

## Newly Detected *PKHD1* Gene Mutation in a Newborn with Fatal Autosomal Recessive Polycystic Kidney Disease

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### ABSTRACT

Autosomal recessive polycystic kidney disease is among the most common inherited ciliopathies and is caused by mutations in the polycystic kidney and hepatic disease 1 (*PKHD1*) gene. Despite its great phenotypic variability, this condition is usually diagnosed during the neonatal and early infantile periods. We report a 37<sup>+3</sup>-gestational-week neonate presenting with fatal autosomal recessive polycystic kidney disease who died at 28 hours of life from severe respiratory failure. The familial history is significant because a previous sibling died in utero at 24<sup>+2</sup> weeks of gestational age and was diagnosed with polycystic kidney disease based on prenatal ultrasonography and autopsy. Our patient's autopsy revealed findings compatible with polycystic kidney disease. In addition, a *PKHD1* gene study of peripheral blood leukocytes identified the compound heterozygote mutation c.274C>T(p.Arg92Trp), as well as the novel heterozygous nonsense mutation c.2770C>T(p.Gln924\*).

**Key Words:** Polycystic kidney, Autosomal recessive, *PKHD1* gene, Mutation

### INTRODUCTION

Congenital kidney and urinary tract malformations comprise 20-30% of all fetal malformations<sup>1)</sup> and cause up to 30-50% of end-stage renal disease (ESRD) cases involving infants and children<sup>2)</sup>. Autosomal recessive polycystic kidney disease (ARPKD) is among the most common hereditary nephropathies and tends to progress to ESRD within the first decade of life<sup>3)</sup>, with up to 30-40% of all patients dying shortly after birth because of respiratory insufficiency caused by pulmonary hypoplasia. Infants who survive the neonatal period display variable clinical features that require close monitoring<sup>4)</sup>. The use of polycystic kidney and hepatic disease 1 (*PKHD1*) gene sequencing data from chromosome 6p21<sup>5)</sup> contributes to an early diagnosis, as well as the genetic counseling of family members with ARPKD.

In this study, we report a newly detected *PKHD1* heterozygous nonsense mutation in a

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newborn presenting with fatal ARPKD.

## CASE REPORT

The infant's mother was a 32-year-old, gravida 2, para 1 Korean woman with asymptomatic premature ventricular contraction that did not require medication. The father was a healthy 32-year-old Korean man. Their first baby died in utero at 24<sup>+2</sup> weeks of gestation in 2010. An autopsy revealed a female fetus (body weight 940 g) with ARPKD accompanied by congenital hepatic fibrosis. At that time, the parents refused genetic testing.

One year later, during the second pregnancy, oligohydramnios began to develop at 22<sup>+3</sup> weeks of gestation, and fetal ultrasonographic findings at 24<sup>+1</sup> weeks of gestation revealed that the fetus had bilateral polycystic kidneys. The mother underwent amnioinfusions of 210 mL, 250 mL, and 350 mL at 24<sup>+1</sup> weeks, 26<sup>+5</sup> weeks, and 28<sup>+5</sup> weeks of gestation, respectively, to treat oligohydramnios. A male newborn was delivered via cesarean section because of shoulder dystocia, and had Apgar scores of 4 at 1 minute and 7 at 5 minutes. The neonate required

immediate intubation due to his poor respiratory efforts and was admitted to the neonatal intensive care unit (NICU). Upon his arrival at the NICU, the patient was lethargic and markedly hypotonic with huge, palpable masses in both flanks. Neither testis was palpable, although bilateral hydroceles and a left inguinal hernia were noted consequent to the increased intra-abdominal pressure. At birth, the patient's growth parameters were as follows: 3.82 kg of body weight (>90<sup>th</sup> percentile), 50 cm of height (50<sup>th</sup>–90<sup>th</sup> percentile), and 36.5 cm of head circumference (>90<sup>th</sup> percentile). The initial blood pressure at admission was 102/93 mmHg (>90<sup>th</sup> percentile). The initial chest and abdominal x-rays showed hypo-aerated lungs and a distended abdomen with no bowel gas (Figure 1). The complete blood-cell count was within the normal range, along with a white blood-cell count of 16,100/ $\mu$ L, hemoglobin level of 15.7 g/dL, and platelet count of 235,000/ $\mu$ L. The initial creatinine, blood urea nitrogen, sodium, and potassium levels were 0.95 mg/dL, 7 mg/dL, 136 mmol/L, and 4.4 mmol/L, respectively. Abdominal ultrasonography showed enlarged bilateral kidneys (right: 10.1 cm in length, left: 10.6 cm in length) with a loss of normal corticomedullary differentiation, increased cortical echogenicity due to the multiple interfaces associated with the microcysts (Figure 2A), and heterogeneous echotexture in the liver (Figure 2B). These findings suggested ARPKD with liver involvement. Echocardiography identified severe pulmonary hypertension, as evidenced by a huge patent ductus arteriosus with a right-to-left shunt, and concentric left ventricular (LV) hypertrophy with LV dysfunction (ejection fraction: 30–40%). The patient underwent continuous renal replacement therapy (CRRT) beginning at 8 hours after birth in an attempt to stabilize him for a nephrectomy and peritoneal dialysis. Unfortunately, the patient died 28 hours after birth from progressive respiratory failure.

