

Original Paper

A New PKD1 Mutation Discovered in a Chinese Family with Autosomal Polycystic Kidney Disease

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Key Words

Autosomal polycystic kidney disease • PKD gene • Frame-shift mutation • Gene screening

Abstract

Background/Aims: Autosomal-dominant polycystic kidney disease (ADPKD), a heterogeneous genetic disorder characterized by massive kidney enlargement and progressive chronic kidney disease, is due to abnormal proliferation of renal tubular epithelium. ADPKD is known to be caused by mutations in PKD1 and PKD2 genes. **Methods:** In the present study, the mutation analysis of PKD genes was performed in a new Chinese family with ADPKD using Long-Range (LR) PCR sequencing and targeted next-generation sequencing (targeted DNA-HiSeq). **Results:** A unique 28 bp deletion (c.12605_12632del28) in exon 46 of the PKD1 gene was identified in two affected family members by LR PCR method, but not in any unaffected relatives or unrelated controls. Higher accuracy and less missing detection presented in LR PCR method compared with targeted DNA-HiSeq. This mutation c.12605_12632del28 (p.Arg4202ProextX146) resulted in a delayed termination of amino acid code, and was highly speculated pathogenic in this ADPKD family. Moreover, this newly identified frame-shift change was compared to the PKD gene database, but no similar mutation was yet reported. **Conclusion:** A novel frame-shift mutation, c. 12605_12632del28, in the PKD1 gene was found in a Chinese ADPKD family. All evidence available suggested that it might be the mutation responsible for the disease in that family.

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Introduction

Autosomal-dominant polycystic kidney disease (ADPKD) is a heterogeneous genetic disorder characterized by massive kidney enlargement and progressive chronic kidney disease, which is due to abnormal proliferation of renal tubular epithelium. The development

of cysts is often found in the kidneys, but also present in other organs like liver, seminal vesicles, pancreas, and arachnoids membrane [1]. Approximately 50% of individuals with ADPKD have end-stage renal disease (ESRD) by the age of 60 years, which account for ~5% of ESRD in North America [2-3]. ADPKD is the most common kidney disorder with a Mendelian inheritance pattern, and the prevalence ranges from 1/400 to 1/1000 worldwide [4]. However, the research in a large cohort in China is still absent.

PKD1 and *PKD2*, identified as causal genes for ADPKD, encode cell membrane proteins polycystin-1 (PC1) and polycystin-2 (PC2), respectively. PC1 interacts with PC2 to function as a cell surface signaling receptor as well as a mechano-sensor in renal primary cilia, which is involved in renal tubular differentiation pathways [5]. In linkage-characterized European populations, mutations in *PKD1* and *PKD2* account for 85% and 15% of cases with ADPKD, respectively [6]. However, more recent population-based series also suggest a relatively higher prevalence of *PKD2* (ranging from 26 to 36%) [7]. *PKD1* patients have larger kidneys and earlier onset of ESRD compared with *PKD2* patients (mean age with ESRD: 53.4 vs. 72.7 years) after adjustment of age and gender [8-11].

PKD1 is a large gene, containing 46 exons and an open reading frame of approximately 13 kb, which encodes a protein with 4,302 amino acids. Particularly, the entire 5' region of *PKD1* (up to exon 33) has been duplicated six times on chromosome 16 [2-3]. In contrast, *PKD2* is a single-copy gene containing 15 exons, encoding a protein with 968 amino acids [2-3]. So far, there are more than 500 and 200 mutations reported in the *PKD1* and *PKD2* genes, respectively, indicating a high level of allelic heterogeneity (Human Gene Mutation Database, HGMD professional release, Cardiff; Polycystic Kidney Disease Mutation Database, PKDB, <http://pkdb.mayo.edu>). Most of these sequence variations result in truncation of the proteins and are unique to a single family [12].

Most recently, it has been reported that effective pharmacologic treatments for ADPKD patients may delay the onset of ESRD in PKD [13]. Therefore, it is important to make early and precise diagnosis of patients with ADPKD. However, clinical diagnostic results of ADPKD by traditional methods were often ambiguous, particularly for patients with an unclear renal phenotype, which makes the need for accurate genetic tests more compelling. Genetic testing of *PKD1* is complicated because of the large transcript size and the complexity of the gene region. In this study, we used a novel mutation screening assay for *PKD1* in a Chinese ADPKD family by directly sequencing long-range (LR) PCR products that cover the entire *PKD1* gene. In the meantime, targeted next-generation sequencing (targeted DNA-HiSeq) was also used for comparison.

