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Original Paper

Novel Mutations in the PKD1 and PKD2 Genes of Chinese Patients with Autosomal Dominant Polycystic Kidney Disease

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Key Words
Autosomal dominant polycystic kidney disease • Mutation • PKD1 • PKD2

Abstract
Background/Aims: Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited kidney disorder with mutations in *PKD1* or *PKD2*. This study aimed to identify novel *PKD1* and *PKD2* mutations in Chinese patients with ADPKD. ***Methods:*** Mutational analyses of both *PKD* genes were performed in 120 Chinese families with inherited ADPKD using long-range PCR and targeted next-generation sequencing approaches. Sanger sequencing was performed to check the positive mutations, while multiplex ligation-dependent probe amplification was adopted to examine those without mutations for the presence of large deletions. ***Results:*** A total of 93 mutations in *PKD1* and *PKD2* were identified in 98 Chinese families with ADPKD inheritance and the detection rate was 81.7% (98/120). The mutation rates of *PKD1* and *PKD2* were 91.4% (85/93) and 8.6% (85/93), respectively. Among the 93 mutations, 59.1% (55/93) were reported for the first time. A total of 65 mutations (26 nonsense, 33 frameshift, 2 large deletion, and 4 typical splicing mutations) were identified as definite pathogenic mutations. The remaining 28 mutations (21 missense, 3 in-frame deletion, and 4 atypical splicing mutations) were determined as probable pathogenic mutations. In addition, 9 *de novo* mutations were found by pedigree analysis. Correlation analysis between genotype and phenotype revealed that patients with *PKD1* mutations or truncating mutations exhibited the most severe clinical outcome. ***Conclusion:*** The newly identified sites for known mutations will facilitate the early diagnosis and prediction of prognosis in patients with ADPKD, and provide fundamental genetic information for clinical intervention to prevent the inheritance of this disease in affected families.

D. Xu and Y. Ma contributed equally to this work.

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Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited kidney disorder with an incidence ranging between 1/400 and 1/1000 [1]. It is a chronic progressive disease characterized by gradually enlarging renal cysts in bilateral kidneys and contributes to approximately 10% of end stage renal disease (ESRD) [2].

ADPKD is a systemic disease that involves different organs [1-4]. However, all ADPKD phenotypes are caused by mutations in 2 genes, *PKD1* (16p13.3) [5] and *PKD2* (4q21) [6], which encode polycystin-1 (PC1) and polycystin-2 (PC2), respectively. Mutations in *PKD1* (85% of all cases) have closer associations with a severe clinical presentation and poorer prognosis than those in *PKD2* (15% of all cases), which suggests a critical role for these genetic factors in predicting the outcomes of patients with ADPKD [7, 8].

At present, the clinical diagnosis of ADPKD is mainly based on renal imaging techniques using the age-related cyst number criteria [9, 10]. However, it is a considerable challenge to provide a definite diagnosis using the current criteria for younger patients without a positive family history or those with *PKD2* mutations. Meanwhile, effective treatment options for this inherited disease are far from sufficient [2]. Recently, we report that blocking gene inheritance through preimplantation genetic diagnosis is the most effective way to reduce the incidence of ADPKD [11]. Therefore, comprehensive mutational information of both *PKD* genes is the key to the early diagnosis of ADPKD and early genetic intervention.

PKD1 consists of 46 exons with a coding sequence of 12912 bp. *PKD2* is composed of 15 exons with a coding sequence of 2907 bp [12]. Both gene sequences are highly variable [13]. To date, a total of 1273 pathogenic *PKD1* and 202 pathogenic *PKD2* mutations have been reported in the Polycystic Kidney Disease Mutation Database (PKDB; http://pkdb.mayo.edu/). However, most mutations are unique as a single-family entity and recurrent mutations account for only 30% of all identified mutations [8]. Thus, it is necessary to investigate a larger number of affected family pedigrees to confirm these known mutations, particularly for missense, in-frame deletion, and atypical splicing mutations. Additionally, most of the currently known mutations listed in PKDB were acquired from studies of Caucasian populations. Mutational data from Asian populations are essential to broaden the representativeness of the database. In the present study, we directly screened *PKD1* and *PKD2* in a total of 120 Chinese families to identify novel mutations that contribute to the development of ADPKD.

Materials and Methods

Patients

Patients who were diagnosed with ADPKD at the Department of Nephrology of the Kidney Institute of the People's Liberation Army, Changzheng Hospital, Second Military Medical University, from January 2016 to August 2017, were enrolled in this study. Diagnostic criteria for ADPKD based on renal ultrasound analysis were adopted as described previously [9, 14]. All enrolled patients provided informed consent and blood samples were obtained from 120 families including at least 1 affected individual. The study was approved by the Ethics Committee of Changzheng Hospital.

