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CFAP47 is Implicated in X-Linked Polycystic Kidney Disease

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Introduction: Autosomal dominant polycystic kidney disease (ADPKD) is a well-described condition in which approximately 80% of all cases have a genetic explanation; and among sporadic cases without a family history, the genetic bases remain unclear in approximately 30% of cases. This study aimed to identify genes associated with polycystic kidney disease (PKD) in patients with sporadic cystic kidney disease in which a clear genetic change was not identified in established genes.

Methods: A next-generation sequencing panel analyzed known genes related to kidney cysts in 118 sporadic cases, followed by whole-genome sequencing (WGS) on 47 unrelated individuals without identified candidate variants. Immunohistology examination was then conducted on both human kidney tissue and kidneys from CFAP47$^{-/Y}$ mice.

Results: Three male patients were found to have rare missense variants in the X-linked gene cilia and flagella-associated protein 47 (CFAP47), none of whom had a family history of the condition. CFAP47 was expressed in primary cilia of human kidney tubules, and knockout (KO) mice exhibited vacuolation of tubular cells and tubular dilation, providing evidence that CFAP47 is a causative gene involved in cyst formation.

Conclusion: This discovery of CFAP47 as a newly identified gene associated with PKD, displaying X-linked inheritance, emphasizes the need for further cases to understand the role of CFAP47 in PKD.

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ADPKD is an autosomal dominant kidney disorder marked by distinctive imaging features and is associated with mutations primarily in 2 genes, PKD1 and PKD2. Typically manifesting in adulthood, the disease is characterized by advancing cyst formation and declining kidney function. Although genetic diagnostic rates vary among studies and centers, PKD1 mutations are found in about 85% to 90% of patients with ADPKD, and PKD2 mutations in about 10% to 15%.[1] One study identified the responsible mutation in 81% of patients diagnosed with clinically typical

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ADPKD[2]; and when ADPKD was studied in a large unselected cohort, only 77% of patients diagnosed with ADPKD in their medical records were identified with variants associated with the disease.[3] In a large ADPKD cohort studied in Taiwan, pathogenic variants in PKD1 or PKD2 were detected in only 69% of families, 7% of which were due to variants in genes other than PKD1 and PKD2.[4] Collectively, these observations suggest that a subset of cases are not explained by mutations in PKD1 and PKD2.

Recent advances in genetic analysis technology have led to the identification of several new genes associated with ADPKD, including GANAB,[5] IFT140,[6] ALG5,[7] ALG9, DNAJB11, and HNF1B, collectively explaining approximately 7% cases of ADPKD.[8,9] In order to identify additional genes associated with this condition we conducted WGS on 47 unrelated patients exhibiting solitary multiple kidney cysts, where no responsible mutation was identified through a panel-based genetic screen for cystic kidney disease. We identified putatively pathogenic variants in the gene, CFAP47, encoding cilia and flagella-associated protein 47 (MIM: 301057), suggesting that it is a responsible gene underlying cystic kidney disease.

METHODS

Patients and Ethics Statement

This research received approval from the Institutional Review Board of Tokyo Medical and Dental University (approval number #G2000-081 and #M2019-324) and was conducted in accordance with the Declaration of Helsinki. All participants in the study provided written informed consent and agreed to the utilization of their DNA and kidney tissues in research aimed at identifying genetic risk variants for kidney function. In addition, all participants consented to the publication of their genetic and medical data in academic journals, provided that the data were anonymized. The patient identifications (PT or K numbers) mentioned within the text are shared exclusively within the research group and are unknown to anyone else.

This is a multicenter cohort study. From 2014 to 2020, patients with sporadic PKD were recruited from 27 Japanese institutions, including Tokyo Medical and Dental University. PKD was defined as having more than 5 cysts in each kidney on both sides, as detected by computed tomography or magnetic resonance imaging (MRI). In assessing family history, the presence or absence of cystic kidney disease in the parents and siblings was carefully determined based on their medical history, including previous illnesses and hospital visits. Adults aged ≥20 years were included in the study. Clinical data were gathered from medical records. The estimated glomerular filtration rate was calculated using the Japanese glomerular filtration rate equation.[10] Total kidney volume was calculated from computed tomography or MRI based on the volume of a modified ellipse for each kidney using the formula: volume = π / 6 × length × width × depth.[11]

Animal Experiments

All animal studies were carried out in accordance with the recommendations of the US National Institutes of Health's Guide for the Care and Use of Laboratory Animals and the guidelines for animal research of Tokyo Medical and Dental University. The study was approved by the animal ethics committee at the School of Life Sciences of Fudan University and the Animal Care and Use Committee of Tokyo Medical and Dental University (approval number: A2023-109A). The investigators took all necessary measures to minimize the pain and suffering of the animals throughout the study. All mice were maintained under standard lightning conditions (12/12 h light/dark cycle). CFAP47-KO MICE ($CFAP47^{-/Y}$) carrying frameshift mutation[12] generated by CRISPR-CAS9 technology were kindly provided by Feng Zhang laboratory in Fudan University, Shanghai, China. The strain background is C57BL/6. Adult mice (aged 40 weeks or older) were used in this study.

Detailed methods of the following items are presented in the Supplementary Methods and Supplementary References.

Genetic Analysis, Immunofluorescence Studies of Human Kidney, Histological Examination of Mouse Kidney, and Statistical Analysis

The gene lists analyzed by targeted sequencing and targeted long-read sequencing (T-LRS) are shown in Supplementary Tables S1 and S2, respectively.

RESULTS

Patient Enrollment and Comprehensive Genetic Analysis

Between 2014 and 2020, we enrolled patients with sporadic PKD from 27 multicenter sites who lacked a positive family history for PKD. A total of 118 individuals were subjected to gene panel screening, encompassing 69 genes (version 1) or 92 genes (version 2) associated with the genetic causes of cystic kidney diseases (Figure 1). The detailed panel screening results of this cohort were reported in Fujimaru T. *et al.*[13,14] Among the 118 cases, 69 (58.5%) had identified causative or candidate pathogenic variants. All cases involved mutations in either PKD1 or PKD2, with only 3 exceptions

