Original Paper

Modification of PCR Conditions and Design of Exon-Specific Primers for the Efficient Molecular Diagnosis of PKD1 Mutations

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Key Words

Autosomal-dominant polycystic kidney disease (ADPKD) • PKD1 • Long-range PCR • Mutation testing

Abstract

Background/Aims: Autosomal-dominant polycystic kidney disease (ADPKD) is a heterogeneous genetic disorder caused by mutations in the *PKD1* and *PKD2* genes. Currently, long-range PCR followed by nested PCR and sequencing (LRNS) is the gold standard approach for *PKD1* testing. However, LRNS is complicated by the high structural and sequence complexity of *PKD1*, which makes the procedure for amplification and analysis of *PKD1* difficult. **Methods:** Here in, we modified the PCR conditions and designed primers for efficient and specific amplification of both the long-range and individual exons of *PKD1*. **Results:** Using the modified system, seven long-range fragments were specifically amplified using two distinct sets of conditions, and all individual exon PCR assays were easily performed using a touch-down PCR method. Seven pathogenic or likely pathogenic variants, including two novel truncated frameshift indels and two novel likely pathogenic missense mutations, were identified in eight unrelated patients with or without histories of ADPKD disease (one variant was observed in two unrelated patients). Using combined bioinformatics tools, two indeterminate missense variants were identified in two sporadic patients. **Conclusion:** Four novel *PKD1* variants were identified in this study. We demonstrated that the modified LRNS method achieves high sensitivity and specificity for detecting pathogenic variants of ADPKD.

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Introduction

Autosomal dominant polycystic kidney disease (ADPKD, MIM#173900) is a common hereditary and genetically heterogeneous kidney disease with a prevalence of approximately 1 in 500 to 1000 people [1]. ADPKD is characterized by bilaterally enlarged kidneys with multiple cysts, as well as additional cysts in the liver, seminal vesicles, pancreas and arachnoid membranes [2, 3]. The chronic progressive development of renal cysts leads to end-stage renal disease in approximately 50% of patients by 60 years of age, leaving hemodialysis or kidney transplant as the only available treatment options at this stage [4].

Clinical studies have indicated that ADPKD is most frequently caused by mutations in two causal genes, *PKD1* (MIM#601313) and *PKD2* (MIM#173910) [5, 6]. Mutations in the *PKD1* gene (located on chromosome 16 at 16p13.3) account for 75% to 85% of ADPKD cases, whereas the remaining 15% of cases are due to mutations in *PKD2* (located on chromosome 4 at 4p21) [7]. ADPKD cases caused by mutations in *PKD1* appear to be more severe than those associated with *PKD2* mutations. For example, an earlier onset of renal failure, at 53.4 years of age, occurs in patients with *PKD1* mutations, whereas the age of onset is 72.7 years for those with *PKD2* mutations [8, 9]. Therefore, genetic testing, particularly within the *PKD1* gene, plays a significant role in ADPKD diagnosis.

Although it has become possible to obtain a molecular diagnosis of ADPKD in recent years [10], directly sequencing ADPKD mutations is more complicated than sequencing for mutations that cause other inherited diseases. One reason for this difficulty is that no mutation hot spots have been identified in the *PKD1* and *PKD2* genes; therefore, the entire protein-coding sequences of these two genes must be fully screened. Another reason for the difficulty encountered during ADPKD molecular diagnosis is the extreme enrichment of GC nucleotides in *PKD1*, which complicates the ability to perform efficient polymerase chain reaction (PCR) assays and sequencing reactions. In addition, the region between exon 1 to exon 33 of *PKD1* has undergone intrachromosomal duplication throughout the human genome, which resulted in the generation of six homologous pseudogenes (*PKD1P1-P6*) that are proximally located between 13 and 16 Mb away from *PKD1*. These homologous pseudogenes share 98-99% sequence similarity with the *PKD1* gene [11], which complicates *PKD1* mutation screening because classic PCR amplifies *PKD1* in addition to these homologous sequences. Therefore, it is critical to accurately identify true mutations in the *PKD1* gene by avoiding contamination of the PCR products by these pseudogenes.

