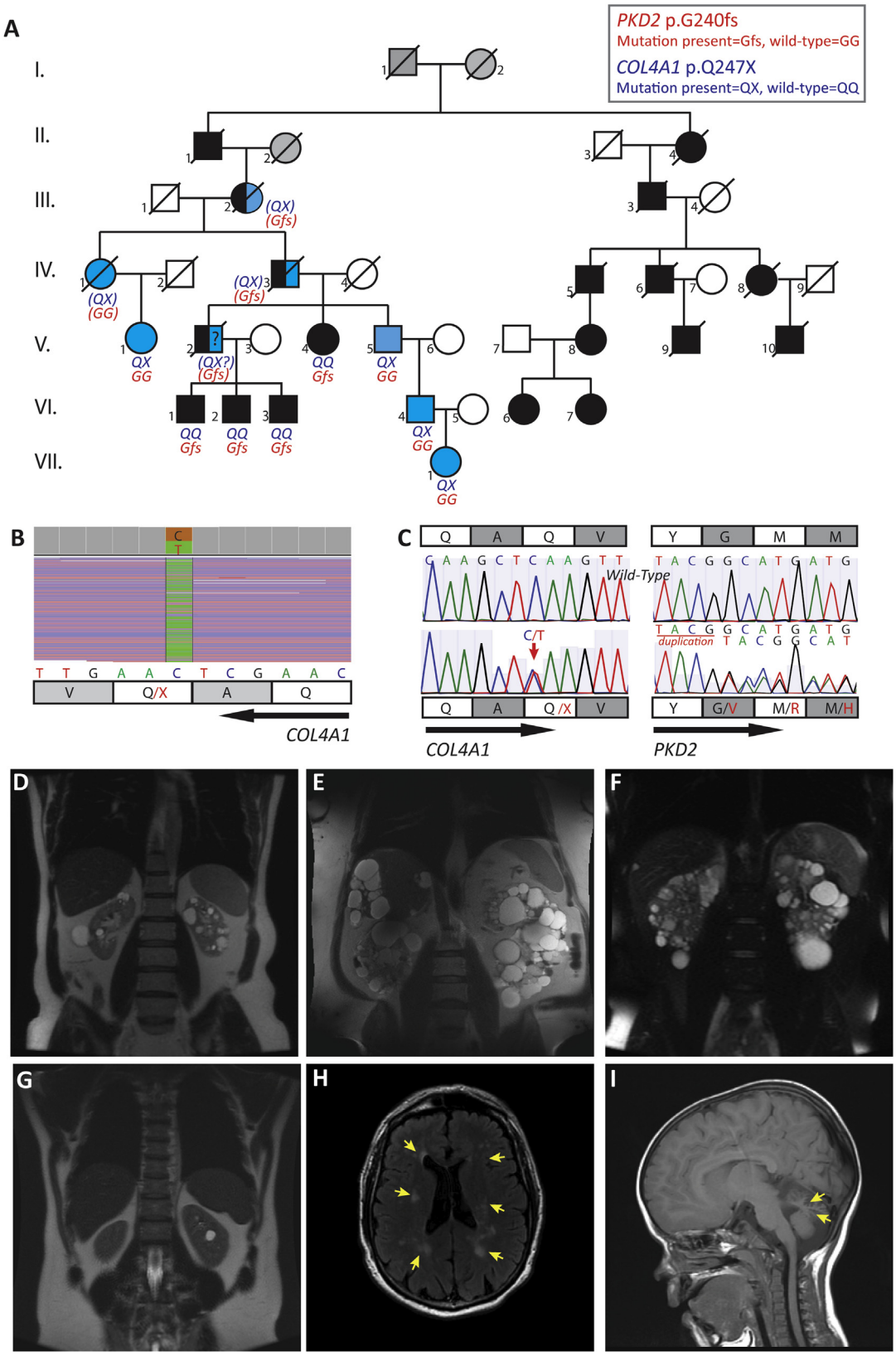

Table 1. Clinical Presentations and Genetic Analyses of 12 Affected Family Members