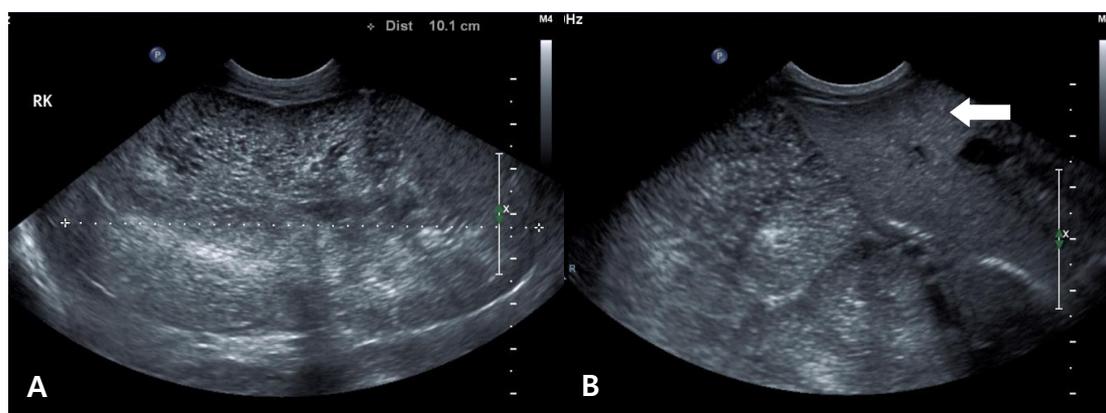
An autopsy was performed and identified marked enlargement of both kidneys (right: 10 cm×6.3 cm×5.7 cm and 188.5 g, left: 10.6 cm×6.3 cm×6 cm and 205.9 g) with spongiotic parenchyma, multiple small cysts, and a slightly enlarged liver (10.3 cm×7.4 cm×4.2 cm and 162.4 g; Figure 3). Genomic deoxyribonucleic acid (DNA) was isolated from the patient's peripheral leukocytes. Sixty-seven exons and corresponding exon-intron boundaries on the *PKHD1* gene were amplified via polymerase chain reaction (PCR). Direct sequencing was subsequently performed using a BigDye<sup>®</sup> Terminator v3.1 Cycle Sequencing Kit and ABI 3130xl Genetic Analyzer (Applied Biosystems,



