Clinical Kidney Journal, 2025, vol. 18, no. 4, sfaf064

https://doi.org/10.1093/ckj/sfaf064
Advance Access Publication Date: 4 March 2025
Original Article

ORIGINAL ARTICLE

The spectrum of diseases, genetic landscape and new mutation sites of hereditary cystic kidney disease

Jingru Bi[1,2], Wenkai Guo[1,2], Pengcheng Ji[2], Shuang Li[2], Peng Wang[2], Qinggang Li[2] and Yuansheng Xie[1,2]

[1]School of Medicine, Nankai University, Tianjin, China and [2]Department of Nephrology, First Medical Center of Chinese PLA General Hospital, State Key Laboratory of Kidney Diseases, National Clinical Research Center for Kidney Diseases, Beijing Key Laboratory of Medical Devices and Integrated Traditional Chinese and Western Drug Development for Severe Kidney Diseases,Beijing Key Laboratory of Digital Intelligent TCM for the Preventionand Treatment of Pan-vascular Diseases,Key Disciplines of National Administration of Traditional Chinese Medicine(zyyzdxk-2023310), Beijing, China

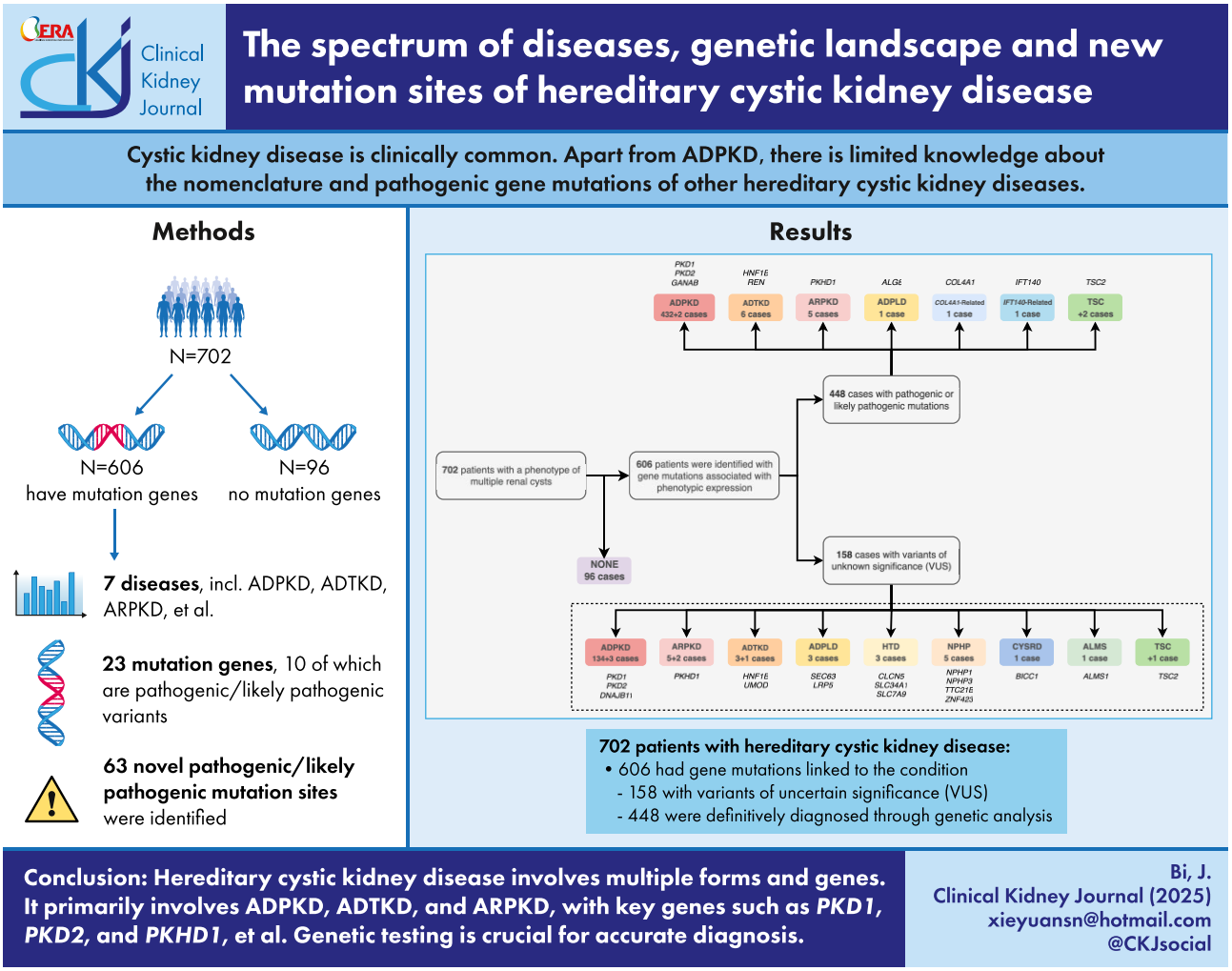
Correspondence to: Yuansheng Xie; E-mail: xieyuansn@hotmail.com

ABSTRACT

Background. Cystic kidney disease is common. Beyond autosomal dominant polycystic kidney disease (ADPKD), knowledge of other hereditary forms of cystic kidney disease remains limited. This study aimed to retrospectively analyse 702 patients with multiple renal cysts from the Chinese PLA General Hospital (September 2015–December 2023).
Methods. Patients suspected of having hereditary cystic kidney disease underwent next-generation sequencing (NGS) and subsequent bioinformatics analysis. Variations were assessed for pathogenicity in accordance with the American College of Medical Genetics and Genomics (ACMG) guidelines. Moreover, the ClinVar and Mastermind databases were used to identify novel mutation sites. Statistical analysis was performed using SPSS 25.0 software.
Results. Of 702 patients, 96 (13.7%) lacked gene mutations associated with cystic kidney disease. In contrast, 606 patients (86.3%) were found to have gene mutations associated with renal cyst phenotypes, involving 23 unique mutated genes. Among these, mutations in 158 patients were categorized as variants of uncertain significance. The remaining 448 patients harboured mutations predicted by the ACMG guidelines to be pathogenic or likely pathogenic, enabling a diagnosis of hereditary cystic kidney disease. These mutations were linked to seven diseases and 10 genes. The most common was ADPKD [434 cases (96.9%)], followed by autosomal dominant tubulointerstitial kidney disease [ADTKD; six cases (1.3%)], autosomal recessive polycystic kidney disease [ARPKD; five cases (1.1%)] and tuberous sclerosis complex [two cases (0.4%)]. One case each was found for autosomal dominant polycystic liver disease, *COL4A1*-related disease and *IFT140*-related disease. The mutated genes included *PKD1*, *PKD2*, *GANAB*, *HNF1B*, *REN*, *PKHD1*, *ALG8*, *IFT140*, *COL4A1* and *TSC2*. Moreover, 63 novel pathogenic or likely pathogenic variants were identified.
Conclusion. In this study we identified 23 mutated genes linked to renal cyst phenotypes, 10 of which had pathogenic or likely pathogenic variants. These findings facilitated the diagnosis of seven hereditary cystic kidney diseases, including ADPKD, ADTKD, ARPKD and others. Furthermore, 63 novel pathogenic or likely pathogenic variants were identified.

