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A de novo *PKD1* mutation in a Chinese family with autosomal dominant polycystic kidney disease

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Abstract

Background: *PKD1,* which has a relatively high mutation rate, is highly polymorphic, and the role of *PKD1* is incompletely defined. In the current study, in order to determine the molecular etiology of a family with autosomal dominant polycystic kidney disease, the pathogenicity of an frameshift mutation in the *PKD1* gene, c.9484delC, was evaluated.

Methods: The family clinical data were collected. Whole exome sequencing analysis determined the level of this mutation in the proband's *PKD1*, and Sanger sequencing and bioinformatics analysis were performed. SIFT, Polyphen2, and MutationTaster were used to evaluate the conservation of the gene and pathogenicity of the identified mutations. SWISS-MODEL was used to predict and map the protein structure of *PKD1* and mutant neonate proteins.

Results: A novel c.9484delC (p.Arg3162Alafs*154) mutation of the *PKD1* gene was identified by whole exome sequencing in the proband, which was confirmed by Sanger sequencing in his sister (II7). The same mutation was not detected in the healthy pedigree members. Random screening of 100 normal and end-stage renal disease patients did not identify the c.9484delC mutation. Bioinformatics analysis suggested that the mutation caused the 3162 nd amino acid substitution of arginine by alanine and a shift in the termination codon. As a result, the protein sequence was shortened from 4302 amino acids to 3314 amino acids, the protein structure was greatly changed, and the PLAT/LH2 domain was destroyed. Clustal analysis indicated that the altered amino acids were highly conserved in mammals.

Conclusion: A novel mutation in the *PKD1* gene has been identified in an affected Chinese family. The mutation is probably responsible for a range of clinical manifestations for which reliable prenatal diagnosis and genetic counseling may be provided.

Abbreviations: ADPKD = autosomal dominant polycystic kidney disease, PCR = polymerase chain reaction, PKD = polycystic kidney disease.

Keywords: bioinformatics, gene mutation, PKD1 gene, polycystic kidney disease, whole exome sequencing

1. Introduction

Polycystic kidney disease (PKD) can be inherited in a dominant and recessive manner. Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common monogenic diseases in humans, with an incidence of 1:1000 to 1:2500 in the general population.[1,2] At present, ADPKD affects up to 12 million individuals in the world, including 1.5 million Chinese population, and is the fourth most common cause for Renal replacement therapy worldwide.[3] ADPKD is the most common monogenic cause of kidney failure, occurring in more than one in 400 to 1000 live births and accordingly represents a major socioeconomic medical problem globally.[4]

ADPKD is most commonly caused by mutations in the genes *PKD1* (in 78% of disease pedigrees) or *PKD2* (in 15% of disease pedigrees), which encode the proteins polycystin-1 (PC1) and -2 (PC2), respectively.[5] PKD genes are highly polymorphic, with different genetic variations reported in *PKD1* and *PKD2*, and the incidence of newly discovered mutations in PKD1 is high. increasing the incidence of sporadic cases.[6] Compared with patients with *PKD1* mutations, those with *PKD2* mutations have a milder phenotype and reach

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end-stage renal disease about 20 years later.[7,8] ADPKD is characterized by progressive development and enlargement of cysts in all nephron segments, but preferentially in the collecting duct. Progressive loss of kidney function takes place over many decades and frequently leads to end-stage renal disease during or after the sixth decade of life.[4,9] Extra-renal manifestations of ADPKD are prevalent, including cysts affecting the liver, pancreas, central nervous system, and genitourinary tract.[10–12] In addition to locus heterogeneity, large variations are observed in the disease phenotype and progression, even within families.

With no hotspot mutation information available for *PKD1*, clinical molecular diagnostic techniques are difficult.[13] To overcome these challenges, the ADPKD family has been investigated using techniques such as polymerase chain reaction (PCR)-single-strand conformation polymorphism, Sanger sequencing, and whole exome sequencing. The study identified a novel c.9484delC (p.Arg3162Alafs*154) mutation of the *PKD1* gene, defining the cause of the family patients, and further genetic counseling and kidney management guidance for the family.

