Research article

Genetic landscape and clinical outcomes of autosomal recessive polycystic kidney disease in Kuwait

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ABSTRACT

Background: Autosomal recessive polycystic kidney disease (ARPKD), a rare genetic disorder characterized by kidney cysts, shows complex clinical and genetic heterogeneity. This study aimed to explore the genetic landscape of ARPKD in Kuwait and examine the intricate relationship between its genes and clinical presentation to enhance our understanding and contribute towards more efficient management strategies for ARPKD.
Methods: This study recruited 60 individuals with suspected ARPKD from 44 different families in Kuwait. The participants were of different ethnicities and aged 0–70 years. Additionally, 33 were male, 15 were female, and 12 had indeterminant sex due to congenital anomalies. Comprehensive clinical data were collected. Mutations were identified by next-generation whole exome sequencing and confirmed using Sanger sequencing.
Results: Of the 60 suspected ARPKD cases, 20 (33.3 %) died within hours of birth or by the end of the first month of life and one (1.7 %) within 12 months of birth. The remaining 39 (65.0 %) cases were alive, at the time of the study, and exhibited diverse clinical features related to ARPKD, including systematic hypertension (5.0 %), pulmonary hypoplasia (11.7 %), dysmorphic features (40.0 %), cardiac problems (8.3 %), cystic liver (5.0 %), Potter syndrome (13.3 %), developmental delay (8.3 %), and enlarged cystic kidneys (100 %). Twelve mutations, including novel truncating mutations, were identified in 31/60 cases (51.7 %) from 17/44 families (38.6 %). Additionally, 8/12 (66.7 %) mutations were in the *PKHD1* gene, with the remaining four in different genes: *NPHP3*, *VPS13P*, *CC2D2A*, and *ZNF423*.
Conclusions: This study highlights the spectrum of clinical features and genetic mutations of patients with ARPKD in Kuwait. It highlights the necessity for personalized approaches to improve ARPKD diagnosis and treatment, offering crucial insights into managing ARPKD.

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1. Introduction

Autosomal recessive polycystic kidney disease (ARPKD) is a rare, inherited, and severe polycystic kidney disease that leads to early end-stage renal disease (ESRD). It is considered the primary cystic disease in childhood and consists of bilateral renal disease and congenital hepatic fibrosis. Its recessive inheritance leads to an early onset with severe symptoms [1,2]. ARPKD and autosomal dominant polycystic kidney disease (ADPKD) are both considered leading inherited forms of renal disease [3–6]. The incidence of ARPKD has been reported to be 1 in 26,500 live births [7]. ARPKD is mainly caused by mutations in the polycystic kidney and hepatic disease 1 (*PKHD1*) gene [8]. The *PKHD1* gene has the longest open reading frame of 67 exons and encodes a fibrocystin protein (of length 4074 amino acids), a membrane protein localized to the sensory cilium of the cortical and medullary collecting ducts and the thick ascending limbs of the loop of Henle; it is also expressed in the biliary and pancreatic tracts [1,2,7,9]. *PKHD1* is widely expressed in the neural tube, primordial gut, bronchi, early ureteric bud, adrenal cortex, mesonephric tubules, and immature hepatocytes during fetal development, indicating its role in organ development and tubular morphogenesis [10].

ARPKD is diagnosed prenatally or neonatally, with rapid progression leading to early mortality, primarily attributed to respiratory compromise and renal failure [2]. It is also seen to be present with oligohydramnios or anhydramnios due to prenatal kidney failure, resulting in the Potter syndrome phenotype, which includes pulmonary hypoplasia due to impaired lung development, dysmorphic facial features, and clubfoot contracted limbs [1,2]. Alternatively, later-onset, milder forms present manageable renal impairment that increases survival to 85 % [2]. Because ARPKD is associated with both the kidney and liver, cystic liver also plays a role in ARPKD, and polycystic liver disease is considered the primary extrarenal manifestation and appears with age [1,2]. Two main liver disease manifestations are seen in ARPKD: portal hypertension (resulting from progressive hepatic fibrosis) and cholangitis [2]. Portal hypertension is associated with multiple complications, including splenomegaly, thrombocytopenia, and esophageal varices that cause severe bleeding [2]. While the disease is autosomal recessive, some heterozygous carriers of a *PKHD1* mutation may have a higher risk of developing polycystic liver disease or mild ARPKD [2]. Its genetic and clinical variability underscores the complexity of ARPKD, necessitating more effective diagnosis and deeper understanding for improved management.