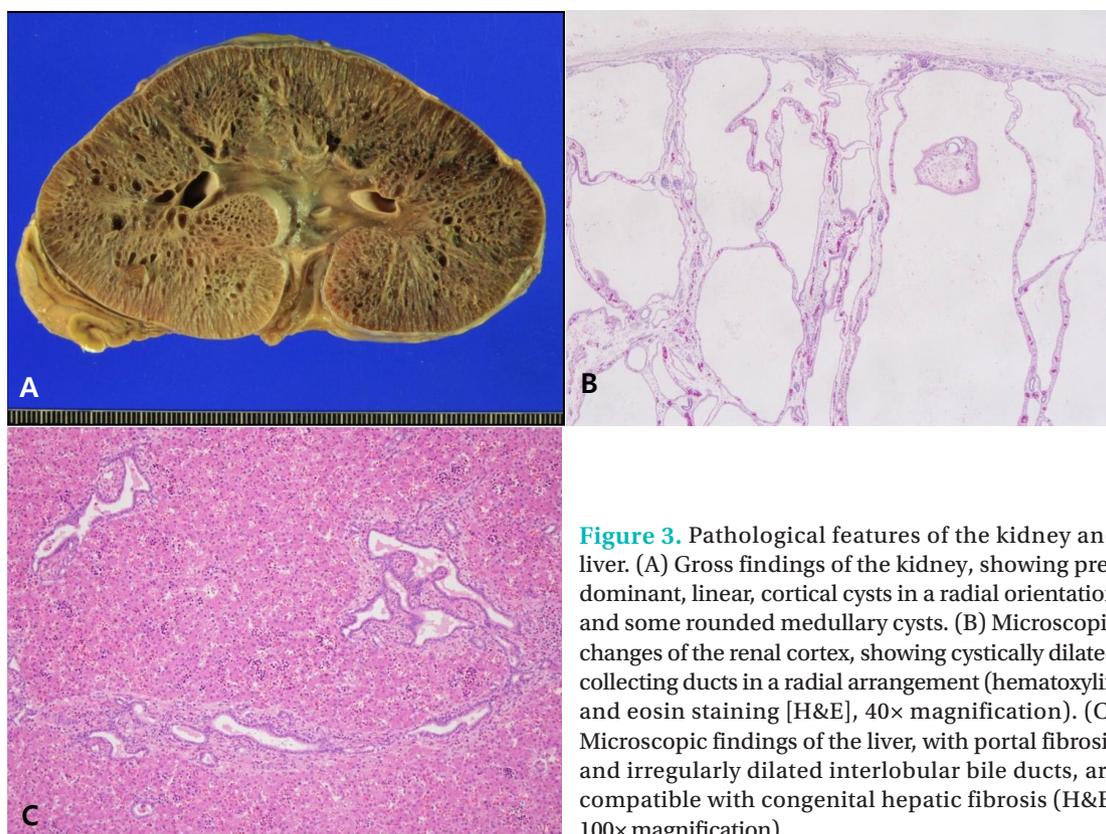
**Figure 1.** Initial chest and abdomen radiographs reveal small, hypo-aerated lungs and a distended non-gaseous abdomen.

Foster City, CA, USA). The patient's *PKHD1* gene contained compound heterozygous c.274C>T(p.Arg92Trp) and c.2770C>T (p.Gln924\*) mutations in exon 4 and exon 26, respectively (Figure 4). The c.274C>T(p.Arg92Trp) mutation was previously reported<sup>6)</sup>; however, the heterozygous nonsense mutation of c.2770C>T(p.Gln924\*) is a newly discovered mutation and has not yet been reported. Following the patient's diagnosis with

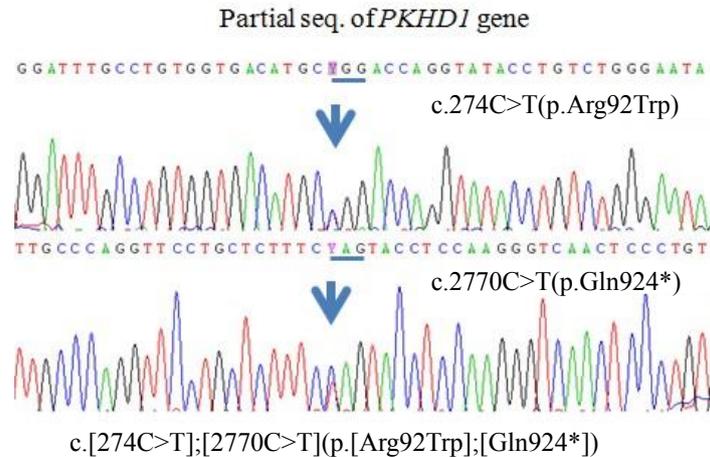
ARPKD by DNA sequencing, the parents underwent *PKHD1* gene mutation analysis. The mother's gene sequencing found a heterozygous c.274C>T(p.Arg92Trp) mutation, and the father was a carrier of the c.2770C>T(p.Gln924\*) mutation (Figure 5).