2. Materials and methods

Written informed consent was obtained from the individual(s) AND/OR minor(s)' legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

2.1. Objects

The present study was approved by the institutional research board of the North Sichuan Medical College (Nanchong, China). A large Chinese family with a history of ADPKD was recruited for this study from dialysis centers and nephrology clinics of the Affiliated Hospital of North Sichuan Medical College in August 2019, and all participants provided written informed consent. In our study, ADPKD in a 62-year-old male proband was initially diagnosed at the age of 59 years during evaluation of gross hematuria, when imaging incidentally found enlarged kidneys with innumerable cysts in both the kidney and liver (Fig. 1). There was self-limited gross hematuria and urolithiasis, but no history of hypertension, hernia, or pain in the flank, back, or abdomen. Blood urea nitrogen was 248.04 mg/dL, serum creatinine 27.04 mg/dL. The patient was normotensive, and the rest of her physical examination was normal.

Pedigree investigation: his sister (II7) had the same ultrasound results as the proband, and there was no obvious abnormality in renal function. Ultrasound results of his brother (II3) and his daughter (III4) indicated a cyst, but no obvious renal function abnormality was found at present. (See Fig. 2 for the prevalence of the proband's family members). On the premise of definite diagnosis, whole exome sequencing was performed on the proband, and Sanger sequencing was performed on the son, grandchildren, and sister (Fig. 3).

2.2. Methods

2.2..1. Specimen collection and genomic DNA extraction. After obtaining informed consent and signature of the proband and family members, gene diagnosis analysis was carried out. Take 2 mL of blood from the proband and other members of the family and conduct anticoagulation with EDTA. DNA extraction was performed using the DNeasy Blood and Tissue kit (ShangHai Baiao Technology Co., Ltd, Shanghai, China) according to the manufacturer's protocol. Briefly, a DNA collection spin tube was used to isolate genomic DNA from blood samples. Meanwhile, a healthy person's blood and a portion of distilled water without nucleic acid were used as controls, and DNA extraction was performed simultaneously. The quality and concentration of the DNA samples were evaluated using spectrophotometry (260/280 nm) and 1% agarose gel electrophoresis.

2.2..2. Pathogenic gene detection. Whole exome sequencing of blood samples from the proband was performed using BGI (Beijing, China). The Beijing Genomics institution (BGI was established in Beijing on September 9, 1999, with the official launch of the "1% Project of the International Human Genome Project") adopted Agilent and other liquid-phase capture systems to efficiently capture and enrich the DNA in the whole exome region of humans, and provides 2 high-throughput sequencing platforms, BGISEQ-500 and Illumina. Whole exome sequencing was used to detect PKD-related genes in proband families. In this method, DNA is fragmented to form a library, then captureding and enriching the DNA of the target gene exon and adjacent shear region by chip, and finally detecting mutations by high-throughput sequencing platform, which can detect all mutations (including point mutations and insertion deletion within 20bp) in exons and their adjacent ± 20 bp intron regions, and can indicate copy number variation at the exon level.

2.2..3. Data and bioinformatics analysis. Data and bioinformatics analysis: the adapter sequences and sequences with low base quality were removed using Trimmomatic with threshold SLIDINGWINDOW:4:15.[14] The quality of the filtered data was evaluated using Fastqc(Fastqc_v0.11.8). The filtered sequences were aligned to the reference sequence of the human genome using the BWA software (reference gene version GRCh37/hgl9). GATK software was used for point mutation identification, quality correction and point mutation filtering with threshold QD>2.0; FS>60.0; MQ>40.0;

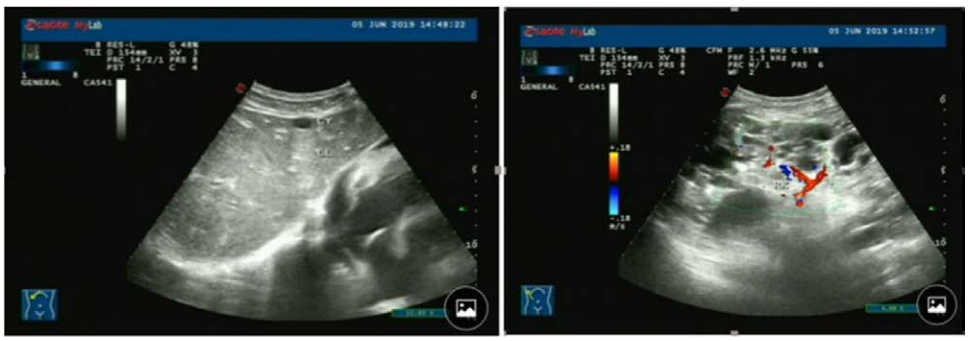

Figure 1. Ultrasound results of the proband: enlarged kidneys with innumerable cysts in both kidney and the liver.