This study aimed to explore the genetic landscape of ARPKD in Kuwait and examine the intricate relationship between its genes and phenotypes to enhance our understanding and contribute towards more efficient management strategies for this condition.

2. Materials and methods

2.1. Participant recruitment and sample collection

Blood samples were collected from patients referred to the Kuwait Medical Genetics Center in ethylenediaminetetraacetic acid (EDTA) blood collection tubes. The cohort comprised 60 patients with suspected ARPKD from 44 different families. The patients with suspected ARPKD were subjected to prenatal screening and ultrasound tests, which detected cystic kidneys that were echogenic with a loss of cortico-medullary differentiation, very early kidney disease, or liver portal hypertension with periportal hepatic fibrosis.

Genetic analyses were performed using the DNA extracted from the collected blood samples. This study collected clinical information on the selected cases from their medical files, including the clinical diagnosis, presence of fluid-filled cysts in the kidney, kidney enlargement, systemic hypertension, pulmonary hypoplasia, dysmorphic features, cardiac problems, cystic liver, potter syndrome, and developmental delay. It also collected demographic information, including a family history of ARPKD and parental consanguinity.

Ethical approval

This study was ethically approved by the Research Ethics Committee of the Ministry of Health (MOH) in Kuwait (2022/1996). All cases provided written consent that is obtained from adult patients or the parents of pediatric or deceased patients in agreement with the ethical guidelines set by the MOH Research Ethics Committee.

2.2. Genetic analysis

a) DNA extraction

DNA was manually extracted from 5 mL of whole blood obtained from suspected ARPKD cases in EDTA blood collection tubes using the phenol/chloroform method.

b) Next-generation whole exome sequencing (WES)

The concentration and purity of the extracted DNA samples were measured using a NanoDrop 2000 spectrophotometer. Next, they were diluted to the desired concentration using a Qubit 3 Fluorometer. Then, WES libraries were prepared for each DNA sample by target amplification using the Ion AmpliSeq Exome RDY Kit (Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer's protocol. Briefly, the primer and HiFi reagent were added to the DNA samples, which were then subjected to PCR amplification using a ProFlex PCR system. Next, the FuPa reagent was added to each sample to fragment the DNA, and then ligase and an adapter were added to label the fragments. Then, the DNA fragments were purified and quantified using quantitative PCR. Finally, the

templates were prepared using the Ion Chef System and sequenced using the Ion Torrent Ion S5 XL semiconductor sequencer (Thermo Fisher Scientific).

2.3. Variant detection

The sequencing of each DNA sample created a variant call format (VCF) file for each case. Disease-causing variants were identified by uploading each patient's VCF file containing all detected variants to the Ion Torrent reporting service.

2.4. Validation of the identified variants by Sanger sequencing

The genotypes determined for the identified variants/mutations by WES were validated using direct Sanger sequencing of the PCR products. Sanger sequencing was performed using the BigDyeTerminator v3.1 Cycle Sequencing FS Ready Reaction Kit (Applied Biosystems), according to the manufacturer's instructions, on an Applied Biosystems 3730xl DNA analyzer (Applied Biosystems).

2.5. Assessment of variant pathogenicity

The identified variants were classified using the ANNOVAR software tool based on their sorting intolerant from tolerant (SIFT) scores and predictions [11], polymorphism phenotyping version 2 (Polyphen2) human variant (HVAR) scores and predictions [12], functional analysis through hidden Markov models (FATHMM) scores and predictions, ensemble logistic regression (MetaLR) scores and predictions [13], PhyloP 100-way vertebrate scores, genomic evolutionary rate profiling (GERP^{++}) rejected substitutions (RS) scores, PhastCons 100-way vertebrate scores [14], and site-specific phylogenetic analysis (SiPhy) 29-way scores to identify variants of interest based on their predicted pathogenicity [15].

