




# Homozygous Missense Mutation on Exon 22 of *PKHD1* Gene Causing Fatal Autosomal Recessive Polycystic Kidney Disease

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## Abstract

Autosomal recessive polycystic kidney disease, described as a congenital hepatorenal fibrocystic syndrome, is a significant inherited cause of end stage renal failure in children with reported incidence of 1 in 20,000 live births. The clinical spectrum is wide. Antenatal findings of echogenic reniform enlarged kidneys associated with evidence of intrauterine renal failure in the form of severe oligoamnios are pathognomonic. Postnatal illness ranges from fatal respiratory failure due to pulmonary hypoplasia in neonates to chronic kidney disease in children, or later presentation of ductal plate malformation and portal hypertension. Advances in genetic diagnostic techniques have allowed recognition of genotypes. We report a novel homozygous missense variant on exon 22 of *PKHD1* gene (chr6:51915067G > A; c.2167C > T) that results in the amino acid substitution of cysteine for arginine at codon 723 (p.Arg723Cys). The affected neonate presented with antenatal anhydramnios, classical radiological features, and severe hypoxic respiratory failure likely due to pulmonary hypoplasia and succumbed. The parents were found to be heterozygous carriers. Detection of the specific variant in the proband facilitated prenatal investigation in the next pregnancy.

## Keywords

- ▶ *ARPKD*
- ▶ *PKHD1*
- ▶ polycystic kidney disease
- ▶ fibrocystin-polyductin complex

## Introduction

Autosomal recessive polycystic kidney disease (ARPKD) is a rare disease with an incidence of approximately 1 in 20,000 live births.<sup>1</sup> The carrier frequency in discrete populations is estimated to be 1 in 70.<sup>2</sup> Genetic confirmation in the proband could impact future pregnancies in the family and counseling.

The *PKHD1* gene on chromosome 6p21.1-p12 that codes for fibrocystin/polyductin (FPC) is implicated primarily.<sup>3</sup> FPC is localized to primary cilia and their basal bodies. The location, concentration, and presence during early ciliogenesis seem to indicate its role in microtubule organi-

zation and mechano-chemosensation (i.e., key functions of primary cilia). This in turn is vital to the maintenance of the structural integrity of various organs- notably renal and hepatic.<sup>4</sup> Several mutations, mostly missense, have been reported.<sup>5</sup> Most patients who have severe clinical features are compound heterozygotes. We report a rare missense homozygous mutation on exon 22 that presented perinatally. To the best of our knowledge, there are only three previous reports of this variant, in compound heterozygous form. Adding to the scientific literature may assist appropriate future pathogenicity classification of this unique variant and aid genetic counseling.

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## Patient Report

Patient was referred to our institute at 31 weeks of gestation (nonconsanguineous union) with severe oligohydramnios and abnormal fetal kidneys on ultrasonography (USG). These findings were further classified as reniform enlargement of bilateral fetal kidneys (lengths of right and left kidneys were 8.6 and 6.5 cm, respectively), diffuse hyperechogenicity, poor corticomedullary differentiation (CMD), and no urine in fetal bladder or discernible liquor pockets. The fetal liver was unremarkable. The family received a provisional diagnosis of ARPKD. The baby was delivered through anhydramnios at 35 weeks of gestation. The neonate, who was critically ill from birth, had features of hypoxic respiratory failure and required high-frequency oscillatory ventilation. A clinical diagnosis of pulmonary hypoplasia due to ARPKD was supported by the rapidly deteriorating clinical course, occurrence of tension pneumothorax, and anuria (►Fig. 1). USG showed enlarged reniform kidneys, of sizes  $9.3 \times 4.6$  cm (right) and  $9.3 \times 3.3$  cm (left), with multiple tiny cysts, altered CMD, no renal pelvis/ureteral dilatation, and no urine in bladder. USG of liver did not reveal any abnormality. The baby succumbed on day 2 of life. The family was counseled by the senior genetic counselor and they sought testing for cystic diseases of kidney.

The sequences obtained after next generation sequencing (NGS) were aligned to human reference genome (GRCh37/hg19) by using BWA program<sup>6,7</sup> and analyzed by using Picard and GATK version 3.6 to identify variants relevant to the clinical indication.<sup>8,9</sup> GATK best practices framework for the identification of variants in the sample were followed. Clinically relevant mutations were annotated by using published variants in the literature and a set of disease databases – ClinVar, OMIM, GWAS, HGMD, and SwissVar.<sup>10–14</sup> Common variants were filtered based on allele frequency in 1000 Genome Phase 3, ExAC, EVS, dbSNP147, 1000 Japanese Genome, and MedGenome Laboratory's internal Indian population database.<sup>15,16</sup> Nonsynonymous variants effect was



**Fig. 1** X-ray of chest and abdomen; right pneumothorax, low volume lung, abdomen almost completely gas less (due to enlarged kidneys filling the abdomen).