Long-range PCR (LR-PCR) amplification, targeted next-generation sequencing, and mutation identification

Approximately 5 mL peripheral blood was obtained from each enrolled individual, and genomic DNA was extracted from lymphocytes by using a DNA extraction kit (QIAamp DNA Blood Mini Kit; QIAGEN, Inc., Valencia, CA). The exons, most of the 5′ and 3′ untranslated regions (UTRs), and the exon-intron boundaries of *PKD1* and *PKD2* were amplified with a total of 6 distinct LR-PCR reactions (5 reactions for *PKD1* and 1 reaction for *PKD2*). The LR-PCR primers chosen for *PKD1* and *PKD2*, LR-PCR amplification reaction

system, and amplification conditions for the various LR-PCR fragments have been described elsewhere [11]. The LR-PCR products for *PKD1* and *PKD2* were purified with Agencourt AMPure XP Beads, followed by quantification and fragmentation using a NEBNext Fast DNA Fragmentation Kit (New England BioLabs, Ipswich, MA). A sequencing library of the LR-PCR products and other Ampliseq multiplex PCR products was constructed with an Ion Plus Fragment Library Kit (Thermo Fisher Scientific, Waltham, MA) and then sequenced with an Ion PGM™ 200 Sequencing Kit (Thermo Fisher Scientific). Mutations identified in *PKD1* and *PKD2* were validated by Sanger sequencing. Patients who had no mutations in *PKD1* and *PKD2* were screened for large deletions by multiplex ligation-dependent probe amplification (MLPA) analysis [15, 16]. All sequencing reads were mapped to the *PKD1/2* reference genome (NM_001009944.2 for *PKD1* and NM_000297.2 for *PKD2*) with Torrent Server TMAP software. The standard nomenclature recommended by HGVS (http://www.hgvs.org/mutnomen/) was adopted to name the mutations identified in this study. The dbSNP138, 1000 Genomes, Exon Sequencing Project (http://evs.gs.washington.edu), HapMap, and PVFD (variant frequency in normal populations) databases were used to filter out polymorphic variants. In accordance with the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk) and PKDB, the remaining mutations were divided into known mutations and novel mutations.

Evaluation of the pathogenicity of sequencing mutations

Nonsense, frameshift, typical splicing, and larger deletion mutations were defined as definite pathogenic mutations; while missense, atypical splicing, and in-frame deletion mutations were considered probable pathogenic mutations in this study [17].

For the known probable pathogenic mutations, we firstly screened PKDB to determine if their pathogenicity was known (highly likely pathogenic or likely pathogenic). Secondly, we used several web-based prediction tools to evaluate the pathogenic potential of these mutations: The PolyPhen-2 (Polymorphism Phenotyping v2; http://genetics.bwh.harvard.edu/pph/) [18] and Sorting Intolerant from Tolerant (SIFT) (http://sift.jcvi.org/) [19] tools were used for missense mutations, and the Mutation Taster (http://mutationtaster.org/) tool [20] was used for missense and in-frame deletion mutations. The PolyPhen-2 tool could divide the mutations into 3 categories (benign, possibly damaging, and probably damaging); the SIFT tool could divide the mutations into 2 categories (tolerated and damaging); and the Mutation Taster could divide the mutations into 2 categories (polymorphism and disease-causing). Finally, segregation analyses were performed by direct sequencing of parental DNA to verify the pathogenicity of those mutations in the pedigree.

For novel probable pathogenic mutations, the PolyPhen-2, SIFT, and Mutation Taster tools were also used to evaluate their potential pathogenicity, and segregation analysis was further performed to verify their pathogenicity in the pedigree.

Mutation domain analysis

Several domain databases were used to analyze the mutation domains of PC1 and PC2, including the Simple Modular Architecture Research Tool (http://smart.embl-heidelberg.de), Pfam (http://pfam.xfam.org/), and PROSITE (http://prosite.expasy.org/). The mutation domains for PC1 and PC2 were determined by the location of the initial mutation site of amino acids.

Statistical analysis

The significance of comparisons was determined by the chi-squared test using SPSS 17.0 software (SPSS, Inc., Chicago, IL). A p-value < 0.05 was considered statistically significant.

Results

General characteristics of the mutations in 120 families

A total of 120 families were enrolled in this study. Among which, 93 mutations in *PKD1* and *PKD2* were found in 98 families, and the remaining 22 families had neither *PKD1* nor *PKD2* mutations. The overall mutation rate of both *PKD* genes was 98/120 (81.7%). Of these 93 mutations, 55 (59.1%) were detected as novel mutations and 38 (40.9%) as known mutations.

Screening of *PKD1* and *PKD2* revealed 85 mutations in *PKD1* (34 known mutations and 51 novel mutations) and 8 mutations in *PKD2* (4 known mutations and 4 novel mutations). The mutation rates of *PKD1* and *PKD2* were 91.4% (85/93) and 8.6% (8/93), respectively. Among the 34 known mutations of *PKD1*, 15 (44.1%) were nonsense mutations, 6 (17.6%) were frameshift mutations, 1 (2.9%) was an in-frame deletion, 10 (29.4%) were missense mutations, 1 (2.9%) was a splice-site mutation, and 1 (2.9%) was a large deletion; 3 (75%) of the 4 known *PKD2* mutations were nonsense mutations and 1 (25%) was a splice-site mutation. Meanwhile, 9 (17.6%) of the 51 novel *PKD1* mutations were nonsense mutations, 26 (51.0%) were frameshift mutations, 2 (3.9%) were in-frame deletions, 9 (17.6%) were missense mutations, 4 (7.8%) were splice-site mutations, and 1 (2.0%) was a large deletion; 1 (25%) of the 4 novel *PKD2* mutations was a nonsense mutation, 2 (50%) were missense mutations, and 1 (25%) was a splice-site mutation (Fig. 1).