2.6. Structural and stability analyses

Four nonsynonymous point mutations in the *PKHD1* gene were selected for these analyses based on the reference fibrocystin amino acid sequence (UniProt accession: P08F94) [16]. The sections of the fibrocystin sequence containing the variants were subjected to highly accurate structural modeling using AlphaFold 2.0 via the Google Colab online cloud service [17]. Each variant's effects on the stability and dynamics of fibrocystin were assessed using DynaMut [18]. The fibrocystin structures containing the variants were visualized using the PyMOL structural analysis software.

3. Results

3.1. Clinical features

The 60 cases with clinically suspected ARPKD were from 44 families, of which 31 were consanguineous. Of the 60 patients, 33 (55.0 %) were male, 15 (25.0 %) were female, and 15 were of indeterminant sex due to congenital anomalies or death soon after birth. ARPKD was diagnosed before the age of 10 years in 57 of the 60 patients (Table 1). The severity of the patients' clinical features varied from highly lethal, causing perinatal deaths, to mild renal impairment, with patients still alive at the age of 70 years. One in three of the

Table 1
ARPKD: autosomal recessive polycystic kidney disease, N: number, %: percentage.

Characteristics	Count of ARPKD cases, N, exhibiting the charateristics	Proportion of study cohort, %, exhibiting the characteristics
ARPKD affected cases	60	100
Males	33	55
Females	15	25
Not specified		
Age of diagnosis	12	20
Birth to 10 years	57	95
>10 years	3	5
Deceased	21	35
Birth- 1st month:	20	33.3
2–12 months:	1	1.7
Alive	39	65
Clinical features		
Systemic hypertension	3	5
Pulmonary hypoplasia	7	11.7
Dysmorphic features	24	40
Cardiac problems	5	8.3
Cystic liver	3	5
Potter syndrome	8	13.3
Developmental delay	5	8.3
Enlarged cystic kidneys	60	100

Table 2
Summary of genotype-phenotype outcomes of ARPKD cases. WES: whole exome sequencing.

Family no.	Pedigree no.	Genotype	Count of affected individuals in the family	Consanguinity	Post-neonatal death	Systemic hypertension	Pulmonary hypoplasia	Dysmorphic features	Cardiac problems	Cystic liver	Potter syndrome	Developmental delay
1	I	PKHD1 c.7638_7639dupTT (p.Ser2547Phe)	1	/	/		/	/	/			
2	II	PKHD1 c.4870C > T (p.Arg1624Trp)	3	/		/				/		
3	III	NPHP3 c. 2694-delA	3	/	/			/		/	/	
4		Unidentified	1	/								
5	IV	PKHD1 c.664A > G (p.Ile222Val) + c.3988del (p.Leu1330Phefs*5)	3		/	/				/		/
6	V	VPS13B c.8030delG (p.Cys2677fs*65)	2	/			/					/
7		Unidentified	1	/								
8	VI	NPHP3 c. 2694-del	3	/	/		/					
9		Unidentified	1	/						/		
10	VII	Unidentified	2	/	/						/	
11	VIII	PKHD1 c.5134G > A (p.Gly1712Arg) + c.6059del (p.Thr2020Ilefs813)	2	/	/		/	/				
12		Unidentified	1									
13		NPHP3 c. 2694-del	1	/	/			/			/	
14	IX	CC2D2A c.3084del (p.Lys1029Argfs*3)	3	/	/			/				/
15	X	PKHD1 c.982C > T	2	/	/			/				
16		Unidentified	1									
17		Unidentified	1	/								
18		Unidentified	1		/			/			/	
19		Unidentified	1	/	/			/				
20		Unidentified	1	/				/				
21		Unidentified	1									
22		ZNF423 c.2738C > T (p.Pro913Leu)	1	/				/				
23	XI	PKHD1 c.3539G > A (p.Gly1180Glu)	2	/	/			/				
24		Unidentified	1	/								
25		NPHP3 c. 2694-del	1	/							/	
26		Unidentified	1					/				
27		PKHD1 c.4870C > T (p.Arg1624Trp)	1	/		/		/				
28		Unidentified	1									
29		Unidentified	1	/			/	/				
30		Unidentified	1									
31		Unidentified	1	/	/			/				

