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REVIEW ARTICLE

A novel PKHD1 mutation identified in a family affected by ARPKD

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ABSTRACT

Objective: Autosomal recessive polycystic kidney disease (ARPKD) is a rare inherited renal cystic disease involving multiple organs. It is caused by mutations in the PKHD1 gene. Here, we investigate the gene mutations in a family affected by ARPKD. **Methods:** Genomic DNA was extracted from peripheral blood leukocytes obtained from the subjects, by means of targeted gene capture and next generation sequencing technologies for mutation screening, and were confirmed by Sanger sequencing. **Results:** Two heterozygous mutations of PKHD1, c.6890T>C (p.Ile2297Thr) and c.11215C>T (p.Arg3739Trp), located in exons 43 and 62, respectively, were identified in the patient. Furthermore, the father and mother were revealed to be carriers of heterozygous c.6890T>C (p.Ile2297Thr) and c.11215C>T (p.Arg3739Trp) mutations, respectively. Mutation of c.11215C>T (p.Arg3739Trp) has been found in the ARPKD Mutation Database (http://www.humgen.rwth-aachen.de) but mutation of c.6890T>C (p.Ile2297Thr) has not been reported. **Conclusions :** Compound heterozygous PKHD1 mutations were elucidated to be the molecular basis of ARPKD in this patient. The newly identified c.6890T > C (p.Ile2297Thr) mutation in the patient expands the mutation spectrum of the PKHD1 gene. Targeted gene capture and next generation sequencing are suitable for genetic diagnosis of single-gene inherited diseases like ARPKD, in which the pathogenic gene is large.

Keywords: *Autosomal recessive polycystic kidney disease; PKHD1 gene; next generation sequencing*

Introduction

Autosomal recessive polycystic kidney disease (ARPKD, OMIM_263200) is a serious genetic renal cystic disease with an incidence of about 1 in 20,000[1,2]. ARPKD is clinically characterized by a significant increase in kidney size, fusiform dilatation of the renal collecting duct and biliary dysgenesis. Clinical characteristics of ARPKD typically appear during neonatal and infantile period. About 30% of affected children die within the first year of life, mainly due to pulmonary hypoplasia and respiratory distress caused by compression of enlarged kidneys[3]. The survivors and a later onset patients gradually progress to end-stage renal disease before adulthood. A minority of patients are only diagnosed in adulthood, with liver-related complications and mild kidney diseases.

ARPKD is caused by mutations in the 472kb-long PKHD1 gene, which is located on 6p12[4]. The PKHD1 gene is mainly expressed in the kidney and liver, as well as the pancreas. Its longest transcript (NM_138694.3) is composed of 67 exons, encoding the multi-domain transmembrane protein fibrocystin/polyductin (FPC), which has 4074 amino acids[5,6]. FPC is a receptor-like transmembrane protein that localizes at primary cilia, and possibly functions as a sensory organelle of renal tubular epithelial cells or bile duct epithelial cells[7].

In ARPKD patients with medullary ectasia alone, cystic lesions may be confused with those associated with medullary sponge kidney. Therefore, an accurate genetic diagnosis is needed for further diagnosis. In this study, for one patient who could not be clearly diagnosed as having ARPKD or medullary sponge kidney by renal ultrasonography, we performed targeted gene “All Exons capture” against approximately 4000 known human genetic disease-related genes and carried out next generation sequencing testing for his family. We confirmed that the patient has ARPKD, and we also found new mutations of the PKHD1 gene in the patient’s family.

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Materials and Methods

Case information

The 5-year-old boy who was the subject of this study had no major complaints about discomfort. However, occasional abdominal ultrasound examination revealed that the size of his left kidney was about 9.4 × 4.4 cm, and the size of his right kidney was about 9.8 × 4.1 cm. Enhanced echogenicity masses were radially distributed along the renal medulla, but it was not clear if these were due to medullary sponge kidney, or if they could be attributed to ARPKD (Figure 1). The patient was not hypertensive. Kidney function tests showed that serum urea concentration was 6.11mmol/L and serum creatinine concentration was 37 μmol/L (in the normal range), serum calcium and phosphorus were also in the normal range, PTH was 26.17 pg/mL (reference range 15–65 pg/mL). A liver ultrasound showed no abnormalities. Both parents of the patient had no family history of genetic diseases and were in a non-consanguineous marriage. Liver and kidney ultrasound examination of both parents showed no abnormalities. The patient had no siblings, and his mother had no history of abnormal pregnancies.