Received: 14.8.2024; Editorial decision: 27.1.2025

GRAPHICAL ABSTRACT



Keywords: autosomal dominant polycystic liver disease, autosomal dominant tubulointerstitial kidney disease, hereditary cystic kidney disease, new mutation sites, polycystic kidney disease

KEY LEARNING POINTS

What was known:

- Cystic kidney disease is common, with autosomal dominant polycystic kidney disease being the most prevalent form; however, knowledge about other types and their pathogenic variants remains limited.
- Research on cystic kidney disease has predominantly focused on polycystic kidney disease.
- A comprehensive understanding of the disease spectrum and genetic landscape of cystic kidney disease is crucial for clinicians to improve diagnostic methodologies, thereby minimizing misdiagnoses and missed diagnoses.

This study adds:

- The identification of seven distinct types of hereditary cystic kidney disease.
- The discovery of 23 mutated genes associated with hereditary cystic kidney diseases, 10 of which included pathogenic or likely pathogenic variants.
- The identification of 63 novel pathogenic or likely pathogenic variants.

Potential impact:

- This study elucidates the primary disease and genetic spectra of hereditary cystic kidney disease, identifying new mutation sites and thus enriching the gene database.
- These findings expand clinicians' understanding and perspectives, potentially leading to enhanced diagnostic accuracy and the development of targeted therapeutic strategies.
- By providing a comprehensive genetic and clinical overview, this study enhances the ability to identify and manage various forms of cystic kidney disease, ultimately contributing to better patient outcomes and reducing the incidence of misdiagnoses and missed diagnoses.

INTRODUCTION

Cystic kidney disease is common, and its prognosis significantly varies depending on the cause. Hereditary cystic kidney disease generally has a poor prognosis, whereas simple kidney cysts have a more favourable prognosis, highlighting the importance of precise and differential diagnoses.

Hereditary cystic kidney disease comprises a group of disorders caused by gene mutations and is characterized by the formation of multiple cysts within the kidneys. Over time, these cysts increase in size and number, potentially resulting in decreased kidney function and progression to end-stage renal disease. These disorders can result from various gene mutations, the most common being autosomal dominant polycystic kidney disease (ADPKD), with gene mutations including *PKD1* and *PKD2* [1]. Additionally, several gene mutations can lead to multiple kidney cysts, but clinicians have limited knowledge of the disease and the genetic landscape associated with hereditary cystic kidney disease.

The Chinese PLA General Hospital is a large, comprehensive hospital and the first medical institution in China to establish a specialty clinic for hereditary kidney disease. Patients come from across the country, providing a rich resource that encompasses a diverse spectrum of diseases and genetic variations. Summarizing the disease spectrum, genetic landscape and new mutation sites in hereditary cystic kidney diseases will help expand clinicians' diagnostic approaches, enrich gene databases and provide additional information on pathogenic sites for future studies.

This study aimed to retrospectively analyse 702 patients with multiple renal cysts from the Chinese PLA General Hospital (September 2015–December 2023).

MATERIALS AND METHODS

Study population

This study retrospectively analysed 702 patients diagnosed with or suspected of having hereditary cystic kidney disease at the Hereditary Kidney Disease Clinic of the Chinese PLA General Hospital between September 2015 and December 2023. The inclusion criteria were as follows: more than two kidney cysts found on ultrasonography, computed tomography or magnetic resonance imaging; or a family history of cystic kidney disease plus at least one kidney cyst; and relevant genetic testing for kidney disease. Genetic testing was approved by the Ethics Committee of the Chinese PLA General Hospital (approval number: 2012-001). The patients provided informed consent before blood sampling for genetic testing.

Study methods

Genetic testing and data collection

We used next-generation sequencing (NGS) to analyse the genomes of patients diagnosed with hereditary cystic kidney disease. The genetic testing for each individual was conducted. Patients with typical manifestations of multiple renal cysts or a dominant family history of cystic kidney disease underwent a genetic panel test targeting 140 known ciliopathy-related genes (Supplementary Table S1). For patients presenting with atypical renal cysts or unexplained renal dysfunction, an expanded panel covering >800 genes associated with kidney diseases was utilized (Supplementary Table S2). In certain cases, whole exome sequencing (WES) was performed.

Data processing and bioinformatics analysis

To ensure the accuracy and comprehensiveness of the sequencing results, rigorous quality control measures and multistep bioinformatics analyses were applied. The detailed process was as follows:

Data preprocessing and quality control. FastQC was employed to evaluate the quality of raw sequencing data. Low-quality reads as well as adapter contamination were filtered out. High-quality reads were aligned to the reference genome (hg19 version) using BWA-MEM, resulting in bam files.

Variant detection and functional annotation. The Genome Analysis Toolkit or SAMtools was used to detect variants, including single nucleotide variants (SNVs) and small insertions/deletions (indels).

Detected variants were functionally annotated using ANNOVAR or Variant Effect Predictor, assessing their potential impact on gene and protein function. The pathogenicity of the variants was classified according to guidelines provided by the American College of Medical Genetics and Genomics (ACMG).

Protein function prediction and splicing effect assessment. For potential pathogenic variants, multiple prediction tools were employed to evaluate protein function. Rare Exome Variant Ensemble Learner (REVEL) was used to predict the pathogenicity of variants, while spliceAI was used to determine whether variants affected splicing.

PKD1 gene analysis. Due to the high sequence similarity (97.7%) between exons 1–33 of the *PKD1* gene and six pseudogenes on chromosome 16, a long polymerase chain reaction amplification strategy was used to avoid pseudogene interference during mutation detection in this region. The amplified products were validated by Sanger sequencing to ensure accurate mutation identification.

Copy number variation (CNV) analysis. CNV analysis was conducted using CNVkit to identify large-scale gene copy number variations. For patients with ambiguous CNV results, further validation was performed using multiplex ligation-dependent probe amplification.

Mitochondrial gene analysis. Mitochondrial gene analysis was performed only for those patients who underwent WES. For other patients, genetic testing primarily focused on nuclear genome variations, and a comprehensive analysis of mitochondrial genes was not included.

MUC1-VNTR analysis. The variable number tandem repeat (VNTR) region of the *MUC1* gene, due to its complex structure, was excluded from routine analysis. Detailed analysis of this region requires specialized methods and was not a focus of this study.