Definite pathogenic mutations

In this study, 69.9% (65/93) of the mutations were detected as definite pathogenic mutations. Of these 65 mutations, 25 (17 nonsense, 6 frameshift, and 1 large deletion) were regarded as known mutations (Table 1), while the other 40 were considered to be novel mutations (Table 2). Among the known mutations, 2 nonsense mutations (*PKD1* c.6472C>T and *PKD1* c.12124C>T) were found in 3 families; 1 nonsense mutation (*PKD1* c.11884C>T) and 1 frameshift mutation (*PKD1* c.6727_6728delCA) were found in 2 families, and the remaining 21 mutations were found in single families. Among the 40 novel mutations identified, 9 were nonsense mutations, 27 were frameshift mutations, 1 was a large deletion, and 3 were typical splicing mutations. Most of these mutations were found in single pedigrees, except for *PKD1* c.10524_10525insT (found in family no. 91 and family no. 93). By using MLPA analysis, a known large deletion mutation (exon 1 of *PKD1*) and a novel large deletion mutation (from exon 7 to exon 15 of *PKD1*) were found in family no. 87 and family no. 44, respectively. The rate of large deletion mutations in *PKD1* was 2.4% (2/85).

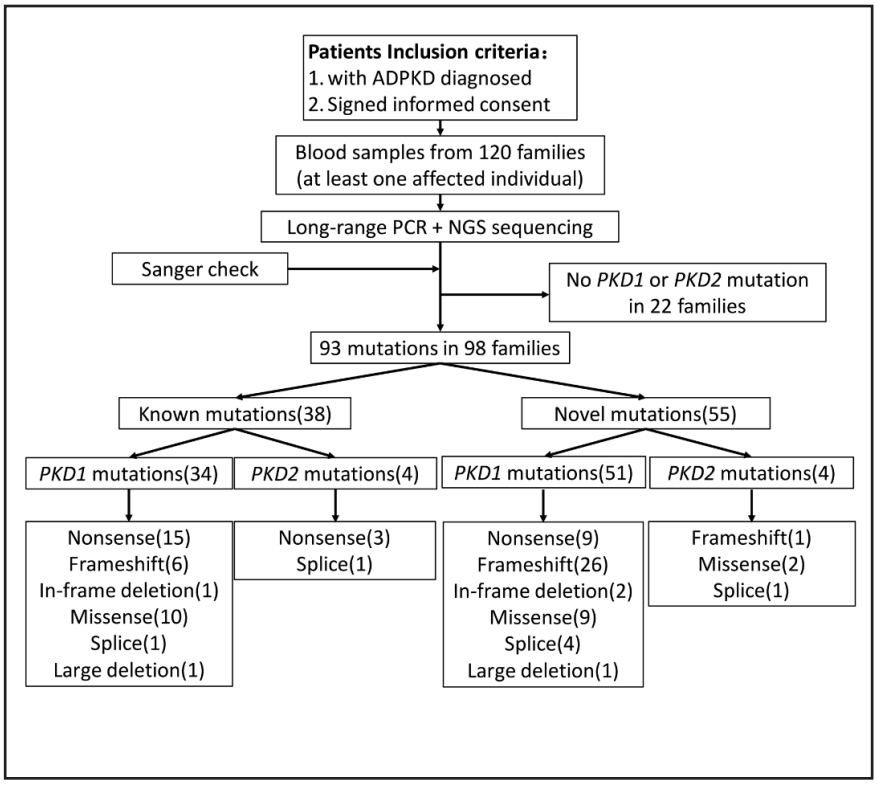

Fig. 1. Flow diagram of mutation detection in PKD1 and PKD2. NGS: the next-generation sequencing; MLPA: multiplex ligation-dependent probe amplification.

Table 1. Previously reported definite pathogenic mutations that were found in this study

No.	Gene	Exon	Codon	cDNA change	Protein change
Nonsense					
42	PKD1	5	189	c.566C>A	p.Ser189*
7	PKD1	5	351	c.1051C>T	p.Gln351*
21	PKD1	10	663	c.1987C>T	p.Gln663*
81	PKD1	15	1172	c.3514C>T	p.Gln1172*
71	PKD1	15	1436	c.4306C>T	p.Arg1436*
29	PKD1	15	1599	c.4797C>A	p.Tyr1599*
62/97/102	PKD1	15	2158	c.6472C>T	p.Gln2158*
18	PKD1	17	2402	c.7204C>T	p.Arg2402*
24/54	PKD1	43	3962	c.11884C>T	p.Gln3962*
8	PKD1	43	3979	c.11935C>T	p.Gln3979*
14	PKD1	44	4004	c.12010C>T	p.Gln4004*
47	PKD1	44	4012	c.12036G>A	p.Trp4012*
39/77/101	PKD1	44	4042	c.12124C>T	p.Gln4042*
19	PKD1	46	4225	c.12673C>T	p.Gln4225*
32	PKD1	46	4231	c.12691C>T	p.Gln4231*
86	PKD2	4	306	c.916C>T	p.Arg306*
22	PKD2	6	464	c.1390C>T	p.Arg464*
20	PKD2	11	742	c.2224C>T	p.Arg742*
Frameshift					
104	PKD1	3	116	c.348_352delTTTAA	p.Asn116fs1*
100	PKD1	15	1408	c.4220dupC	p.Phe1408Valfs22*
98	PKD1	15	1672	c.5014_5015delAG	p.Arg1672Glyfs97*
105	PKD1	15	2192	c.6574_6580delACCGCCA	p.Thr2192Alafs17*
50/95	PKD1	15	2243	c.6727_6728delCA	p.Gln2243Glufs17*
99	PKD1	26	3082	c.9240_9241delAT	p.Ala3082Cysfs95*
Large deletion					
87	PKD1	1	1	Exon1del(1-215del)	p.Met1fs