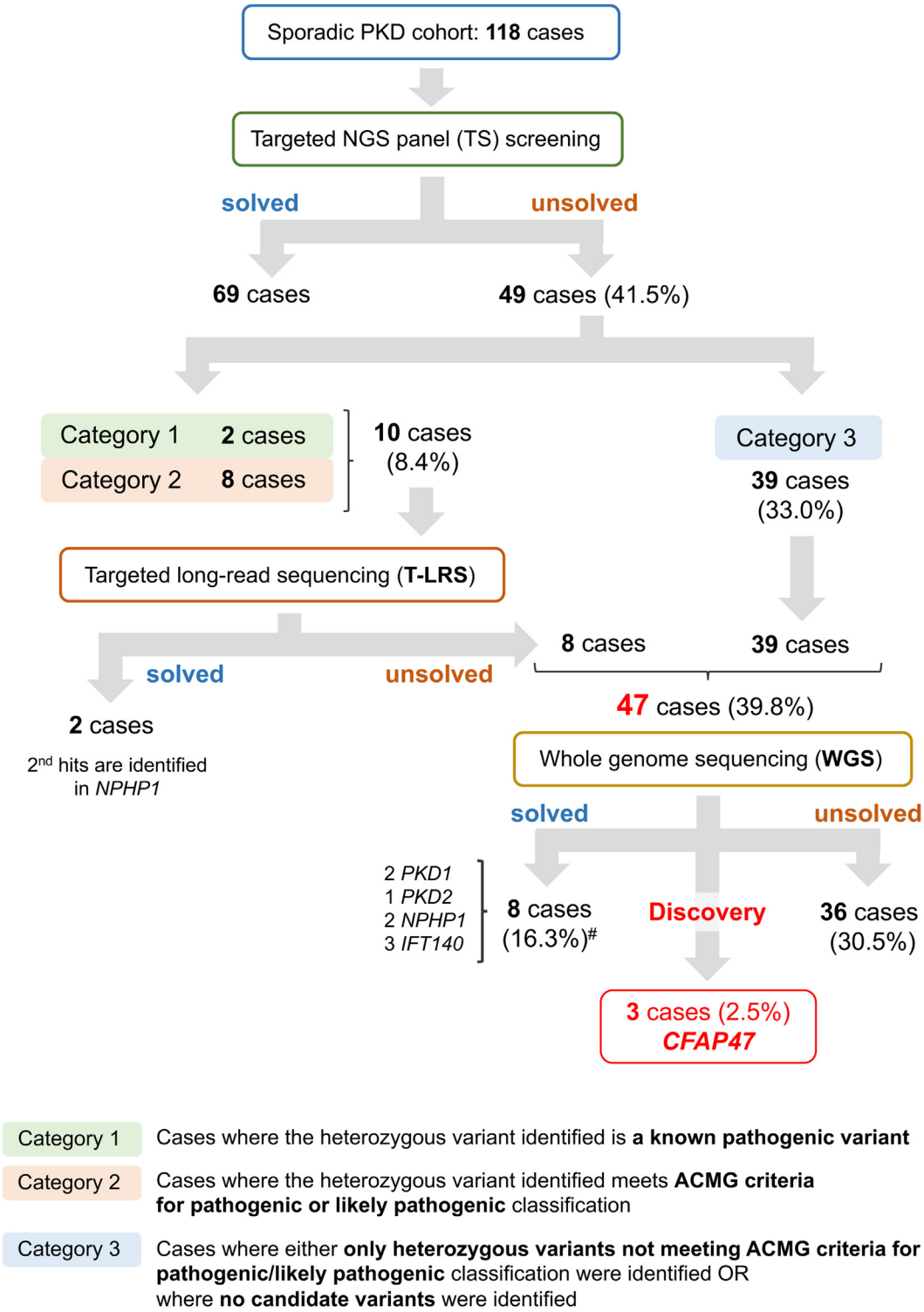

Figure 1. Summary of genetic analysis for a cohort of 118 cases with sporadic PKD. Targeted next generation sequencing panel analysis covering cystic kidney disease-related genes was followed by targeted long-read sequencing (T-LRS) and whole-genome sequencing (WGS). This approach identified CFAP47 mutations in 3 cases, constituting 2.5% of the total cohort. #The proportion within the 49 unsolved cases. PKD, polycystic kidney disease.

(NPHP4, PKHD1, and OFD1). The 49 cases (42%) that remained unresolved after primary screening with targeted next generation sequencing panels were divided into 3 categories based on the type of variants detected. Briefly, the group in which only a mono-allelic variant of possible pathological significance in a recessive genetic disease was detected was subdivided into category 1 (previously reported pathological mutations) and category 2 (pathogenic or likely-pathogenic based on the American College of

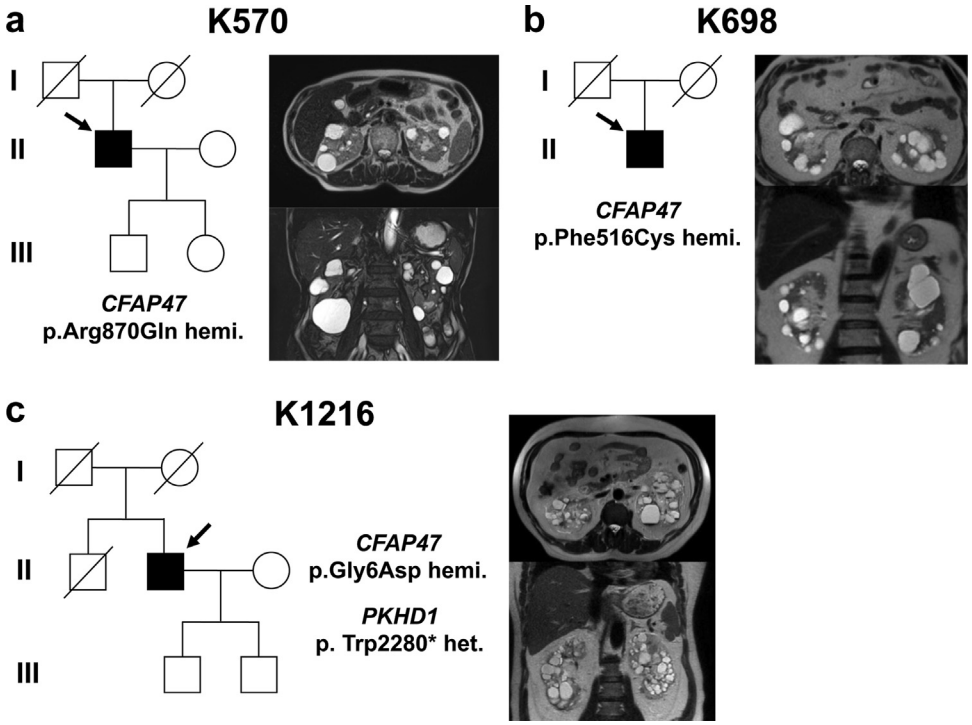

Figure 2. Family tree and MRI of polycystic kidneys. Family trees, genetic mutations, and abdominal MRI images of K570 (case 1), K698 (case 2), and K1216 (case 3) are shown in Panels A, B, and C, respectively. MRI, magnetic resonance imaging.