Discovery of novel mutation sites

While analysing known genes with pathogenic variants, particular attention was paid to novel mutation sites that had not been previously reported. Novel variations were screened by searching the ClinVar database and Mastermind genomic literature. These newly discovered variations were subjected to in-depth bioinformatic and functional analyses to evaluate their potential effects on kidney disease phenotypes.

Statistical analyses

All statistical analyses were performed using SPSS Statistics version 25.0 (IBM, Armonk, NY, USA) and included descriptive statistics, normality tests and *t*-tests.

RESULTS

Disease spectrum

The genetic testing results of the 702 enrolled patients were analysed, including 336 female and 366 male patients, indicating a nearly equal prevalence between the genders. The median age of the patients was 38.6 years (range 0–77.2). This analysis covered 30 provincial-level administrative regions in China, excluding Taiwan, Hong Kong, the Macau Special Administrative Region and the Tibet Autonomous Region (Supplementary Fig. S1). Among these patients, 531 underwent a genetic panel test targeting 140 ciliopathy-related genes, 118 received an expanded panel covering over 800 genes associated with kidney diseases and 53 underwent WES. A total of 606 patients carried mutations in genes related to the kidney cyst phenotype, accounting for 86.3% (606/702) of the cases, whereas cases without mutations in genes for cystic kidney disease accounted for 13.7% (96/702).

A total of 23 mutated genes associated with hereditary cystic kidney disease were identified in 606 patients.

The ACMG guidelines classified pathogenic or likely pathogenic variants in 448 patients, involving 10 genes and seven hereditary diseases. The most common diagnosis was ADPKD, observed in 434 cases (96.9%), followed by autosomal dominant tubulointerstitial kidney disease (ADTKD) in six cases (1.3%) and autosomal recessive polycystic kidney disease (ARPKD) in five cases (1.1%). Additionally, tuberous sclerosis complex (TSC) was diagnosed in two cases (0.4%), while autosomal dominant polycystic liver disease (ADPLD), *COL4A1*-related disease and *IFT140*-related disease were each identified in one case (0.2%) (Fig. 1, Table 1).

In addition to the 448 patients, another 158 patients were found to carry gene mutations associated with phenotypes, which were classified as variants of uncertain significance (VUS) according to the ACMG guidelines. The pathogenicity of these mutations requires further evidence, advanced computational tools or functional studies for validation. The implicated mutated genes and their potentially related diseases are detailed in Fig. 1 and Table 1.

The clinical information of a subset of patients with a single pathogenic mutation, excluding *PKD1* and *PKD2* mutations, are presented in Table 2.

Genetic landscape

Among the 702 patients, 606 had gene mutations related to cystic kidney disease, including 448 patients whose mutation sites were classified as pathogenic or likely pathogenic by the ACMG. In total, there were 459 distinct mutations spanning 364 mutation sites (Supplementary Table S3). Based on the inheritance patterns of the gene mutations, the diseases are divided into dominant and recessive inheritance. Dominant inheritance included six diseases involving nine gene mutations, totalling 443 patients (441 had one gene mutation detected and two had two gene mutations detected). Recessive inheritance included one disease involving one gene mutation, totalling five patients (Table 1).

ADPKD

There were 356 cases with *PKD1* mutations, including 354 cases with only *PKD1* mutations [median age 37.6 years (range 3.0–69.8)]. Among the remaining three cases, two had co-occurring *TSC2* mutations. A total of 360 mutations were detected, 317 of which were located in exons [88.1% (317/360)]. The most common type of mutation was a point mutation, accounting for 47.8% (172/360) of the cases, including 57 missense mutations and 115 nonsense mutations. Frameshift mutations were the second most common, accounting for 32.5% (117/360) of all mutations. Exon 15 had the highest frequency of point mutations,

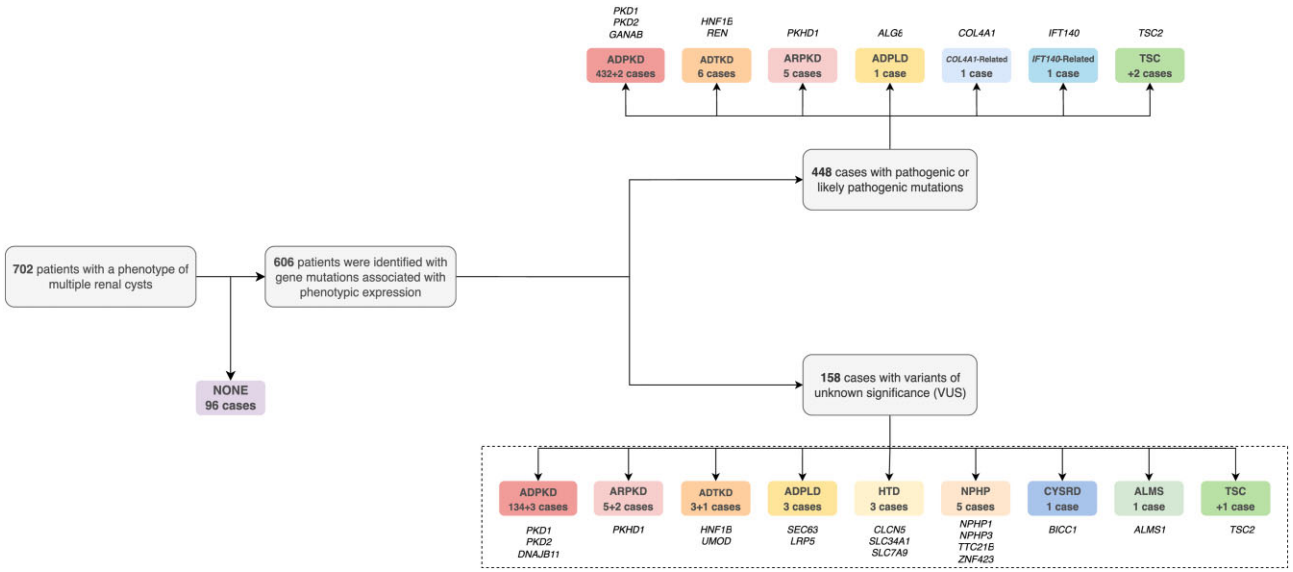


Figure 1: Spectrum of hereditary cystic kidney disease. Among 702 patients with cystic kidney disease, 96 had no detectable pathogenic gene mutations while 606 patients exhibited gene mutations associated with phenotypes. Of these 606 patients, 448 had mutations classified as pathogenic or likely pathogenic, involving seven diseases and 10 mutated genes. The remaining 158 patients had mutations classified as VUS. The dashed box highlights diseases and mutated genes with unclear diagnoses. NONE: the absence of detectable pathogenic gene mutations; +: the number of patients with multiple gene mutations, including specific gene mutations.

accounting for 15.7% (27/172) of all point mutations, which was significantly higher than in the other exons.

