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ORIGINAL ARTICLE

Novel *PKD1* and *PKD2* mutations in Taiwanese patients with autosomal dominant polycystic kidney disease

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Autosomal dominant polycystic kidney disease (ADPKD) is a heterogeneous disease caused by mutations in *PKD1* and *PKD2*. The genotype–phenotype correlations are not completely understood. We performed direct PCR-sequencing plus multiplex ligation-dependent probe amplification for *PKD1* and *PKD2* in 46 unrelated patients. Disease-causing mutations were identified in 30 (65%) patients: 23 (77%) patients have mutations in *PKD1* and 7 (23%) have mutations in *PKD2*. Nonsense, splicing or frame-shifting mutations were found in 18 patients, exon duplication in 1 and missense mutations in 11 patients. Two likely *PKD1* hypomorphic alleles (p.Arg2477His and p.Arg3439Trp) segregated with mild disease in a family. A total of 34 mutations were identified and 17 (50%) of which are novel. The median age at onset of dialysis was significantly earlier in patients with *PKD1* mutations (52 years) than in patients with *PKD2* mutations (65.5 years) and those with an undetermined genotype (67 years) by survival analysis (log-rank test, $P=0.014$). Patients carrying *PKD1*-truncating mutations have a trend toward earlier initiation of dialysis compared with carriers of non-truncating mutations (52 years vs 57 years, $P=0.061$). A family history of dialysis before 55 years was more common in PKD1 patients than in others ($P<0.05$). In conclusion, this study identified novel mutations in *PKD1* and *PKD2* and demonstrated the presence of *PKD1* hypomorphic alleles in Taiwanese patients. Patients carrying *PKD1* mutations, especially those with truncating mutations, could have a more rapidly progressive disease than others. These results might have implications for diagnosis and risk stratification in patients with ADPKD.
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INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD) is a common inherited disorder resulting from mutations in *PKD1* (MIM#601313) and *PKD2* (MIM#173910) genes.[1] In clinically defined populations, mutations in *PKD1* and *PKD2* account for 85% and 15% of cases, respectively.[2] This slowly progressive disease is not only associated with multiple kidney cysts but also causes systemic manifestations, such as liver cysts and cerebral aneurysms.[3] The protein products of *PKD1* and *PKD2* are polycystin-1 (PC-1) and PC-2, respectively. These two proteins are critical for maintaining normal renal tubular structure during kidney development.[4–6] PC-1 and PC-2 have been found in primary cilia to act as a mechanosensor[7,8] and between cells or at cell–matrix attachments to mediate cell adhesion.[9,10] End-stage renal disease (ESRD) is expected in nearly 50% of ADPKD patients at the age of 60 years.[11] Patients with *PKD1* mutations usually have an average age at onset of ESRD earlier than patients with *PKD2* mutations (53.4 years vs 72.7 years).[12,13] Both locus and allelic heterogeneity in the *PKD* genes may influence the rate of disease progression,[14,15] but the genotype/phenotype correlations are not entirely clear.

Molecular diagnostics for ADPKD has become available and increasingly important in recent years.[2] Traditional diagnostic approach is based on renal ultrasound that cannot exclude the disease in at-risk individuals until the age of 40 years, especially in families with mutations in *PKD2*.[16] PCR and direct sequencing analysis can provide direct evidence of *PKD* mutations, while multiplex ligation-dependent probe amplification (MLPA) may detect large deletion/duplication mutations that cannot be detected by direct sequencing.[17,18] However, there are no mutation hot spots in *PKD* genes.[2] Furthermore, interpretation of the identified missense

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mutations can be challenging, because multiple sequence variants exist in *PKD1* and *PKD2*.[19] Recent studies have demonstrated the existence of incompletely penetrant *PKD1* mutations.[20–22] The combination of these hypomorphic *PKD1* alleles may cause typical-to-severe cystic disease, whereas one such allele may cause unusually mild disease.[23] The Polycystic Kidney Mutation Database (PKDB; http://pkdb.mayo.edu) is a continually updated online reference describing all published *PKD* mutations and variants.[24,25] A collection of more mutation data of *PKD* genes from different populations would allow better interpretation of the results of genetic testing.

Although *PKD* mutations from different countries have been reported, data in the Taiwanese population are still lacking.[19,26] Here we report on our single-center experience of direct sequencing and MLPA for *PKD1* and *PKD2* in 46 unrelated Taiwanese patients with an ultrasound diagnosis of ADPKD. The specific purpose was to examine the characteristics of mutations in our cohort and their correlations to disease progression.

PATIENTS AND METHODS

Patients

A total of 46 unrelated patients with a clinical diagnosis of ADPKD at the Chang Gung Memorial Hospital, Linkou, Taiwan were enrolled between October 2008 and May 2011. All participants signed written informed consent. Approval for this work had been obtained from the Institutional Review Board Committee at the Chang Gung Memorial Hospital. The study was conducted in adherence to the Declaration of Helsinki. The diagnosis of ADPKD was made according to the renal ultrasound criteria.[27] In the absence of a family history of ADPKD, bilateral renal enlargement and at least 10 cysts in each kidney were required for diagnosis.[28] Blood samples were obtained from these patients and their available families members. Control DNA was obtained from a database of healthy volunteers.