Materials and Methods

Ethics statement

This study was approved by the institution review board of Union Hospital, Tongji Medical College, Huazhong University of Science & Technology. We initiated this research in accordance with the defined protocols. The design and performance of current study involving human subjects were clearly described. Written informed consent was obtained from all participants.

Subjects

The proband, a 50-year-old man, presented at nephrology outpatient clinic because of resistant hypertension, chronic flank pain and frequent macroscopic hematuria (Fig. 1). Physical examination found two abdominal masses at palpation of lateral quadrants. Serum creatinine level was 8.75 mg/dL with an estimated glomerular filtration rate (GFR) of 10.2 mL/min/1.73 m². Serum hemoglobin was 89 g/L. Renal ultrasound examination showed bilateral enlarged kidneys with multiple cysts of variable sizes. Cysts

Table 1. PKD1 Primers for LR PCR

Fragment	Primer sequence (5'–3')	Temp. (°C)	Size (bp)
Exon 1	CGCAGCCTTACCATCCACCT	64	2278
Exon 2-7	CCCCGAGTAGCTGGAACCTACAGTTACACACT	70	4041
Exon 8-12	ACGTCTGCGAGCTGCAGCCC	70	3893
Exon 13-15	TGGAGGGAGGGACGCCAATC	65	4391
Exon 15-21	ATCCCTGGGGGTCTTACCATCTCTTA	68	5253
Exon 22-26	ATGCTTAGTGAGGAGGCTGTGGGGGTCCA	70	3276
Exon 27-34	CGGGTCACCGGTTGTGGCA	70	3916
Exon 35-41	CAAGAGGCTCAAGAACTGCCCCG	68	2632
Exon 42-46	GAGTAGTTCTCCAGGAGTGCCG	63	2370

were also found in both livers. The diagnosis of ADPKD was established and a genetic conformation was recommended. Moreover, the hereditary pedigree was further investigated.

Mutation analysis

Genomic DNA was extracted from peripheral blood lymphocytes using standard DNA extraction methods. Due to the complexity of the PKD1 gene, we used a novel LR PCR plus directly sequencing assay in PKD1 mutation screening in this study, which was confirmed to be more sensitive, specific and accurate compared with other analysis system such as SURVEYOR-WAVE method. The LR PCR was performed as previously described with minor modification [14]. Briefly, the entire coding region (5' and 3' untranslated regions, and exon-intron boundaries of PKD1) were amplified in nine distinct LR PCRs (Gene1, Gene2-7, Gene8-12, Gene13-15, Gene15-21, Gene22-26, Gene27-34, Gene35-41, and Gene42-46), using PCR primers (Table 1) anchored in either the rare mismatched region with the HGs or the single-copy region of *PKD1*. The details for amplification, purification and sequence analysis of LR PCR products were referenced as described by Tan et al [14].

Meanwhile, targeted DNA-HiSeq was also performed. A custom capture array (NimbleGen, Roche) was designed to capture all exons (3093), splice sites and the immediately adjacent intron sequences of 222 genes involved in more than 100 different genetic diseases, including ADPKD [15]. Target DNA capture, enrichment and elution were performed as previously described [15]. The potential disease-causing mutations identified were further confirmed by PCR and sequence analyses in the proband and his family (Fig. 1). In addition, for *PKD2*, all 15 exons including adjacent 30–50 bp intronic sequence were directly amplified by PCR. Primers flanking 15 exons of *PKD2* gene were designed from genomic sequence (GenBank Acc. No. NT_016354) using the program PRIMER3 (http://zeon.well.ox.ac.uk/git-bin/primer3_www.cgi). The details for direct sequencing and data analysis performed were described previously [16].

Results

Pedigree investigation

The family history was further investigated after the proband was diagnosed with ADPKD. His father died 10 years ago because of uremia. Unfortunately, no special examinations were performed and the actual etiology of renal failure was unclear. The proband has no children, but he has a brother two years older than him. The proband's brother was suggested to do relevant examination, and the results showed that he had the same disease and his blood creatinine had increased to 2.26 mg/dL without obvious symptoms and signs. The proband's brother has a 20-year-old son and he refused to do the examinations relevant to ADPKD. The proband's mother is healthy to date. So we speculated that these two brothers' disease was inherited from their father, and the pedigree drawing was showed in the Fig. 1.

