

## Case Report

# Novel mutations of *PKHD1* and *AHI1* identified in two families with cystic renal disease

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**Abstract:** *Objective:* To report newly identified mutations in two families in China with cystic renal disease. *Case presentations:* Two fetuses were found by prenatal ultrasound to have symmetrically enlarged kidneys with increased echogenicity and cystic changes. We isolated fetal and parental genomic DNAs from umbilical cord blood and circulating leukocytes, performed next generation sequencing for mutations, followed by Sanger sequencing for confirmation. We discovered two new heterozygous mutations in *PKHD1*: c.2507\_2515delTGAAGGAGG (p.Val836\_Glu838del) in exon 24 among the fetus and father, as well as c.6840G>A (p.Trp2280\*) in exon 42 among the fetus and mother. A mutation of c.2507\_2515delTGAAGGAGG caused deletion of three amino acids. Two heterozygous mutations in *AHI1*, c.1304G>A (p.Arg435Gln), and c.3257A>G (p.Glu1086Gly) were identified in the second fetus, while the former was also found in the mother. The mutated locus in *AHI1* is highly conserved among humans, dogs, mice, and monkeys. *Conclusions:* We report two newly identified mutations in *PKHD1* and *AHI1*. An accurate genetic diagnosis is crucial for genetic counseling of parents with offspring carrying cystic renal disease.

**Keywords:** Cystic renal disease, *PKHD1* gene, *AHI1* gene, autosomal recessive polycystic kidney disease, Joubert syndrome

### Introduction

Cystic renal disease is classified into four types: autosomal recessive polycystic kidney disease (ARPKD)/infantile polycystic kidney disease, renal cystic dysplasia, autosomal dominant polycystic kidney disease (ADPKD), and cystic/hydronephrotic kidneys caused by prolonged obstruction [1]. However, cystic renal disease may be an important manifestation of other inherited disorders with renal involvement, such as ciliopathy syndromes, or tuberous sclerosis, and von Hippel-Lindau disease.

When cases with cystic renal disease are discovered, it is crucial that we make an accurate genetic diagnosis in order to facilitate subsequent prenatal diagnosis, genetic counseling, and to administer appropriate treatment. In this case series, we sequenced two probands with cystic renal disease and their parents from our institute, and report novel mutation loci.