Clinical assessment

Patients were interviewed for family and medical histories. A family history of early dialysis (before the age of 55 years) and late dialysis (after the age of 65 years or no need for dialysis) due to ADPKD was recorded.[13] Clinical and laboratory data were obtained from medical records. An estimated glomerular filtration rate (eGFR) was calculated according to the Modification of Diet in Renal Disease study equation.[29]

Mutation analysis of *PKD1* and *PKD2*

Genomic DNA was extracted from lymphocytes harvested from venous blood samples. Direct sequencing of exonic and flanking introgenic regions of all *PKD1* and *PKD2* exons was performed as previously published methods.[25,30] For amplifying the duplicated part of *PKD1* (exons 1–34), long-range PCR was first performed. Seven distinct long-range PCR fragments (exon 1, exons 2–7, exons 8–12, exons 13–15, exons 15–21, exons 22–28 and exons 29–34) were amplified using *PKD1*-specific primers.[25,30] Exons 35–46 of *PKD1* and exons 1–15 of *PKD2* were amplified from genomic DNA according to previously described PCR primers and conditions.[30,31] The PCR products were purified and sequenced on an ABI3730 autosequencer (Applied Biosystems, Foster City, CA, USA). The pathogenicity of missense variants were evaluated using SIFT, PolyPhen and GVGD prediction programs as described previously.[20] The 1000 Genomes Project data (http://browser.1000genomes.org), the Exome Sequencing Project (http://evs.gs.washington.edu/EVS) and the Single-Nucleotide Polymorphism Database were checked for the reported sequence variants. The Human Splicing Finder software (http://www.umd.be/HSF/) was used to predict splicing signal.[32] PKDB (http://pkdb.mayo.edu) was consulted for the previously reported mutations. The recommendation of HGVS (Human Genome Variation Society) for mutation nomenclature was followed. Nucleotide changes were numbered according to the coding sequences of *PKD1* (NM_001009944.2) and *PKD2* (NM_000297.3).

MLPA

Screening for *PKD1/PKD2* exon deletion/duplication was performed using MLPA according to the manufacturer's instructions. The MLPA kit (SALSA MLPA KIT P351-B1/P352-B1 *PKD1-PKD2*) was purchased from MRC-Holland (Amsterdam, The Netherlands).[33] It contains *PKD1* probes for 37 of the 46 exons and 15 *PKD2* probes covering all *PKD2* exons. Data were analyzed using the Coffalyser MLPA analysis tool (MRC-Holland, Amsterdam, The Netherlands).

Real time quantitative PCR (Q-PCR)

MLPA revealed one family with significant copy number variation of probe for *PKD1* exon 1. To validate the results of MLPA, a real-time Q-PCR was performed using Custom Taqman Copy Number Assays (Applied Biosystems, Carlsbad, CA, USA). A set of commercially designed primers against the binding site of MLPA *PKD1* exon 1 probe was used (Assay ID: PKD1E1_CCS07DN). The reaction was performed in triplicate for each sample.

Statistical analysis

Continuous variables are expressed as mean ± s.d. and compared using one-way analysis of variance. Categorical variables were analyzed using the Fisher's exact or Chi-square test. Cumulative renal survival curves were generated by the Kaplan–Meier method and compared with the log-rank test. *P*-values <0.05 were considered statistically significant. All analyses were performed using the Graphpad Prism 5.1 (Graphpad, La Jolla, CA, USA).

RESULTS

Clinical characteristics and genotyping

We performed a complete mutational analysis using direct sequencing and MLPA analysis of *PKD1* and *PKD2* in 46 unrelated patients with the diagnosis of ADPKD by ultrasound. We found disease-causing mutations in 30 (65%) patients: 23 (77%) patients have *PKD1* mutations and 7 (23%) have *PKD2* mutations. The remaining 16 (35%) were categorized as undetermined genotype either because no mutations were detected or the identified variants were interpreted as nonpathogenic variants.

Table 1 summarizes the clinical characteristics in the study cohort according to their genotypes. There were 24 male and 22 female patients. The mean age was 47.5 ± 13.0 years. ADPKD family history was reported in 35 (76%) patients. Family history of ADPKD with early dialysis (age $\leqslant$55 years) was found in 11 (24%) patients, whereas late dialysis (age $>$65 years) was reported in 8 (17%) patients. Hypertension was noted in 31 (67.4%) patients while gross hematuria, urinary tract infection and urolithiasis were found in 13 (28.3%), 16 (34.8%) and 19 (41.3%) patients, respectively. Subarachnoid hemorrhage was noted in 1 (2.2%) patient and two patients reported documented cerebral aneurysms. Mitral valve prolapse was noted in 1 (2.2%) patient. Extrarenal cysts in the liver, pancreas and spleen were detected in 29 (63%) patients. In patients with *PKD1* mutations, the ages at recruitment tended to be younger than the other two groups ($P=0.09$). A family history of dialysis after 65 years was more frequent in patients with *PKD2* mutations, whereas a family history of dialysis before 55 years was more frequent in patients with *PKD1* mutations ($P<0.05$). Other clinical parameters and antihypertension medication use were not significantly different between genotypes.