Medical Genetics and Genomics criteria[15]). The group with no candidate variants or likely-benign variants was categorized as category 3. The clinical information on the 49 cases unresolved in the primary screening is presented in Supplementary Table S3. For categories 1 and 2, T-LRS on the Oxford Nanopore platform was employed to identify variants in the contralateral allele.[16] In 2 cases (PT1049 and PT1192), an initially undetected deletion variant in NPHP1 was identified, ultimately contributing to the diagnosis (Supplementary Table S4). WGS was performed on 47 cases, including 8 that remained unresolved after T-LRS analysis and 39 category 3 cases. T-LRS analysis and WGS were performed in collaboration with the University of Washington Center for Rare Disease Research. Details regarding the analysis procedures, cutoff values used in various filtering steps, and other specifics are described in the Methods section and Supplementary Information. The details of the pathogenic variants detected are presented in Supplementary Table S4, including 2 cases with PKD1 pathogenic variants and 1 with a PKD2 pathogenic variants, both of which were deletion variants. While the 3 IFT140 mutations had been identified during the primary panel screening phase, there was no consensus at that time that monoallelic variants in IFT140 were a cause of PKD.[6] These 3 cases were redefined as having PKD due to the pathogenic variant in IFT140.

In addition to evaluating known genes, we sought to discover responsible genes for PKD using WGS. As the cohort involved simplex cases, candidate variants were filtered assuming either autosomal recessive (homozygous or compound heterozygous variants) or X-chromosome inheritance. This identified a rare hemizygous missense variant in CFAP47, an X-linked gene, in 3 male subjects (Figures 1 and 2). The clinical course of these 3 patients is outlined below, and key clinical findings are summarized in Table 1. The pedigree and abdominal MRI images are presented in Figure 2.

Case Presentation

All 3 individuals were Japanese men with no family history of PKD or chronic kidney disease. The mean age ± SD at diagnosis for the 3 patients was 70.7 ± 11.4 years, and kidney function in terms of estimated glomerular filtration rate averaged 31.8 ± 18.8 ml/min per 1.73 m^2. The mean volume of both kidneys was 668.3 ± 71.3 ml, only mildly enlarged compared to the

Table 1. Clinical characteristics of the 3 patients

Patient ID	Age, yr	Sex	Hypertension	PLD	eGFR, ml/min per 1.73 m^2	TKV, ml
PT570 (Case 1)	74	Male	+	–	53.5	587
PT698 (Case 2)	80	Male	+	–	21.8	698
PT1216 (Case 3)	58	Male	+	–	20.2	720

eGFR, estimated glomerular filtration rate; PLD, polycystic liver disease; TKV, total kidney volume.
The term "sex" is used to refer specifically to biological and physiological differences.

normal volume of 404 ml in males.[17] All patients presented with no liver cysts, and all of them were undergoing treatment for hypertension. A summary of each case is provided in the next section.

Case 1 (PT570)

A 74-year-old man, under treatment with angiotensin-2-receptor blockers for hypertension, maintained a regular blood pressure around 130/80 mm Hg. He presented with bilateral kidney cysts and mild proteinuria [urine total protein-to-creatinine ratio (UPCR): ∼0.8 g/g Cr]. Throughout the disease course, urine occult blood remained consistently negative. Magnetic resonance angiography of the head revealed no cerebral aneurysm, and hepatic cysts were absent (Figure 2a). Kidney function had remained stable for several years, with serum creatinine ranging from 1.05 to 1.15 mg/dl. Although the patient's father passed away at the age of 62 due to cerebral hemorrhage, no kidney disease was detected. Furthermore, the eldest son and daughter showed no signs of kidney cysts.

Case 2 (PT698)

An 80-year-old man with a history of urinary tract stone disease, benign prostatic hyperplasia, and aortic stenosis. He was prescribed angiotensin-2-receptor blockers and calcium channel blockers for hypertension, maintaining a blood pressure of 125/75 mm Hg. There was no family history of kidney disease, including cystic kidney disease. His family doctor reported kidney dysfunction, noting a decreased estimated glomerular filtration rate of 50.4 ml/min per 1.73 m^2 and UPCR of 0.5 g/g Cr at the age of 72 years. At the age of 80 years, his kidney function deteriorated further to blood urea nitrogen 28 mg/dl, creatinine 2.31 mg/dl, estimated glomerular filtration rate 22.1 ml/min per 1.73 m^2, and UPCR 1.5 g/g Cr. UPCR increased to about 1 g/g Cr, but urinary occult blood remained negative. Abdominal MRI revealed multiple cysts in both kidneys, with no hepatic cysts (Figure 2b). Echocardiography indicated no left ventricular contractility issues, only mild aortic stenosis.

Case 3 (PT1216)

A 58-year-old man with hypertension, prescribed angiotensin-2-receptor blocker and calcium channel blockers to maintain a stable blood pressure around 130/80 mm Hg. He is also taking warfarin for persistent atrial fibrillation and has a history of bilateral kidney stones. Urine analysis revealed mild proteinuria with a UPCR of approximately 0.6 g/g Cr and mild urine occult blood (2+ in qualitative analysis). Abdominal MRI displayed bilateral multiple kidney cysts but no liver cysts (Figure 2c). His father passed away from lung cancer, with no kidney cysts noted. His mother did not have any documented kidney disease. His older brother passed away shortly after birth, and the cause remains unknown. No kidney cysts were observed in the 2 sons. Echocardiography revealed no left ventricular contractility problems and no apparent valvular disease.

Interpretation of the Pathogenicity of CFAP47 Variants

CFAP47 encodes cilia and flagella associated protein 47, a protein that plays a role in the formation and function of cilia and flagella.[12,18] Given the association between genes underlying cystic kidney disease and genes encoding cilia-related proteins,[19] we considered CFAP47 as a high-priority candidate gene.

We identified 3 rare missense variants in CFAP47 (NM_001304548.2): c.2609G>A; p.(Arg870Gln), c.1547T>G; p.(Phe516Cys), c.17G>A; p.(Gly6Asp) in cases 1 to 3, respectively (Table 2). The affected individuals were all males with hemizygous X-chromosome mutations, exhibiting an allele frequency of <0.0005 in gnomAD. *In silico* pathogenicity prediction scores, CADD and REVEL, generally favored the damaging variant.