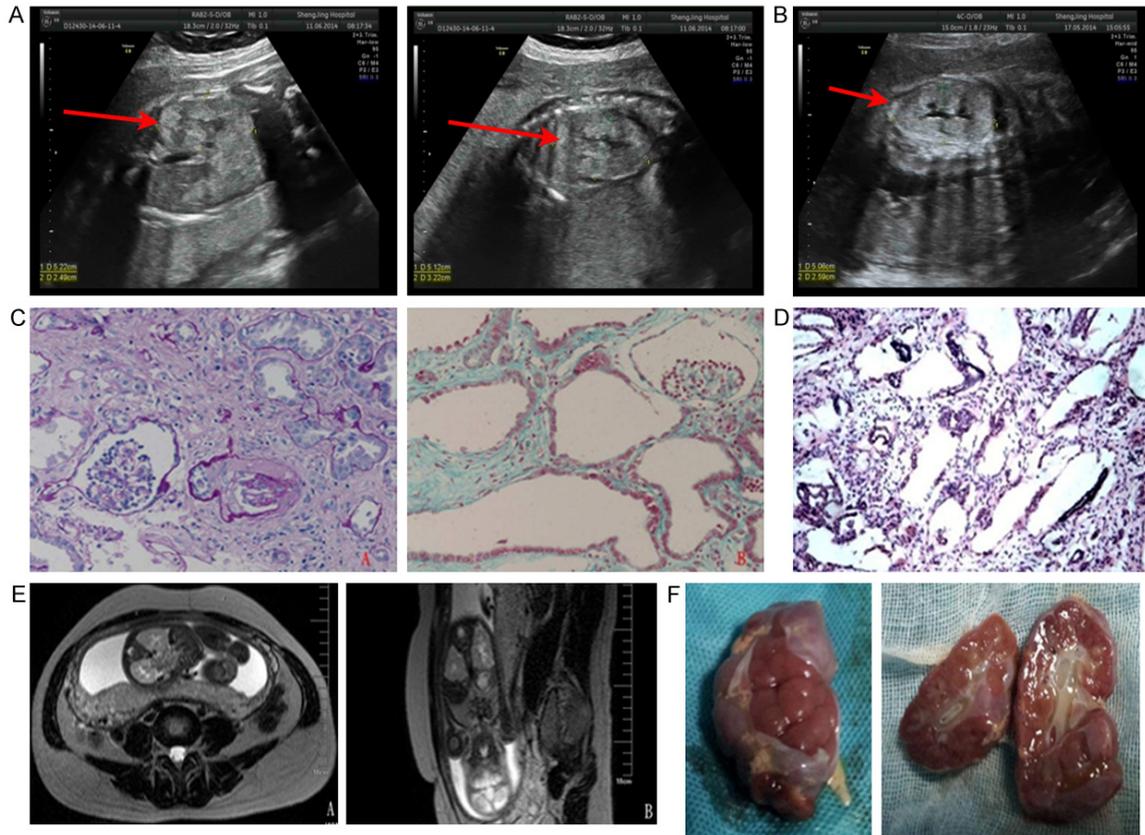
### Case presentation

All procedures performed in this study were approved by the Ethics Committee of Shengjing

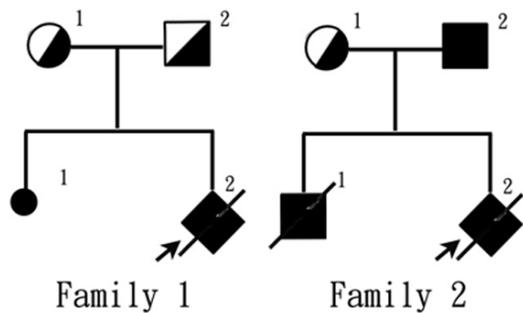
Hospital of China Medical University (2016-PS30J), and informed written consent from the probands' parents was obtained.

A 25-year-old pregnant woman with one prior unsuccessful pregnancy due to early embryonic death at 80 days was found to have a fetus with bilateral enlarged hyperechoic kidneys at the gestational age of 27 weeks. Fetal ultrasonography showed a second-trimester pregnancy with oligohydramnios, enlargement of fetal kidneys, and hyperechoic renal parenchyma. Infantile polycystic kidney disease was considered (**Figure 1A**). Renal biopsy found fusiform dilation of the renal collecting ducts and distal renal tubules with low-flat epithelium, and epithelial cell exfoliation of partial tubules (**Figure 1D**). A decision was made to terminate the pregnancy immediately.

Another woman had one prior successful pregnancy, and her first male baby developed end-stage renal disease at the age of 11 months, had obvious developmental retardation, and eventually died. Ultrasonography of his urinary system showed a 7.6 cm × 4.1 cm left kidney, a 7.3 cm × 3.9 cm right kidney, and with generally



**Figure 1.** (A) Fetal ultrasonography of the first proband showed bilateral kidney enlargement and enhanced echogenicity of the renal parenchyma (arrow). (B) Fetal ultrasonography of the second proband showed similar findings. (C) Renal biopsy of the second proband's older brother showed massive cystic dilation of the proximal tubules with low-flat epithelium, epithelial cell exfoliation of partial tubules, as well as massive interstitial fibrosis (A: PAS 200 ×; B: Masson 200 ×). (D) Renal pathology of the first proband showed fusiform dilation of collecting ducts and distal renal tubules (light microscope, HE 100 ×). (E) Fetal magnetic resonance imaging of the second proband showed enlargement of both kidneys and mild dilation and deformation of the renal pelvis and calices (A: T2-weighted cross-sectional view, B: T2-weighted coronal view). (F) Gross examination of the fetal kidneys from the second proband, showing cystic enlargement.