To specifically amplify the *PKD1* gene, a long-range PCR was performed using unique primers that target the slight sequence variations between the *PKD1* pseudogenes and *PKD1*. Using these unique and lengthy amplification products as templates, nested PCR to amplify each individual exon was followed by sequencing [12-14]. Although a variety of methods are used to screen *PKD1* mutations, such as high-resolution melt analysis [15], DNA high-performance liquid chromatography (DHPLC) [16], direct sequencing of long-range PCR products [17] and next-generation sequencing [18], long-range then nested PCR and sequencing (LRNS) is the predominant method in many laboratories. However, the efficient application of LRNS to amplify various regions of the targeted fragments has been hampered by complicated PCR conditions, variations in polymerases and the use of additional additives, such as betaine [2, 17]. Moreover, individual exon amplification using the diluted long-range PCR products as templates does not eliminate the possibility of pseudogene fragment contamination because the remaining minimal homologous sequence may be amplified by non-specific individual exon primers. In the present study, we modified the PCR conditions and designed exon-specific primers for both long-range fragment and individual exon amplification to develop an easy and efficient procedure for amplifying the entire *PKD1* gene. In our modified system, only two sets of PCR conditions are required to perform long-range PCR, one set of conditions is specific to exon 1 and the other is used to amplify the remaining six lengthy fragments. Meanwhile, the individual exon amplification of *PKD1* and *PKD2* can be performed using a single touch-down PCR assay. For the sequencing reaction, exon-specific primers were indexed using a universal M13 sequence, which further simplified the sequencing procedure.

Materials and Methods

Ethics statement and patient recruitment

This research conformed to the principles outlined in the Declaration of Helsinki and was approved by the Ethics Committee of the Third Affiliated Hospital of Guangzhou Medical University. All enrolled patients and their family members provided written informed consent.

The clinical assessment of ADPKD patients was performed using standard diagnostic ultrasound criteria if there was a known family history of disease [19]. In the absence of a family history, ADPKD diagnosis required the detection of bilateral renal enlargement with multiple cortical and medullary cysts [20]. A total of 10 unrelated probands (5 men and 5 women) and more than 10 family members participated in this study. All of the 10 probands were diagnosed as having ADPKD based upon renal ultrasound findings in accordance with the criteria mentioned above. Among those 10 patients, 6 reported a family history of ADPKD disease and 4 reported no family history of ADPKD. Genomic DNA derived from whole blood was extracted and purified from the patients and their available family members using the Qiagen DNeasy Tissue Kit according to the manufacturer's instructions (Qiagen, Hilden, Germany).

Long-range and individual exon PCR primer design

Using the free online website hosted by the University of California Santa Cruz (UCSC, <http://genome.ucsc.edu/>), we evaluated the targeted fragments of the *PKD1* gene (NM_001009944) sequence using the BLAT program to find the minimal region that included nucleotide differences compared with the six pseudogenes. The mismatched sequences were used to carry out specific long-range and individual exon primer design. In this study, the annealing temperature for all primers was near 60°C to ensure that all fragment amplifications could be subjected to similar PCR conditions. In addition, to simplify the sequencing procedure, a universal M13 sequence was indexed to the 5' end of the exon-specific primers.

Modifications of the PCR conditions

The previously reported PCR conditions varied for each long-range fragment and individual exon amplification. Moreover, variations in the GC nucleotide contents of each exon required additional additives, which complicated the PCR procedures. To overcome these limitations, we modified the PCR conditions for long-range PCR by optimizing the annealing temperatures of the primers. To efficiently amplify the GC-rich long fragments, a long and accurate PCR kit was used in the present study (TaKaRa, Otsu, Shiga, Japan). For the individual exon amplifications, a robust hot start ready mix (Kapa Biosystems, MA, USA) was used to perform quick and specific amplification of *PKD1* and *PKD2* using the selected touch-down PCR conditions. In addition, to efficiently sequence the PCR products, particularly within the GC-rich exons, a 5-minute denaturing step at 98°C was included prior to the sequencing reaction to melt the complex structure of the fragments. The amplification products were purified and sequenced using an ABI 3500XL system (Applied Biosystems, Foster City, CA, USA).