Application of the American College of Medical Genetics and Genomics criteria[15] classified all 3 variants as variants of unknown significance (VUS), and there was no record of any of these variants in ClinVar.[20] In case 3 (PT1216), a heterozygous nonsense mutation (PKHD1 [NM_138694.4]: c.6840G>A: p.[Trp2280*]),[21] previously reported as a disease-causing mutation in

Table 2. Profile of the candidate variants in the 3

Patient ID	Gene[a]	Variant	Zygosity	gnomAD[b]	CADD[c]	REVEL[d]	ACMG criteria	Reports
PT570	CFAP47	c.2609G>A; p.(Arg870Gln)	hemi.	0.0003823	19.2	0.106	VUS	none
PT698	CFAP47	c.1547T>G; p.(Phe516Cys)	hemi.	0.000006981	23.8	0.346	VUS	none
PT1216	CFAP47	c.17G>A; p.(Gly6Asp)	hemi.	0.0003047	18.9	0.025	VUS	none
	PKHD1	c.6840G>A : p.(Trp2280*)	het.	N/A	37.0	N/A	Pathogenic	Hou *et al.* 2018[21]

ACMG, American College of Medical Genetics and Genomics; hemi., hemizygous; het., heterozygous; N/A, not available; VUS, variant of unknown significance.
[a]NCBI accession in CFAP47 and PKHD1 are NM_001304548.2 and NM_138694.4, respectively.
[b]Genome Aggregation Database, v4.0 or v2.1.1.[36]
[c]Combined Annotation-Dependent Depletion phred score.[37]
[d]REVEL score.[38]

PKHD1, the gene associated with autosomal recessive PKD (ARPKD), was identified. We used T-LRS to exclude a second pathogenic variant in the contralateral allele. This variant is also interpreted as pathogenic (PVS1, PM2, PP3, and PP5) by the American College of Medical Genetics and Genomics criteria and is considered to have pathological significance. However, given that it is a mono-allelic variant and ARPKD is an autosomal recessive disease, the extent to which the phenotype is affected, if at all, is unclear.

Basic Examination of the Effects of CFAP47 on Kidney Phenotype

Considering the numerous uncertainties in the molecular biology of CFAP47, including its kidney localization, and the interpretation of VUS on a candidate gene, we chose to conduct a basic experimental validation to confirm whether CFAP47 is indeed the causative gene. Our investigation confirmed the expression of CFAP47 in human kidney tubular cilia through double staining with antibodies to CFAP47 and acetylated alpha-tubulin, a marker of primary cilia (Figure 3a). Incidentally, immunohistochemistry for CFAP47 was also attempted in mouse kidneys; however, despite using multiple antibodies and employing various techniques, it proved unattainable. Subsequently, we examined the kidney phenotype of CFAP47-KO mice ($CFAP47^{-/Y}$). This mouse model, previously reported by Chunyu Liu and Feng Zhang *et al*. as a pathological model of human asthenoteratozoospermia,[12] served as the basis for our collaborative study analysis. As illustrated in Figure 3b and c, the kidneys of 40-week-old KO mice were slightly but significantly larger than those of wild-type (WT) ($CFAP47^{+/Y}$) mice, although no gross macromorphological cyst formation was evident. Histological examination using hematoxylin and eosin staining revealed scattered areas with sparing hematoxylin and eosin staining in the kidneys of the KO mice, particularly in the subcortical and corticomedullary borders (Figure 3d, left). Similar changes were consistently observed in all 3 individual KO mice. At higher magnification, vacuolation of tubular cells was evident in KO mice, along with the fusion of some vacuoles, displaying tubular dilatation-like features (Figure 3d, right). Vacuolation of tubular epithelial cells was not observed in WT mice. The vacuolation and tubular dilatation were considered to contribute to the mild enlargement of the kidney volume. This suggests that the loss of CFAP47 function induces cyst-like changes in kidney tubules.

To further investigate the location of vacuolation within tubular segments, we performed differential staining on kidney tissues from KO mice, using anti-AQP1 for proximal tubules and anti-NCC for distal tubules (Figure 4a). The results revealed that vacuolation occurred exclusively in the proximal tubules. Subsequently, to examine the effect of the CFAP47 mutation on polycystin (PC) 1 or 2 behavior, we stained cilia in kidney tissues of KO and WT mice using α-acetylated tubulin (Figure 4b). Results showed significantly shorter cilia in KO mice compared to WT (Figure 4c, $P = 0.004$). Cilia shortening can impair PC1's and PC2's mechanosensory functions, resulting in dysregulated cell proliferation and fluid secretion, which contribute to cyst development.[22] This suggests that the CFAP47 mutation may affect PC1 or PC2 behavior.

Estimated Prevalence of PKD Associated With CFAP47 Mutation

Recent advancements have enabled the estimation of genetic prevalence using publicly available minor allele frequency (MAF) data. Using the genetic prevalence estimator (GeniE, https://genie.broadinstitute.org), we assessed the genetic prevalence of loss-of-function variants in CFAP47, including missense and nonsense mutations. We used gnomAD v4.1.0 for the MAF database. Estimating the genetic prevalence of X-linked inheritance using MAF data poses practical challenges, as discussed later. Consequently, we conducted the analysis assuming a recessive inheritance mode. The estimated global genetic prevalence was determined to be 1 in 7,401,904. The prevalence of ADPKD in the Japanese population is reported to be approximately 1 in 730 to 1471 individuals at most,[23] which is comparable to the prevalence of 1 in 1075 individuals reported in a US population sequencing study.[24] Although we have calculated the genetic prevalence using gnomAD, it is important to note that genetic prevalence correlates with, but is not equivalent to, disease prevalence.[25] Using our cohort data, we estimated the prevalence of CFAP47. Assuming ADPKD affects approximately 1 in 1000 individuals (100 per 100,000), with 15% being sporadic cases, we calculated: 100×0.15 (sporadic cases) $\times$ 0.025 (CFAP47 patients)/100,000 = 0.375 per 100,000. This equates to approximately 1 in 267,000 individuals.