**Figure 2.** The genealogical tree of the two probands in this case series.

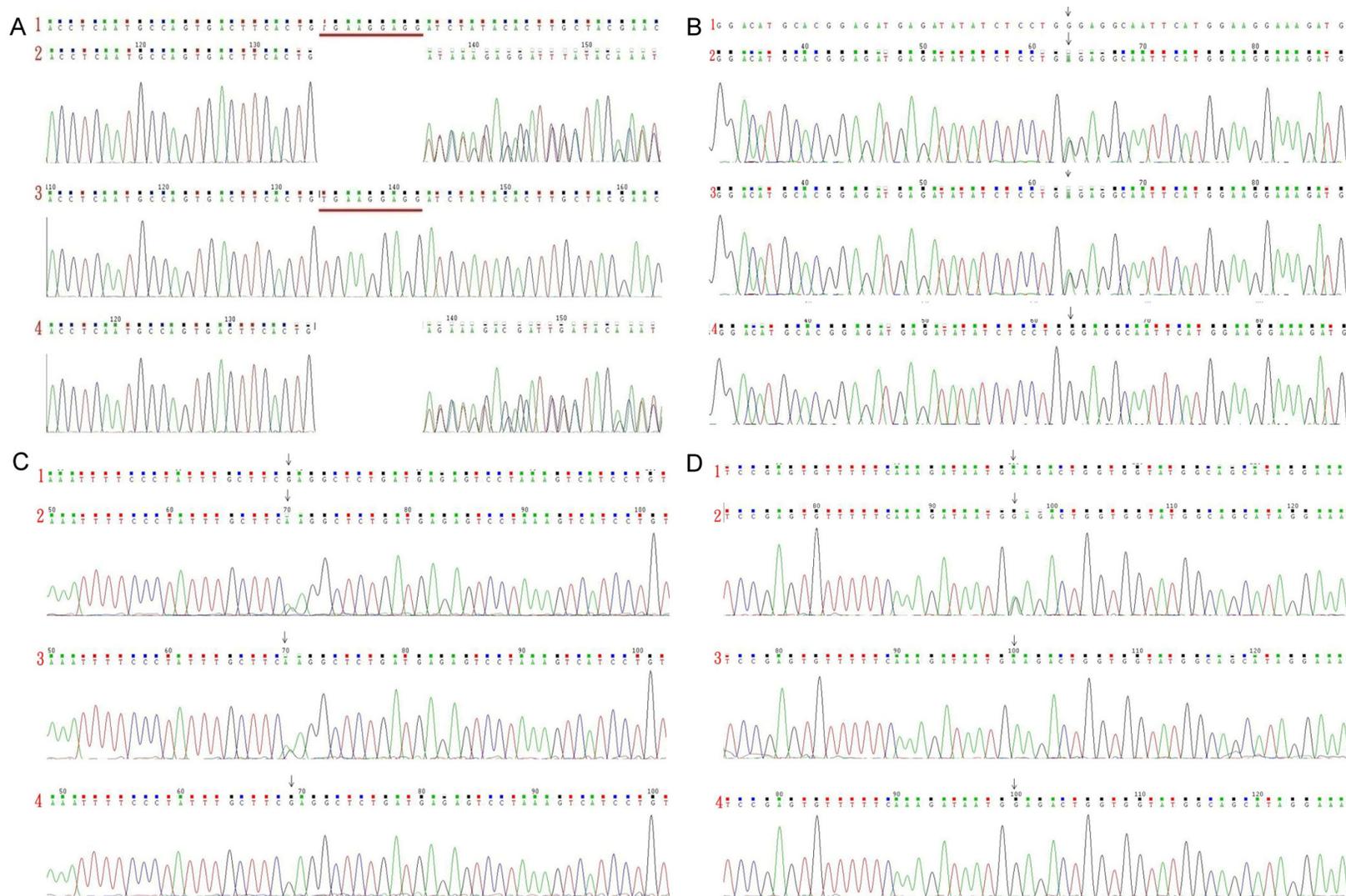
hyperechoic parenchyma and poor corticomedullary differentiation.