Missense mutations

A total of 21 missense mutations (10 known and 11 novel) were found in this study. The characteristics of the 10 known missense mutations are described in Table 3. All 10 of the known missense mutations were in *PKD1* and were defined as highly likely pathogenic or likely pathogenic mutations by PKDB. In these 10 missense mutations, 8 were identified as "probably damaging," 1 as "possibly damaging" (*PKD1* c.9136C>T), and 1 as "benign" (*PKD1* c.385T>C) by the PolyPhen-2 tool. Using the SIFT and Mutation Taster tools, 9 of the 10 missense mutations were demonstrated to be pathogenic mutations, except for *PKD1* c.11156G>A. Pedigree analysis revealed that 5 missense mutations (*PKD1* c.385T>C, *PKD1* c.3722T>A, *PKD1* c.6832G>A, *PKD1* c.9409C>T, and *PKD1* c.9412G>A) were pathogenic and co-segregated with ADPKD, while 2 missense mutations (*PKD1* c.11156G>A and *PKD1* c.11257C>T) were *de novo* mutations. Provided with incomplete pedigree information for families no. 2 and no. 46, the pathogenicity of another 2 missense mutations (*PKD1* c.7483T>C and *PKD1* c.9136C>T) was not determined by pedigree analysis.

The mutational characteristics of the 11 novel missense mutations are described in Table 4. There were 9 and 2 mutations in *PKD1* and *PKD2*, respectively. Among which, 6/9 *PKD1* mutations were classified as "probably damaging," 1/9 *PKD1* mutations and 2/2 *PKD2* mutations were classified as "possibly damaging," and 2/9 *PKD1* mutations were classified as "benign" by the PolyPhen tool. Meanwhile, 7/9 *PKD1* mutations and 1/2 *PKD2* mutations were classified as "damaging" by SIFT. According to the Mutation Taster tool, 7/9 *PKD1* mutations and 2/2 *PKD2* mutations were classified as "disease-causing." Pedigree analysis revealed that 6/9 *PKD1* mutations and 2/2 *PKD2* mutations were pathogenic mutations, 2/9 *PKD1* mutations (c.2897G>C and c.11541C>A) were *de novo* mutations, and 1/9 *PKD1* mutations (c.11541C>A) was indeterminate due to incomplete pedigree information for family no. 15. Two missense mutations (*PKD1* c.4984G>A and *PKD1* c.7544G>C), which were demonstrated to be benign by the prediction tools (more than 2/3 prediction tools suggested they were non-pathogenic), were classified as pathogenic mutations in pedigree analysis.

Table 2. Novel pathogenic mutations that were found in this study LRRNT, leucine rich repeat N-terminal domain; REJ, receptor for Egg Jelly; PKD, PKD repeats; N/D, no domain defined; GPS, CL-1–like GPS; PLAT, Polycystin-1, Lipoxygenase, Alpha-Toxin domain; TM, transmembrane region; Lectin, C-type lectin domain

No.	Gene	Exon/ Intron	Codon	cDNA change	Protein change	Protein domain
Nonsense						
76	PKD1	4	144	c.430C>T	p.Gln144*	N/D
63	PKD1	5	331	c.993T>A	p.Tyr331*	PKD I
65	PKD1	15	1958	c.5873G>A	p.Trp1958*	PKD XIII
35	PKD1	15	2078	c.6232C>T	p.Gln2078*	PKD XV
103	PKD1	23	2824	c.8470C>T	p.Gln2824*	REJ
91/93	PKD1	35	3509	c.10524_10525insT	p.Glu3509*	N/D
43	PKD1	39	3734	c.11202C>A	p.Tyr3734*	TM
64	PKD1	43	3922	c.11765G>A	p.Trp3922*	TM
85	PKD1	45	4083	c.12248C>A	p.Ser4083*	TM
Frameshift						
78	PKD1	1	4	c.10delG	p.Ala4Profs68*	N/D
55	PKD1	1	6	c.15_31delGCCCGCCCGCCTGGCGC	p.Pro6Glyfs101*	N/D
84	PKD1	1	42	c.118_124dupGGCCCAG	p.Ala42Glyfs73*	LRRNT
83	PKD1	5	400	c.1198delC	p.Arg400Glufs64*	N/D
69	PKD1	12	956	c.2865dupC	p.Val956Argfs144*	PKD II
1	PKD1	15	1187	c.3553_3558delinsTGCACCTA	p.Gly1185Cysfs13*	PKD IV
30	PKD1	15	1200	c.3597_3601delTGCGG	p.Ala1200Glyfs8*	PKD IV
10	PKD1	15	1513	c.4537dupG	p.Val1513Glyfs9*	PKD VIII
67	PKD1	15	1648	c.4943delA	p.His1648Profs73*	PKD X
72	PKD1	15	1786	c.5357delC	p.Pro1786Argfs15*	PKD XI
93	PKD1	15	2263	c.6786delT	p.Gly2263Alafs50*	REJ
26	PKD1	17	2364	c.7088_7089dupTG	p.Pro2364Cysfs18*	REJ
68	PKD1	17	2365	c.7087_7093dupGTGCCCA	p.Ile2365Serfs56*	REJ
74	PKD1	17	2401	c.7199_7202dupCCAA	p.Lys2401Asnfs19*	REJ
73	PKD1	25	2992	c.8974_8975delCA	p.His2992Serfs75*	N/D
79	PKD1	25	3055	c.9164delT	p.Leu3055Profs18*	GPS
89	PKD1	36	3577	c.10729_10732delGGTG	p.Gly3577Argfs6*	N/D
45	PKD1	42	3859	c.11576delC	p.Pro3859Argfs85*	TM
4	PKD1	42	3898	c.11692_11693delTC	p.Ser3898Alafs61*	TM
66	PKD1	42	3899	c.11695dupC	p.Leu3899Profs61*	TM
23	PKD1	43	3928	c.11772_11775dupGGAA	p.Trp3928Alafs33*	TM
36	PKD1	45	4055	c.12163delC	p.Leu4055Serfs142*	TM
88	PKD1	45	4123	c.12367delG	p.Glu4123Serfs74*	N/D
9	PKD1	45	4148	c.12444delG	p.Glu4148Aspfs49*	N/D
95	PKD1	46	4151	c.12453_12454delCA	p.His4151Glnfs4*	N/D
75	PKD1	46	4244	c.12730delC	p.His4244Thrfs113*	N/D
80	PKD2	4	365	c.1092delC	p.Ala365Leufs9*	TM
Large deletion						
44	PKD1	7	462	Exon7-Exon15del(1386-6915)	p.Arg462fs	Lectin
Typical splicing						
3	PKD1	Intron 29-30	3309	c.9924-2A>G	p.Ser3309fs	-
90	PKD1	Intron 36-37	3607	c.10821+1G>A	p.Lys3607fs	-
27	PKD1	Intron 43-44	4002	c.12004-1G>C	p.Ala4002fs	-