DISCUSSION

A comprehensive panel screening of causative genes for cystic kidney disease was conducted on a cohort of 118 patients with sporadic cases of cystic kidney disease and no familial history. WGS was performed on 47 patients who did not exhibit an identified responsible variant. Rare hemizygous missense variants in CFAP47 were identified in 3 men within this subgroup. Basic verification, guided by histological evaluation, confirmed the localization of CFAP47 to the primary

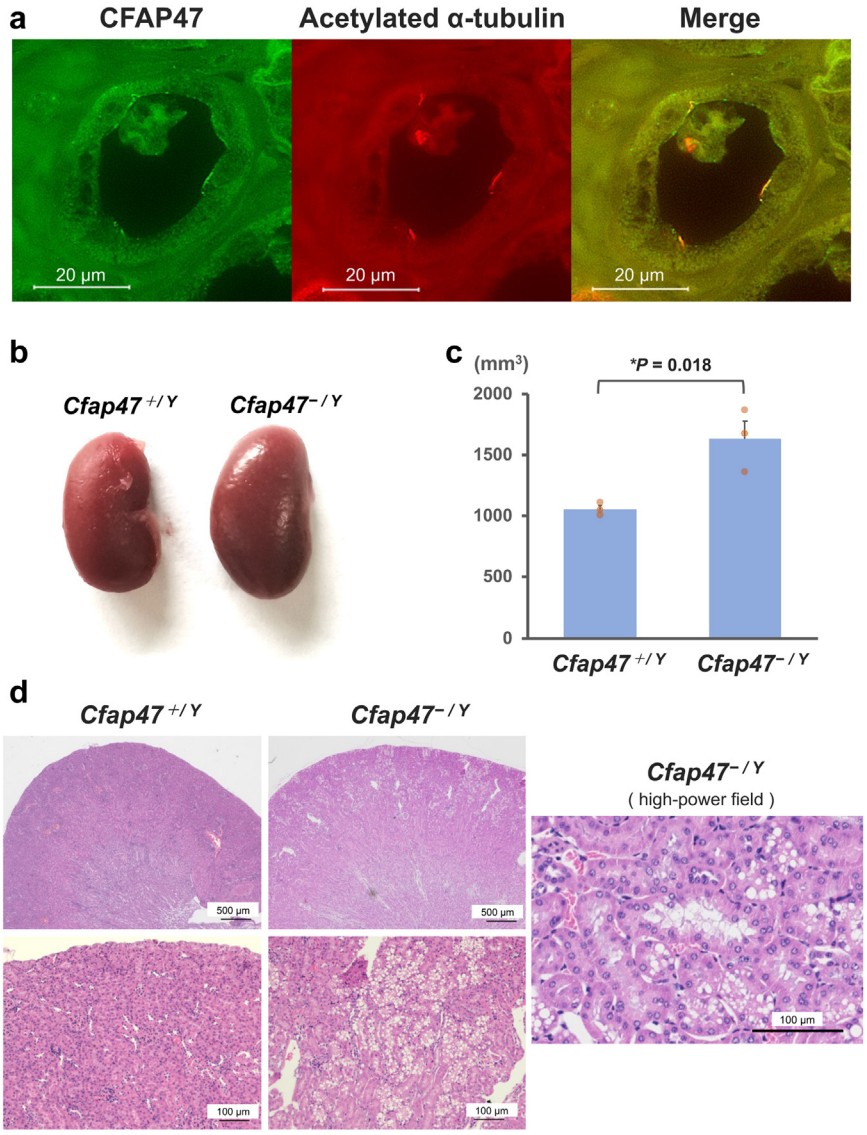

Figure 3. Evaluation of CFAP47 expression sites in human kidney tubules and kidney morphology of CFAP47$^{-/Y}$ mice. (a) Human kidney normal tubules coimmunostained with acetylated alpha-tubulin antibody (red) and CFAP47 antibody (green); (b) CFAP47$^{+/Y}$ AND CFAP47$^{-/Y}$ mouse kidney macro-morphology images; (c) kidney volume comparison using the ellipsoid formula: (length × width × [π/6])[2] (d) Low and high magnification images with hematoxylin and eosin stain.

cilia of human kidney tubular epithelial cells. Notably, tubular epithelial cells in CFAP47-KO mice exhibited evidence of vacuolation and tubular dilation. These findings suggest that CFAP47 serves as a causative gene for X-linked cystic kidney disease.

The genetic background analysis of sporadic PKD in our cohort has been previously reported.[13,14] As presented in Figure 1, among the unsolved cases, the 47 individuals constituted 39.8% of the total 118 cases, and the 3 individuals carrying the CFAP47 pathogenic variants accounted for approximately 2.5% of the entire cohort. As a result, diagnostic variants were identified in 71 cases (61%) in the cohort.

The global genetic and disease prevalence of PKD with CFAP47 mutation show a discrepancy. As noted on the GeniE website (https://genie.broadinstitute.org), predicting the prevalence of X-linked inherited diseases using MAF databases is generally complex and difficult to evaluate accurately. One reason for this is that the genetic prevalence of X-linked inheritance may be underestimated. This is likely due to a combination of the following factors: (i) failure to account for the one-third *de novo* mutation rate of X-linked diseases and (ii) female carriers of X-linked rare diseases often have milder symptoms, making them less likely to participate in studies included in gnomAD or meet recruitment criteria. This leads to fewer symptomatic carriers and a lower observed allele frequency of pathogenic variants in gnomAD. Including more biobanks may help address the second issue. However, much work is still needed to improve the quality of genetic prevalence estimates for X-linked diseases.