Table 2 (*continued*)

Family no.	Pedigree no.	Genotype	Count of affected individuals in the family	Consanguinity	Post-neonatal death	Systemic hypertension	Pulmonary hypoplasia	Dysmorphic features	Cardiac problems	Cystic liver	Potter syndrome	Developmental delay
32		Unidentified	1	/					/			
33	XII	Unidentified	1	/								
34		Unidentified	1						/			
35	XIII	PKHD1 c.3539G > A (p. Gly1180Glu)	1	/					/			
36		Unidentified	1									
37		Unidentified	1	/						/		
38		Unidentified	1						/			
39	XIV	Unidentified	2		/		/	/			/	
40	XV	PKHD1 c.7638_7639dupTT (p.Ser2547Phe)	1	/								
41		Unidentified	1	/						/		
42		Unidentified	1									
43	XVI	NPHP3 c. 2694-del	1	/	/							
44		Unidentified	1	/								

patients died within the first month of life. The documented clinical features included systematic hypertension (3/60, 5 %), pulmonary hypoplasia (7/60, 11.7 %), dysmorphic features (24/60, 40 %), cardiac abnormalities (5/60, 8.3 %), cystic liver (3/60, 5 %), Potter syndrome (8/60, 13.3 %), developmental delay (5/60, 8.3 %), and enlarged cystic kidneys (60/60, 100 %) (Table 1).

3.2. Genetic analysis

Examination of the WES data for all genes from the 60 suspected cases identified 12 mutations in *PKHD1* (n = 8), nephrocystin 3 (*NPHP3*; n = 1), vacuolar protein sorting 13 homolog B (*VPS13B*; n = 1), coiled-coil and C2 domain containing 2A (*CC2D2A*; n = 1), and zinc finger protein 423 (*ZNF423*; n = 1) genes in 31/60 (51.7 %) cases from 17 of the 44 (38.6 %) families (Table 2; Fig. 1). No genetic mutations were identified in the remaining 29 patients (48.3 %). Two of the mutations in *PKHD1* (c.7638_7639dupTT [p. Ser2547Phe] and c.6059del [p.Thr2020Ilefs813]) and one in *VPS13B* (c.8030delG [p.Cys2677fs*65]) were novel variants that truncate the encoded protein.

Eight of the 12 mutations were homozygous in patients from consanguineous families, and the remaining four were heterozygous. Four of these homozygous mutations were observed in more than one family: families 1 and 40 harbored the same homozygous mutation in *PKHD1* (c.7638_7639dupTT [p.Ser2547Phe]); families 2 and 27 harbored the same homozygous mutation in *PKHD1* (c.4870C > T [p.Arg1624Trp]); families 3, 8, 13, 25, and 43 harbored the same homozygous mutation in *NPHP3* (c. 2694-del); and families 23 and 35 harbored the same homozygous mutation in *PKHD1* (c.3539G > A [p.Gly1180Glu]). Additionally, the patients from two consanguineous families (5 and 11) were found to be compound heterozygous for distinct mutations in *PKHD1*: those in family 5 had the c.664A > G (p.Ile222Val) and c.3988del (p.Leu1330Phefs*5) mutations, and those in family 11 had the c.5134G > A (p. Gly1712Arg) and c.6059del (p.Thr2020Ilefs813)] mutations (Table 2).

Nine of the 12 mutations were found to be fatal since they were often seen in patients who had died postneonatally (Table 3): seven in *PKHD1*, one in *CC2D2A*, and one in *NPHP3*.