The first male baby received a renal biopsy, which showed subacute interstitial nephritis

and cystic dilations of the proximal tubules (**Figure 1C**), raising suspicion of congenital cystic renal diseases. He received no genetic diagnosis at that time due to limited resources. Her second fetus was found to have bilateral enlarged echogenic kidneys at the gestational age of 29 weeks, accompanied by situs inversus viscerum, mirror-image dextrocardia, and bilateral renal enlargement with enhanced echogenicity and infantile polycystic kidney disease was suspected (**Figure 1B**).

Magnetic resonance imaging (MRI) indicated an enlargement of the fetal kidneys and mild dilation and deformation of the renal pelvis and calices (**Figure 1E**). According to the clinical presentations of the second proband and his older brother, ARPKD was proven clinically, and the fetus was terminated soon after diagnosis.

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**Figure 3.** A: Sequencing results of *PKHD1* exon 24 in the first family. B: Sequencing results of *PKHD1* exon 42 in the first family. C: Sequencing results of *AHI1* exon 9 in the second family. D: Sequencing results in *AHI1* exon 24 in the second family. (1) Reference sequence; (2) Sequence of the proband; (3) Sequence of the proband's mother; (4) Sequence of the proband's father.

Gross examination of the kidney showed cystic enlargement (**Figure 1F**). The babies and their parents were referred for genetic diagnoses.

A detailed evaluation of both fetuses' parents found no clinical signs of renal abnormality (**Figure 2**). We collected umbilical cord blood from both probands (at the time of induction) as well as their parental blood, sending the samples to the pediatric laboratory of Peking University First Hospital for genetic analysis of any cystic renal diseases. Mutation screening was performed using next generation sequencing (NGS) technologies, with results confirmed by Sanger sequencing.

The sequencing results of the first proband showed a heterozygous deletion mutation in the *PKHD1* gene (NM\_138694.3)-c.2507\_2515delTGAAGGAGG (p.Val836\_Glu838del) located in exon 24 (**Figure 3A**)-causing deletion of three amino acids. This mutation was thus considered pathogenic. Another heterozygous missense mutation-c.6840G>A (p.Trp2280\*) located in exon 42 (**Figure 3B**)-was also discovered causing protein truncation and the production of a peptide consisting of 2,279 amino acids only (normally 4,074 amino acids). This mutation was also considered pathogenic.

A database search (<http://www.humgen.rwth-aachen.de>) did not find these two pathogenic mutations in *PKHD1*. A heterozygous mutation in exon 42-c.6840G>A (p.Trp2280\*)-was found in the first proband's mother (**Figure 3B**), and a heterozygous mutation in exon 24-c.2507\_2515delTGAAGGAGG (p.Val836\_Glu838del)-was found in the first proband's father (**Figure 3A**). Since his parents each had a heterozygous mutation in *PKHD1* without phenotypes, while the proband had a compound heterozygous mutation with phenotypes, we believed that the inheritance pattern of this case was likely autosomal recessive, suggesting the diagnosis of ARPKD.

On the other hand, the sequencing results of the second proband showed two heterozygous missense mutations of *AHI1* gene (NM\_001134830.1): c.1304G>A (p.Arg435Gln) in exon 9 (**Figure 3C**) and c.3257A>G (p.Glu1086Gly) in exon 24 (**Figure 3D**). The heterozygous mutation of c.1304G>A (p.Arg435Gln) was also found in the second proband's mother (**Figure 3C**), and this mutation could cause substitution

of the 435<sup>th</sup> arginine by glutamine. This locus is highly conserved among humans, dogs, mice, and monkeys, and this mutation has not been reported in the existing genomic sequencing databases including 1000 Genomes Database, dbSNP database, ESP6500 database, and BGI internal database of the general population. This mutation was predicted to be neutral according to software (EnsCondelPred) analysis. In addition, a homozygous mutation of c.3257A>G (p.Glu1086Gly) was found in the second proband's father (**Figure 3D**) which has been reported to be pathogenic (HGMD, 2008) by causing the substitution of the 1,086<sup>th</sup> glutamine by glycine.