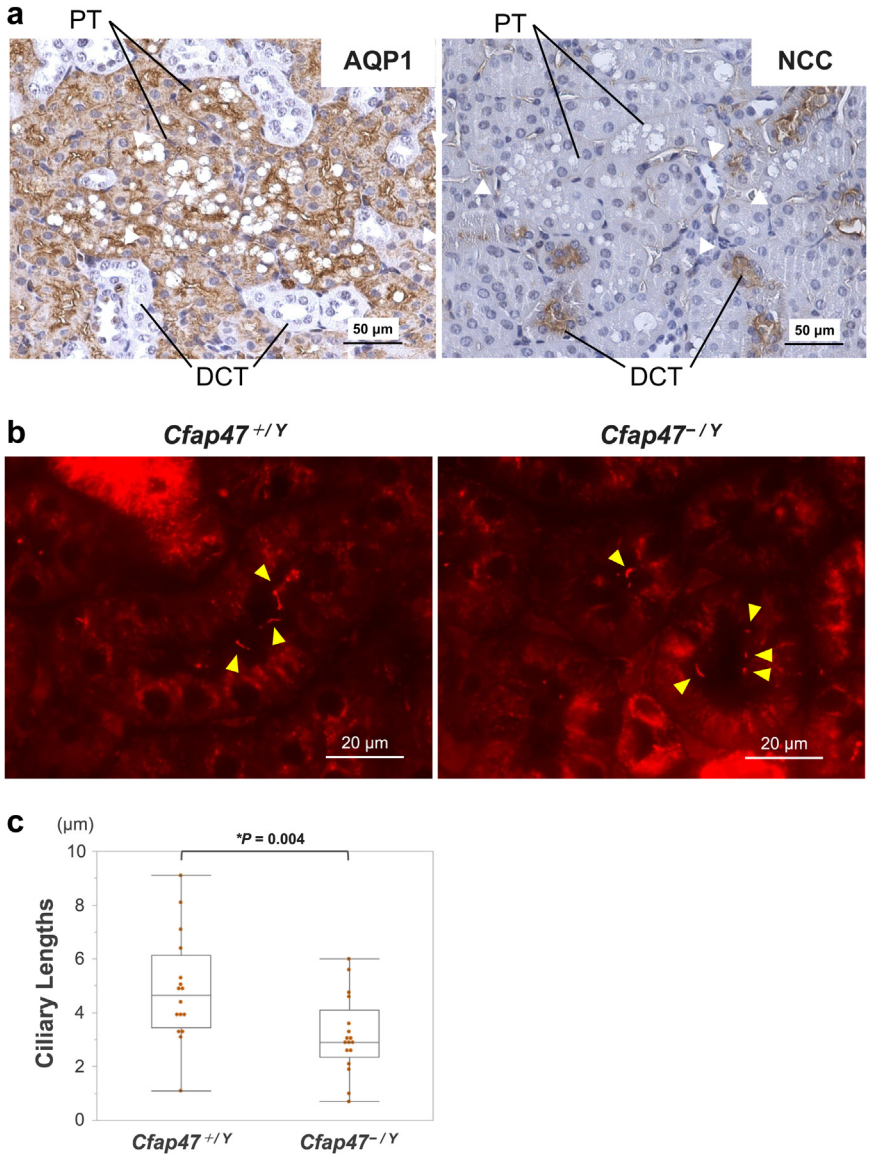

Figure 4. Evaluation of vacuolated cell localization, and primary cilia length in CFAP47$^{-/Y}$ mice. (a) Paraffin sections of Cfap47$^{-/Y}$ mouse kidneys were stained using an enzyme antibody method with proximal tubule marker (AQP1) and distal tubule marker (NCC [SLC12A3]). (b) Fluorescent immunostaining was performed using acetylated alpha-tubulin antibody (red) to detect primary cilia in the proximal tubules of kidneys from CFAP47$^{+/Y}$ AND CFAP47$^{-/Y}$ mice. Arrowheads indicate primary cilia. (c) The length of primary cilia stained in (b) was compared between CFAP47$^{+/Y}$ and CFAP47$^{-/Y}$ and presented as a box plot. DCT, distal convoluted tubule; PT, proximal tubule.

ARPKD has an estimated prevalence of 1 in 10,000 to 40,000 individuals.[23,26] PKD caused by CFAP47 mutations is believed to be even rarer than ARPKD. However, this estimate is based on a small-scale cohort study and may not accurately reflect true epidemiological data.

X-linked cystic kidney disease has previously been associated with oral-facial-digital syndrome type 1 (OFD1), characterized by malformations in the face, oral cavity, and digits. In addition, the clinical phenotype often involves mental retardation and kidney functional impairment.[27] In contrast, the 3 patients with CFAP47 mutations in this study exhibited no facial deformities or mental retardation and lacked extra-renal manifestations. The patient is elderly, and because both parents have already passed away, it is challenging to conduct familial analysis. However, if the mutation is not *de novo*, the mother of the proband is likely to be an obligate carrier of the same mutation. In females with X-linked inheritance, the pathogenic impact of the gene mutation is further influenced by X chromosome inactivation, leading to diverse phenotypic expressions. In the 3 cases of this study, the mothers did not exhibit clear symptoms of kidney cysts or kidney failure, which is consistent with the variability in the manifestation of X-linked inheritance in females.

The pathological significance interpretation of the detected mutations based on the American College of Medical Genetics and Genomics criteria was all classified as VUS. In addition, due to the incomplete supporting evidence from *in silico* pathogenicity prediction scores, observations of the kidneys in CFAP47-KO mice were conducted. There is ongoing discussion regarding the interpretation of VUS,[28] especially in cases of low-penetrance and recessive disease-associated variants, or when the associated phenotypes only partially overlap with the originally reported disease phenotypes.[29] In fact, reconsideration attempts of VUS classification have shown that some were not interpretable as benign.[30] The variants identified in cases 1 and 3 do not necessarily exhibit ultra-rare MAFs in gnomAD (0.00038 and 0.00030, respectively). However, given the mild expression phenotype, it is possible that these variants may not have been recognized as chronic kidney disease or PKD until old age.

The relationship between vacuolation of tubular epithelial cells and the development of large cysts remains unclear; however, vacuolation itself has been reported to occur due to various factors such as ischemia, hyperosmolarity, and lipid accumulation.[31-33] Although this study cannot completely negate some of the aforementioned background factors, the absence of any tissue changes in the littermate WT mice ($CFAP47^{+/Y}$) suggests that the observed alterations are associated with the loss of CFAP47 function. In general, mouse models of cystic kidney disease, even with the same genetic background, exhibit phenotypes that are milder and progress more slowly than the actual phenotypes observed in humans. This is particularly true for recessive forms of the model, adding further complexity to the interpretation.[34] Considering the presence of partially fused vacuoles and areas displaying dilatation of tubular lumens, it is conceivable that vacuolation may contribute to the initiation of cyst formation. Our study of 40-week-old KO mice revealed vacuolation and fusion in proximal tubule cells, but no distinct gross cysts. This might be due to the mice's relatively young age. Considering that the patient is approximately 70 years old, gross cysts might have been observable in older mice, perhaps those aged 60 to 70 weeks.

In the context of considering the mechanism underlying cyst formation due to CFAP47 mutations, previously reported minor causative gene groups of ADPKD and most autosomal dominant polycystic liver disease proteins are involved in the folding and transport of proteins in the endoplasmic reticulum. Among these proteins, PC1 has been identified as particularly sensitive to the reduction in the dosage of these proteins causative for cystic diseases.[5,8,35] For example, GANAB, a causative gene for ADPKD, encodes the glucosidase II subunit α, which is necessary for the maturation, surface expression, and ciliary localization of ADPKD proteins PC1 and PC2.[5] Our study revealed significantly shorter cilia in the proximal tubules of CFAP47-KO mouse kidneys. Although we could not directly assess PC1 and PC2 due to a lack of reliable commercial antibodies, this finding suggests that the CFAP47 mutation may influence PC1 and PC2 behavior.