Pt	Sex	eGFR[a]	HTN[b]	Microscopic Hematuria[b]	Proteinuria[b,c]	Kidney Morphology	Myalgia[b]	Elevated CK[b,d]	CNS Involvement	Cause of Death	Other Conditions[b]	Genetic Results[e]	Diagnosis
IV.1	F	ESRD (71 y)	Y (50 y)	Y (70 y)	Y (70 y)	US at 71 y: atrophic RK (6.6 cm), 2 cysts in LK, 5 in RK	NA	NA	CSVD (CT, 71 y), TIA (36 y), recurrent ischemic strokes (70 y)	Stroke in 1995 (79 y)	NA	NA	HANAC-like syndrome[f]
V.1	F	33 (67 y)	N (67 y)	Y (25 y)	Y (62 y): 0.7	US at 59 y: atrophic RK (7.7 cm), LK (9.3 cm), 2 cysts	Y (NA)	NA	Vertigo (64 y), normal non-enhanced CT (67 y)	—	Hypothyroidism (21 y), gout (67 y)	*COL4A1:* Q247* *PKD2:* WT	HANAC-like syndrome
V.5	M	47 (67 y)	Y (57 y)	Y (14 y)	N (66 y)	MRI at 61 y: >15 cysts/kidney, no liver cyst, HtTKV[g] = 200	Y (65 y)	NA	Migraines, dizzy spells since 63 y; CSVD (MRI 58 y)	—	Gout (52 y), DM (65 y), carotid endarterectomy (57)	*COL4A1:* Q247* *PKD2:* WT	HANAC-like syndrome
VI.4	M	112 (32 y)	N (35 y)	Y (35 y)	N (35 y)	MRI at 35 y: 6 cysts in LK, 2 in RK	Y (32 y)	Y (32 y): 527	Migraines since 30 y, normal enhanced CT (32 y)	—	None	*COL4A1:* Q247*	HANAC-like syndrome
VII.1	F	129 (3 y)	N (4 y)	Y (3 y)	N (3 y)	NA	NA	NA	Global developmental delay, hypotonia, absence epilepsy, MRI: thin cerebellar folia (4 y)	—	None	*COL4A1:* Q247*	*COL4A1*-related CNS disorder
III.2	F	ESRD (48 y)	NA			NA	NA	NA	NA	ESRD (46 y)	NA	NA	Likely *PKD2/COL4A1*[e]
IV.3	M	ESRD (51 y)	N (53 y)	Y (50 y)	Y (50 y)	Pyelography: enlarged kidneys, numerous renal cysts	Y (50 y)	NA	Migraines (50 y)	ESRD (51 y)	Tortuosity of the retinal arteries (50 y), Raynaud phenomena (50 y)	NA	Likely *PKD2/COL4A1*[f]
V.2	M	ESRD (51 y)	Y (NA)	NA	NA	Nonenhanced CT at 56 y: enlarged polycystic kidneys, polycystic liver	Y (56 y)	Y (55 y): 206	Normal nonenhanced CT (56 y)	ID (57 y)	Gout (<50 y), hearing loss (56 y), aseptic necrosis of femoral head (56 y), spontaneous cecum perforation (56 y)	NA	ADPKD-PKD2[f]; possible *PKD2/COL4A1*

(Continued)

Table 1 (Cont'd). Clinical Presentations and Genetic Analyses of 12 Affected Family Members

Pt	Sex	eGFR[a]	HTN[b]	Microscopic Hematuria[b]	Proteinuria[b,c]	Kidney Morphology	Myalgia[b]	Elevated CK[b,d]	CNS Involvement	Cause of Death	Other Conditions[b]	Genetic Results[e]	Diagnosis
V.4	F	30 (66 y)	N (59 y)	NA	N (59 y)	US: enlarged polycystic kidneys, polycystic liver	N	NA	None	—	None	*PKD2:* c.715_718dupTACG *COL4A1*: WT	ADPKD-PKD2
VI.1	M	21 (54 y)	Y (25 y)	N (55 y)	Y (53 y): 0.8	MRI at 53 y: enlarged polycystic kidneys, HtTKV[g]=1,953	N	NA	None	—	T2DM (52 y); obesity, with BMI 40 kg/m^2	*PKD2:* c.715_718dupTACG *COL4A1*: WT	ADPKD-PKD2
VI.2	M	56 (50 y)	Y (41 y)	NA	N (50 y)	MRI at 46 y: enlarged polycystic kidneys, HtTKV[g]=470	N	NA	None	—	None	*PKD2:* c.715_718dupTACG *COL4A1*: WT	ADPKD-PKD2
VI.3	M	36 (47 y)	Y (24 y)	N (47 y)	Y (34 y)	US at 31 y: LK 14.5 cm, RK 13.3 cm, >15 cysts/kidney	Y (47 y)	Y, under statins (33 y)	None	—	T1DM (11 y), MN (31 y)	*PKD2:* c.715_718dupTACG *COL4A1*: WT	ADPKD-PKD2

Abbreviations and definitions: ADPKD, autosomal dominant polycystic kidney disease; BMI, body mass index; CK, creatine kinase; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; CNS, central nervous system; CSVD, cerebral small-vessel disease; CT, computed tomography; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; HANAC, hereditary angiopathy with nephropathy, aneurysms, and muscle cramps; HTN, hypertension; HtTKV, height-adjusted total kidney volume; ID, infectious disease; LK, left kidney; MN, membranous nephropathy; MRI, magnetic resonance imaging; NA, not available; pt, patient; RK, right kidney; T1(2)DM, type 1 (2) diabetes mellitus; TIA, transient ischemic attack; US, ultrasound; WT, wild-type.