### Discussion

ARPKD is a severe, early-onset form of cystic renal disease, and affected fetuses often have oligohydramnios and massively enlarged kidneys. Renal findings in ARPKD patients include bilateral kidney enlargement, hyperechogenicity with poor corticomedullary differentiation, and multiple tiny cysts originating from distal renal tubules and collecting ducts [2]. The clinical diagnosis relies upon imaging results and pathological examinations, but the molecular diagnosis (i.e. *PKHD1* mutation) is also of great importance for the clinical management, genetic counseling, and prenatal diagnosis of ARPKD patients and their families.

*PKHD1* is located at 6p12 and has a length of 472 kb [3]. Its longest open reading frame consists of 67 exons that encodes a type I transmembrane protein consisting of 4,074 amino acids, called fibrocystin/polyductin (FPC) [4]. Evidence indicates that FPC has multiple alternative splicing variants [5].

At present, 750 mutations have been catalogued in the ARPKD mutation database (<http://www.humgen.rwth-aachen.de>), but *PKHD1* does not contain mutational hotspots [6]. Moreover, most patients may have compound heterozygous *PKHD1* mutation. A genotype-phenotype correlation study found that (in terms of collecting duct dilatation without portal fibrosis) the phenotypes of fetal and neonatal ARPKD were significantly associated with the presence of two truncating mutations [7]. However, exceptions do exist, such as a case of homozygous *PKHD1* mutation due to a large

deletion that reportedly survived the neonatal period [8]. In addition, the phenotype may be highly variable among patients with the same genotype, probably due to gene modification [9].

The first proband was considered to have infantile polycystic kidneys, based on ultrasonographic findings. The pathological examination identified fusiform dilation of distal renal tubules and collecting ducts. In addition, we found compound heterozygous changes of *PKHD1* including a deletion mutation (c.2507\_2515delTGAAGGAGG) and a truncating mutation (c.6840G>A), which were also found separately in his mother and father. This is consistent with the autosomal recessive inheritance pattern seen in ARPKD.

For the second proband, using renal pathology results from his older brother showing cystic dilations of proximal tubules, the primary clinical diagnosis of ARPKD was highly likely. However, gene sequencing in this proband did not find any mutation in *PKHD1* but instead two heterozygous missense mutations (c.1304G>A and c.3257A>G in *AHI1*), one of pathogenic genes of Joubert syndrome (JBTS) [10] type 3 and located on chromosome 6q23.3 [11]. JBTS is a rare autosomal recessive ciliopathy, characterized by developmental delay and oculomotor abnormalities, with renal manifestations similar to those of ARPKD [12].

We believe that the proband and his older brother might have JBTS3. The missense mutation of c.3257A>G in the second proband was transmitted from his father who was homozygous for the mutation but had no phenotypes of renal cystic disease. This mutation site has been reported by Kroes in 2008, and it is located in exon 24 of *AHI1* and encodes SH3 protein binding domain [13]. The father in this family similarly did not have renal involvement, consistent with the above report. Homozygosity of mutation in his father, in combination with the heterozygous mutation in his mother and a compound heterozygous mutation in the proband, is compatible with autosomal recessive inheritance pattern of JBTS.

In conclusion, we found that autosomal recessive ciliopathy JBTS is difficult to distinguish from ARPKD in term of renal presentations only. Therefore, a genetic analysis provides a more accurate diagnosis of JBTS. Newly discov-

ered mutations of ARPKD expand the mutation spectrum of this gene, assisting subsequent prenatal diagnosis and genetic counseling of these two families.

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### Disclosure of conflict of interest

None.

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