The kidney sizes as estimated by ultrasound were not different between genotypes. However, patients with *PKD1* mutations in our study tended to be younger than patients with *PKD2* mutations, suggesting that kidney enlargement may occur earlier in PKD1 patients than in PKD2 patients.

Renal survival analysis

We then performed a Kaplan–Meier survival curve analysis to compare the age at onset of ESRD between different genotypes. As shown in Figure 1a, PKD1 patients ($n=23$) have a significantly lower renal survival curve than PKD2 patients ($n=7$) and patients with an undetermined genotype ($n=16$) (log-rank test, $P=0.014$). The median age of ESRD was 52 years in patients with *PKD1* mutations ($n=7$, range 40–58 years), 65.5 years in patients with *PKD2* mutations ($n=2$, range 64–67 years) and 67 years in patients with an undetermined genotype ($n=6$, range, 41–79 years). We then investigated whether the type of *PKD1* mutation influenced the age at onset of ESRD. We found a trend of earlier initiation of dialysis in patients with *PKD1*-truncating mutations ($n=14$) compared with patients with *PKD1* missense mutations ($n=9$) as shown in Figure 1b (log-rank test, $P=0.061$). The median age of ESRD in patients with and without *PKD1*-truncating mutations was 52 ($n=5$) and 57 ($n=2$) years, respectively.

Characteristics of mutations

Using direct PCR sequencing, we found 15 definite pathogenic mutations (12 in *PKD1* and 3 in *PKD2*) in 17 (35%) probands (Tables 2 and 3). Among these mutations, 9 (60%) were nonsense mutations, 5 (33%) were frame-shifting and 1 (7%) was splicing mutation. We identified two novel *PKD2* missense mutations (p.Try392Asp and p.Ala389Val) predicted to be likely pathogenic (Table 3). Fifteen *PKD1* missense mutations, which were predicted to be likely pathogenic, were identified in nine patients (Table 4). A deletion mutation in *PKD1* intron 38 (c.IVS38-100_98del3) predicted to generate new splice sites was found in one patient who started maintenance dialysis at the age of 40 years (Table 4). One patient (CG44) was found to have a *PKD1*-truncating mutation (p.Glu2810X) and four likely pathogenic missense mutations (Table 4). She experienced right middle cerebral artery infarction at the age of 45 years and an aneurysm was found in the right cranial internal carotid artery by angiography. She had advanced chronic kidney disease (eGFR 15 ml per min per 1.73 m^2) at the age of 50 years. In total, we identified 119 different sequence variants of *PKD1* and *PKD2*: 15 (13%) are definite pathogenic mutations, 17 (14%) are likely pathogenic mutation, 50 (42%) are likely non-pathogenic missense mutation and 37 (31%) are silent polymorphism.

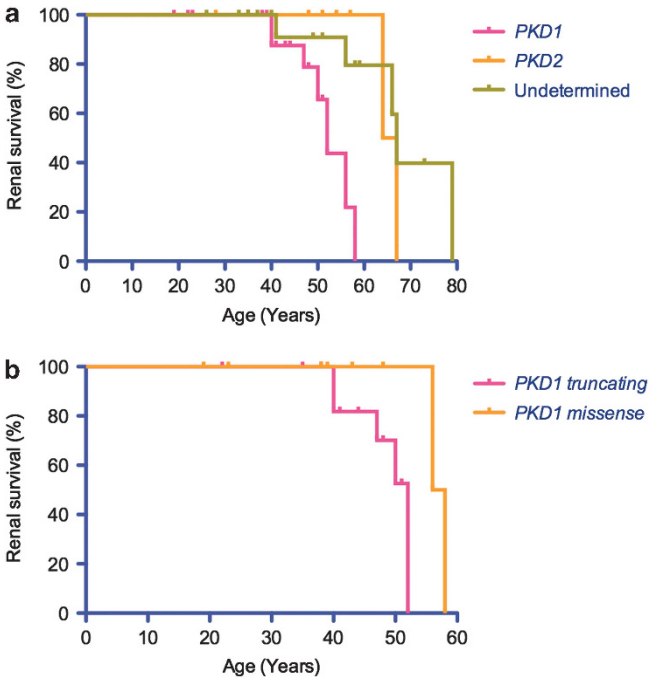


Figure 1 Kaplan–Meier survival curves of the age at onset of ESRD according to *PKD* genotypes. (**a**) Comparisons between patients with *PKD1* ($n=23$) and *PKD2* mutations ($n=7$) and patients with an undetermined genotype ($n=16$) ($P=0.014$, log-rank test). (**b**) Comparisons between patients with truncating mutations ($n=14$) and missense mutations ($n=9$) in *PKD1* in the study cohort ($P=0.061$, log-rank test). Tick marks denote censored patients.