[a]Expressed in mL/min/1.73 m^2 on the basis of the last data available; obtained with the CKD-EPI equation for adults and the bedside Schwartz formula for the child.

[b]Values in parentheses are age when first reported if present or when last available data available if not present.

[c]When available, proteinuria is expressed in g/g urinary creatinine.

[d]When available, expressed in IU/L.

[e]Q247*: frameshift leading to stop codon instead of glutamine at amino acid 247; c.715_718dupTACG: a duplication of the indicated 4-nucleotide sequence, predicted to lead to a frameshift at the glycine amino acid 240.

[f]The patient is likely a digenic carrier considering the familial history and the dominant inheritance of each condition.

[g]Calculated by stereology and expressed in mL/m.

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Case Report

The index case, V.5, in pedigree M625 (Fig 1A) had experienced microscopic hematuria since age 14 years and PKD was diagnosed at 21 years (Table 1). His family history was significant for ADPKD in 4 generations. At 56 years of age, he had more than 20 cysts per kidney, no liver cysts (Fig 1D), and an estimated glomerular filtration rate of 47 mL/min/1.73 m^2 by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. The patient and 3 affected relatives participated in the HALT-PKD clinical trial; VI.2 was a participant in Study A (estimated glomerular filtration rate > 60 mL/min/1.73 m^2 at enrollment) and V.4, V.5, and VI.1 were participants in Study B (estimated glomerular filtration rates of 25-60 mL/min/1.73 m^2).[12,13] Molecular analysis[3] of *PKD1* and *PKD2* led to the identification of a frameshifting variant of *PKD2* (c.715_718dupTACG [a duplication of the indicated 4-nucleotide sequence], predicted to lead to a frameshift at the glycine amino acid 240 [p.Gly240fs]) in individuals V.4, VI.1 and VI.2, but was not detected in V.5 (Fig 1A and C). As opposed to that seen in the 3 mutation-positive individuals, height-adjusted total kidney volume was normal in V.5 (Table 1; Fig 1D-F). At age 57 years, V.5 had proximal stenosis of the left carotid artery diagnosed and underwent endarterectomy. At re-evaluation 1 year later for recurrent spells of dizziness and an episode of memory loss and confusion, gadolinium-enhanced magnetic resonance imaging showed periventricular and subcortical leukoencephalopathy, consistent with chronic cerebral small-vessel disease (Fig 1H). Magnetic resonance angiography of the carotid arteries was at this time unremarkable.

We expanded the genetic analyses by rescreening individual V.5 by targeted next-generation sequencing to analyze all the exons and flanking intronic sequences of *PKD1*, *PKD2*, *PKHD1*, *GANAB*, *HNF1B*, *UMOD*, *PRKCSH*, *SEC63*, *LRP5*, *TSC1*, *TSC2*, *COL4A1*, *SEC61B*, and 52 candidate genes. Libraries were enriched using custom capture baits (SureSelect; Agilent) and paired-end sequencing of 150 base pairs was performed on an Illumina HiSeq4000. After alignment of the resulting FASTQ files to the human genome 19 (hg19) reference sequence and realignment and recalibration (using Genome Analysis Toolkit [GATK]), multisample variant calling was performed (with GATK Haplotype Caller) and variants were filtered with Variant Quality Score Recalibration. Variant mining was performed with Golden Helix SNP & Variation Suite, version 8. From this analysis, we identified a cytosine to thymine substitution at nucleotide 739 of the coding sequence (c.739C>T) in exon 13 of *COL4A1*, predicted to lead to a nonsense mutation (introduction of a premature stop codon) at the glutamine at amino acid 247 (p.Gln247*) (Fig 1B). The nucleotide variant was confirmed by Sanger sequencing (Fig 1C). The diagnosis of HANAC-like syndrome was consistent with the clinical presentation, associating longstanding microscopic hematuria, renal cysts, and cerebral small-vessel disease. The patient also reported intermittent myalgia.