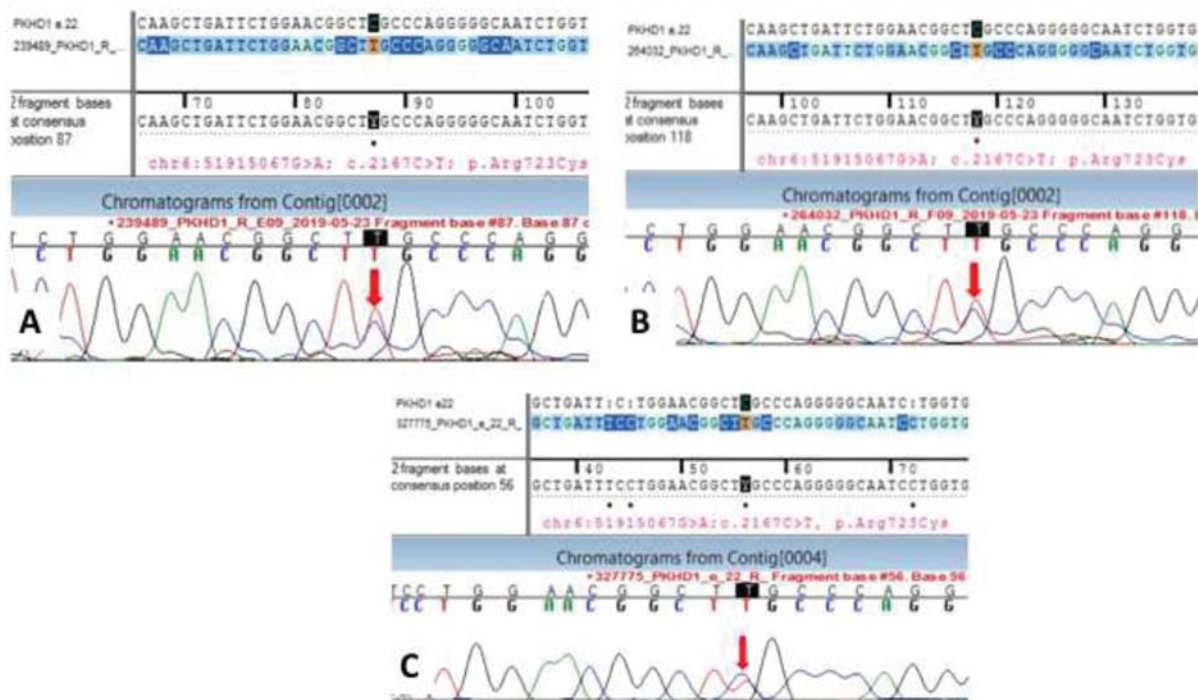
calculated by using multiple algorithms such as PolyPhen-2, sorting intolerant from tolerant (SIFT), Mutation Taster2, Mutation Assessor, and likelihood ratio test (LRT). A homozygous missense variation in exon 22 of the *PKHD1* gene (chr6:51915067G > A; c.2167C > T) that results in the amino acid substitution of cysteine for arginine at codon 723 (p. Arg723Cys) was identified in the index patient, which was further validated by Sanger sequencing. A same variant affecting the same codon has previously been reported as one of the compound heterozygous variants in a patient affected with autosomal recessive polycystic kidney disease.<sup>17</sup> The in silico prediction of the variant is benign by SIFT, LRT, and MutationTaster2. The reference codon is conserved across mammals. The variant was classified as variant of uncertain significance (VUS).

The same variant was detected in the heterozygous state in both parents on Sanger sequencing. The kidneys and liver of both parents were screened by USG and found to be normal, indicating that they were asymptomatic carriers. The family visited with their second pregnancy 2 years later. After the couple was counseled regarding prenatal testing of a VUS, they decided to go for amniocentesis. The fetus was found to be heterozygous for the variant identified in the index child. Follow-up scans were normal and a healthy female baby was delivered at term. Her postnatal USG was reported normal and she remains well on follow-up. Sequence chromatograms of the parents and second fetus are represented in ►Fig. 2.