Table 1 Comparison of clinical characteristics between 46 ADPKD patients according to genotypes

		Genotype			
	All patients	*PKD1* (n = *23*)	*PKD2* (n = *7*)	*Undetermined* (n = *16*)	P-*value*
Gender, M/F	24/22	14/9	2/5	9/7	0.32
Age (years)	47.5 ± 13.0	43.4 ± 10.81	52.9 ± 13.0	51.1 ± 14.8	0.09
eGFR (ml per min per 1.73 m^2)	60.2 ± 45.1	54.8 ± 41.5	58.6 ± 39.4	68.61 ± 53.2	0.65
Positive family Hx	35 (76%)	18 (78%)	6 (86%)	11 (69%)	0.64
Family Hx of HD ⩽ 55 y/o	11 (24%)	9 (39%)	0 (0%)	2 (13%)	0.04*
Family Hx of HD > 65 y/o	8 (17%)	1 (4%)	3 (43%)	4 (25%)	0.04*
Diagnosis < 30 y/o	7 (15%)	5 (22%)	1 (14%)	1 (6.25%)	0.41
Hypertension	31 (67%)	17 (74%)	5 (71%)	9 (56%)	0.50
Gross hematuria	13 (28%)	8 (35%)	1 (14%)	4 (25%)	0.54
Urinary tract infection	17 (37%)	10 (43%)	3 (43%)	4 (25%)	0.47
Urolithiasis	19 (41%)	9 (39%)	3 (43%)	7 (44%)	0.96
Subarachnoid hemorrhage	1 (2%)	1 (5%)	0 (0%)	0 (0%)	0.60
Mitral valve prolapse	1 (2%)	1 (5%)	0 (0%)	0 (0%)	0.60
Extrarenal cysts (liver, spleen, pancreas)	29 (63%)	16 (70%)	5 (71%)	8 (50%)	0.41
Mean kidney length (cm)	14.8 ± 2.3	14.9 ± 2.4	14.7 ± 1.9	14.8 ± 2.3	0.99
ACEI/ARB	28 (61%)	15 (65%)	4 (57%)	9 (56%)	0.37

Abbreviations: ACEI, angiotensin-converting enzyme inhibitor; ADPKD, autosomal dominant polycystic kidney disease; ARB, angiotensin II receptor blocker; eGFR, estimated glomerular filtration rate; HD, hemodialysis; Hx, history; M, male; F, female.
Data are mean ± s.d. or number (percentage). *$P<0.05$.

Table 2 Definite pathogenic mutations in *PKD1* found in the study

Exon/intron	*cDNA change*	*Protein change*	*Variant effect*	*Family no. (Patient no.)*	*Proband ID*	*Previous description*
5	c.1198C>T	p.Arg400X	Truncation	1	CG9	PKDB[48]
15	c.3375_3376ins16	p.Ser1125fs15X	Frameshift	1	CG25	Novel
15	c.4447C>T	p.Gln1483X	Truncation	1	CG78	PKDB[39]
15	c.5014_5015delAG	p.Arg1672fs97X	Frameshift	1	CG24	PKDB[25]
17	c.7204C>T	p.Arg2402X	Truncation	1	CG67	PKDB[49]
22	c.8161+1G>C		Splicing	1	CG13	Novel
23	c.8428G>T	p.Glu2810X	Truncation	1	CG44	PKDB[19]
23	c.8566C>T	p.Gln2856X	Truncation	1	CG8	Novel
33	c.10281ins_C	p.Pro3426fs5X	Frameshift	1	CG40	Novel
35	c.10528_10529delAC	p.Thr3509fs136X	Frameshift	1	CG29	Novel
43	c.11831delT	p.Leu3943fs40X	Frameshift	1	CG5	Novel
45	c.12391G>T	p.Glu4131X	Truncation	1 (2)	CG7	Novel

Abbreviations: ID, identification; PKDB, Polycystic Kidney Mutation Database.
Large duplication is not included in the table.

Table 3 Five *PKD2* mutations found in the study

Exon	*cDNA change*	*Protein change*	*Variant effect*	*Family no. (Patient no.)*	*Proband ID*	*Previous description*
2	c.681C>A	p.Tyr227X	Truncation	1	CG62	PKDB[36]
9	c.1960C>T	p.Arg654X	Truncation	1 (2)	CG22	PKDB[24]
13	c.2407C>T	p.Arg803X	Truncation	3 (9)	CG2,CG32,CG73	PKDB[36,37]
5	c.1166C>T	p.Ala389Val	Substitution	1	CG42	Novel[a]
5	c.1174T>G	p.Tyr392Asp	Substitution	1	CG10	Novel[a]

Abbreviations: ID, identification; PKDB, Polycystic Kidney Mutation Database.
[a]Predicted to be likely pathogenic by PolyPhen.