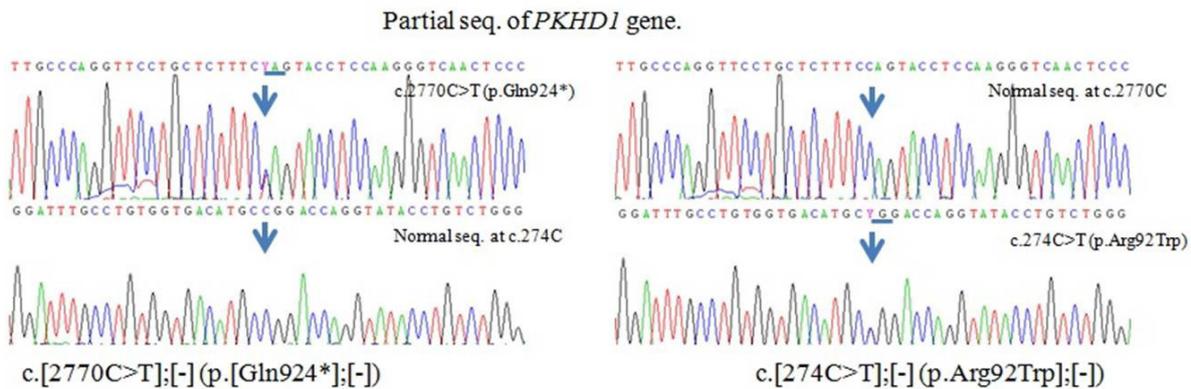
**Figure 2.** Ultrasonographic images of the patient during the first day of life. (A) The right kidney is enlarged, with a loss of normal corticomedullary differentiation and increased cortical echogenicity due to multiple interfaces associated with microcysts (right kidney: 10.1 cm, left kidney: 10.6 cm in length). (B) The liver (arrow) exhibits a heterogeneous echo texture.



**Figure 3.** Pathological features of the kidney and liver. (A) Gross findings of the kidney, showing predominant, linear, cortical cysts in a radial orientation and some rounded medullary cysts. (B) Microscopic changes of the renal cortex, showing cystically dilated collecting ducts in a radial arrangement (hematoxylin and eosin staining [H&E], 40× magnification). (C) Microscopic findings of the liver, with portal fibrosis and irregularly dilated interlobular bile ducts, are compatible with congenital hepatic fibrosis (H&E, 100× magnification).



**Figure 4.** Electrophoretogram of *PKHD1* gene exon 4 and 26 sequencing in this patient. A novel heterozygous nonsense mutation, c.2770C>T (p.Gln924\*), was identified in exon 26. Abbreviation: *PKHD1*, polycystic kidney and hepatic disease 1.



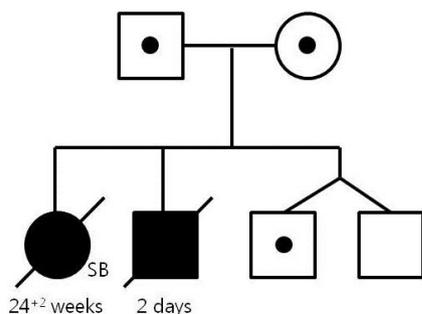
**Figure 5.** Electrophoretogram of *PKHD1* gene exon 4 and 26 sequencing in both parents. The father is a heterozygous carrier of the c.2770C>T(p.Gln924\*) mutation, and the mother is a heterozygous carrier of the c.274C>T(p.Arg92Trp) mutation. Abbreviation: *PKHD1*, polycystic kidney and hepatic disease 1.

## DISCUSSION

ARPKD is an autosomal recessive disease characterized by the cystic dilatation of the renal collecting duct, as well as congenital hepatic fibrosis. The cystic changes in the kidney begin during the fetal stage and progress from the renal medulla toward the renal cortex. Histopathology of the fetal kidneys may reveal slender, dilated collecting ducts extending radially from the medulla to the nephrogenic zone, whereas the neonatal kidneys may exhibit cystic changes in the dilated tubules, which are radially arrayed in the cortex. Older patients with ARPKD (infants and children) might have dilated and elongated cortical collecting ducts, with large ectatic medullary collecting ducts.

Contrarily, a patient with ARPKD will exhibit congenital hepatic fibrosis with portal and interlobular fibrosis and biliary duct hyperplasia<sup>7</sup>. The incidence of ARPKD has been reported to range from 1/10,000 to 1/40,000<sup>7,8</sup>.