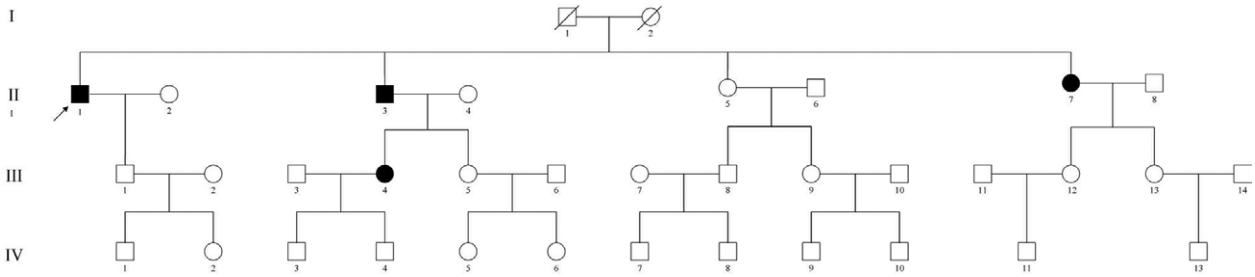

Figure 2. Pedigree of an Chinese family with ADPKD: The proband is indicated with an arrowhead. The shape described the sex (circles were identified as female and square were identified as male), the solid black shapes represent the individuals of autosomal dominant polycystic kidney disease. ADPKD = autosomal dominant polycystic kidney disease.

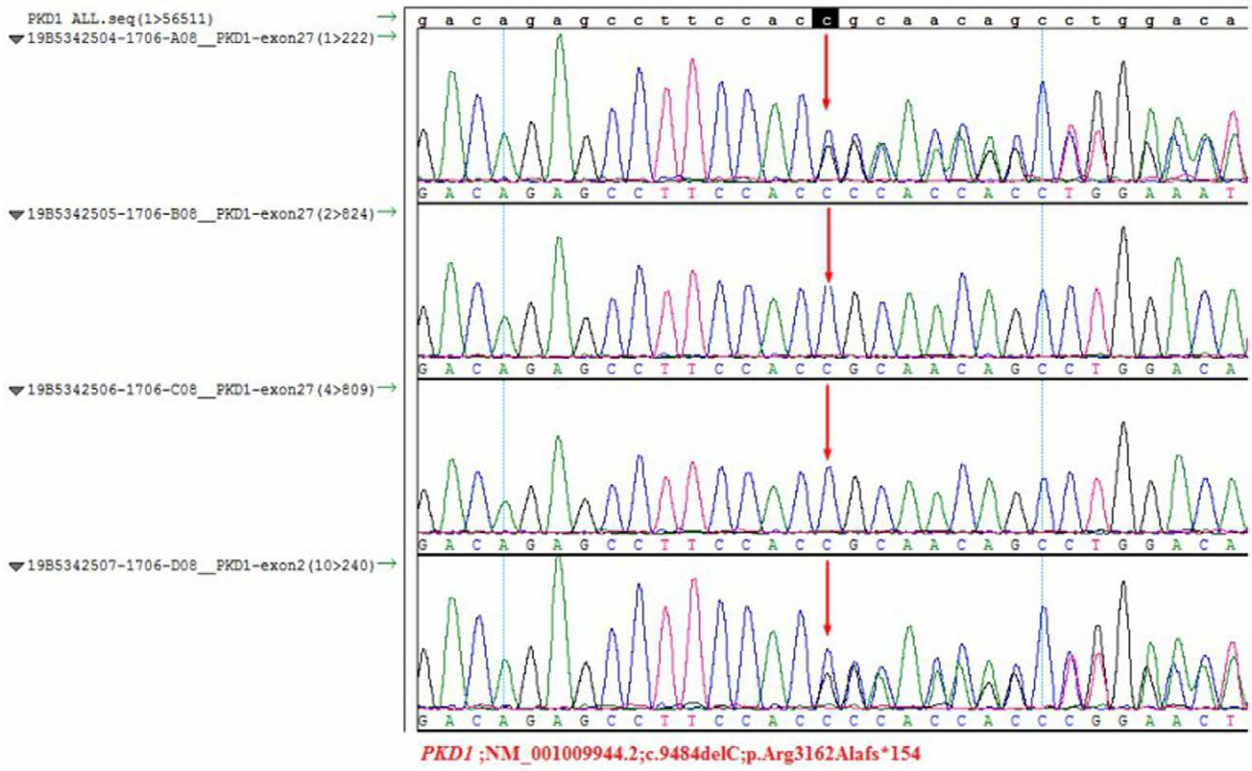

Figure 3. Sanger sequencing was performed on the proband, grandchildren and sister. The sequence is II1, III1, IV1, II7.II1, and II7 have c.9484delC.