Table 3. Characteristics of the known missense mutations. Protein domain: LRRNT, leucine rich repeat N-terminal domain; REJ, receptor for Egg Jelly; PKD, PKD repeats; N/D, no domain defined; GPS, CL-1–like GPS; PLAT, Polycystin-1, Lipoxygenase, Alpha-Toxin domain; TM, transmembrane region. PolyPhen: B, benign; PoD, possibly damaging; PD, probably damaging. PKDB: Polycystic Kidney Disease Mutation Database (http:// pkdb.mayo.edu); HLP: highly likely pathogenic; LP: likely pathogenic

No.	Gene	Exon	Codon	cDNA change	Protein change	Protein domain	PolyPhen	SIFT	Mutation Taster	PKDB	Number of patients/affected/total family members
52	PKD1	4	129	c.385T>C	p.Cys129Arg	LRRNT	B	Damaging	Disease-Causing	HLP	2/2/3
38	PKD1	15	1241	c.3722T>A	p.Ile1241Asn	PKD V	PD	Damaging	Disease-Causing	LP	3/3/6
34	PKD1	15	2278	c.6832G>A	p.Gly2278Arg	REJ	PD	Damaging	Disease-Causing	HLP	2/2/5
46	PKD1	18	2495	c.7483T>C	p.Cys2495Arg	REJ	PD	Damaging	Disease-Causing	HLP	1/1/3
6	PKD1	23	2858	c.8572G>A	p.Gly2858Ser	N/D	PD	Damaging	Disease-Causing	HLP	1/3/4
2	PKD1	25	3046	c.9136C>T	p.Arg3046Cys	GPS	PoD	Damaging	Disease-Causing	LP	1/1/3
41	PKD1	27	3137	c.9409C>T	p.His3137Tyr	PLAT	PD	Damaging	Disease-Causing	LP	2/2/3
61	PKD1	27	3138	c.9412G>A	p.Val3138Met	PLAT	PD	Damaging	Disease-Causing	LP	3/3/5
70	PKD1	38	3719	c.11156G>A	p.Arg3719Gln	TM	PD	Tolerated	Polymorphism	HLP	1/1/3
11	PKD1	39	3753	c.11257C>T	p.Arg3753Trp	TM	PD	Damaging	Disease-Causing	HLP	1/1/5

Table 4. Characteristics of the novel missense mutations. WSC, WSC domain; Lectin, C-type lectin domain; PKD, PKD repeats; REJ, receptor for Egg Jelly; PLAT, Polycystin-1, Lipoxygenase, Alpha-Toxin domain; TM, transmembrane region; N/D, no domain defined. PolyPhen: B, benign; PoD, possibly damaging; PD, probably damaging

No.	Gene	Exon	Codon	cDNA change	Protein change	Protein domain	PolyPhen	SIFT	Mutation Taster	Number of patients/affected/total family members
37	PKD1	5	214	c.640T>G	p.Cys214Gly	WSC	B	Damaging	Disease-Causing	2/2/5
31	PKD1	7	522	c.1565G>C	p.Cys522Ser	Lectin	PD	Damaging	Disease-Causing	4/4/6
6	PKD1	12	966	c.2897G>C	p.Arg966Pro	PKD II	PD	Damaging	Disease-Causing	1/1/4
59	PKD1	15	1662	c.4984G>A	p.Val1662Ile	PKD X	B	Tolerated	Polymorphism	4/4/8
48	PKD1	19	2515	c.7544G>C	p.Arg2515Pro	REJ	PoD	Tolerated	Polymorphism	2/2/4
57	PKD1	21	2668	c.8003T>C	p.Leu2668Pro	REJ	PD	Damaging	Disease-Causing	3/3/5
17	PKD1	22	2698	c.8093T>C	p.Leu2698Pro	REJ	PD	Damaging	Disease-Causing	3/3/6
15	PKD1	28	3204	c.9611A>T	p.Asp3204Val	PLAT	PD	Damaging	Disease-Causing	1/1/3
33	PKD1	42	3847	c.11541C>A	p.Ser3847Arg	TM	PD	Damaging	Disease-Causing	1/1/3
56	PKD2	9	662	c.1984A>C	p.Thr662Pro	TM	PoD	Damaging	Disease-Causing	3/3/10
35	PKD2	13	832	c.2495G>C	p.Ser832Thr	N/D	PoD	Tolerated	Disease-Causing	2/2/4