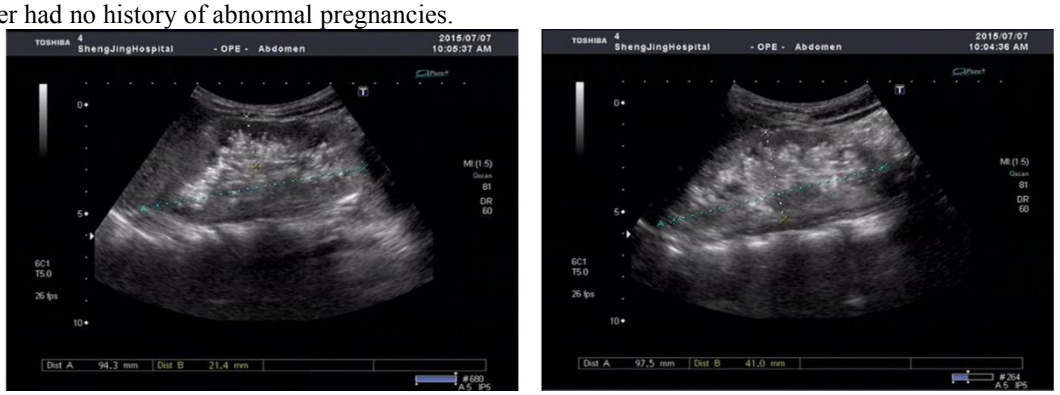

Figure 1. Renal ultrasound results of the patient show enlarged bilateral kidneys and visibly enhanced echogenicity masses radially distributed along the renal medulla

Sample collection and DNA extraction

A volume of 2 mL of peripheral whole blood (EDTA anticoagulant) was collected from each member of the patient's family, after obtaining signed informed consent from the patient and parents. The study was approved by the Ethics Committee of Shengjing Hospital of China Medical University (2016PS30J). Genomic DNA was extracted using the BloodGen Midi Kit (CWBIO, China) according to the manufacturer's instructions (the extraction of genomic DNA and subsequent tests were completed by Beijing Deyi Clinical Laboratory).

Next generation sequencing

With reference to the literature and the OMIM database, targeted gene All Exons capture was performed using customized Roche NimbleGen capture probes, which were specific for genomic exons of about 4000 known human genetic disease-related genes.

Preparation of DNA library

1) Genomic fragmentation: genomic DNA was broken into pieces of about 200bp using a sonicator (Cavoris). 2) Blunt-end repair: fragmented DNA was blunt-end repaired by Klenow Fragment, T4 DNA polymerase and T4PNK. 3) 3'-end polyadenylation: an adenine base was added to the 3'-end of the repaired product of the previous step by polymerase, to prepare for the subsequent ligation step. 4) Ligation: the T4DNA ligase reaction was set-up and left for a certain time at a suitable temperature in a Thermo mixer, in order to ligate the adapter and the adenine from the previous step. 5) Amplification: the ligation product was amplified using ligation-mediated (LM)-PCR for 4–6cycles. 6) Hybridization: the DNA library and probes were mixed together in the hybridization system for 60–68 hours at 65 °C. 7) Bead wash and DNA elution: Streptomycin beads were incubated with the hybridization mixture, then eluted by an elution solution. 8) Amplification of elution product: the elution product was amplified using LM-PCR for 10 cycles.

Illumina sequencing

Illumina sequencing was performed by following the instructions on Illumina hiseq 2500 platform standardized operating procedures, and original image data were acquired, Illumina official basecall analysis software "BclToFastq" was then used to obtain raw data.

Basic information analysis

Raw data were subjected to statistical analysis, ligation contamination and poor quality data were discarded. Sequences were aligned against reference sequences (alignment software BWA), and hg19 genome was used as the reference genome. “Samtools” analysis software was used for SNP detection and annotation, and “pindel” software was used for Indel detection and annotation. The detected SNP and Indel were screened according to the depth of sequencing and mutation quality in order to obtain high quality and reliable information on mutations. Based on their locations in the PKHD1 gene, effects on amino acid changes, cleavage sites, UTR and intron mutations were analyzed. Based on homologous alignments and conserved protein structure algorithms, effects of screened mutations on overall protein were predicted using “SIFT”. Cleavage hazard was also predicted for point mutations near cleavage sites.