Identification of the pathogenic PKD1 and PKD2 mutations

The Blood samples were collected from the proband, his elder brother, his mother and healthy controls. Total genomic DNA from whole blood was extracted according to the manufacturer's standard procedure using the QIAamp DNA Blood Midi Kit (Qiagen, Hilden, Germany).

Using targeted DNA-HiSeq, no large deletion or duplication was found in either *PKD1* or *PKD2* gene. Only one missense change (p. Lys4151Thr) was detected in the coding region of *PKD1*, which was a known polymorphism in the *PKD* gene mutation database. No sequence variants including frame-shift, nonsense, missense, or shear variants were detected in *PKD2* gene. The quality report chart was showed in the Fig. 2, the exon sequencing coverage reached more than 99% except the first, 2, 11, 42 exon of the *PKD1* gene and the first exon of *PKD2* gene. The mean sequencing depth of every exon was quite close to the median depth, which indicates the randomness of sequencing was good.

Using LR PCR method, the missense change (p.Lys4151Thr) was also found in the coding region of *PKD1* (Fig. 3A). Moreover, a frame-shift mutation c.12605_12632del28 (p.Arg4202ProextX146) was detected in exon 46 of *PKD1* gene (Fig. 3B). This frame-shift variant induces a delayed termination of amino acid code, which is probably pathogenic, although no related reports of this mutation have been found before. The result indicates that LR PCR method is more accurate and comprehensive in *PKD1* gene screening compared with targeted DNA-HiSeq. However, LR PCR method is expensive, complex, and highly labor intensive, which hinders the usage of LR PCR in routine clinical practices.

In addition, the same frame-shift mutation (c. 12605_12632del28) was found in the DNA sample of the proband's brother, and no gene mutations were found in the blood sample of their mother and healthy controls (Fig. 3B). Thus, this frame-shift mutation was highly speculated as the pathogenic mutation in this ADPKD family.

Discussion

ADPKD is an important inherited nephropathy with high prevalence. The clinical significance of genetic testing in ADPKD is well established. However, *PKD1* lies in a complex region that is duplicated six times on chromosome 16. The presence of these highly homologous pseudogenes has made genetic analysis of *PKD1* challenging, thereby discrimination of these pseudogenes from *PKD1* requires locus-specific amplification methods. Although some researchers performed denaturing high-performance liquid chromatography (DHPLC) and polymerase chain reaction-single-strand conformation polymorphism (PCR-SSCP) to screen mutations in ADPKD for research and clinical purposes [17-18], direct sequencing is considered to be a better choice [18]. Recently, Tan *et al* proposed a novel LR PCR Sequencing Method [14]. By using this tool, the entire *PKD1* coding region was amplified by nine reactions, which generated product sizes from 2 to 6 kb and circumvented the needs for specific PCR amplification of individual exons. In the present

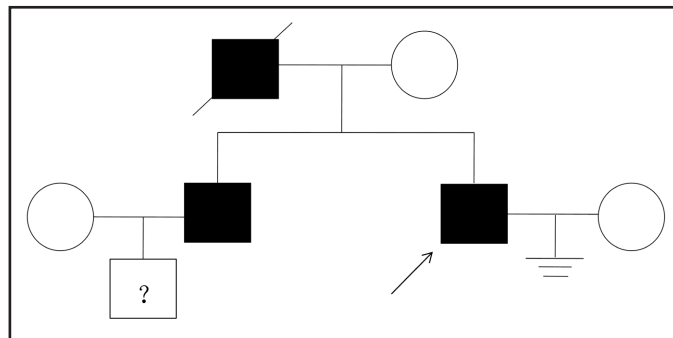


Fig. 1. Family pedigree. The arrow indicates the proband. Question mark means the disease status is unclear without the evidence of medical examination.