3.3. Pathogenicity analysis

Of the eight mutations in the *PKHD1* gene, two were non-truncating with conflicting annotation for pathogenicity, one was truncating and likely pathogenic, and the remaining five were truncating pathogenic mutations. The *CC2D2A* mutation was a frameshift caused by a deletion and was classified as truncating and pathogenic. The *ZNF423* mutation was a nonsynonymous point mutation and was classified as truncating and pathogenic. The *VPS13B* mutation was a frameshift caused by a deletion and was classified as truncating and pathogenic. The *NPHP3* mutation was a frameshift caused by a deletion and was classified as truncating and pathogenic. Patients with mutations in these genes, except *ZNF423*, were often observed to die at an early age (Table 3). While nine of the 12 mutations were annotated as pathogenic, Three in *PKHD*1 (p.Gly1180Glu, p.Arg1624Trp, and p.Gly1712Arg) were annotated as either likely pathogenic or conflicting pathogenicity (Table 2).

3.4. Structural and stability analysis

Protein structural stability analysis indicated that while the p.Gly1180Glu stabilizes the structure, the remaining three mutations destabilize the structure (Fig. 2).

4. Discussion

ARPKD is a rare and fatal monogenic polycystic kidney disease that leads to chronic kidney disease and early ESRD during childhood. Consistent with observations on rare genetic disorders, its incidence is higher in the Middle East due to consanguineous marriages [19]. This study presents the first genetic landscape of ARPKD in one Middle Eastern state, Kuwait, examining the clinical
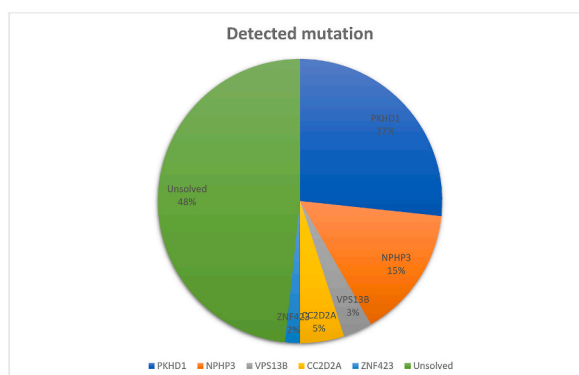

Fig. 1. Genes affected that were detected in the study cohort.

Table 3

Details of ARPKD mutations, Chr: chromosome.

Family no.	Pedigree no.	Gene	Chr	Exon	c.DNA variant	Protein variant	Ref. gene	SIFT score and prediction[1]	Polyphen2 HVAR Score and Prediction[2]	FATHMM Score and Prediction[3]	MetaLR Score and Prediction[4]	PhyloP 100way Vertebrate[5]	GERP++ RS[6]	PhastCons 100way Vertebrate[7]	SiPhy 29way[8]	Mutation type	Functional effect	Pathogenicity	ARPKD Variant Database	dbSNP ID
2, 27	II	PKHD1	6	32	c.4870C>T	p.Arg1624Trp	NM_138694	0.002 Damaging	Possibly damaging	-1.11 Tolerated	0.222 Tolerated	4.822	4.43	1	0.57	Non-synonymous	Non-truncating	Conflicting pathogenicity	Yes	rs200391019
5	IV	PKHD1	6	9	c.664A>G	p.Ile222Val	NM_138694	0 Damaging	Benign	-1.93 Damaging	0.38 Tolerated	4.437	5.18	1	0.578	Non-synonymous	Truncating	Pathogenic	Yes	rs369925690
5	IV	PKHD1	6	32	c.3988del	p.Leu1330Phefs*5	NM_138694									frameshift	Truncating	Pathogenic	No	
11	VIII	PKHD1	6	32	c.5134G>A	p.Gly1712Arg	NM_138694	0 Damaging	Damaging	-3.77 Damaging	0.918 Damaging	3.611	4.68	0.906	0.583	Non-synonymous	Non-truncating	Conflicting pathogenicity	yes	rs141103838
11	VIII	PKHD1	6	37	c.6059del	p.Thr2020Ilefs813	NM_138694									Frameshift	Truncating	Pathogenic	No	
14	IX	CC2D2A	4	25	c.3084del	p.Lys1029Argfs*3	NM_001080522									Frameshift	Truncating	Pathogenic	No	
15	X	PKHD1	6	14	c.982C>T		NM_138694					1.097	3.83	1	0.651	Nonsense	Truncating	Pathogenic	Yes	rs398124503
22		ZNF423	16	2	c.2738C>T	p.Pro913Leu	NM_001330533	0.01 Damaging	Damaging	2.84 Tolerated	0.098 Tolerated	9.994	4.81	1	0.888	Non-synonymous	Truncating	Pathogenic	Yes	rs200585917
23, 35	XI, XIII	PKHD1	6	30	c.3539G>A	p.Gly1180Glu	NM_138694	0 Damaging	Damaging	-2.47 Damaging	0.779 Damaging	3.301	4.81	0.992	0.601	Non-synonymous	Truncating	Likely pathogenic	Yes	rs636692
1, 40	I, XV	PKHD1	6	48	c.7638_7639dupTT	p.Ser2547Phe	NM_138694									Frameshift	Truncating	Pathogenic	No	
6	V	VPS13B	8	43	c.8030delG	p.Cys2677fs*65	NM_017890									Frameshift	Truncating	Pathogenic	No	
3, 8, 13, 25, 43	III, VI, XVI	NPHP3	3	20	c. 2694-del		NM_153240									Frameshift	Truncating	Pathogenic	Yes	rs751527253