MQRankSum>-12.5; ReadPosRankSum>-8.0; SOR>3.0 to obtain the candidate point mutation data set. Annovar was used in the 1000 Genomes Project and ExAC, and sites with a frequency of less than 0.01 or with strong site conservation and severe influence on protein structure or function were selected for the ClinVar, HGMD, and OMIM databases linked from PubMed. Annovar was used to predict the site pathogenicity and protein function of candidate site mutations with SIFT, Polyphen 2, and MutationTaster. Conservative prediction of phastConsElements46way was made by Annovar.

2.2..4. Sanger sequencing. According to the results of whole exome sequencing of the proband, the detected mutation sites were verified by Sanger sequencing, and the corresponding sites of II1, II7, III1, and IV1 were analyzed. Premier software (version 5.0) was used to design amplification primers for mutation sites, and the primer design avoided the interference of pseudogenes. PCR was performed to amplify *PKD1* c.9484delC (forward, 5′-CGC TCT TCA TGG CTT GGC AG-3′, and reverse, 5′-CTG GCC TCA TTC CTG CCT CA-3′). The primers were commissioned by Bioengineering (ShangHai) Co., LTD. to synthesize. After purification and recovery of the amplified products, all PCR products were directly sequenced using an ABI3730XL DNA sequencer. The sequencing results were simultaneously inputted into the Seqman sequence analysis software with the NCBI BLAST nucleic acid sequences for alignment analysis.

2.2..5. Co-segregation analysis. To confirm the association between mutation and disease pathogenicity, in addition to the analysis of 4 potentially affected family members in the family by direct sequencing (Sanger method as previously described), 100 clinically diagnosed patients with renal failure and 100 normal populations were used as controls to study their mutation status in c.9484delC for further analysis of this mutation site.

3. Results

3.1. Studied family suffers from ADPKD.

A family with ADPKD, including 23 individuals from 4 generations, was studied. The family's medical history was further

investigated following the diagnosis of the proband with PKD. The proband was a 62-year-old man who was diagnosed with bilateral PKD and polycystic liver disease at the age of 59 years. In the following 3 years, the proband had recurrent hematuria and a sharp decline in renal function. The patients were treated many times in the affiliated hospital of North Sichuan Medical College, and kidney stones appeared during the course of the disease and were treated with surgery. According to the pedigree (Fig. 2), 4 patients in the family were found to have multiple renal cysts on ultrasound, including 2 in women and 2 in men. The diagnosed patients in the family were siblings of the proband and the daughters of the brothers (Fig. 2); however, III1 (34-year-old) and IV1 (5-year-old) did not exhibit PKD. Collectively, the results indicated that the family analyzed suffered from ADPKD.

3.2. Novel deletion mutation of PKD1 gene

Capture and high-throughput sequencing of all exons, exons, and intron junctions of proband (II1) showed c.9484delC (p.Arg3162Alafs*154) variation in the proband (Fig. 4). The Sanger sequencing validation results were consistent (Fig. 3). The proband's sister (II7) carried the same mutation (Fig. 3), while the proband's son and grandson did not have the mutation. At the same time, the mutation was not detected in the 100 random normal population and 100 patients with end-stage renal disease. The arrows (Fig. 4) indicate the heterozygous frameshift in the PKD1 gene identified by whole exon sequencing. The reading length of 6 negative chains is listed in the figure, and about half of the sites are missing "G", its complementary strand corresponding base is "C". This chain is a normal chain, resulting in a frameshift, early termination of translation, and a new protein encoding only 3314 amino acids.