Two *PKD1* missense mutations (c.2897G>C and c.8572G>A) were found in family no. 6. The novel *PKD1* missense mutation c.2897G>C at exon 12 resulted in the substitution of a basic arginine residue for a non-polar hydrophobic proline residue at position 966 (p.Arg966Pro). All 3 prediction tools identified c.2897G>C to be a pathologic mutation. Moreover, pedigree analysis revealed that c.2897G>C was a *de novo* mutation in family no. 6. The known *PKD1* missense mutation c.8572G>A at exon 23 led to the replacement of a non-polar hydrophobic glycine residue with a polar neutral serine residue at position 2858 (p.Gly2858Ser). All 3 prediction tools and PKDB identified c.8572G>A as a pathologic mutation. However, pedigree analysis revealed that the c.8572G>A mutation in the proband was inherited from the unaffected father, which was also found in an unaffected brother, indicating that c.8572G>A was not a pathologic mutation (Fig. 2).

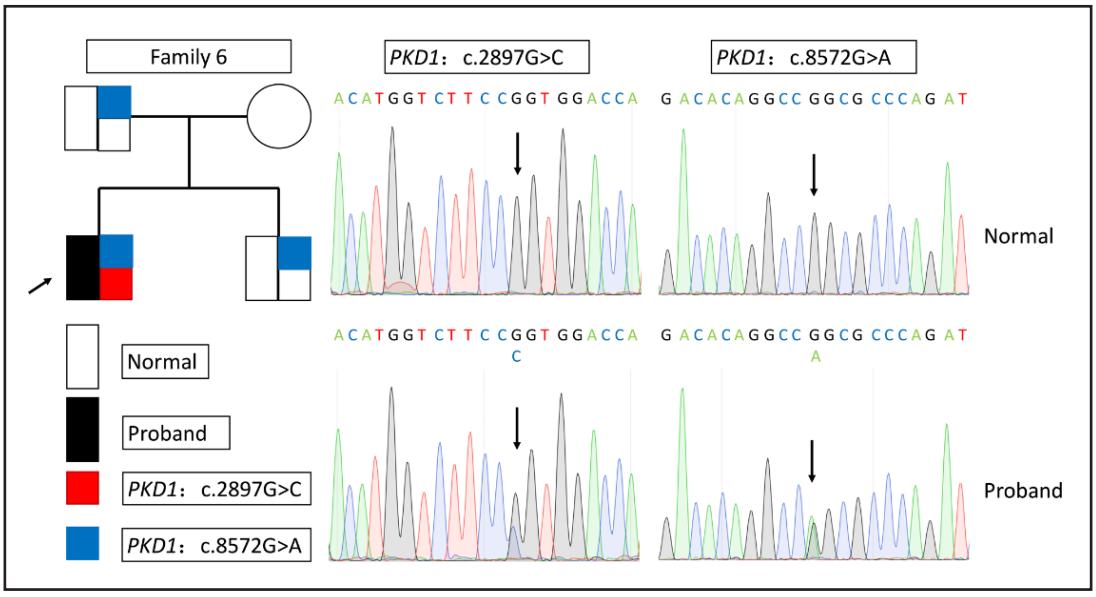

Fig. 2. Two mutations in family no. 6. Two mutations (PKD1 c.2897G>C and PKD1 c.8572G>A) were detected in family no. 6. The PKD1 c.2897G>C mutation was revealed as a de novo mutation; the PKD1 c.8572G>A mutation did not co-segregate with the PKD1 disease phenotype in this family.

In-frame deletion mutations

Three in-frame deletion mutations of *PKD1* were found in this study (Table 5). One of the in-frame deletion mutations (*PKD1* c.7825_7827delATC) was reported previously as a pathogenic mutation within Mutation Taster and PKDB. Moreover, the pathogenicity of this mutation was verified by pedigree analysis. The remaining 2 in-frame deletion mutations were novel. One mutation (*PKD1* c.8318_8326delCCCTGACGC) was demonstrated to be a pathogenic mutation by Mutation Taster and pedigree analysis, while the pathogenicity of another mutation (*PKD1* c.12399_12407delCCTGCGCAG), which was demonstrated to be a polymorphism by Mutation Taster, was defined as indeterminate in pedigree analysis due to the lack of complete pedigree information.

Atypical splicing mutations

The characteristics of the 4 atypical splicing mutations are described in Table 5. Among which, 2 mutations (*PKD1* c.11017-10C>A and *PKD2* c.1094+3_+6delAAGT) were reported previously and classified as highly likely pathogenic mutations by PKDB. They were also defined as pathogenic mutations by pedigree analysis. The remaining 2 mutations (*PKD1* c.10822-3C>G and *PKD2* c.1095-16_1095-8del9TTTCCTTTG) were novel atypical splicing mutations, which were further found to co-segregate with ADPKD in families no. 16 and no. 28 by pedigree analysis, suggesting the pathogenic nature of these atypical splicing mutations.

De novo mutations

A total of 9 *de novo* mutations of *PKD1* were identified in this study (Table 6). Among which, 3 (33.3%) were frameshift mutations, 4 (44.5%) were missense mutations, 1 (11.1%) was a nonsense mutation, and 1 (11.1%) was a typical splice mutation. Two of the missense mutations (c.11257C>T and c.11156G>A) had been reported previously as pathologic mutations. The remaining 2 missense mutations (c.2897G>C and c.11541C>A), which occurred *de novo* in the probands, were novel mutations found in this study and demonstrated to be pathologic mutations by the prediction tools.