PKD1 and PKD2 mutation analysis

The raw sequencing data were input into the Mutation Surveyor Software (Soft Genetics, State College, PA) for initial variation screening, followed by a careful inspection of the electropherograms for quality assurance purposes. All nonsense mutations, frameshift indels, missense mutations and splicing mutations were submitted to the ADPKD Mutation Database (<http://pkdb.mayo.edu>), the 1000 Genomes Project (<http://browser.1000genomes.org/index.html>), the Exome Sequencing Project (<http://evs.gs.washington.edu/EVS/>) and the Single Nucleotide Polymorphism database to determine if the mutations were previously reported or novel. During variant analysis, nonsense mutations, frameshift indels that produced a premature STOP codon and typical splicing mutations were considered to be definitely pathogenic. Missense mutations were computationally evaluated using a combination of the Polyphen-2, SIFT and Mutation Taster, Align Grantham Variation Grantham Deviation and mutation assessor programs [21-25]. A highly likely pathogenic mutation was predicted if all five programs assessed the variant as pathogenic. A variant would be considered to be likely pathogenic if at least three of the five programs predicted the variant to be pathogenic or if the variant segregated from affected family members. For mutation segregation analysis in single patients with no family history, a missense mutation was considered to be indeterminate if at least two programs predicted the variant to be pathogenic. If no mutations were found after sequencing the

Table 1. Long-range PCR PKD1 gene primer information

Fragments	Primer Sequence(5' to 3')	Fragments Position and Size		PMT*	GC #
PKD1 L1F	TAGCTGGGATTATAGGTGCCTG	chr16:2185086-2187158	2073 bp	60.5	66.7
PKD1 L1R	CAGACACCTCCTGGAACCTTC			60.3	
PKD1 L2-8F	TTGCTATGCTGTCCATCCTG	chr16:2166315-2170525	4211 bp	59.8	64.0
PKD1 L2-8R	TTCAGGAATAACTCACACACGC			60.2	
PKD1 L9-12F	TTCCACCGGAGTCTTCCTC	chr16:2163066-2166280	3215 bp	60.2	65.9
PKD1 L9-12R	GCAGCATGAAGCAGAGCAG			61.0	
PKD1 L13-15F	TTCTGCTCTGCTTCATGCTG	chr16:2159866-2163086	3221 bp	60.4	67.2
PKD1 L13-15R	TGGTAAATGGCTCGGAGG			59.6	
PKD1 L15-21F	AGAGGCAGTTTGTGCAGCTC	chr16:2155145-2161005	5861 bp	60.7	66.0
PKD1 L15-21R	ACACAGGACAGAACGGCTG			59.9	
PKD1 L22-26F	TCCAGTCAAGTGGGCTCTC	chr16:2151325-2154769	3445 bp	58.9	63.3
PKD1 L22-26R	CAATGAAGAGGAAAGCAGCAC			60.0	
PKD1 L27-34F	TTGTGGCATGACTGAGGC	chr16:2146985-2150884	3900 bp	59.3	60.0
PKD1 L27-34R	GCTCTTTCCACAGACAACAGAG			59.1	

*Primer Melting Temperatures; # GC Contents of PCR Products

entire *PKD1* and *PKD2* genes, large deletions or duplications were identified using the multiplex ligation-dependent probe amplification (MLPA) method (<http://www.mlpa.com>). The mutations were all named using the standard nomenclature guidelines recommended by the Human Genome Variation Society (HGVS, <http://www.hgvs.org/>).

Results

Specific primer design for long-range and individual exon PCR

After inputting the targeted region sequence of *PKD1* into the BLAT program and identifying the mismatched sequence in the homologous pseudogenes, we designed seven long-range PCR primers (exon 1, exon 2-8, exon 9-12, exon 13-15, exon 15-21, exon 22-26, exon 27-34) that specifically amplified the region between exon 1 and exon 34 of *PKD1*. In addition, 56 primer pairs that amplified individual exons were also designed following the same principle applied during long-range primer design. All of the long-range and individual exon primers were evaluated using the UCSC In-Silico PCR program and were predicted to produce unique amplification products. The fragments of the long-range PCR products ranged in size from 2.1 kb to 5.8 kb, and the individual exon PCR products were approximately 500 bp each. In this study, all of the primers were designed to have a melting temperature near 60°C to ensure that all of the PCR reactions could be performed under similar conditions. For the individual exon PCR primers, an M13 sequence, tgtaaaacgacggccagt or caggaaacagctatgacc, was indexed to the forward or reverse primer, respectively. The details of the specific primers used during long-range PCR are listed in Table 1, and the individual exon PCR primers are available upon request.