The cysts in PT1216 exhibited some morphological differences compared to the other 2 individuals, who displayed numerous small cysts, and more impaired kidney function. In this case, along with the CFAP47 mutation, a previously reported mutation in PKHD1, responsible for ARPKD, was identified. The coexistence with CFAP47 mutations may have had some impact on the more severe phenotype in this case.

Cases 1 and 3 had children, suggesting that their reproductive abilities were unaffected. Missense mutations reported as causes of asthenoteratozoospermia, namely S1742G, I2385N, and P2890T, are localized at the C-terminal end.[12] The difference in the localization of mutations may be associated with differences in phenotype.

The limitations of this study lies in the selection of sporadic cases; as mentioned earlier, it is primarily based on medical history rather than necessarily on the imaging evaluation of all parents. This approach may include cases that do not strictly qualify as true sporadic cases. Although the KO mice provide an optimal model for studying the loss of function in CFAP47, it is important to note that they do not directly replicate the pathological effects associated with the specific missense mutations investigated in this study. It should be noted that the CFAP47 antibody used in this study was not suitable for staining mouse kidneys due to sensitivity issues, making detailed histological examinations in mouse kidneys challenging.

In conclusion, CFAP47 is a newly discovered gene associated with PKD, demonstrating X-linked inheritance, a trait uncommon in cases of PKD. CFAP47 should be considered as one of the causes of sporadic cases of PKD. Nevertheless, the pathophysiological evidence supporting CFAP47 as a cyst-causing gene remains limited. To strengthen this evidence, more cases with causative mutations in CFAP47 need to be documented, and more comprehensive basic studies should be conducted.

APPENDIX

List of Members of the University of Washington Center for Rare Disease Research

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DISCLOSURE

DEM is on a scientific advisory board at Oxford Nanopore Technologies (ONT), is engaged in a research agreement with ONT, and they have paid for his travel to speak on their behalf. DEM holds stock options in MyOme. All the other authors declared no competing interests.

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Data Availability Statement

The data underlying this article cannot be shared publicly due to the privacy of individuals that participated in the study. The data will be shared on reasonable request to the corresponding author.

AUTHOR CONTRIBUTIONS

Conceptualization was done by TM, TF, SU, and ES. Data curation was done by TM, TF, KP, DEM, MZ, and UW-CRDR. Formal analysis was done by TM, TF, KP, DEM, MZ, UW-CRDR, and ES. Funding acquisition was done by TM, TF, SU, and ES. Investigation was done by TM, TF, CL, KP, KY, TS, MC, DEM, MZ, UW-CRDR, FZ, and ES. Methodology was done by TM, TF, SU, and ES. Resources were provided by TM, TF, CL, KP, KY, TS, MC, SM, FA, YM, HK, KS, UW-CRDR, JXC, MJB, Y-QT, FZ, SU, and ES. Supervision was done by TM, Y-QT, FZ, SU, and ES. Writing of the original draft was by TM, TF, and ES. Writing: review and editing was done by TM, TF, CL, KP, KY, TS, MC, DEM, MZ, SM, FA, YM, HK, KS, UW-CRDR, JXC, MJB, Y-QT, FZ, SU, and ES.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplementary Methods.

Supplementary References.

Table S1. Targeted genes and disease categories included in the panels.

Table S2. Target regions for adaptive sampling (T-LRS).

Table S3. Clinical information on cases unresolved by panel screening.

Table S4. List of cases and variants profiles in which the responsible mutation was identified by WGS or T-LRS in known genes.

REFERENCES

1. Rossetti S, Consugar MB, Chapman AB, et al. Comprehensive molecular diagnostics in autosomal dominant polycystic kidney disease. *J Am Soc Nephrol*. 2007;18:2143–2160. https://doi.org/10.1681/ASN.2006121387
2. Mallawaarachchi AC, Lundie B, Hort Y, et al. Genomic diagnostics in polycystic kidney disease: an assessment of real-world use of whole-genome sequencing. *Eur J Hum Genet*. 2021;29:760–770. https://doi.org/10.1038/s41431-020-00796-4
3. Chang AR, Moore BS, Luo JZ, et al. Exome sequencing of a clinical population for autosomal dominant polycystic kidney disease. *JAMA*. 2022;328:2412–2421. https://doi.org/10.1001/jama.2022.22847
4. Yu CC, Lee AF, Kohl S, et al. PKD2 founder mutation is the most common mutation of polycystic kidney disease in Taiwan. *NPJ Genom Med*. 2022;7:40. https://doi.org/10.1038/s41525-022-00309-w
5. Porath B, Gainullin VG, Cornec-Le Gall E, et al. Mutations in GANAB, encoding the glucosidase IIα subunit, cause autosomal-dominant polycystic kidney and liver disease. *Am*