Table 4 Likely pathogenic mutations in *PKD1* found in this study and prediction of their pathogenicity

			SIFT[a]		*SIFT*[b]		*PolyPhen*[a]		*AlignGVGD*[b]					
Exon/intron	*cDNA change*	*Protein change*	*MG*	*VS*	*MG*	*VS*	*MG*	*VS*	*MG*	*VS*	*Family no. (Patient no.)*	*Proband ID*	*Frequency control*	*Previous description*
5	c.1142G>T	p.Gly381Val	B	0	B	0	B	2.40	N	C0	1[c]	CG44	0/50	dbSNP ID: rs2519261
5	c.1211C>T	p.Pro404Leu	B	0	N	0.11	B	2.72	N	C0	1 (2)	CG3	0/50	Novel
5	c.1261C>T	p.Arg421Cyc	C	0.01	C	0.01	B	2.22	N	C0	1	CG53	0/50	Novel
7	c.1543G>A	p.Gly515Arg	C	0.03	C	0.02	B	2.17	N	C0	1[c]	CG44	0/50	PKDB
15	c.6341A>G	p.Tyr2114Cyc	B	0	B	0	B	2.76	B	C65	1	CG1	0/50	Novel
17	c.7172G>A	p.Gly2391Asp	N	0.66	B	0.00	B	2.05	B	C65	1 (3)	CG52	0/87	PKDB[39]
18	c.7430G>A	p.Arg2477His	B	0	I	0.05	C	1.92	N	C0	1 (2)[d]	CG30	7/131	PKDB[34]
23	c.8291T>C	p.Met2764Trp	B	0	N	0.26	B	2.72	N	C0	1[c]	CG44	0/47	Novel
23	c.8447T>C	p.Leu2816Pro	C	0.01	N	0.11	B	2.04	C	C35	1[c]	CG44	0/47	PKDB[50]
25	c.9157G>A	p.Ala3053Thr	N	0.18	B	0	C	1.54	B	C55	2 (2)	CG4, CG41	0/50	Novel
28	c.9583T>C	p.Trp3195Arg	B	0	B	0	B	3.90	B	C65	1	CG35	0/50	Novel
30	c.10043G>A	p.Arg3348Gln	I	0.06	B	0.00	C	1.75	C	C35	1 (2)	CG22	0/59; 9/11826[e]	dbSNP ID: rs146494724[51]
31	c.10102G>A	p.Asp3368Asn	I	0.05	C	0.03	C	1.61	N	C0	2 (3)	CG7, CG22	NA	PKDB[30]
33	c.10315C>T	p.Arg3439Trp	C	0.01	B	0.00	B	2.51	I	C25	1 (2)[d]	CG30	0/50; 1/12972[e]	ESP ID: TMP_ESP_16_2147410
38	c.11157-100_98del3	Aberrant splicing	Predicted to introduce new splice sites								1	CG37	NA	Novel
39	c.11257C>T	p.Arg3753Trp	I	0.09	C	0.04	B	2.00	N	C0	1	CG18	NA	PKDB[25]

Abbreviations: dbSNP, database for single-nucleotide polymorphism; ESP, Exome Sequencing Project; ID, identification; MG, mutation group (B: highly likely; C: likely; I: indeterminate; N: neutral); NA, not analyzed; PKDB, Polycystic Kidney Mutation Database; VS, variant score.
[a]Default alignment (human, mouse, rat, chicken, xenopus, fugu).
[b]Polycystin-1 orthologue alignment (human, mouse, rat, chicken, xenopus, fugu, dog, opossum, tetraodon nigroviridis).
[c]Found with p.E2810X in CG44.
[d]Occurred simultaneously.
[e]Allele frequencies in ESP.

Large duplication mutation

To identify large deletion or duplication mutation that cannot be detected by direct sequencing, we then performed a copy number analysis using MLPA on all patients in this cohort. There were no multiple exon deletions found. Duplication of *PKD1* exon 1 (relative peak ratio 1.66) was identified in a 47-year-old woman, and a similar result was found in her son (relative peak ratio: 1.58) (Figure 2a). Repeated MLPA gave similar results. These results were further

validated using real-time Q-PCR (Figure 2b). The pedigree of this family and clinical data are shown in Figure 2c.

Hypomorphic alleles

In one family, the concurrence of *PKD1* p.Arg2477His and p.Arg3439Trp variants segregated with the presence of polycystic kidney disease in two sisters (Figure 3a). To our surprise, their parents and two other siblings, each of them carries *PKD1* p.Arg3439Trp or p.Arg2477His, were negative for renal cysts. These results suggested that p.Arg3439Trp and p.Arg2477His could be hypomorphic alleles, as both were incompletely penetrant. *PKD1* p.Arg2477His has been previously reported in a Han Chinese family as a pathogenic mutation,[34] but whether or not it coexisted with other missense mutations is not known. *PKD1* p.Arg3439Trp is predicted to be highly likely pathogenic by computer programs, and its allele frequency in the Exome Sequencing Project was 1/12972 (Table 4). Amino-acid multi-alignments demonstrate that the positions of mutations are highly conserved across species in p.Arg3439Trp and partially conserved in p.Arg2477His (Figure 3b).