Modified PCR conditions for efficient and simple long-range and individual exon PCRs

Compared with the previously reported complex PCR conditions, only two sets of PCR conditions were required to perform specific and accurate amplification of seven long-range fragments in this study. One set of conditions was specific to the amplification of exon 1 because of the extremely high GC content in this exon. The other six long-range fragments were amplified using a single PCR reaction system and one set of amplification conditions. In brief, the 50 µl total volume reaction mixture contained the following: 25 µl 2X GC buffer I, 8 µl dNTP (2.5 mM each), 0.5 µl long and accurate Taq enzyme, 1 µl forward primer and 1 µl reverse primer (20 pmol/µl each), 1 µl genomic DNA (100 ng/µl) and 13.5 µl ddH₂O. The PCR conditions are listed in Table 2. The reaction mixture contents for the long-range fragment of exon 1 were identical to the contents mentioned above, except that the 2X GC buffer I was

Table 2. PCR conditions for long-range (LR) and individual exon amplification

PKD1 Sequence	PCR Conditions
Exon 1 (L01)	94°C for 1 minute; followed by 30 cycles of 94°C for 30 seconds, 63°C for 30 seconds, 72°C for 90 seconds; with an extension step at 72°C for 7 minutes
Exon 2-8 (L02)	94°C for 1 minute; followed by 30 cycles of 94°C for 30 seconds, 60°C for 30 seconds, 72°C for 3 minutes; with an extension step at 72°C for 7 minutes
Exon 9-12 (L03)	Same as described above
Exon 13-15 (L04)	Same as described above
Exon 15-21 (L05)	Same as described above
Exon 22-26 (L06)	Same as described above
Exon 27-34 (L07)	Same as described above
Individual Exon PCR	A touch-down protocol was designed for individual exon amplification: 95°C for 3 minutes; followed by 10 cycles of 95°C for 10 seconds, 66°C for 10 seconds, with a decrease of 0.5°C per cycle and 72°C for 10 seconds; followed by 20 cycles of 95°C for 10 seconds, 61°C for 10 seconds, 72°C for 10 seconds; with a final extension step at 72°C for 7 minutes

Fig. 1. Modified conditions for simultaneous long-range PCR amplification. Using specific PCR primers, all seven long-range fragments were efficiently amplified using two sets of conditions. One set of conditions was specific to exon 1 (L01) and the other conditions were used to amplify the remaining six fragments (L02: exon 2-8; L03: exon 9-12; L04: exon 13-15; L05: exon 15-21; L06: exon 22-26; L07: exon 27-34). All long-range fragments ranged in size from 2.1 kb to 5.8 kb.

Fig. 2. Design of exon-specific primers for the efficient amplification of individual *PKD1* exons. By identifying the mismatched sequence between the homologues pseudogenes and the individual *PKD1* exon sequence, 56 primer pairs for individual exon amplification were designed. All of the individual *PKD1* exons were specifically amplified using a single touch-down PCR method. In addition, the PCR procedure is rapid, requiring only 40 minutes to complete.

replaced with 2X GC buffer II due to the high GC content of this exon. The PCR conditions for exon 1 amplification also slightly differed from those of the other fragments (Table 2). Using these specific and modified conditions, all seven long fragments were easily and efficiently amplified (Figure 1).

For individual exon amplification, 56 pairs of specific primers for *PKD1* and 16 primer pairs for *PKD2* were used in a robust enzyme mixture PCR system. The components of the 12.5 µl reaction volume included the following: 6.25 µl 2X KAPA2G hot start Ready Mix, 1 µl each forward and reverse primers, 0.75 µl (30 ng/µl) 1:100 diluted long-range products for *PKD1* exons 1 to 34 or 0.75 µl (30 ng/µl) genomic DNA to amplify the remaining *PKD1* exons and all *PKD2* exons. A touch-down PCR assay was set for all *PKD1* and *PKD2* individual exon amplifications (Table 2). All of the products were specifically amplified within 40 minutes (Figure 2).

Table 3. Details of the *PKD1* variants identified using the modified method

Patient ID	Exon	cDNA Change	Protein Change	PolyPhen-2	SIFT	Mutation Taster	Align GVGD		Score	Mutation Assessor	Final Prediction
							GV	GD			
1#	15	c.4839het_dupT	E1614*	/	/	/	/	/	/	/	DP
2	24	c.8823C>CA	Y2941*	/	/	/	/	/	/	/	DP
3#	40	c.11329het_dupA	S3777Kfs*39	/	/	/	/	/	/	/	DP
4	13	c.3101A>AT	N1034I	PRO D	D	D	0	148.91	C65	Medium	HLP
10#	37	c.10838T>C	L3613P	PRO D	D	D	0	97.78	C65	Medium	HLP
5/6	36	C.10678 G>GA	G3560R	PRO D	T	D	0	125.13	C65	Medium	LP
7#	17	c.7184A>G	N2395S	POS D	T	D	0	46.24	C45	Low	LP
8	10	c.2039A>AT	Y680F	PRO D	T	P	0	21.61	C15	Medium	I
8/9	35	c.10529C>CT	T3510M	POS D	T	P	0	81.04	C65	Low	I