## Discussion

The suspicion of ARPKD is based on clinical and radiological findings in the proband with normal renal status in both parents. Presence of typical radiological findings (i.e., antenatally or postnatally) with evidence of hepatic involvement, normal parental renal status, and genetic/pathological confirmation is required to classify as ARPKD.<sup>18</sup> Renal disease may present in neonates as nephromegaly, hypertension, and renal dysfunction. The majority of patients progress rapidly to end stage renal disease requiring renal replacement therapy. Sick neonates have features of pulmonary hypoplasia, and approximately 30% die in infancy.<sup>19</sup>

As advances in renal replacement therapy and kidney transplantation improve long-term survival, it is likely that clinical hepatobiliary disease will become a major feature of the natural history of ARPKD. In addition, a subset of affected individuals are identified later with hepatosplenomegaly; in whom the renal disease is often mild and may be discovered incidentally during imaging studies of the abdomen. In our patient, typical clinical and radiological presentation left little doubt about the diagnosis. Presentation as liver disease may be the case in older children.<sup>20</sup> Some patients develop histologic liver involvement, which is reflected by the disease term “polycystic kidney and hepatic disease 1 (PKHD1)”. Defective remodeling of the ductal plate leads to biliary atresia, and hepatic fibrosis that can result in complications such as hypersplenism and esophageal varices.



**Fig. 2** Sequence chromatograms and alignment to the reference sequence showing the variation in exon 22 of the *PKHD1* gene (chr6:51915067G > A; c.2167C > T; p.Arg723Cys) detected in heterozygous condition. (A) Mother, (B) father, (C) and fetus of second pregnancy (sibling-to-be) of the index patient.

Discovery of the genetic basis of the disease as *PKHD1* gene mutations in the 1950s was a milestone. The principal role of the coded protein, FPC seems to be localized to the primary cilia and basal body and might be involved in microtubule organization.<sup>4</sup> The protein is considered to undergo post-translational modifications, but the function is not entirely known. Mutations implicated in ARPKD are evenly distributed along the length of the *PKHD1* gene. Therefore, it is hypothesized that a certain amount of full-length functional protein is required for function. Another proposed mechanism is that mutations disrupt the relative abundance of functional isoforms of FPC, which disturb their required stoichiometric ratios.<sup>2</sup>

Presence of two truncating mutations is associated with severe clinical presentation during the neonatal period. Some missense mutations can, however, be as severe as truncating mutations. This may be related to as yet unidentified modifying genes (including those in noncoding intronic regions), complex transcriptional profiles, and epigenetic influences. Recently, mutations in *DZIP1L* (which codes for ciliary TZ protein) have been described. These patients usually follow a moderate clinical course.<sup>2</sup>

In our case, a homozygous missense variation was identified in exon 22. This variant has been previously reported as one of the compound heterozygous variants in a patient affected with autosomal recessive polycystic kidney disease.<sup>21</sup> Szabo et al<sup>17</sup> reported a neonate who presented with respiratory failure with compound heterozygous variants on exon 22 c.2167C > T and exon 58 c.8870 T > C. Gunay-Aygun et al<sup>5</sup> included survivors beyond 6 months of age and identified 77 *PKHD1* variants, they found one

Caucasian nonperinatal patient with compound heterozygous variant in exon 22 c.2167C > T and c.34669C > G.

Deleterious effects of any variation consist of damage to protein structures and gene regulation. Alterations in the protein sequence may ultimately affect protein charge, geometry, hydrophobicity, dynamics, translation, or inter/intraprotein interaction. In silico prediction algorithms use this information to predict the damaging effect of a particular variation. This variant “c.2167C > T” in exon 22 of *PKHD1* gene has been classified as benign by SIFT, Polyphen-2 and Mutation Taster2. Gunay-Aygun et al also classified it to be probably benign. We have observed this variant to be phenotypically associated with typical clinical and radiological presentation in homozygous state in the deceased proband in our case. Segregation in the parents, and the carrier status in the healthy younger sibling has been confirmed by Sanger sequencing. The previous reports and our case indicate that this variant is probably damaging and may be associated with the disease. When more case reports and/or experimental evidences based on targeted mutagenesis become available in the future, it is possible that the classification of this variant may change.

## Conclusion

Homozygous missense variation on exon 22 of *PKHD1* gene described here was associated with typical features of perinatal ARPKD. This is a case report that adds to the disease database.

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None.

**Conflict of Interest**

None declared.

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