Table 5. Characteristics of the in-frame deletion and atypical splicing mutations. PKDB: Polycystic Kidney Disease Mutation Database (http:// pkdb.mayo.edu); HLP, highly likely pathogenic; LP, likely pathogenic; DP, definitely pathogenic

No.	Gene	Exon/ Intron	Codon	cDNA change	Protein change	Mutation Taster	PKDB	Novel /known	Number of patients/affected/ total family members
In-frame deletion									
40	PKD1	20	2609	c.7825_7827delATC	p.Ile2609del	Disease-Causing	LP	Known	2/2/4
25	PKD1	23	2773	c.8318_8326delCCCTGACGC	p.Pro2773_Thr2775del	Disease-Causing		Novel	3/3/5
13	PKD1	45	4133	c.12399_12407delCCTGCGCAG	p.Phe4133_Arg4136delinsLeu	Polymorphism	-	Novel	1/1/3
Atypical splicing									
16	PKD1	Intron 36	3607	c.10822-3C>G	p.Lys3607fs	-	-	Novel	3/3/5
49	PKD1	Intron 37	3672	c.11017-10C>A	p.Arg3672fs	-	HLP	Known	2/2/3
96	PKD2	Intron 4	365	c.1094+3_+6delAAGT	p.Ala365fs	-	HLP	Known	2/2/5
28	PKD2	Intron 4	365	c.1095-16_1095-8del9TTTCCTTTG	p.Ala365fs	-	-	Novel	2/2/4

Table 6. De novo mutations that were found in this study. PolyPhen: B, benign; PoD, possibly damaging; PD, probably damaging. PKDB: Polycystic Kidney Disease Mutation Database (http:// pkdb.mayo.edu); HLP, highly likely pathogenic; LP, likely pathogenic; DP, definitely pathogenic

No.	Gene	Exon/ Intron	Codon	cDNA change	Protein change	Type	Poly-Phen	SIFT	Mutation Taster	PKDB	Novel/know n
55	PKD1	1	6	c.15_31delGCCCGCCCGCCTGGCGC	p.Pro6Glyfs101*	Frameshift	-	-	-	-	Novel
67	PKD1	15	1648	c.4943delA	p.His1648Profs73*	Frameshift	-	-	-	-	Novel
4	PKD1	42	3898	c.11692_11693delTC	p.Ser3898Alafs61*	Frameshift	-	-	-	-	Novel
21	PKD1	10	663	c.1987C>T	p.Gln663*	Nonsense	-	-	-	DP	Known
6	PKD1	12	966	c.2897G>C	p.Arg966Pro	Missense	PD	Damaging	Disease-Causing	-	Novel
70	PKD1	38	3719	c.11156G>A	p.Arg3719Gln	Missense	PD	Tolerated	Polymorphism	HLP	Known
11	PKD1	39	3753	c.11257C>T	p.Arg3753Trp	Missense	PD	Damaging	Disease-Causing	HLP	Known
33	PKD1	42	3847	c.11541C>A	p.Ser3847Arg	Missense	PD	Damaging	Disease-Causing	-	Novel
27	PKD1	Intron 43	4002	c.12004-1G>C	p.Ala4002fs	Typical splicing	-	-	-	-	Novel

Table 7. Association between genotype and phenotype. ESRD: end stage renal disease; * Two affected individuals have experienced ESRD at the age of 68 and 69, respectively

	ESRD before age 60	Non-ESRD before age 60	P
Total	42/73 (57.5%)	31/73 (42.5%)	
Gene			
PKD1	41/67 (61.2%)	26/67 (38.8%)	
PKD2	1/6 (16.6%)	5/6 (83.3)	0.035
Mutation types of PKD1			
Truncating mutations of PKD1	34/49 (69.4%)	15/49 (30.6%)	
Nosense	14	7	
Frameshift	20	7	
Large deletion	0	1	
Non-truncating mutations of PKD1	7/18 (38.9%)	11/18 (61.1%)	0.023
Missense	6	9*	
In-frame deletion	1	2	

Association between genotype and phenotype

Approximately 50% of ADPKD patients progress to ESRD before the age of 60 years. To evaluate the association between genotype and phenotype, we analyzed the rate of ESRD before 60 years of age in the available pedigrees. A total of 73 pedigrees were enrolled with 67 mutations in *PKD1* and 6 mutations in *PKD2*. Of these pedigrees, 42/73 (57.5%, involving 41 *PKD1* mutations and 1 *PKD2* mutation) had individuals who developed ESRD before 60 years of age. The ESRD rate for the *PKD1* mutations (41/67, 61.7%) was significantly higher than that of the *PKD2* mutations (1/6, 16.6%) ($p = 0.035$). A total of 49 truncating mutations and 18 non-truncating mutations were found in the 67 *PKD1* mutational pedigrees. Of these mutations, 34/49 (69.4%) truncating mutations (14 nonsense and 20 frameshift) and 7/18 (38.9%) non-truncating mutations (6 missense and 1 in-frame deletion) were identified in individuals who developed ESRD before 60 years of age. The ESRD rate for the truncating mutations was significantly higher than that of the non-truncating ones ($p = 0.023$) (Table 7).

Discussion

Mutation analysis of *PKD1* and *PKD2* in Chinese ADPKD patients has been reported previously [21-24], although with limited numbers of ADPKD families (less than 100 in all previous studies). Recently, Jin et al. [25] reported mutation analysis of *PKD1* and *PKD2* among 148 ADPKD patients. However, the lack of pedigree analysis in their study limited the possibility to evaluate the pathogenicity of the identified mutations, especially for those probably pathogenic mutations. In the present study, a total of 120 Chinese families with ADPKD were enrolled and 93 *PKD1* and *PKD2* mutations were detected. Pedigree analyses were performed for all found mutations to evaluate their pathogenicity.