Note: abbreviations; PRO D: probably damaging; POS D: possibly damaging; B: benign; T: tolerated; D: disease causing; P: polymorphism; DP: definitely pathogenic; HLP: highly likely pathogenic; LP: likely pathogenic; I: indeterminate; #: Novel identified variants

Fig. 3. Two novel frameshift mutations identified in ADPKD patients. Using the modified LRNS method, two novel definitely pathogenic frameshift mutations, c.4839het_dupT and c.11329het_dupA, were identified in our small patient cohort.

After Exo-Sap purification, 0.8 μ l of the purified PCR products, 1 μ l M13 primer (5 pmol/ μ l) and 4.2 μ l pure water were combined to perform a 5-minute heat-denaturing step at 98°C prior to the sequencing reaction; this denaturing step facilitated the sequencing of exon 1 and that of the other high GC exons (data not shown).

Mutation evaluation

In this study, a total of 10 unrelated probands whom have a documented ADPKD were recruited from the Third Affiliated Hospital of Guangzhou Medical University. Although the patient sample size is markedly small, the modified LRNS method was demonstrated to be efficient and specific for ADPKD molecular diagnosis. In 3 unrelated patients who reported an ADPKD disease family history, three truncating pathogenic mutations, including two novel frameshift mutations (c.4839het_dupT and c.11329het_dupA) and one previously reported nonsense mutation (c.8823C>CA), were discovered (Table 3, Figure 3). These mutations segregated with the affected family members and were not observed in healthy family members. In the remaining 7 unrelated patients, 6 possible pathogenic missense variants were discovered, including one variant that was observed in 2 unrelated patients (Table 3). Among those variants, 2 were predicted to show a high likelihood of pathogenicity (Figure 4), 2 were predicted to be likely pathogenic and 2 were indeterminate. The indeterminate variants were only found in 2 patients who reported no familial disease history, indicating that there were no family members from which the mutation could segregate. In summary, a total of 9 mutations were identified using the modified LRNS method, 7 (77.8%) were considered to be associated or likely associated with disease, and 2 indeterminate variants require additional experiments to confirm whether they are disease associated or not.

Discussion

To date, linkage analysis [26] and gene-based mutation screening [17, 27-30] are the two main methods that have been developed to carry out ADPKD disease mutation screening.

The major disadvantage of linkage analysis is that this method cannot be performed without a sufficient number of affected family members or when the proband is suspected of having a *de novo* mutation [31]. Although various gene-based screening methods are available for ADPKD molecular testing, such as the LRNS method, HRM method, and even next-generation sequencing methods, direct mutation sequencing remains the preferred choice for making a clinical diagnosis of ADPKD disease [12, 14, 16, 32-34].

The molecular diagnosis of ADPKD mutations is technically challenging because of the characteristics of the region involved: its large size, extremely high GC content and extensive allelic heterogeneity, as well as the existence of six homologous *PKD1* pseudogenes. To efficiently screen authentic *PKD1* variants, avoiding pseudogene contamination and ensuring the specific amplification of true *PKD1* fragments are critical. Although the traditional LRNS method can specifically amplify the large sizes of the true *PKD1* fragments [13, 35], the full procedure necessary to perform long-range and individual exon PCRs is complicated [17]. For example, efficiently amplifying various long-range fragments requires multiple denaturing times, multiple annealing temperatures and multiple set of cycling parameters, resulting in a complex and time-consuming procedure [17]. Meanwhile, even using 1:10⁵ dilutions of the long-range PCR fragments as templates for the nested PCR cannot completely exclude the possibility of homologous pseudogene contamination because the *PKD1* homologous genes may be amplified when non-specific primers are used to amplify the diluted long-range PCR templates. Pseudogene sequence contamination has always complicated variant analysis and data interpretation during ADPKD diagnosis. To overcome these limitations, a two-step specific primer system was designed to ensure efficient *PKD1* molecular testing in this study. The first step specifically amplifies the long-range fragments, and the next step specifically amplifies the individual exons. Our results demonstrated that the sequencing data specifically matched the actual *PKD1* gene, as none of the six *PKD1* pseudogenes were detected in any of our patient samples (data not shown). These results suggest that this two-step specific primer system was able to maximally reduce the adverse effects of pseudogene contamination, which may aid in the efficient and correct diagnosis of ADPKD disease.