Undetermined genotype

There were 16 patients who had no *PKD* variants found or the identified sequence variants were interpreted as non-pathogenic.

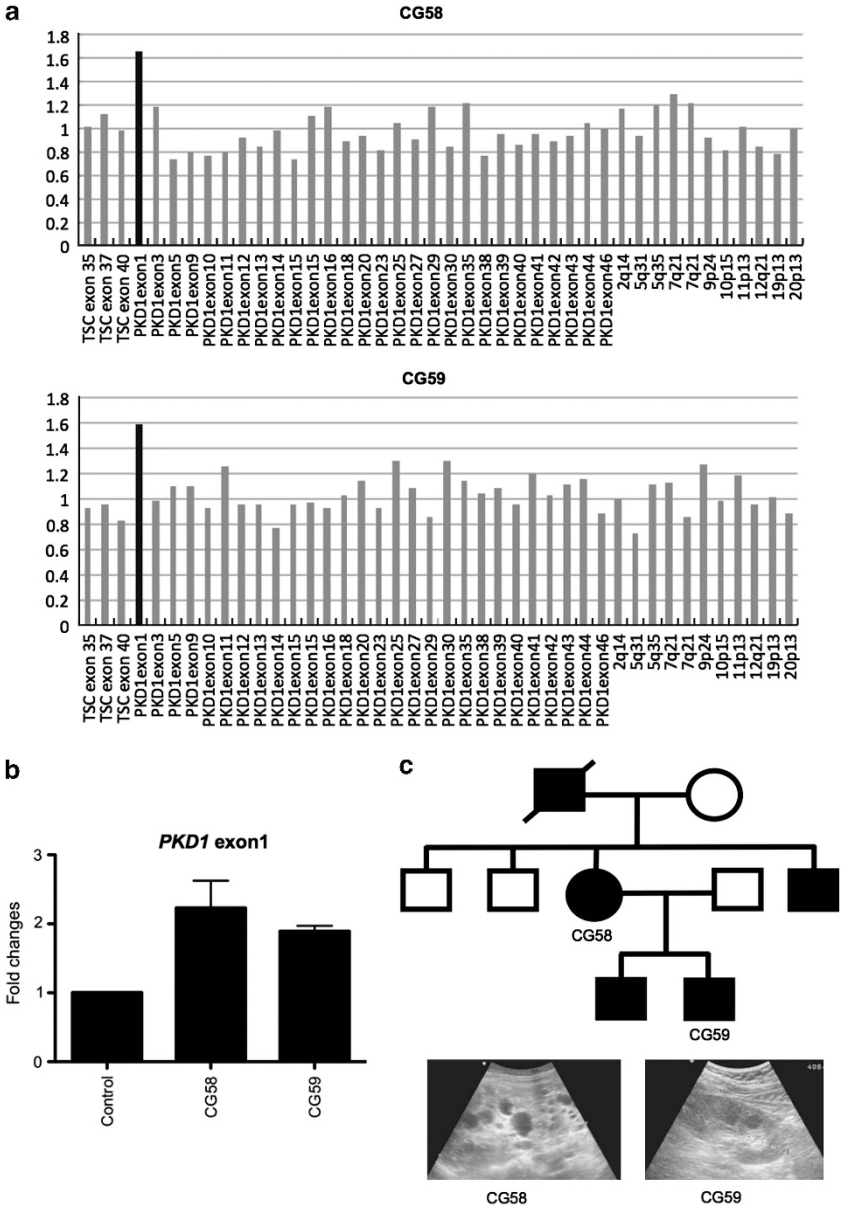
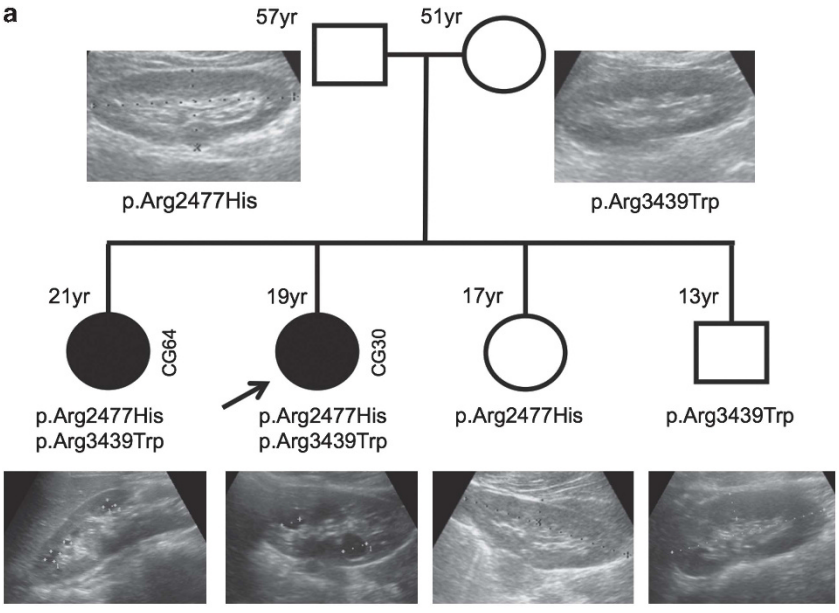