3.3. Bioinformatics analysis and mutation identification

The gene sequencing results showed that the *PKD1* exon 27 lacks a C heterozygous frameshift mutation located in the PLAT/LH2 domain. PLAT/LH2 is a polycystin-1 domain. Polycystins are a large family of membrane proteins composed of multiple domains that are present in fish, invertebrates, mammals, and humans that are widely expressed in various cell types and whose biological functions remain poorly defined. In humans, mutations in *PKD1* and *PKD2* have been shown to be a cause ADPKD. The generally proposed function of PLAT/LH2 domains is to mediate interactions with lipids or membrane-bound proteins. Because of the frameshift mutation, the protein sequence was shortened from 4302 amino acids to 3314 amino acids, the protein structure changed greatly, and the PLAT/LH2 domain was destroyed.

Searching PubMed, HGMD, and other databases, no related reports about the pathogenicity judgment of c.9484delC:p.R3162fs was found. The mutation frequency and protein function of c.9484delc: p.r3162fs were predicted. The frameshift mutation was not found in 1000 Genomes Projects and ExAC, which suggests a frequency far lower than 0.01. The prediction analysis of SIFT, Polyphen 2, and MutationTaster indicated that c.9484delC might affect the structure and function of the protein. Phast Cons Elements 46 made conservative predictions with a score of 625, indicating that this site was highly conservative. Meanwhile, BLAST function in NCBI was used to carry out a conservative comparison of the amino acid level of c.9484delC in different species, and the results showed that the mutation site was highly conserved in different species (Fig. 5). Furthermore, according to ACMG-AMP guidelines for interpretation of variants (https://doi.org/10.1038/gim.2015.30), the evidence may be classified as moderate or supporting evidence: PVS1 (null variants), PM2 (absent from controls), PP1 (cosegregation), and PP3 (computational evidence conservation); therefore, the novel frameshift mutation may be classified as pathogenic.

In conclusion, due to the frameshift mutation, the protein sequence was shortened from 4302 amino acids to 3314 amino acids, and the protein structure and function were greatly altered. The PKDl result of the species conservation alignment of the mutant amino acid sequence, the amino acid sequence conservation analysis showed that the amino acid sequence in this region was highly conserved, and the mutation site region indicated by the arrow was arginine (R) in different species.

Figure 4. Analysis of DNA sequencing results: the arrows indicate the heterozygous frameshift mutation was found by sequencing c.9484delC, the reading length of 6 negative chains is listed in the figure, and about half of the sites are missing "G", its complementary strand corresponding base is "C". This chain is a normal chain, resulting in frameshift, early termination of translation, and the new protein encoding only 3314 amino acids.

NewProtein	3121	EILVKTGWGRGSGTTAHVGIMLYGVDSRSGHRHLDGDRAFHATAWTSSGSPPRTAWVACG	3180	Homo sapiens
NP_000287.4	3121	EILVKTGWGRGSGTTAHVGIMLYGVDSRSGHRHLDGDRAFHRNSLDIFRIATPHSLGSVW	3180	Homo sapiens
NP_001009944.3	3121	EILVKTGWGRGSGTTAHVGIMLYGVDSRSGHRHLDGDRAFHRNSLDIFRIATPHSLGSVW	3180	Homo sapiens
XP_011520830.1	3139	EILVKTGWGRGSGTTAHVGIMLYGVDSRSGHRHLDGDRAFHRNSLDIFRIATPHSLGSVW	3198	Homo sapiens
XP_024306066.1	3161	EILVKTGWGRGSGTTAHVGIMLYGVDSRSGHRHLDGDRAFHRNSLDIFRIATPHSLGSVW	3220	Homo sapiens
XP_024306068.1	3091	EILVKTGWGRGSGTTAHVGIMLYGVDSRSGHRHLDGDRAFHRNSLDIFRIATPHSLGSVW	3150	Homo sapiens
XP_024306067.1	3137	EILVKTGWGRGSGTTAHVGIMLYGVDSRSGHRHLDGDRAFHRNSLDIFRIATPHSLGSVW	3196	Homo sapiens
XP_024089797.1	2822	EILVKTGWGRGSGTTAHVGIMLYGVDSRSGHRHLDGDRAFHRNSLDIFRIATPHSLGSVW	2881	Pongo abeli
XP_030654794.1	3120	EILVKTGWGRGSGTTAHVGIMLYGVDSRSGHRHLDGDRAFHRNSLDIFRIATPHSLGSVW	3179	Nomascus leucogenys
XP_024205850.1	3119	EILVKTGWGRGSGTTAHVGIMLYGVDSRSGHRHLDGDRAFHRNSLDIFRIATPHSLGSVW	3178	Pan troglodytes
XP_032000766.1	3098	EILVKTGWGRGSGTTAHVGIMLYGVDSRSGHRHLDGDRAFHRNSLDIFRIATPHSLGSVW	3157	Hylobates moloch
XP_007980387.1	3120	EILVKTGWGRGSGTTAHVGIMLYGVDSRSGHRHLDGDRAFHRNSLDIFQIATPHSLGSVW	3179	Chlorocebus sabaeus
XP_012997058.1	3113	EILVKTGWGRGSGTTAHVGIMLYGADSRSGHRHLDGDRAFHRNSLDIFRIATPHSLGNVW	3172	Cavia porcellus