There were 76 cases of *PKD2* mutations, all of which exclusively involved *PKD2* mutations [median age 48.8 years (range 15.2–76.6)]. A total of 76 mutations were detected, with point mutations being the most common, accounting for 50.0% (38/76), including 34 nonsense and four missense mutations. Frameshift mutations were the second most common, representing 32.9% (25/76) of all mutations.

There were two cases of *GANAB* mutations. Although there is ongoing debate regarding the classification of *GANAB* as ADPKD or ADPLD, it is more appropriately classified as ADPKD since the gene is involved in the maturation of polycystin-1 (PC1).

Other autosomal dominant cystic kidney diseases

This study included six cases of ADTKD involving two genetic subtypes. The distribution was as follows: five cases with *HNF1B* mutations and one case with a *REN* mutation. All patients exhibited multiple kidney cysts, with some experiencing reduced kidney function and liver cysts. The median patient age was 24.4 years (range 2.9–37.8), with a male:female ratio of 5:1.

In addition, one patient with an *ALG8* mutation was diagnosed as having ADPLD. Another patient with a dominant *IFT140* mutation was identified, which was potentially associated with ciliopathy. Furthermore, a single case with a *COL4A1* mutation was reported.

Additionally, two cases of *TSC2* mutations with large segment deletions were reported, all of which involved mutations in the *PKD1* gene.

ARPKD

Five cases of *PKHD1* mutations were identified [median age 26.0 years (range: 17.3–53.3)]. Among the five patients, only one carried a homozygous mutation, whereas the remaining four had compound heterozygous mutations. Nine mutation sites were identified, eight of which were missense mutations (88.9%). In one compound heterozygous patient, a mutation site was categorized as a VUS by ACMG. The patient reported no family history; however, the possibility of autosomal dominant inheritance with incomplete penetrance cannot be ruled out.

Age analysis in PKD patients revealed a statistically significantly lower average age of onset in patients with *PKHD1* mutations compared with those carrying *PKD1* and *PKD2* mutations (Bonferroni post hoc test; $P < .05$). Specifically, the average age of onset was found to follow the sequence *PKHD1* < *PKD1* < *PKD2*. Among patients carrying *PKD1* and *PKD2* mutations, no statistically significant difference in average age of onset was observed ($P > .05$). Gender distribution analysis revealed no significant differences in male:female ratios among the three groups (chi-squared test; $P = .907$). Despite the limited sample size, patients with *PKHD1* mutations demonstrated a clear trend of younger age at onset.

VUS in genes associated with phenotypes

In addition to the 448 patients harbouring pathogenic or likely pathogenic mutations, 158 carried phenotype-associated mutations classified as VUS under the ACMG guidelines.

The identified mutations span 18 genes and are potentially associated with nine distinct diseases, including ADPKD (associated genes: *PKD1*, *PKD2*, *DNAJB11*), ARPKD (associated gene: *PKHD1*), ADTKD (associated genes: *HNF1B*, *UMOD*), ADPLD (associated genes: *SEC63*, *LRP5*), hereditary tubulopathies (HTD; associated genes: *SLC34A1*, *SLC7A9*, *CLCN5*), nephronophthisis (NPHP; associated genes: *TTC21B*, *ZNF423*, *NPHP1*, *NPHP3*), cystic renal dysplasia (CYSRD; associated gene: *BICC1*), Alström syndrome (ALMS; associated gene: *ALMS1*) and TSC (associated gene: *TSC2*) (Fig. 1, Table 1). Further details on mutation sites are available in Supplementary Table S4).

Table 1: Genetic landscape of hereditary cystic kidney disease.

Characteristics	Disease	Mutant gene	Cases, *n*	Inheritance pattern
Mutations in genes associated with phenotypes and pathogenicity	ADPKD	*PKD1*	354 + 2	AD
		PKD2	76	AD
		GANAB	2	AD
	ADTKD	*HNF1B*	5	AD
		REN	1	AD
	ARPKD/AD	*PKHD1*	5	AR
	ADPLD	*ALG8*	1	AD
	IFT140-related	*IFT140*	1	AD
	COL4A1-related	*COL4A1*	1	AD
	TSC	*TSC2*	+2	AD
	Multiple gene mutations	*PKD1* + *TSC2*	2	
Mutations in genes associated with phenotypes with VUS pathogenicity	ADPKD	*PKD1*	121 + 5	AD
		PKD2	10 + 2	AD
		DANJB11	1	AD
	ARPKD	*PKHD1*	5 + 2	AR
	ADTKD	*HNF1B*	2	AD
		UMOD	1 + 1	AD
	ADPLD	*SEC63*	1	AD
		LRP5	2	AD
	HTD	*SLC34A1*	1	AD
		SLC7A9	1	AD
		CLCN5	1	AD
	NPHP	*TTC21B*	2	AD/AR
		ZNF423	1	AD/AR
		NPHP1	1	AR
		NPHP3	1	AR
	CYSRD	*BICC1*	1	AD
	ALMS	*ALMS1*	1	AR
	TSC	*TSC2*	+1	AD
	Multiple gene mutations	*PKD1* + *PKD2*	2	
		PKD1 + *TSC2*	1	
		PKD1 + *PKHD1*	1	
		PKD1 + *PKHD1* + *UMOD*	1	

Proportion of different diseases among hereditary cystic kidney disease patients. +: the number of patients with multiple gene mutations, including a specified gene mutation. AD: autosomal dominant inheritance; AR: autosomal recessive inheritance. ADPKD, ADTKD, ARPKD, NPHP, ADPLD, HTD, TSC, CYSRD and ALMS are defined as shown in Fig. 1.

No pathogenic mutations in genes detected

In 96 patients, no clear gene mutations were detected or the gene mutations found were benign or possibly benign based on ACMG pathogenicity analysis and REVEL protein function.

Novel pathogenic or likely pathogenic mutation sites in genes

Through the ClinVar and Mastermind genomic literature databases, 63 novel mutations sites were identified among those classified as pathogenic or likely pathogenic by the ACMG guidelines.

Among *PKD1* mutation patients, 360 mutations were detected, involving 288 mutation sites. Among these, 49 novel mutation sites were identified (Fig. 2, Supplementary Table S5).

Among *PKD2* mutation patients, 76 mutations were detected involving 56 mutation sites. Among these, 10 novel mutation sites were identified (Fig. 3, Supplementary Table S6).

Four additional novel mutations were detected in other genes (Table 3).

DISCUSSION

To date, this is the most comprehensive study on hereditary cystic kidney disease and its mutations in genes. We systematically organized the genetic testing results of 702 patients diagnosed with hereditary cystic kidney disease at the hereditary kidney disease clinic over the past nine years. We found that 606 (86.3%) patients harboured gene mutations associated with cystic kidney disease, involving 23 mutated genes and >500 mutation sites. Among these, 158 patients harboured mutations classified as VUS, while 448 patients were definitively diagnosed based on genetic findings. These diagnoses encompassed seven

Table 2: Mutated genes and associated clinical data in a subset of patients without *PKD1* or *PKD2* mutations.