J Hum Genet. 2016;98:1193–1207. https://doi.org/10.1016/j.ajhg.2016.05.004

6. Senum SR, Li YSM, Benson KA, et al. Monoallelic IFT140 pathogenic variants are an important cause of the autosomal dominant polycystic kidney-spectrum phenotype. *Am J Hum Genet*. 2022;109:136–156. https://doi.org/10.1016/j.ajhg.2021.11.016
7. Lemoine H, Raud L, Foulquier F, et al. Monoallelic pathogenic ALG5 variants cause atypical polycystic kidney disease and interstitial fibrosis. *Am J Hum Genet*. 2022;109:1484–1499. https://doi.org/10.1016/j.ajhg.2022.06.013
8. Cornec-Le Gall E, Olson RJ, Besse W, et al. Monoallelic mutations to DNAJB11 cause atypical autosomal-dominant polycystic kidney disease. *Am J Hum Genet*. 2018;102:832–844. https://doi.org/10.1016/j.ajhg.2018.03.013
9. Besse W, Chang AR, Luo JZ, et al. ALG9 Mutation carriers develop kidney and liver cysts. *J Am Soc Nephrol*. 2019;30:2091–2102. https://doi.org/10.1681/ASN.2019030298
10. Matsuo S, Imai E, Horio M, et al. Revised equations for estimated GFR from serum creatinine in Japan. *Am J Kidney Dis*. 2009;53:982–992. https://doi.org/10.1053/j.ajkd.2008.12.034
11. Cadnapaphornchai MA, McFann K, Strain JD, Masoumi A, Schrier RW. Prospective change in renal volume and function in children with ADPKD. *Clin J Am Soc Nephrol*. 2009;4:820–829. https://doi.org/10.2215/CJN.02810608
12. Liu C, Tu C, Wang L, et al. Deleterious variants in X-linked CFAP47 induce asthenoteratozoospermia and primary male infertility. *Am J Hum Genet*. 2021;108:309–323. https://doi.org/10.1016/j.ajhg.2021.01.002
13. Fujimaru T, Mori T, Sekine A, et al. Kidney enlargement and multiple liver cyst formation implicate mutations in PKD1/2 in adult sporadic polycystic kidney disease. *Clin Genet*. 2018;94:125–131. https://doi.org/10.1111/cge.13249
14. Fujimaru T, Kawanishi K, Mori T, et al. Genetic background and clinicopathologic features of adult-onset nephronophthisis. *Kidney Int Rep*. 2021;6:1346–1354. https://doi.org/10.1016/j.ekir.2021.02.005
15. 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
16. Miller DE, Sulovari A, Wang T, et al. Targeted long-read sequencing identifies missing disease-causing variation. *Am J Hum Genet*. 2021;108:1436–1449. https://doi.org/10.1016/j.ajhg.2021.06.006
17. Cheong B, Muthupillai R, Rubin MF, Flamm SD. Normal values for renal length and volume as measured by magnetic resonance imaging. *Clin J Am Soc Nephrol*. 2007;2:38–45. https://doi.org/10.2215/CJN.00930306
18. Liao HQ, Guo ZY, Huang LH, et al. WDR87 interacts with CFAP47 protein in the middle piece of spermatozoa flagella to participate in sperm tail assembly. *Mol Hum Reprod*. 2022;29. https://doi.org/10.1093/molehr/gaac042
19. Ma M, Ma M. Cilia and polycystic kidney disease. *Semin Cell Dev Biol*. 2021;110:139–148. https://doi.org/10.1016/j.semcdb.2020.05.003
20. Landrum MJ, Lee JM, Riley GR, et al. ClinVar: public archive of relationships among sequence variation and human phenotype. *Nucleic Acids Res*. 2014;42:D980–D985. https://doi.org/10.1093/nar/gkt1113
21. Hou L, Du Y, Zhang M, Su P, Zhao C, Wu Y. Novel mutations of PKHD1 and AHI1 identified in two families with cystic renal disease. *Int J Clin Exp Pathol*. 2018;11:2869–2874.
22. Ma M, Gallagher AR, Somlo S. Ciliary mechanisms of cyst formation in polycystic kidney disease. *Cold Spring Harb Perspect Biol*. 2017;9. https://doi.org/10.1101/cshperspect.a028209
23. Nishio S, Tsuchiya K, Nakatani S, et al. A digest from evidence-based Clinical Practice Guideline for Polycystic Kidney Disease 2020. *Clin Exp Nephrol*. 2021;25:1292–1302.
24. Tsimaratos M, Cloarec S, Roquelaure B, et al. Chronic renal failure and portal hypertension–is portosystemic shunt indicated? *Pediatr Nephrol*. 2000;14:856–858. https://doi.org/10.1007/s004679900268
25. Xiao Q, Lauschke VM. The prevalence, genetic complexity and population-specific founder effects of human autosomal recessive disorders. *NPJ Genom Med*. 2021;6:41. https://doi.org/10.1038/s41525-021-00203-x
26. Salman MA, Elgebaly A, Soliman NA. Epidemiology and outcomes of pediatric autosomal recessive polycystic kidney disease in the Middle East and North Africa. *Pediatr Nephrol*. 2024;39:2569–2578. https://doi.org/10.1007/s00467-024-06281-0
27. Feather SA, Woolf AS, Donnai D, Malcolm S, Winter RM. The oral-facial-digital syndrome type 1 (OFD1), a cause of polycystic kidney disease and associated malformations, maps to Xp22.2-Xp22.3. *Hum Mol Genet*. 1997;6:1163–1167. https://doi.org/10.1093/hmg/6.7.1163
28. Aklilu AM, Gulati A, Kolber KJ, Yang H, Harris PC, Dahl NK. The VUS challenge in cystic kidney disease: a case-based review. *Kidney360*. 2024;5:152–159. https://doi.org/10.34067/KID.0000000000000298
29. Houge G, Laner A, Cirak S, de Leeuw N, Scheffer H, den Dunnen JT. Stepwise ABC system for classification of any type of genetic variant. *Eur J Hum Genet*. 2022;30:150–159. https://doi.org/10.1038/s41431-021-00903-z
30. Tsai GJ, Rañola JMO, Smith C, et al. Outcomes of 92 patient-driven family studies for reclassification of variants of uncertain significance. *Genet Med*. 2019;21:1435–1442. https://doi.org/10.1038/s41436-018-0335-7
31. Zhou C, Yool AJ, Nolan J, Byard RW. Armanni-Ebstein lesions: a need for clarification. *J Forensic Sci*. 2013;58:S94–S98. https://doi.org/10.1111/j.1556-4029.2012.02274.x
32. Zhou C, Vink R, Byard RW. Hyperosmolarity induces Armanni-Ebstein-like renal tubular epithelial swelling and cytoplasmic vacuolization. *J Forensic Sci*. 2017;62:229–232. https://doi.org/10.1111/1556-4029.13235
33. Parai JL, Kodikara S, Milroy CM, Pollanen MS. Alcoholism and the Armanni-Ebstein lesion. *Forensic Sci Med Pathol*. 2012;8:19–22. https://doi.org/10.1007/s12024-011-9264-3
34. Sieben CJ, Harris PC. Experimental models of polycystic kidney disease: applications and therapeutic testing. *Kidney360*. 2023;4:1155–1173. https://doi.org/10.34067/KID.0000000000000209

35. Fedeles SV, Tian X, Gallagher AR, et al. A genetic interaction network of five genes for human polycystic kidney and liver diseases defines polycystin-1 as the central determinant of cyst formation. *Nat Genet*. 2011;43:639–647. https://doi.org/10.1038/ng.860

36. Chen S, Francioli LC, Goodrich JK, et al. A genomic mutational constraint map using variation in 76,156 human genomes. *Nature*. 2024;625:92–100. https://doi.org/10.1038/s41586-023-06045-0

37. Kircher M, Witten DM, Jain P, O'Roak BJ, Cooper GM, Shendure J. A general framework for estimating the relative pathogenicity of human genetic variants. *Nat Genet*. 2014;46(3):310–315. https://doi.org/10.1038/ng.2892

38. Ioannidis NM, Rothstein JH, Pejaver V, et al. REVEL: an ensemble method for predicting the pathogenicity of rare missense variants. *Am J Hum Genet*. 2016;99(4):877–885. https://doi.org/10.1016/j.ajhg.2016.08.016