Figure 5. Conservative analysis of proteins: PKDl result of the species conservation alignment of the mutant amino acid sequence, the amino acid sequence conservation analysis showed that the amino acid sequence in this region was highly conserved, and the mutation site region indicated by the arrow was arginine (R) in different species.

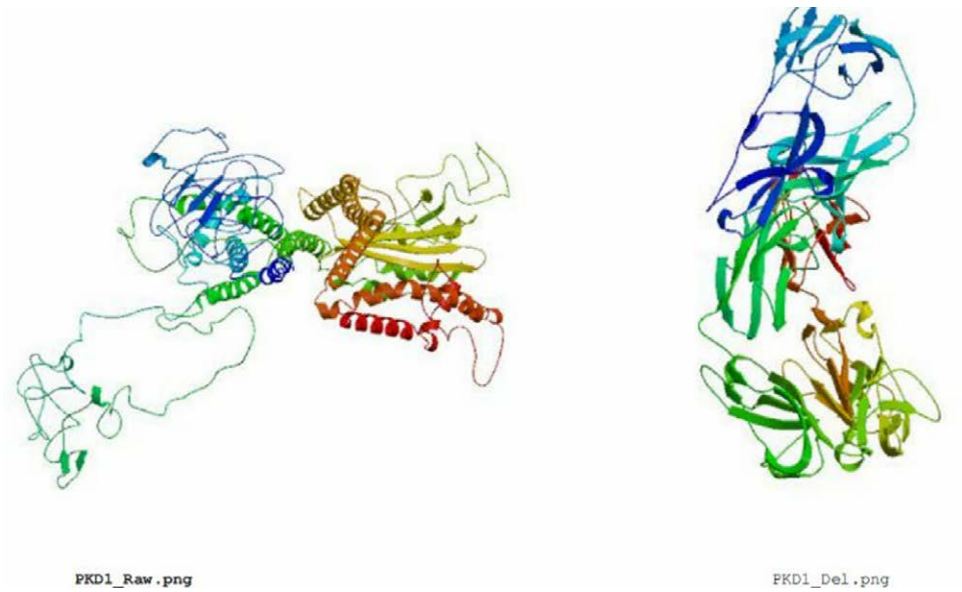

Figure 6. Protein structure diagram: the right picture shows the normal *PKD1* encoded protein. The left picture is the new protein of *PKD1*c.9484delC. The frameshift mutation of c. 9484delC shorted the protein sequence from 4302 amino acids to 3314 amino acids, greatly changing the protein structure.

3.4. Protein structure analysis

The *PKD1* and novel proteins of frameshift mutations were predicted, and the protein structure was drawn using SWISS-MODEL (https://swissmodel.expasy.org/interactive) (Fig. 6). The polycystic protein *PKD1* contains A chain (275–354) and B chain (3049–4169), however, the mutated protein contains Down's Syndrome Cells Adhesion Molecule isoform 1.30.30, N-terminal 8 Ig domains. The right picture shows the normal

PKD1 encoded protein, and the left picture shows the new protein *PKD1*c.9484delC. The frameshift mutation of c. 9484delC shorted the protein sequence from 4302 amino acids to 3314 amino acids, greatly changing the protein structure.

4. Discussion

ADPKD is characterized by multiple renal cysts with progressive enlargement of the bilateral kidneys, with high heterogeneity and delayed.[15] A majority of ADPKD patients have a family history, but about 10% of the patients are due to new mutations in the gene.[16] The current study suggests that ADPKD is mainly caused by mutations in the *PKD1* and *PKD2* genes. However, recent studies have found that mutations in GANAB and DNAJB11 exist in a small group of patients,[17] and the mutation phenotype is not exactly the same as that caused by *PKD1* or *PKD2*.