Sanger validation

Primers were designed based on verified mutation sequences of the PKHD1 gene (Table 1); PCR was carried out to amplify the DNA using the following conditions: pre-denaturation at 95 °C for 5 min; denaturation at 95 °C for 30 sec, annealing at 60 °C for 30 sec, chain elongation at 72 °C for 30 sec, amplification for a total of 30 cycles, then elongation at 72 °C for an extra 10 min; total PCR reaction volume was 50 μL. Sequencing was performed using the ABI 3730XL sequencer, and sequencing primers were the same as the original PCR primers. Sequence analysis and comparisons were performed using the “DNASTAR” software.

Table 1. Primer sequences of PKHD1 gene exon 43 and 62

Exon	Primers	Sequences	Length of products(bp)	Tm(℃)
PKHD1-exon43	Forward	5’-AGGGACTAAATGTCTATTTTTGTGTTT-3’	720	60
	Reverse	5’-GTAAAATACACACTGGGTTAAGAAG-3’		
PKHD1-exon62	Forward	5’-CGAGTATTAAACACTAAAAGGGATGGG-3’	427	60
	Reverse	5’-CGATGTACGATGAATCGAGATTTCTG-3’		

Ethical Statement

With the approval by the Ethics Committee of Shengjing Hospital of China Medical University (2016PS30J), and the informed written consent from the parents was obtained.

Results

From the sequencing results, the patient was shown to have compound heterozygous mutations in the PKHD1 gene, namely c.6890T > C (p.Ile2297Thr) (Figure 2) and c.11215C > T (p.Arg3739Trp) (Figure 3) located in exon 43 and 62, respectively. Mutation of c.6890T > C leads to the replacement of the 2297th isoleucine by threonine, and mutation of c.11215C > T causes the replacement of the 3739th arginine by tryptophan in the protein, both resulting in dysfunctional protein structure predicted by “SIFT” software. The c.11215C > T (p.Arg3739Trp) mutation is present in the mutation database (http://www.humgen.rwth-aachen.de), while the c.6890T > C (p.Ile2297Thr) mutation has not yet been reported and can therefore be regarded as a newly discovered pathogenic mutation. The father was the carrier of c.6890T > C (p.Ile2297Thr) (Figure 2), and the mother was the carrier of c.11215C > T (p.Arg3739Trp) (Figure 3). Both parents of the patient were shown to have a heterozygous point mutation on the PKHD1 gene, but both had a normal phenotype. However, the patient himself was shown to have compound heterozygous mutations with an abnormal phenotype, which is consistent with the ARPKD autosomal recessive inheritance law.

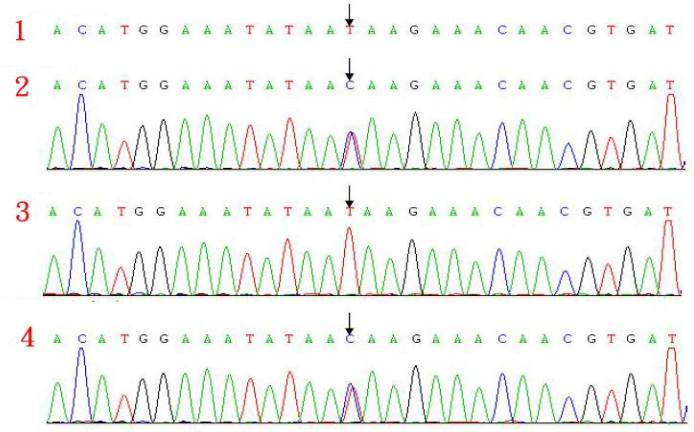

Figure 2. PKHD1 gene exon 43 sequencing results (1. NCBI reference sequence；2. Sequence of the patient；3. Sequence of the patient’s mother；4. Sequence of the patient’s father)

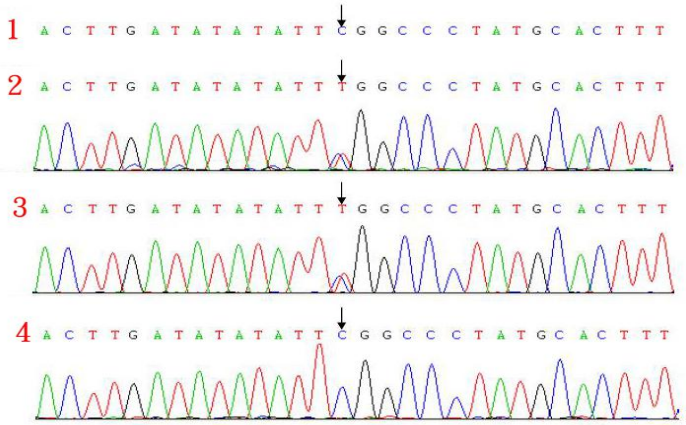

Figure 3. PKHD1 gene exon 62 sequencing results (1. NCBI reference sequence; 2.Sequence of the patient; 3. Sequence of the patient's mother; 4.Sequence of the patient's father)