Figure 2 MLPA analysis showing duplication of *PKD1* exon 1 in a Taiwanese family of ADPKD. (**a**) *PKD1* and reference probes are listed on the *x* axis. The relative peak area of the amplification product (peak ratio) is depicted on the *y* axis. Note that the peak ratios of *PKD1* exon 1 probe (black bars) are increased in patient CG 58 (1.63) and patient CG59 (1.58). (**b**) Pedigree and ultrasonography of the family. CG58 (mother) had bilateral enlarged kidneys with multiple cysts at the age of 47 years (eGFR: 77 ml per min per 1.73 m^2). Her father required dialysis at the age of 64 years and died after 7 years. CG59 had a few cysts at the age of 18 years with normal renal function. Other family members were unavailable for genetic testing. (**c**) Real-time Q-PCR using Custom Taqman Copy Number Assays with primers against the binding site of the MLPA exon 1 probe. Results shown are means ± s.d. from two independent experiments done in triplicate.



Variant	SIFT[a]		SIFT[b]		PolyPhen[a]		AlignGVGD[b]	
	MG	VS	MG	VS	MG	VS	MG	VS
p.Arg2477His	B	0	I	0.05	C	1.92	N	C0
p.Arg3439Trp	C	0.01	B	0.00	B	2.51	I	C25

Figure 3 Two *PKD1* missense mutations (p.Arg2477His and p.Arg3439Trp) segregated with ADPKD in a Taiwanese family. (**a**) The pedigree and renal ultrasound data of the family. These mutations segregated in two affected daughters with multiple renal cysts. The parents and two other siblings had normal renal function, and the findings of renal ultrasound were normal. The proband (CG 30) presented with recurrent urinary tract infection at the age of 19 years and bilateral renal cysts and ovarian cysts were found (eGFR: 89 ml per min per 1.73 m^2). Her sister (CG 63) presented with bilateral renal cysts at the age of 21 years (eGFR: 109 ml per min per 1.73 m^2) and had a history of acute pyelonephritis. (**b**) Multiple sequence alignments for the *PKD1* changes p.Arg2477His and p.Arg3439Trp. The results of pathogenicity prediction using web-based computer programs (SIFT, Polyphen and AlignGVGD) are shown at the bottom of the figure. [a]Default alignment (human, mouse, rat, chicken, xenopus, fugu); [b]Polycystin-1 orthologue alignment (human, mouse, rat, chicken, xenopus, fugu, dog, opossum, tetraodon nigroviridis); MG, mutation group (B: highly likely; C: likely; I, indeterminate: N, neutral); VS, variant score.

These patients tended to have a slower decline of renal function than the patients with *PKD1* mutations (Figure 1a). Other clinical characteristics were not significantly different in patients with an undetermined genotype compared with others (Table 1).

DISCUSSION

We performed a comprehensive clinical and mutational analysis in a cohort of ADPKD patients. This is the first study to describe the characteristics of *PKD1* and *PKD2* mutations in Taiwan. Worldwide, there have been 1923 sequence variants for *PKD1* and 241 for *PKD2* as described in the PKDB. Among these variants, 929 of *PKD1* and 167 of *PKD2* are pathogenic mutations. In the current study, 50% (17/34) of mutations are novel and 50% (17/34) are recurrent mutations reported in the literature. The 17 novel mutations we found in the Taiwanese population would expand the spectrum of *PKD* mutations and contribute to the global references for genetic counseling of ADPKD patients.

Among the 34 mutations we identified, only three mutations were present in more than one unrelated patient. This is consistent with the findings that no single mutation accounts for >2% of unrelated ADPKD patients in previous studies.[2,35] Therefore, ADPKD patients who seek for the genetic diagnosis need to undergo a complete mutation analysis of *PKD1* and *PKD2* unless the causative mutations have been identified in other family members. Nevertheless, a recurrent mutation *PKD2* p.R803X was found in three families in Taiwan and five families in other populations.[30,36–39] This frequent mutation could be related to its location in the CpG dinucleotide site that is susceptible to

mutations, or it could be a founder mutation that derived from the common ancestor.[35]

The frequency of large rearrangements reported in a large ADPKD cohort ($n = 202$) was 4%.[17] In this study, we identified duplication of *PKD1* exon 1 by MLPA and Q-PCR in 1 out of 46 cases (2%). A previous study has reported duplication of *PKD1* IVS17 and exon 18 in one patient.[17] Overall, deletion and duplication mutations are rare in ADPKD patients, and MLPA can be reserved for those mutation-negative patients after direct PCR sequencing.