ADPKD, with genetic heterogeneity, is caused by a mutation in *PKD1* on chromosome 16p13.3 in 85% of patients. *PKD1* is located in the 16p13.3 region, with a length of approximately 52 KB, containing 46 exons and encoding polycystic protein (polycystin-1, PC1). According to the current research, the *PKD1* gene is highly heterogeneous. The 32th to 46th exon region at the 3'-terminal is a single copy region, while the 1st to 32th exon region at the 5'-terminal is replicated 6 times on the 16th chromosome, forming 6 pseudogenes, and the sequence similarity between pseudogenes and true genes is as high as 97.7%,[17] It cannot be excluded that there are new pseudogene sequences in the *PKD1* gene in the Chinese population due to differences in ethnicity and individuals; therefore, the molecular diagnosis of ADPKD is extremely difficult because of the complexity of the *PKD1* gene and the high heterogeneity of *PKD1* and *PKD2* genes. According to domestic and foreign reports, PKDl, with a wide range of gene mutations, does not have mutation hotspots.

In this study, we found that exon 27 of the *PKD1* gene in the proband had a heterozygous shift mutation of c.9484delC via detection of pathogenic genes for 1 ADPKD family, which caused the early termination of the protein sequence due to the shift of the new protein sequence from 3162 (p.Arg3162Alafs*154). We did not find any reports about the pathogenicity of the mutation by searching many databases, such as PubMed and HGMD, on this account, we consider it a new mutation. Furthermore, Sanger sequencing verified that the mutation exists in this family, while healthy family members and 100 healthy controls and patients with end-stage kidney disease had no mutations. Hence, the possibility of polymorphism was preliminarily ruled out. Meanwhile, The BLAST function in NCBI was used to compare the conservation of c.9484delC at the amino acid level in different species. The results showed that the region of the mutation site was highly conserved among different species, which suggested that the mutation of the 27th exon c.9484delC may be pathological and is closely related to the clinical manifestations of ADPKD in this family. A heterozygous frameshift mutation of C missing from the 27th exon of the *PKD1* gene in this family is located in the PLAT/LH2 domain.

It is generally thought that the function of the PLAT/LH2 domain mediates its interaction with lipid or membrane-binding proteins. The frameshift mutation of c. 9484delC shorted the protein sequence from 4302 amino acids to 3314 amino acids, greatly changing the protein structure, and destroying the PLAT/LH2 domain. Through the SWISS - MODEL (https://swissmodel.expasy.org/interactive) predict new protein of mutant gene and the protein structured of PKD (Fig. 6). The polycystic protein *PKD1* contains A chain (275–354) and B chain (3049–4169), however, the mutated protein contains Down's Syndrome Cells Adhesion Molecule isoform 1.30.30, N-terminal 8 Ig domains. Therefore, this frameshift mutation may affect the function of the PLAT/LH2 domain, resulting in a change in the efficiency of *PKD1* gene encoding protein binding to form a complex, which affects the subsequent signal transduction pathway, causing a series of changes in cellular biological behavior, leading to the occurrence of PKD. Combined with the results of segregation analysis. and bioinformatics analysis suggested that this mutation may be a new pathogenic mutation.

In the follow-up of this family, we found that in addition to the members included in testing, the proband's elder brother, the daughter of his elder brother, and the daughter of his sister showed multiple cysts on color ultrasound, but there were no clinical symptoms. However, other members were reluctant to accept genetic testing because of the psychological effects of genetic testing. Although we found that the early diagnosis of ADPKD has the benefit of preventing complications and protecting kidney function of patients by the follow-up of many polycystic kidney families, the adverse consequences (such as occupational, educational, emotional, and insurable problems) caused by acquiring positive diagnosis before symptoms far outweigh the benefits. Since there is currently no effective treatment for such diseases, the decision to screen for the disease for people at risk should be made depending on the patient's preferences and trade-offs after a full understanding of the benefits and adverse consequences of diagnosis.

Author contributions

Conceptualization: Ting Wei.
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Formal analysis: Ting Wei, Yueyang Zhang.
Funding acquisition: Qingsong Liu.
Methodology: Bing Zhang.
Project administration: Ting Wei, Wei Tang.
Resources: Bing Zhang.
Supervision: Xin Li.
Validation: Xin Li.
Writing – original draft: Ting Wei, Tao Tang.
Writing – review & editing: Qingsong Liu.s

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