1 SIFT scores range from 0 to 1. Scores <0.05 are predicted to be damaging.
2 Polyphen2 HVAR score predictions as follows; Probably damaging (0.909-1), Possibility damaging (0.447-0.908) and Benign (0-0.446).
3 FATHMN scores less than -1.5 are predicted as Damaging while scores higher than -1.5 are predicted as Tolerated.
4 MetaLR scores ranges from (0-1). Deleterious threshold >0.5.
5 Phylop 100way Vertebrate: Negative scores indicate faster-than expected evolution, while positive values indicate conservation. Deleterious threshold > 1.6.
6 GERP++ RS: Deleterious threshold > 4.4.
7 PhastCons 100way Vertebrate: scores range from 0-1. Scores higher than 0.5 are more likely to be conserved.
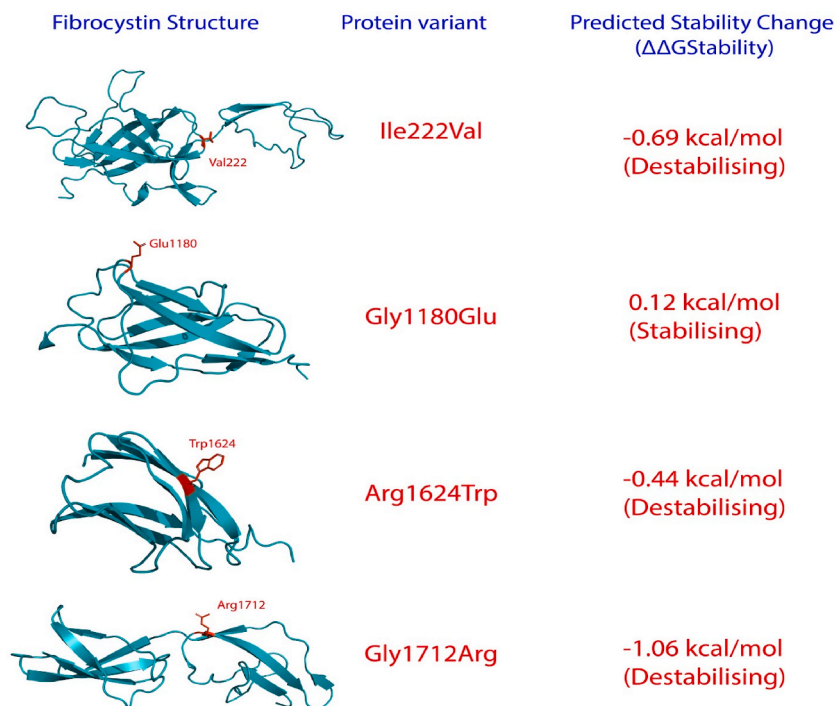8 SiPhy 29way: Deleterious threshold > 12.17.