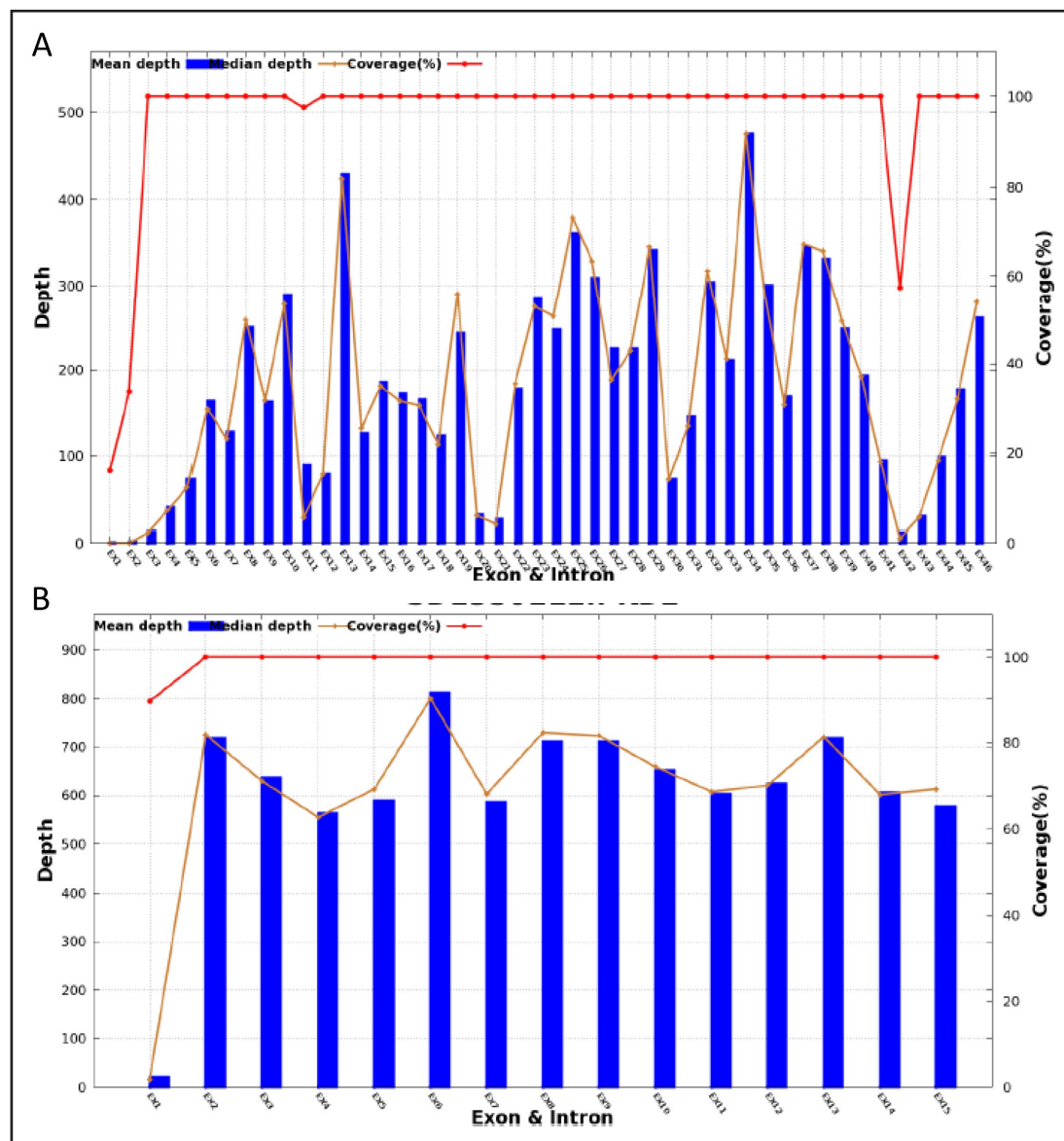
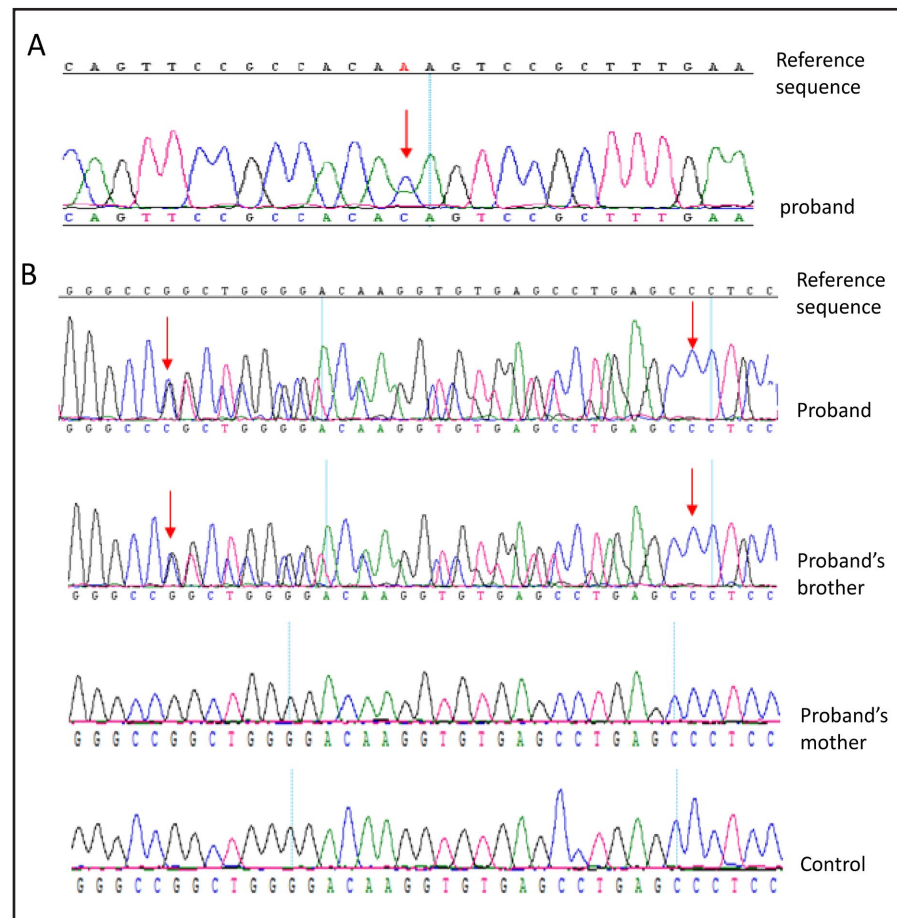