No.	Gene	Variation	Age of onset (years); symptoms	Age (years)	Gender	Renal cyst	Maximum cyst size	Creatinine level (μmol/l)	eGFR slopes (ml/min/1.73 m^2/year)	Other diseases
439	*PKHD1*	NM_13 8694 (*PKHD1*): c.3089C>T (p.Ala1030Val); NM_13 8694 (*PKHD1*): c.2341C>T (p.Arg781Ter)	19; elevated creatinine levels	33	Female	Multiple cysts in both kidneys	Medium (2–4 cm)	200	7.2	Liver fibrosis, portal hypertension
440	*PKHD1*	NM_13 8694 (*PKHD1*): c.2240dupT (p.Ala748GlyfsTer18); NM_13 8694 (*PKHD1*): c.2507T > C (p.Val836Ala)	17; renal cyst and elevated creatinine levels	20	Male	Multiple cysts in both kidneys	–	276	33.3	Hyperuricaemia
442	*PKHD1*	NM_13 8694 (*PKHD1*): c.572G > C (p.Cys191Ser); NM_13 8694 (*PKHD1*): c.2507T > C (p.Val836Ala)	54; renal cyst	60	Male	Multiple cysts in both kidneys	Small (≤2 cm)	Normal	–	Liver cyst, prostate stones, colonic mucinous tubular adenoma, colon polyps
427	*ALG8*	NM_024079.5 (*ALG8*): c.129G > A (p.Trp43Ter)	22; renal cyst	25	Female	Multiple cysts in both kidneys	Medium (2–4 cm)	Normal	–	–
436	*IFT140*	NM_014 714 (*IFT140*): c.1524 + 1G > T (splicing)	55; renal cyst	64	Male	Multiple cysts in both kidneys	Large (>4 cm)	Normal	–	Kidney stones
151	*PKHD1*	NM_13 8694 (*PKHD1*): c.1675C > T (p.Arg559Trp); NM_13 8694 (*PKHD1*): c.11525G > A (p.Arg3842Gln)	12; renal cyst	24	Female	Multiple cysts in both kidneys	Large (>4 cm)	700	50	Hyperuricaemia, history of gout attacks
153	*PKHD1*	NM_13 8694 (*PKHD1*): c.2880G > T (p.Gln960His); NM_13 8694 (*PKHD1*): c.8687T > C (p.Ile2896Thr)	9; renal cyst	23	Female	Multiple cysts in both kidneys	Small (≤2 cm)	Normal	–	Liver cyst, liver fibrosis, portal hypertension
120	*NPHP1*	NM_000 272 (*NPHP1*): dup	32; renal cyst	41	Female	Multiple cysts in both kidneys	Large (>4 cm)	Normal	–	–
118	*LRP5*	NM_002335.4 (*LRP5*): c.1310C > T (p.Thr437Met)	25; renal cyst	50	Female	Multiple cysts in both kidneys	Large (>4 cm)	Normal	–	Kidney stones, urinary tract infection, bone hyperplasia
119	*LRP5*	NM_002335.4 (*LRP5*): c.3656G > A (p.Arg1219His)	18; elevated creatinine levels and renal cyst	28	Male	Multiple cysts in both kidneys	Large (>4 cm)	Normal	–	Hyperuricaemia, history of gout attacks
121	*SEC63*	NM_007214 (*SEC63*): c.1605del (p.Lys535Asnfs)	20; haematuria (microscopic)	27	Male	Multiple cysts in both kidneys	Small (≤2 cm)	Normal	–	Kidney stones, hyperuricaemia
114	*CLCN5*	NM_000084.5 (*CLCN5*): c.205G > C (p.Gly69Arg)	38; renal cyst and renal failure	39	Male	Multiple cysts in both kidneys	Small (≤2 cm)	900	–	Rickets
123	*SLC7A9*	NM_014 270 (*SLC7A9*): c.829G > A (p.Val277Met)	34; elevated creatinine levels	42	Female	Multiple cysts in both kidneys	Small (≤2 cm)	120	0.7	Hyperuricaemia
113	*BICC1*	NM_001080512.3 (*BICC1*): c.496A > G (p.Ile166Val)	31; renal cyst	32	Male	Multiple cysts in both kidneys	Small (≤2 cm)	136.5	15	Liver cyst

The patient numbers (No.) in the table correspond to Supplementary Tables S3 and S4. Among them, the first five patients (439, 440, 442, 427, 436) have genetic mutations classified as pathogenic or likely pathogenic, as indicated in Supplementary Table S3. In contrast, the subsequent nine patients (151, 153, 120, 118, 119, 121, 114, 123, 113) carry genetic mutations categorized as VUS, as indicated in Supplementary Table S4.