Fibrocystin Structure	Protein variant	Predicted Stability Change ($\Delta\Delta G$Stability)
	Ile222Val	-0.69 kcal/mol (Destabilising)
	Gly1180Glu	0.12 kcal/mol (Stabilising)
	Arg1624Trp	-0.44 kcal/mol (Destabilising)
	Gly1712Arg	-1.06 kcal/mol (Destabilising)

Fig. 2. structural and stability analysis that shows structure of the four selected point mutation in the *PKHD1* gene, and their protein stability in the gene.

and genetic heterogeneity in a cohort of 60 patients diagnosed with ARPKD from 44 families. Most of the cohort (57/60, 95 %) were diagnosed between birth and the age of 10 years, indicating the early onset of the disease. The cohort showed the common clinical features of ARKPD, including systemic hypertension, pulmonary hypoplasia, dysmorphic features, cardiac problems, cystic liver, potter syndrome, developmental delay, and enlarged cystic kidneys. The disease severity varied from being highly lethal, causing postneonatal death, to mild renal impairment in patients still alive at the age of 70 years. Notably, 21/60 (35 %) of the cases were deceased due to kidney failure, respiratory failure, or poor organ development. It has been previously reported that 30%–50 % of ARPKD cases die shortly after birth due to respiratory deficiency resulting from pulmonary hypoplasia and thoracic compression caused by the massive enlargement of the kidneys [20]. It has also been estimated that the survival rate increases to 85 % once the ARPKD cases pass the perinatal period [2]. Another study in North America confirms that infants surviving the perinatal period have a better long-term prognosis [21]. Early detection and diagnosis can significantly increase the survival rate of symptomatic cases via treatments that can slow disease progression [1].

This study observed 12 mutations (of which three are novel) in its cohort. Eight of the 12 mutations were observed in the *PKHD1* gene, which is unsurprising since *PKHD1* has a very long open reading frame of 67 exons. The observed mutations in *PKHD1* were in exons 9, 14, 20, 30, 32, 37, and 48, with the one in exon 32 often present in patients with ARPKD. Nine of the 12 detected mutations were annotated as pathogenic, with the remaining three located in *PKHD1* and annotated as likely pathogenic (p.Gly1180Glu) or conflicting pathogenicity (p.Arg1624Trp and p.Gly1712Arg). A 2009 study by Gunay-Aygun et al. confirmed that the p.Gly1180Glu mutation is likely pathogenic. In addition, a 2016 study by Edrees et al. found the p.Arg1624Trp mutation to be pathogenic. Moreover, the study by Gunay-Aygun et al. suggested that the p.Gly1712Arg mutation was likely pathogenic.

Except for the two variants in *PKHD1* that were annotated as conflicting pathogenicity (p.Arg1624Trp and p.Gly1712Arg), all mutations identified in *PKHD1* truncated the protein. Among the 12 detected variants, nine were lethal since those that inherited them frequently died neonatally: seven in the *PKHD1* gene, one in the *NPHP3* gene, and one in the *CC2D2A* gene. NPHP3 is present in the cilia of renal cells. The mutations found in the *NPHP3* gene are often deletions that cause a malfunction in the renal cilia, leading to ARPKD [22]. CC2D2A is a coiled-coil and C2 domain protein responsible for renal cilia formation. Mutations in the *CC2D2A* gene can affect cilia formation, causing ARPKD [23]. The *ZNF423* gene encodes a nuclear protein that functions as a DNA-binding transcription factor. This gene has been previously associated with nephronophthisis-14 and Joubert syndrome-19, and mutations in this gene can cause a ciliopathy, causing ARPKD [24]. Because *PKHD1* encodes fibrocystin, it is required to regulate the proliferation and differentiation of renal and biliary epithelial cells, with its dysfunction causing abnormal ciliary signaling [25].