We broadened the familial investigation and identified several other family members with *COL4A1*-related phenotypes. Individual V.1 and her mother IV.1 had microscopic hematuria, mild proteinuria, atrophic cystic kidneys, and decreased kidney function/kidney failure (Table 1). The latter had a transient ischemic stroke at age 36 years and recurrent strokes after 70 years. Individual VII.1, a child, had developmental delay associated with ataxia, global hypotonia, and absence epilepsy. She had moderate cerebellar hypoplasia (Fig 1I) and proved positive for the *COL4A1* mutation, likely underlying her neurologic phenotype. In her father, VI.4, HANAC-like syndrome was subsequently diagnosed; he had muscle cramps and elevated creatine kinase concentrations since age 32 years. Urinalysis revealed microhematuria, and magnetic resonance imaging of the abdomen showed bilateral kidney cysts (Fig 1G). Considering the dominant inheritance of *PKD2* and *COL4A1*-related disease, the family history, and lack of phenotype in IV.4, IV.3, who died at age 51 years from end-stage kidney disease, was an obligate carrier for the *COL4A1* mutation and likely had the *PKD2* mutation. Although retrograde pyelography showed enlarged cystic kidneys, ADPKD was considered "unlikely to be the only cause to his renal insufficiency" because he had systemic symptoms, including severe Raynaud phenomenon, migraines, and myalgia, and his kidneys were "not massively enlarged." Fundus examination showed "a curly-cue extension of many of the arterioles bilaterally, probably congenital." His mother, III.2, who died from uremia at age 46 years, was also likely carrying both the *PKD2* and the *COL4A1* mutations. Individual V.2 had ADPKD and started hemodialysis therapy at age 51 years. Medical history was also significant for myalgia with an elevated creatine kinase concentration, hearing loss, and gout. The ADPKD course was significantly more severe in V.2 than in his 3 sons and his sister, all with ADPKD-*PKD2* and negative for the *COL4A1* variant, suggesting that V.2 inherited both variants (Table 1).

Discussion

In the setting of a single family with multiple individuals fulfilling the ADPKD diagnosis criteria,[8] we describe how targeted next-generation sequencing revealed 2 separate genetic causes, ADPKD-*PKD2* and *COL4A1*, clarifying the disease heterogeneity over 6 generations.

COL4A1 and *COL4A2* encode procollagen type IV α1 and α2, which assemble to form the heterotrimeric helix α1/α1/α2 and are present in almost all basement membranes. In the kidney, α1/α1/α2 is expressed in Bowman capsule and tubular basement membranes, but replaced by the heterotrimer α3/α4/α5 in glomerular basement membranes after embryogenesis. Consistent with this expression pattern, *COL4A1*-related phenotypes encompass cerebrovascular, ophthalmologic, renal, cardiac, and muscular abnormalities.[14] Fewer than 80 *COL4A1* pedigrees have been reported to date, including 8 with the full HANAC

phenotype.[10,11,14,15] The disease presentation in the 4 HANAC-like patients reported here is consistent with these previous descriptions. Interestingly, tortuosity of the retinal arteries, a hallmark of the COL4A1 vascular phenotype, was described in this family more than 50 years ago. Unexpectedly, identifying the cause of individual V.5's renal phenotype led to an etiologic diagnosis in his 4-year-old granddaughter, affected by developmental delay and hypotonia. Simultaneous intrafamilial occurrence of these divergent phenotypes was not previously reported.[11] HANAC syndrome is classically attributed to missense mutations affecting glycine residues within the COL4A1 triple-helix domain (a dominant-negative mechanism), while rare truncating mutations (haploinsufficiency), as in our pedigree, have mostly been reported with cerebrovascular disease.[14,16,17] Of note, we identified 3 deceased individuals with suggested (although unconfirmed) digenic *PKD2*/*COL4A1* disease. All reached end-stage kidney disease at around age 50 years, significantly earlier than usually observed for either monogenic disorder, suggesting an aggravating effect of the *COL4A1* variant on the PKD2 phenotype.[6,10,14,15,17] Only a handful of digenic cystic diseases have been reported, involving *PKD1*, *PKD2*, or *HNF1B*,[18,19] but these cases are likely underdiagnosed, illustrating the value of targeted next-generation sequencing of multiple cystic genes.

As specific therapies become available for ADPKD, obtaining a precise diagnosis is a prerequisite to provide appropriate treatment. However, ADPKD imaging-based diagnostic criteria require a positive familial history, lacking in 10% to 25% of patients, and genetic diagnostics are presently rarely performed in patients with ADPKD, although they are part of the diagnostic algorithm of other kidney diseases.[6] In our study, although the proband met the imaging criteria, he was affected by a totally different condition, phenocopying ADPKD. Inclusion of cases as presented here in clinical trials, along with others with slowly progressing renal phenotypes, can reduce the likelihood of a positive outcome,[20] supporting systematic genetic screening of patients with ADPKD considered for treatment or inclusion in trials. Although the high cost of ADPKD genetic testing has long been a disincentive, improvement of the screening protocols, together with the rapid development of specific next-generation sequencing approaches, will undoubtedly increase its accessibility.[6] The routine analysis of multiple cystic genes in patients with ADPKD may eventually reveal the full genetic and phenotypic spectrum of cystic kidney diseases.

Article Information

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