In the current study, the overall detection rate of *PKD* mutations was 81.7%, which was consistent with the range of 52.3–85.8% reported in previous studies among Chinese populations [21-25]. Neither *PKD1* nor *PKD2* mutations were found in 22 probands with a clinical manifestation of ADPKD. A similar phenomenon was also reported in previous studies [22, 25]. Although no *PKD* mutations were found in these patients, the protein levels of PC1 and PC2 were probably down-regulated by other mechanisms. Previous studies had reported that microRNA-17 and microRNA-93 could bind directly to the 3′ UTR of the *PKD* genes and post-transcriptionally down-regulate their expression [26-29]. In addition, Zheng et al. found that far upstream element-binding protein 1 could also bind to the 3′ UTR of *PKD2* to suppress its translation [30]. These mechanisms might explain the absence of *PKD* mutations in some ADPKD patients.

The mutation rates of *PKD1* and *PKD2* in the present study were 91.4% and 8.6%, respectively; while most studies among Caucasian populations reported the proportion of *PKD1* and *PKD2* mutations to be approximately 85% and 15%, respectively [8]. In fact, most of the probands enrolled in this study were below 40 years of age, which might partially explain the small difference in the distribution of *PKD* mutations. Among these 93 mutations, 59.1% were reported for the first time, and the remaining 40.9% were recurrent mutations. The recurrence rate was higher than in previous reports, ranging from 16.7% to 30% in Caucasian populations [8, 17, 31].

Definite pathogenic mutations accounted for 69.9% of all identified mutations in the present study, which was comparable to the results from the previously reported 45.1–66.6% in Caucasian populations [8, 17, 31], but higher than the 27.7–52.2% reported in Asian populations [25, 32]. Among the definite pathogenic mutations, the large deletion mutation was a special one that was detected by MLPA analysis [15]. Previous studies reported that the detection rate of large deletions accounted for a maximum of 4% of all *PKD1* mutations and even fewer among all *PKD2* mutations [16]. In our study, 2 large deletion mutations were found in *PKD1* with a detection rate of 2.4%, which was consistent with the data reported previously.

It is challenging to evaluate the pathogenicity of missense mutations with inadequate pedigree information. However, not all pedigree information needed for pathogenicity evaluation is easily available to researchers. Several prediction tools (PolyPhen-2, SIFT, and Mutation Taster) have been developed to assist the evaluation of the pathogenicity of missense mutations. Although the pathogenicity of most of the missense mutations evaluated by the prediction tools used in this study was consistent with the results of pedigree analysis, 2 mutations (*PKD1* c.4984G>A and *PKD1* c.7544G>C), which were demonstrated as benign mutations by the prediction tools, were identified as pathogenic mutations through pedigree analysis. A similar phenomenon was also found in several other related studies [33, 34]. Due to the nature of ADPKD as a single-gene inherited disease, segregation analysis is recommended as a gold standard to evaluate the pathogenicity of missense mutations rather than prediction tools. Additionally, a missense mutation (*PKD1* c.8572G>A) was reported as a highly likely pathogenic mutation in PKDB [35], while it was demonstrated to

be a non-pathogenic mutation by pedigree analysis in our study. This may suggest additional information from different pedigrees is required to evaluate the pathogenicity of missense mutations.

The high level of allelic heterogeneity in both *PKD1* and *PKD2*, as well as the prevalence of private mutations in ADPKD patients, suggest that there is a high frequency of *de novo* mutations in this disease. In our study, a total of 9 *de novo* mutations were found in 120 families (7.5%), which is larger than that reported in previous studies, ranging from 0.9% to 3.1% [17, 36, 37]. A complete collection of pedigree information from all of the involved families in our study may partially explain this difference.

A previous study reported that the average age of onset of ESRD for *PKD1* and *PKD2* mutation carriers was 54.3 and 74.0 years, respectively [7]. Consistently, we found that *PKD1* mutation carriers had an earlier age of onset of ESRD than those with *PKD2* mutations. Moreover, patients with truncating mutations were found to have a more severe clinical outcome than those with non-truncating mutations, which confirmed the strong correlation between mutation type and median age at ESRD onset identified in previous studies [34, 38].

There are some limitations of our study that merit consideration. Firstly, most of the novel mutations were found only in single pedigrees, whose pathogenicity needs further verification by different pedigree data, especially for the probably pathogenic mutations. Secondly, although we collected as much pedigree information as possible, some of the novel missense and in-frame deletion mutations are still without complete pedigree information; thus, the pathogenicity of these mutations remains to be determined. Finally, although the pathogenicity of the atypical splicing mutations in our study was evaluated by pedigree analysis, it would be better to analyze their pathogenicity further using minigene splicing assays.

Conclusion

This comprehensive search for mutations in *PKD1* and *PKD2* using 120 Chinese ADPKD families found a total of 93 *PKD1* (91.4%) and *PKD2* (8.6%) mutations in 98 Chinese families with ADPKD. Among the 93 mutations, 59.1% were reported for the first time and 40.9% were found to be recurrent mutations. The increased number of known mutations will facilitate the early diagnosis and prognostic prediction of ADPKD patients and can serve as a genetic background for clinical interventions to block disease inheritance in affected families.

Disclosure Statement

The authors declare that they have no competing interests.

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