Because of the high GC content and complicated structure of the *PKD1* gene, it is difficult to efficiently amplify all of the fragments within *PKD1* under standard PCR conditions. Consequently, obtaining optimal mutation testing results requires various PCR reactions with respect to the conditions or inclusion of additional additives [14, 17]. Although the products amplified by our primers all had GC contents that exceeded 60% and the GC content of exon 1 was 81%, the long-range and individual exon amplifications were efficiently achieved using relatively simple conditions (compared to previous methods). For example, GC-rich

Fig. 4. Identification of a highly likely pathogenic variant in an ADPKD patient. (A): A heterozygous missense variant (c.10838 T>C, p.L3613P) was identified in a patient with ADPKD. The upper column represents the reference sequence; the lower column is the sequence data from the patient. (B) Multiple sequence alignments for the *PKD1* change p.L3613P reveal a highly conserved sequence at chromosome16:2143723, which is where the substitution mutation is found (red arrow). (C) PolyPhen-2, an online bioinformatics tool, predicted that the p.L3613P mutation is a most likely damaging variant with a score of 1.00.

fragments can be efficiently and quickly (the entire procedure to perform individual exon PCR requires 40 minutes) amplified using a robust enzyme mix with a single touch-down PCR method. In addition, we recommended a 98°C denaturing step prior to the sequencing reaction in our modified LRNS method. A denaturing step has been demonstrated to be effective for most difficult templates; heat denaturing alone has been shown to be sufficient for sequencing the majority of difficult regions when the GC content is below 70% [36]. Our modified system did not require any additives in the PCR reactions; all of the products, even the 81% GC-rich exon 1, were able to be efficiently sequenced by adding a denaturing step, suggesting that a denaturing step may be beneficial for sequencing highly structured genes.

Due to the small sample size, we did not discover any mutations in *PKD2*, splice variants or large duplication/deletions in this study. According to the screening data generated from the 10 patients using our modified LRNS method, all 10 patients presented disease-associated or likely disease-associated variants, demonstrating the high sensitivity and specificity of our modified method. Among the 10 patients, 6 reported familial ADPKD disease histories and all presented segregated pathogenic variants, resulting in a pathogenic variant detection level of 100% among the patients with a positive family history. Two indeterminate missense (but not clearly pathogenic) variants were identified in two sporadic patients. Previous studies have also reported a low level of definitely pathogenic variant detection in patients who did not report a familial ADPKD disease history, suggesting that pathogenic variants are more frequently found in patients with a family history of ADPKD than in those without a family history [10, 14]. Due to the lack of family members available for variant segregation and the technical difficulty of functional analysis, it is difficult to predict whether the missense variants are pathogenic or non-pathogenic, and missense variants are generally evaluated using several bioinformatics programs [10]. In this study, we used five bioinformatics tools to evaluate the missense variants and filter out the numerous benign non-synonymous missense variants. Making functional predictions of indeterminate variants is controversial using single-patient cases and requires a large sample cohort for further clarification.

Although only 10 patient samples were analyzed using our modified LRNS method, two novel truncated frameshift indels (c.4839het_dupT and c.11329het_dupA) and one previously reported nonsense mutation were discovered within this small cohort. Among the five definitely and highly likely pathogenic variants, 60% (3/5) truncation mutations and 40% (2/5) frameshift mutations found in association with ADPKD in this study are consistent with the findings of previous reports [14, 27, 37], which further demonstrates that the modified LRNS method can be used to efficiently and specifically make an ADPKD diagnosis.

Conclusions

In summary, we used a modified LRNS method for the efficient, sensitive and specific molecular diagnosis of ADPKD disease. Using exon-specific primers and modified PCR conditions, the pathogenic variant detection level exceeded 77.8%, and four novel mutations were identified in our limited patient group. We demonstrated that the modified method offered an improvement over traditional methods, which will enable efficient, specific and simple screening of the entire *PKD1* gene.

Disclosure Statement

The authors declare that there are no conflicts of interest.

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