Knowing whether a family carries mutations in *PKD1* or *PKD2* may have prognostic value, because PKD2 patients typically develop ESRD two decades later than PKD1 patients.[26] Furthermore, the mutation type and location in *PKD1* have been shown to influence renal survival in previous studies.[15] The position of the mutation in *PKD1* has been shown to predict the occurrence of intracranial aneurysms.[40] In a recent report from 519 pedigrees in the Genkyst cohort, carriers of a *PKD1*-truncating mutation have a significantly earlier age at onset of ESRD than patients with a non-truncating mutation (55 years vs 67 years).[14] Our data support the notion that a more severe phenotype can be expected in PKD1 patients, especially in those with a *PKD1*-truncating mutation. Therefore, genetic testing for ADPKD patients would be helpful to clarify the disease status and provide prognostic information.[2] Robinson *et al.*[26] proposed that direct *PKD2* mutation testing can be offered to identify the subgroup less likely to progress to ESRD. The prognostic information could be important for guiding the choice of potential treatments for ADPKD that might have side effects after long-term use.[28,41]

Hypomorphic *PKD1* alleles are increasingly recognized in recent genotype–phenotype studies.[20–22] These missense variants have reduced PC-1 function and cause mild disease when present alone or severe disease when they occur simultaneously. In our cohort, we found two likely hypomorphic alleles, *PKD1* p.Arg2477His and p.Arg3439Trp, which segregated with the disease in one family. To date, nine *PKD1* hypomorphic alleles have been described in different populations as collated in PKDB.[21,22] For example, a compound heterozygote for the *PKD1* variants p.Arg3105Trp and p.Arg2765Cys caused multiple cysts in childhood, whereas another family member heterozygous for p.Arg2765Cys developed only one cyst.[20] Patients heterozygous for *PKD1* p.Arg3277Cys had only few cysts, but those homozygous for p.Arg3277Cys developed multiple renal cysts and ESRD.[20] *PKD1* p.Arg2220Trp and p.Arg3277Cyc *in trans* co-segregated with early-onset ADPKD, whereas no cysts were found in the heterozygous parents.[21] These findings support the concept of a critical cellular threshold of PCs in the pathogenesis of ADPKD.[22,42] A valid functional study for these missense variants is still lacking.[22,43]

It is worth noting that a careful history taking for the age of ESRD in affected family members may provide a simple means of predicting the mutated gene in clinical practice.[13] Our results support the use of family history information as an aid in risk stratification. For those whose relatives have developed ESRD before the age of 50 years, the family history is highly suggestive of carrying a *PKD1* mutation.[13,26] For those whose affected members developed ESRD aged >70 years or continued to have sufficient renal function, the presence of a *PKD2* mutation is highly likely.[13]

In previous studies, disease-causing mutations were identified in approximately 64–90% of ADPKD patients by direct PCR sequencing.[35,39] Another study using denaturing high-performance liquid chromatography found mutations in only 52% of ADPKD patients.[44] The possible causes of the relative low mutation detection rate in our study may include cohort size, methods of mutational analysis, sequencing technique, the presence of large deletion/duplication, deep intron sequence, mosaicism or mutations in other genes. In addition, some missense mutations that were incompletely penetrant could be interpreted as sequence variants. Furthermore, patients without a family history of ADPKD were included in our study. It seems that definite pathogenic mutations are found more frequently in patients with a positive family history of ADPKD than in those without.[2,35]

Several potential limitations of this study merit consideration. First, we only screened for *PKD1* and *PKD2* mutations and therefore cannot exclude the existence of other mutations such as *HNF1β*, *PRKCSH*, *SEC63* or *PKHD1* that may contribute to the cystic phenotype.[2] Second, the expensiveness of exon by exon DNA sequencing precludes us from recruiting more cases. Relatively small sample size may limit the ability to detect a small phenotypic difference between genotypes. Nevertheless, we were still able to detect phenotype differences in our study cohort. Finally, the likely pathogenic mutations identified in our study need to be confirmed in future studies involving more ADPKD families.

As potential therapies for ADPKD are being developed, molecular testing will likely become increasingly important.[2,28] Another important indication is to determine the disease status of potential living related donors with equivocal imaging findings.[19,45] A recent study has shown great promise of next-generation sequencing for genetic PKD diagnostics, because the time and effort needed to detect pathogenic mutations are markedly reduced.[46] Therefore, more efficient and low-cost genetic testing can be expected in the next decade, with the advance of DNA sequencing technique.[35,46] In addition to genetic testing, a simple family history enquiry for the age of hemodialysis may yield prognostic information by estimating *PKD1* (early dialysis) and *PKD2* (late dialysis) genotypes.[13] Many clinical trials of different mechanism-based drug treatment for ADPKD are currently undergoing.[47] It would be interesting to know whether patients with different *PKD* mutations may respond differently to these novel treatments.

CONCLUSIONS

We presented the first complete clinical and mutational analysis of *PKD1/PKD2* in Taiwanese patients with ADPKD. We identified 17 novel mutations of *PKD1* and *PKD2* and demonstrated the presence of *PKD1* hypomorphic alleles. Patients carrying *PKD1* mutations, especially those with truncating mutations, could have a more rapidly progressive disease than others. These results might have implications for diagnosis and risk stratification in patients with ADPKD.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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