Discussion

ARPKD-causing mutations have been identified along the entire length of the PKHD1 gene, and multiple mutation types are pathogenic. Almost 750 pathogenic mutations have been catalogued in the ARPKD mutation database (same URL as above), of which half are missense mutations. The most common mutation is a missense mutation in exon 3, c.107C > T (p.Thr36Met), which accounts for about 20% of all mutant alleles[8]. In general, the PKHD1 gene does not have mutational hotspots, and mutations are specific for a single family[9], with the majority of patients having compound heterozygous mutations. The patient in this study also has compound heterozygous mutations in the PKHD1gene.

Based on genotype-phenotype studies, it has been found that the type of mutations, rather than the site of mutations, affects the phenotype. In a study of fetuses and neonates with ARPKD, it was found that, after adjusting for gestational age, the extent of dilatation of the collecting duct, but not portal fibrosis, significantly correlated with the presence of two truncating mutations (severe genotype)[10]. In general, patients with two truncating mutations often have a severe phenotype and die in perinatal stage[9]. However, there are exceptions, for example, it has been reported that a child homozygous for a large PKHD1 deletion was able to survive well past the neonatal period[11].In addition, patients with the same genotype may also have significantly different phenotypes, suggesting that genetic modifiers modulate disease expression[8]. Thus, the two mutations found in the patient in this study are both missense mutations, which is likely to be the molecular mechanism of the later onset and milder phenotype.

The multi-domain transmembrane protein fibrocystin/polyductin (FPC) is the protein product encoded by the PKHD1 gene. FPC is a single transmembrane-spanning protein with a long extracellular N-terminus and short cytoplasmic tail[12]. FPC undergoes notch-like proteolytic processes, resulting in the shedding of the extracellular domain into the tubular lumen; meanwhile the C-terminal domain translocates into the nucleus for transcription regulation[13]. During embryonic development, the PKHD1 gene is widely expressed in neural tubes, bronchi, primordial guts and early ureteric buds, mesonephric tubules, adrenal cortex and immature hepatocytes, which suggests that it plays an important role in organ development and tubular morphogenesis. In adult tissues, FPC is mainly expressed in kidneys (primarily in collecting ducts and thick ascending loops of Henle), and in ductal epithelial cells of the liver and in the pancreas[12,14]. FPC is mainly localized to primary cilia, and is currently believed to be the "sensation antenna" of kidney epithelial cells or bile duct cells[7]. Incorrect protein structure of FPC leads to loss of polarity in kidney tubular epithelial cells or biliary epithelial cells, and cysts are easily formed.

Renal ultrasound results of ARPKD patients show typical symptoms, such as an increased size of bilateral kidneys, hyperechogenicity, poor corticomedullary differentiation, and multiple small cysts in distal renal tubules and collecting ducts[15]. Lifton RP and Somlo S highlighted, in the book "Genetic Disease of the kidney", that kidneys of children with ARPKD grow to a maximum size when patients are 1–2 years old, then gradually decrease and reach a steady state when patients are 4–5 years old (relative to the children's body size)[16]. As patients grow older, the renal medulla echogenicity gradually increases, concomitant with the formation of small cysts with diameters of less than 2 cm. These small cysts and progressed renal fibrosis may change the morphology of kidneys, sometimes causing older patients to be misdiagnosed with autosomal dominant polycystic kidney disease (ADPKD)[17]. ARPKD patients have a 25% and 50% chance to have bilateral renal pelvicaliectasis and renal calcification, respectively[18,19]. If ARPKD patients only present with simple renal medullary ectasia alone, then it is very hard to distinguish it from medullary sponge kidney. Renal ultrasound results of the patient in this study were not sufficient to identify the disease (between ARPKD and medullary sponge kidney) and, therefore, we adopted the genetic diagnostic method to confirm that the patient had ARPKD and, as a result, we discovered a new mutation site of the PKHD1 gene, c.6890T > C (p.Ile2297Thr), thus expanding the gene mutation database.

Conclusion

Next generation sequencing technology, combined with subsequent Sanger sequencing validation, is the common method currently used to detect large genes such as PKHD1. This is not only an economical means for detection, but also significantly reduces detection time, and gives reliable results. This method is a reliable basis for clinical molecular diagnosis, as well as providing a basis for prenatal genetic diagnosis and genetic counseling.

Disclosure

All the authors declare that there are no competing interests.

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