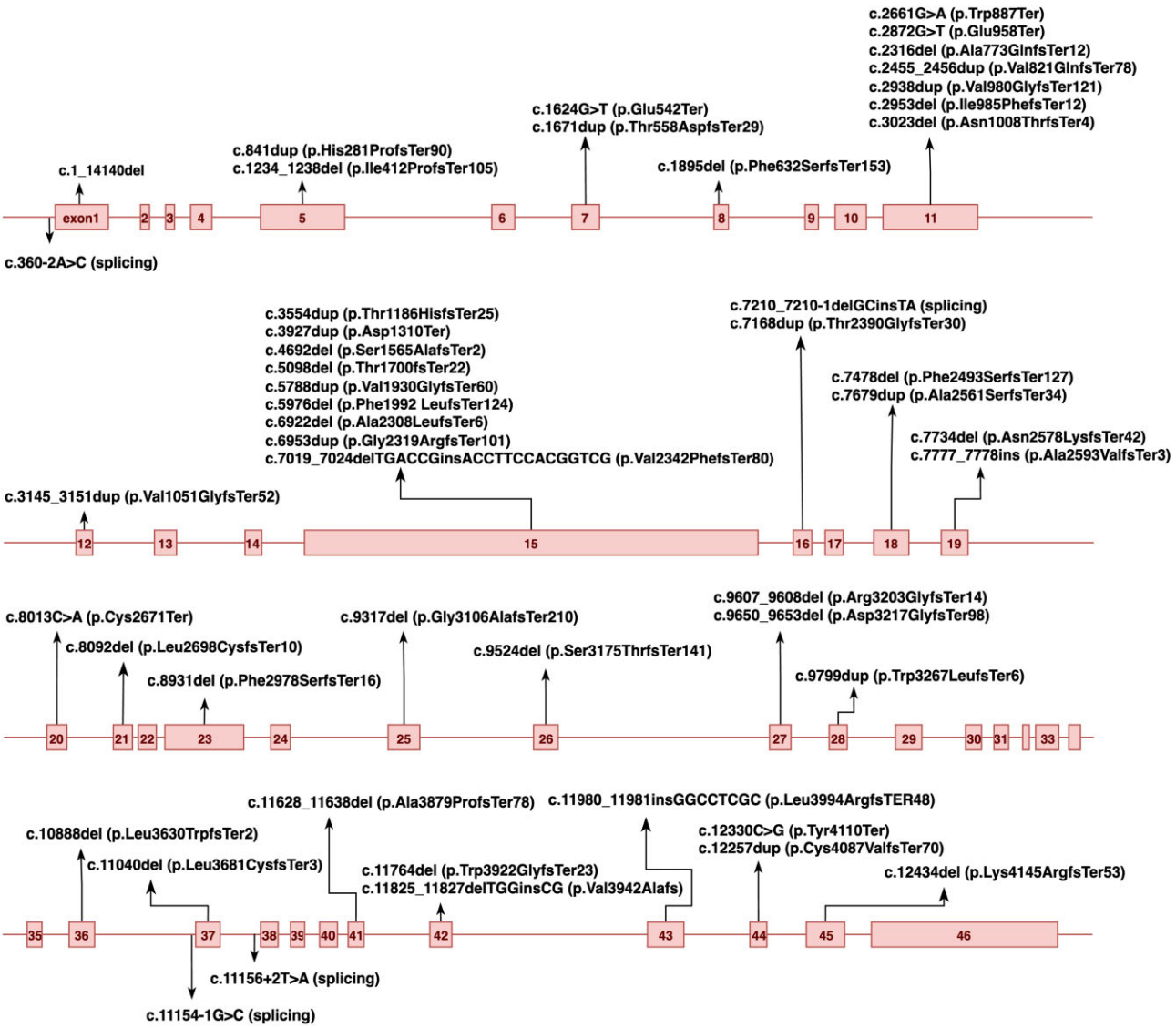


Figure 2: Novel mutation sites in *PKD1*. *PKD1* mutations involved 49 novel mutation sites, including five nonsense mutations, 40 frameshift mutations and four splice site mutations.

distinct diseases associated with 10 pathogenic genes, including the identification of 63 novel mutation sites. This study clarified the primary disease and genetic spectra of hereditary cystic kidney disease, enriched the genetic database and broadened clinicians' diagnostic perspectives.

ADPKD and cystic renal dysplasia

ADPKD is the most common hereditary cystic kidney disease and mainly involves mutations in *PKD1* and *PKD2*. These two genes encode PC1 and polycystin-2 (PC2), respectively. These two proteins interact to form new calcium ion channels and regulate the structure and function of renal tubules [2]. PC1 and PC2 are co-distributed in the primary cilia. Gene mutations can disrupt the mechanosensory capabilities of the cilia, leading to abnormal signal transduction and cell dysfunction, thus they can be classified as ciliopathies [3, 4].

Mutations in *GANAB* and *DNAJB11* are also associated with ADPKD. The *GANAB* gene encodes the catalytic subunit of α-glucosidase II, which is involved in quality control during glycoprotein folding [5]. *DNAJB11* encodes a glycoprotein that resides in the endoplasmic reticulum and acts as a co-factor of GRP78 (HSPA5), which is responsible for the correct folding and assembly of proteins [6]. Mutations in *GANAB* or *DNAJB11* lead to a significant reduction in the membrane surface expression of PC1, further affecting ciliary function. In this study, all four mutated genes were reported, with *PKD1* being the most common among all mutated genes, followed by *PKD2*. The pathogenicity of mutations in *DNAJB11* has yet to be fully elucidated.

Additionally, this study reported a case of renal cysts associated with a *BICC1* gene mutation, with the mutation site classified as a VUS. This gene co-localizes with PC2 at the basal body of the primary cilia and regulates the expression of *PKD2* [7, 8]. Mutations can also lead to CYSRD. Along with *GANAB* and *DNAJB11*, this gene is involved in the regulation of proteins encoded by ADPKD gene mutations. However, the former regulates *PKD2*, whereas the latter two regulate *PKD1*. Currently it is not classified as ADPKD.

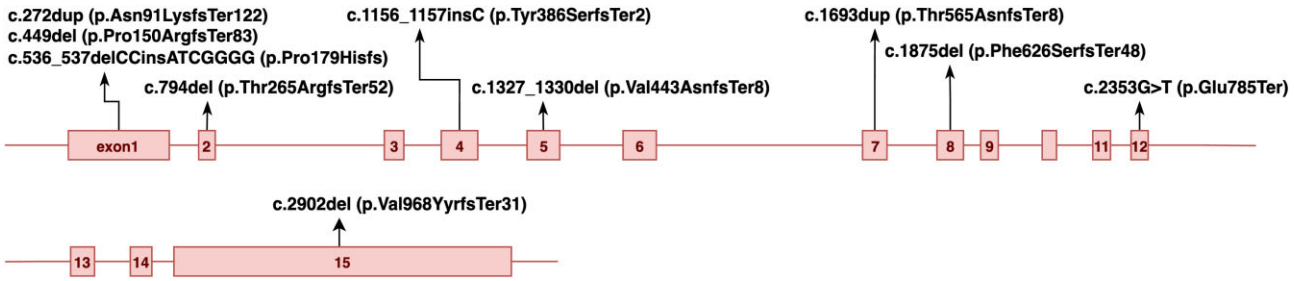


Figure 3: Novel mutation sites in *PKD2*. *PKD2* mutations involved 10 novel mutation sites, including eight frameshift mutations, one nonsense mutation and one deletion mutation.

Table 3: Novel mutation sites.

Gene	Variation	Mutation type	ACMG Criteria	ACMG Classification
PKHD1	NM_138694 (*PKHD1*): c.2240dupT (p.Ala748GlyfsTer18)	Frameshift	PVS1 + PM2 + PM3	Pathogenic
GANAB	NM_198335 (*GANAB*): c.2730G>A (p.Trp910Ter)	Point (nonsense mutation)	PVS1 + PM4 + PM2	Pathogenic
COL4A1	NM_001845 (*COL4A1*): c.3819G>A (p.Trp1273Ter)	Point (nonsense mutation)	PVS1 + PM2	Likely pathogenic
ALG8	NM_024079.5 (*ALG8*): c.129G>A (p.Trp43Ter)	Point (nonsense mutation)	PVS1 + PM2	Likely pathogenic

Other autosomal dominant inherited diseases

ADTKD is characterized by the structural and functional impairment of the renal tubules and interstitium. Currently, six genotypes are known: *HNF1B*, *MUC1*, *UMOD*, *REN*, *SEC61A1* and *DNAJB11* [9, 10]. The first four genes were summarized in the 2015 Kidney Disease: Improving Global Outcomes guidelines, whereas the latter two genes were discovered and reported in recent years. The current study aimed to classify the *DNAJB11* gene as ADPKD. In this study, mutations were detected in all five genes except *SEC61A1*. Among these, some mutation sites in *HNF1B* and all mutation sites in *REN* cases were classified as pathogenic or likely pathogenic, while some mutation sites in the *HNF1B* and all mutation sites in *UMOD* cases were categorized as VUS. These patients may develop kidney cysts, which are usually small and limited in number.