Fig. 2. Variation identified by targeted DNA-HiSeq method. Panel A: *PKD1* gene. Panel B: *PKD2* gene. The quality report chart shows the sequencing coverage reached more than 99% except the first, 2, 11, 42 exon of the *PKD1* gene and the first exon of *PKD2* gene. The mean sequencing depth of every exon was quite close to the median depth.

study, we use both LR PCR and targeted DNA-HiSeq to locus specific mutations of *PKD1* gene, and higher accuracy and less missing detection presented in the LR PCR method.

To date, over 600 of different mutations have been reported in *PKD1* and *PKD2* gene, however, their pathogenic consequences remains unclear, which has created a major difficulty or uncertainty for clinical use. Therefore, for clinical practice, it is very important to know whether an observed mutation is pathogenic or not. To this end, all the identified variations need to be carefully studied, especially when no other family member is available for segregation analysis. In our study, the mutation analysis in this ADPKD family identified a

Fig. 3. Variation identified of the proband by LRPCR method. Panel A shows a missense change (p. Lys-4151Thr) in the coding region of PKD1. Panel B shows a frame-shift mutation c. 12605-12632del28 (p. Arg4202ProextX146) in exon 46 of PKD1.



previously undescribed mutation in *PKD1* gene. This mutation results in a frame-shift variant c.12605_12632del28 (p.Arg4202ProextX146) in exon 46, leading to a delayed termination of amino acid code. In general, protein-truncating (because of frame-shift deletion/insertion, nonsense changes, or splice defects) mutations are considered to be pathogenic. This deletion is predicted to produce a mutant polycystin protein with a frame-shift of its C terminal and 146 amino acids longer than the wild-type protein due to a delayed termination codon. Therefore, it does not produce a new protein, but only the C-terminal of the PKD1 is changed. This mutation has never been reported before, and no similar frame-shift *PKD1* gene mutations were published. Several published studies have demonstrated that large genomic deletions in the *PKD1* gene are relatively rare with a frequency of ~4% [19-20]. Further *in vitro* and *in vivo* functional studies are needed to confirm the pathogenicity of Arg4202ProextX146. So far, multiple mutations in *PKD1* and *PKD2* genes have been identified, however, the genotype-phenotype relationship of these mutations with ADPKD remains largely unknown. Recent studies reported that renal survival associated with *PKD2* mutations was approximately 20 years longer than that associated with *PKD1* mutations. The median age at onset of ESRD was 58 years for *PKD1* mutation carriers and 79 years for *PKD2* mutation carriers [21]. Moreover, Dedoussis et al. found that PKD patients with both *PKD1* mutations and *PKD2* mutations might have a more severe phenotype than those with only *PKD1* mutations [22]. Our finding of the new mutation in *PKD1* will contribute to understand the genotype-phenotype correlation in future studies.

Conclusions

Our study has found a novel unique 28 bp deletion, c. 12605_12632del28, in the *PKD1* gene in a Chinese ADPKD family. All evidence available suggest that it might be the mutation causing the disease in this family. For patients with ADPKD, direct gene diagnosis and prenatal diagnosis in clinical practice would be helpful.

Disclosure Statement

The authors declare that there are no conflicts of interest.

Acknowledgement

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