All the identified mutations were found to be truncating and pathogenic, except for two that were classified as non-truncating with conflicting pathogenicity in the *PKHD1* gene. One of these non-truncating variants was lethal since those homozygous for it died

neonatally. Therefore, non-truncating variants can be lethal in some cases for many reasons, including the presence of modifier genes. Two of the detected mutations (c.3988del [p.Leu1330Phefs*5] in *PKHD1* and c.3084del [p.Lys1029Argfs*3] in *CC2D2A*) are frameshift deletions and were not found in the "Human Gene Mutation Database (https://www.hgmd.cf.ac.uk/). The human ARPKD/PKHD1 mutation database (http://www.humgen.rwth-aachen.de/index.php) contains 748 unique *PKHD1* variants, with almost 45 % considered missense mutations that cause the substitution of conserved amino acids resulting in partial or complete fibrocystin dysfunction [26]. Non-truncating mutations are more common in patients who survive the neonatal period than in patients showing severe phenotypes [27]. The genotype-phenotype relationships in our study show that not only truncating mutations but also non-truncating mutations can result in a severe ARPKD phenotype, leading to death soon after birth.

Our study identified a putative causal mutation in only 51.7 % (31/60) of the included ARPKD cases. This low detection rate could be explained by the appreciable heterogeneity of ARPKD, which may be caused by mutations in other recessive cytogenes. Therefore, a much larger ARPKD cohort must be examined. However, the mutations detected in our study could be used as a genetic tool for diagnosing ARPKD and detecting possible clinical outcomes in the Kuwaiti population. This tool could be used to improve patient care by guiding therapy in treating and managing the clinical features.

The main treatments for patients with ARPKD are pediatric dialysis and kidney transplantation [28]. Current ARPKD management in Kuwait includes managing the disease complications to slow disease progression, reducing the morbidity and mortality for patients who survive after birth. About 50 % of ARPKD cases require kidney replacement therapy in their first two decades of life [29]. Previous studies have shown higher survival rates of 70%–100 % in patients who received combined kidney and liver transplantation [30]. It has been previously observed that patients with ARPKD tend to develop hepatic disease later in life, which can cause portal hypertension and cholangitis [31]. Sodium intake must be managed in patients with ARPKD since restricting dietary sodium intake in ARPKD rats slowed the rapid progression of kidney failure and cyst formation [32].

One major limitation of our study was its small cohort size, which was dictated by the rarity of ARPKD in the general population. In addition, our study could not identify putative causal mutations for ARPKD in 29 of the 60 patients, likely due to its heterogeneity and rarity or the inability of WES to examine most intronic variants, which could cause the disease.

5. Conclusions

ARPKD is a rare and severe monogenic kidney disease with heterogeneous clinical and genetic features. Our study provides the first insights into the genetic landscape of ARPKD in Kuwait. Diagnosing ARPKD early through detecting genetic mutations is vital to start treating its symptoms and complications, slow disease progression, and increase survival.

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CRediT authorship contribution statement

Mariam E. Alhaddad: Writing – original draft, Investigation, Formal analysis, Data curation. **Anwar Mohammad:** Visualization, Software, Resources, Methodology, Investigation. **Khadija M. Dashti:** Writing – review & editing, Software. **Sumi Elsa John:** Investigation. **Yousif Bahbahani:** Investigation. **Mohamed Abu-Farha:** Resources, Investigation, Data curation. **Jehad Abubaker:** Validation, Resources, Investigation. **Thangavel Alphonse Thanaraj:** Resources, Methodology. **Laila Bastaki:** Writing – review & editing. **Fahd Al-Mulla:** Writing – review & editing, Validation. **Mohammad Al-Ali:** Formal analysis, Data curation, Conceptualization. **Hamad Ali:** Supervision, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no conflicts of interest regarding this study.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e33898.

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