ADPLD is the most common extrarenal manifestation of ADPKD. In addition to the liver cysts caused by ADPKD, several gene mutations have been associated with ADPLD, including *PRKCSH*, *SEC35*, *ALG8*, *LRP5* [11] and *SEC63* [12]. This study identified *ALG8* as a gene with potential pathogenicity and *LRP5* and *SEC63* as genes classified as having VUS. *LRP5* encodes a transmembrane protein that regulates Wnt signalling through endocytosis. *ALG8* encodes α-3-glucosyltransferase, which is involved in proper protein folding and function, leading to cyst formation by affecting the glycosylation and cell surface transport of PC1 [13]. The protein encoded by *SEC63* is involved in the correct folding and transport of proteins in the endoplasmic reticulum [14]. These gene mutations further confirm the association between specific genes and ADPLD and multiple renal cysts. However, research indicates that various types of mutations in these genes do not significantly influence the clinical phenotype of ADPLD, including the disease's manifestations and severity. Thus, while these gene mutations contribute to disease pathogenesis, specific mutation types or locations are insufficient to predict the clinical presentation or severity in affected individuals [15]. Additionally, certain heterozygous mutations in the *LRP5* gene may be associated with the development of ADPKD, suggesting a potential genetic interaction between ADPLD and ADPKD [16].

Additionally, one case of a ciliopathy-related *IFT140* compound heterozygous mutation was reported. The protein encoded by *IFT140* is mainly located at the base and tip of the cilia [17]. Its functions include cilia formation and maintenance, retrograde ciliary transport and the regulation of cilia-dependent signal transduction. *IFT140* mutations exhibit two inheritance patterns: one associated with ciliopathies in an autosomal recessive manner and another, more recently discovered pattern, in which monoallelic truncating or obligatory splicing site mutations result in an ADPKD phenotype [18]. In this case, the patient has a splicing site mutation, characterized by a large renal cyst phenotype linked to ciliopathy, and is currently considered autosomal dominant.

Another patient was found to have a *COL4A1* mutation. Mutations in this gene have high clinical heterogeneity and can affect multiple organ systems, including the nervous system and kidneys. This may be related to the phenotype of multiple kidney cysts [19]. The patient presented with clinical phenotypes of multiple liver and kidney cysts and bilateral hydronephrosis. However, the relationship between the gene mutation and the phenotype remains unclear.

The *TSC2* pathogenic gene encodes the protein tuberin, which regulates the mammalian target of rapamycin signalling pathway and affects cell proliferation and metabolism [20]. Mutations can lead to the occurrence of multisystem benign tumours and cysts. *TSC2* is adjacent to *PKD1*, and all three patients with *TSC2* mutations reported by us had *PKD1* gene mutations, including two cases with large segment deletions, possibly related to *PKD1-TSC2* contiguous gene syndrome. We also identified one patient with a single *TSC2* gene mutation without a kidney cyst phenotype; therefore, the association between *TSC2* and the kidney cyst phenotype is unclear.

ARPKD

The ARPKD pathogenic gene *PKHD1* encodes fibrocystin, which is mainly expressed in the kidneys and pancreas. Mutations lead to the loss of fibrocystin function, causing ciliary dysfunction and mitotic defects [21]. Although typically associated with an autosomal recessive inheritance pattern, it may occasionally

manifest in an autosomal dominant form with incomplete penetrance. In this study, *PKHD1* was more commonly associated with compound heterozygous mutations, with only one of the five patients having a homozygous mutation.

Mutations in *PKD1*, *PKD2* and *PKHD1* lead to the development of polycystic kidney disease, with no statistically significant differences in gender distribution noted among patients with the three mutation types. However, the average age of onset in patients carrying *PKHD1* mutations was significantly earlier than that observed in patients with *PKD1* and *PKD2* mutations, aligning with findings from previous studies.

Others

This study identified 158 patients carrying genetic variants linked to phenotypes classified as VUS under the ACMG guidelines. Predicting the pathogenicity of these mutations relies heavily on existing gene and disease databases. However, these databases primarily contain mutation frequency and phenotype association data derived from Western populations, resulting in limited study of rare or newly identified variants due to insufficient database support. Furthermore, inconsistencies among bioinformatics tools underscore the constraints of current prediction models. Consequently, these 158 mutations and their associated diseases require further investigation.

In addition to the VUS mutations mentioned, this study identified three patients with single-gene mutations in HTD, presenting with renal cystic phenotypes. HTD mutations primarily disrupt renal tubular pump function. Although the relationship between HTD mutations and renal cystic phenotypes is poorly understood and sparsely documented in the literature, their potential association cannot be disregarded, considering their impact on renal tubular function.

Additionally, two patients were identified as carrying *TTC21B* mutations and one with a *ZNF423* mutation. These genes are implicated in NPHP and play essential roles in cilia development. Previous studies have reported that mutations in these genes display both dominant and recessive inheritance patterns.

This study also identified four other autosomal recessive gene mutations—*NPHP1*, *NPHP3* and *ALMS1*—all of which are directly related to ciliary function. The first two are classified as NPHP, a disease that leads to kidney failure in children and adolescents. In addition to kidney phenotypes, extrarenal manifestations such as liver fibrosis, situs inversus and cardiac anomalies may also occur. It is associated with various syndromes, including Senior–Løken syndrome (SLSN1), Joubert syndrome (JBTS1) and Meckel–Gruber syndrome (MKS1). Associated gene mutations are located in the cilia and related structures [22].

ALMS is an autosomal recessive disease. The *ALMS1*-encoded protein participates in the structure and function of the centrioles, which are primarily located at the base of the cilia. It manifests as obesity, retinal pigment degeneration, cardiomyopathy and diabetes and is less directly associated with kidney cysts.

Notably, 13.6% of the patients in this study were not found to harbour any associated genetic mutations, highlighting the need for further exploration. Advancements in genetic testing will uncover more pathogenic mutations in genes, enrich the genetic landscape of hereditary cystic kidney diseases and elucidate the molecular mechanisms underlying physiological and pathological processes.

However, this study had some limitations. This was a retrospective, single-centre study, which might have limited the representativeness of the sample. Predictions of the pathogenicity of novel mutations mainly rely on bioinformatics, potentially leading to erroneous pathogenicity predictions. The scope of genetic testing is limited, as most patients do not undergo whole genome or whole exome sequencing, thereby possibly missing known or unknown pathogenic mutations in genes. Disease grouping may change based on future studies as genes are reclassified, such as the recent shift in *GANAB* from ADPLD to ADPKD.