Various clinical symptoms of ARPKD may begin to appear during the perinatal period, neonatal period, infancy, and childhood. These various time points of symptom onset are thought to be caused by different expression levels of the same mutated gene, rather than variations in different genes<sup>9</sup>. Most cases of ARPKD are detected as a bilateral flank mass during the perinatal period and may present with oligohydramnios, lung hypoplasia, and respiratory distress. Of the affected patients, 30-50% will die from sepsis and respiratory failure within



**Figure 6.** The patient's pedigree demonstrates the heritability of autosomal recessive polycystic kidney disease.

a few days to a few weeks<sup>10</sup>. Alternatively, those who survive the neonatal period with fewer renal cystic changes and those with delayed symptom onset have better prognoses<sup>11</sup>. In some reports, 56-67% of post-neonatal surviving patients lived for up to 15 years without progression to ESRD, with reported survival durations up to 55 years<sup>12,13</sup>. According to a study of 164 patients with ARPKD who survived after the neonatal period, the 1- and 10-year survival rates were 85% and 82%, respectively, and the 5-, 10-, and 20-year survival rates without renal replacement therapy were 86%, 71%, and 42%, respectively<sup>14</sup>. A cohort study of 78 patients who survived longer than 6 months found no families with two truncating mutations<sup>6</sup>.

ARPKD is often diagnosed through molecular genetic diagnostic testing of the *PKHD1* gene, which is known to be associated with disease prevalence in approximately 85% of patients with ARPKD<sup>9,11,14,15</sup>. The large *PKHD1* gene, which is located at 6p21.1-p12<sup>7,9</sup> and has a genomic DNA weight of 470 kilobases, encodes the protein known as polyductin or fibrocystin<sup>16</sup>. The role of fibrocystin in the normal kidney is not well-known, although it is expressed in the cytoplasm and on the cell surfaces of renal collecting duct epithelial cells in the cortex and medulla, as well as on epithelial cells of the bile duct and pancreatic duct<sup>17</sup>. Fibrocystin is thought to be a receptor protein involved in cell attachment, repulsion, and proliferation, as well as renal collecting duct and hepatobiliary duct elongation and regulation<sup>16,18</sup>. *PKHD1* gene mutations are varied and scattered, rather than clustered in a particular location. As of May 2015, 748 different *PKHD1* mutations have been reported in a locus-specific database<sup>19</sup>. A case involving a *PKHD1* gene mutation was newly reported in Korea in 2014. This was a frameshift mutation at c.889\_931del43, and the patient did not report a family history<sup>20</sup>.

Controversies regarding the genotype-phenotype correlation

have been reported; however, current reports published by other researchers regarding patients with nonsense mutations have described severe phenotypes<sup>21</sup>. As mutations usually differ by family, approximately one-third of these mutations are unique to a single pedigree. The parents of the affected patients often harbor complex heterozygous *PKHD1* gene mutations. A C→T transition mutation at the *PKHD1* cDNA nucleotide position 107 (Thr36Met) is the most frequently encountered mutation, accounting for approximately 15-20% of all mutated alleles, and is found in families from many human racial groups<sup>8,16,18</sup>. In our patient, the *PKHD1* gene contained compound heterozygous c.274C>T(p.Arg92Trp) and c.2770C>T(p.Gln924\*) mutations. The c.2770C>T(p.Gln924\*) mutation from the father is a nonsense mutation that had not previously been reported. Therefore, we suspect that this c.2770C>T(p.Gln924\*) mutation, alone or in combination, might have been responsible for the lethal consequences experienced by this patient and his aborted sibling. The patient's mother became pregnant in late 2014 with twins following a pre-implantation genetic diagnosis and in vitro fertilization. She underwent chorionic villus sampling at 14 weeks of gestation, which demonstrated that one fetus had a normal *PKHD1* gene, whereas the other fetus was a heterozygous carrier of the c.2770C>T(p.Gln924\*) *PKHD1* gene mutation. She gave birth to apparently healthy male infants with birth weights of 2.9 kg and 3.2 kg in July 2015 (Figure 6).

In conclusion, we report the case of a newborn with fatal ARPKD whose *PKHD1* gene study revealed a compound heterozygous c.274C>T(p.Arg92Trp) mutation and a novel heterozygous nonsense mutation of c.2770C>T(p.Gln924\*).

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