CONCLUSIONS

In this study, 23 gene mutations associated with renal cyst phenotypes were identified, of which 10 included pathogenic or likely pathogenic variants. These findings enabled the diagnosis of seven hereditary cystic kidney diseases, including ADPKD, ADTKD, ARPKD and others. Furthermore, 63 novel pathogenic or likely pathogenic variants were identified. This clarified the main disease spectrum and genetic landscape of hereditary cystic kidney disease and new mutation sites, enriched the gene database and broadened clinicians' perspectives.

SUPPLEMENTARY DATA

Supplementary data are available at *Clinical Kidney Journal* online.

ACKNOWLEDGEMENTS

The authors extend their gratitude to the staff and patients of the Hereditary Kidney Disease Clinic and the Genetic Testing Laboratory at the PLA General Hospital for their cooperation in completing this study.

FUNDING

This work was supported by grants from the National Basic Research Program of China (2011CB944004), National Key Research and Development Program of China (2016YFC0901502) and National Natural Science Foundation of China (82174115).

AUTHORS' CONTRIBUTIONS

Y.X. and J.B. contributed to the research and study design. J.B. was responsible for data acquisition, analysis and interpretation. W.G. provided supervision and mentorship support. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

DATA AVAILABILITY STATEMENT

The datasets generated and/or analysed in the current study are available from the corresponding author upon request.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

REFERENCES

1. Lanktree MB, Chapman AB. New treatment paradigms for ADPKD: moving towards precision medicine. *Nat Rev Nephrol* 2017;**13**:750–68. https://doi.org/10.1038/nrneph.2017.127

2. Tsiokas L, Kim E, Arnould T *et al*. Homo- and heterodimeric interactions between the gene products of *PKD1* and *PKD2*. *Proc Natl Acad Sci USA* 1997;**94**:6965–70. https://doi.org/10.1073/pnas.94.13.6965
3. Nauli SM, Alenghat FJ, Luo Y *et al*. Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. *Nat Genet* 2003;**33**:129–37. https://doi.org/10.1038/ng1076
4. Hanaoka K, Qian F, Boletta A *et al*. Co-assembly of polycystin-1 and -2 produces unique cation-permeable currents. *Nature* 2000;**408**:990–4. https://doi.org/10.1038/35050128
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 Hum Genet* 2016;**98**:1193–207. https://doi.org/10.1016/j.ajhg.2016.05.004
6. Cornec-Le Gall E, Olson RJ, Besse W *et al*. Monoallelic mutations to *DNAJB11* cause atypical autosomal-dominant polycystic kidney disease. *Am Hum Genet* 2018;**102**:832–44. https://doi.org/10.1016/j.ajhg.2018.03.013
7. Kraus MR, Clauin S, Pfister Y *et al*. Two mutations in human *BICC1* resulting in Wnt pathway hyperactivity associated with cystic renal dysplasia. *Hum Mutat* 2012;**33**:86–90. https://doi.org/10.1002/humu.21610
8. Leal-Esteban LC, Rothé B, Fortier S *et al*. Role of bicaudal C1 in renal gluconeogenesis and its novel interaction with the CTLH complex. *PLoS Genet* 2018;**14**:e1007487. https://doi.org/10.1371/journal.pgen.1007487
9. Eckardt KU, Alper SL, Antignac C *et al*. Autosomal dominant tubulointerstitial kidney disease: diagnosis, classification, and management—a KDIGO consensus report. *Kidney Int* 2015;**88**:676–83. https://doi.org/10.1038/ki.2015.28
10. Econimo L, Schaeffer C, Zeni L *et al*. Autosomal dominant tubulointerstitial kidney disease: an emerging cause of genetic CKD. *Kidney Int Rep* 2022;**7**:2332–44. https://doi.org/10.1016/j.ekir.2022.08.012
11. Cnossen WR, te Morsche RH, Hoischen A *et al*. Whole-exome sequencing reveals *LRP5* mutations and canonical Wnt signaling associated with hepatic cystogenesis. *Proc Natl Acad Sci USA* 2014;**111**:5343–8. https://doi.org/10.1073/pnas.1309438111
12. Van Keimpema L, De Koning DB, Van Hoek B *et al*. Patients with isolated polycystic liver disease referred to liver centres: clinical characterization of 137 cases. *Liver Int* 2011;**31**:92–8. https://doi.org/10.1111/j.1478-3231.2010.02247.x
13. Besse W, Dong K, Choi J *et al*. Isolated polycystic liver disease genes define effectors of polycystin-1 function. *J Clin Invest* 2017;**127**:1772–85. https://doi.org/10.1172/JCI90129
14. 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**:639–47. https://doi.org/10.1038/ng.860
15. Van Keimpema L, De Koning DB, Van Hoek B *et al*. Patients with isolated polycystic liver disease referred to liver centres: clinical characterization of 137 cases. *Liver Int* 2011;**31**:92–8. https://doi.org/10.1111/j.1478-3231.2010.02247.x
16. Cnossen WR, Te Morsche RH, Hoischen A *et al*. *LRP5* variants may contribute to ADPKD. *Eur J Hum Genet* 2016;**24**:237–42. https://doi.org/10.1038/ejhg.2015.86
17. Perrault I, Saunier S, Hanein S *et al*. Mainzer-Saldino syndrome is a ciliopathy caused by *IFT140* mutations. *Am Hum Genet* 2012;**90**:864–70. https://doi.org/10.1016/j.ajhg.2012.03.006
18. 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 Hum Genet* 2022;**109**:136–56. https://doi.org/10.1016/j.ajhg.2021.11.016
19. Shan LD, Peng J, Xiao H *et al*. Clinical features and COL4A1 genotype of a toddler with hereditary angiopathy with nephropathy, aneurysms and muscle cramps syndrome. *Zhongguo Dang Dai Er Ke Za Zhi* 2019;**21**:754–60.
20. Ozcan U, Ozcan L, Yilmaz E *et al*. Loss of the tuberous sclerosis complex tumor suppressors triggers the unfolded protein response to regulate insulin signaling and apoptosis. *Mol Cell* 2008;**29**:541–51. https://doi.org/10.1016/j.molcel.2007.12.023
21. Ward CJ, Hogan MC, Rossetti S *et al*. The gene mutated in autosomal recessive polycystic kidney disease encodes a large, receptor-like protein. *Nat Genet* 2002;**30**:259–69. https://doi.org/10.1038/ng833
22. Hoff S, Halbritter J, Epting D *et al*. ANKS6 is a central component of a nephronophthisis module linking NEK8 to INVS and *NPHP3*. *Nat Genet* 2013;**45**:951–6. https://doi.org/10.1038/ng.2681

Received: 14.8.2024; *Editorial decision:* 27.1.2025