Kidney Medicine

Primary Cilia Elongation in Early-Onset Polycystic Kidney Disease with 2 Hypomorphic *PKD1* Alleles: A Case Report

Yohei Taniguchi, Kenichiro Miura, Yoko Shira, Takuya Fujimaru, Eisei Sohara, Yutaka Yamaguchi, and Motoshi Hattori

Recent studies have described several children with very early-onset polycystic kidney disease (PKD) that mimicked autosomal recessive polycystic kidney disease because of 2 hypomorphic *PKD1* gene variants. However, no reports have described pathological changes in the primary cilia in these cases. We analyzed the primary cilia in the kidney tubules of an early elementary school child who had very early-onset PKD and a history of large, echogenic kidneys in utero. There was no family history of autosomal dominant PKD. The patient developed kidney failure and received a living-donor kidney transplant from his father. Genetic analysis revealed compound heterozygous variants in the *PKD1* gene: c.3876C>A (p. Phe1292Leu) and c.5957C>T (p. Thr1986Met). These variants were likely pathogenic based on in silico analysis. The absence of kidney cysts in the parents suggested that these variants were hypomorphic alleles. Pathological examination of the patient's excised kidney showed prominent dilatation of the proximal and distal tubules. Immunofluorescence staining for α-tubulin showed pronounced elongation of the primary cilia. These findings suggest that the hypomorphic *PKD1* variants expressed in this patient with very early-onset PKD were pathogenic.

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INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD) and autosomal recessive polycystic kidney disease (ARPKD) are ciliopathies caused by molecular defects in the primary cilia.[1] Although ADPKD is frequently asymptomatic until middle age, there are a wide variety of phenotypes, ranging from severe neonatal disease to adequate kidney function into old age.[2] There are several reports of children diagnosed in utero with severe polycystic kidney disease (PKD) that mimicked ARPKD related to the inheritance of abnormal *PKD1* genes from symptomatic and asymptomatic parents.[3,4] Furthermore, in several of these families with ARPKD-like severe fetal PKD, 2 hypomorphic *PKD1* gene abnormalities were inherited from 2 asymptomatic parents.[4]

Patients with ARPKD typically have cystic dilatation of the collecting ducts,[5] whereas those with ADPKD have dilatation of the entire kidney tubule.[5] In patients with fetal PKD with 2 hypomorphic *PKD1* genes, histological examination showed cysts arising from all areas of the nephron, including the glomerulus, which was consistent with *PKD1* gene abnormalities.[3,4] However, pathological changes in the primary cilia, where the membrane receptor encoded by the *PKD1* gene is expressed, have not been described in these cases.

Here, we describe a patient with severe PKD mimicking ARPKD resulting from 2 hypomorphic *PKD1* gene abnormalities inherited from 2 asymptomatic parents. We also describe morphological changes in the primary cilia of the patient's kidney tubules.

CASE REPORT

The patient was an early elementary school child who had large, echogenic kidneys that were identified in utero by ultrasound imaging. There was no family history of ADPKD (Fig 1A). The child was born by vaginal delivery at 35 weeks. He did not have respiratory distress at birth and did not require resuscitation. His birth weight was 3,300 g (>99.9th percentile), which was large for gestational age because of his enlarged kidneys. After birth, he was clinically diagnosed with ARPKD. A *PKHD1* gene search was performed, but no genetic abnormality was found.

Kidney failure was observed from infancy, and he was treated conservatively. As kidney dysfunction progressed, he was referred to our department for pre-emptive living-donor kidney transplantation. His abdominal computed tomography (CT) scan showed swelling of both kidneys with scattered cysts of different sizes but no liver complications, which was not typical of ARPKD (Fig 1B). The patient received a living-donor kidney transplant from his father, and his right kidney was removed. Abdominal CT of the donor showed no cysts, and allograft biopsy at the time of transplantation showed no cystic dilatation of the tubules. Two years after transplantation, the allograft function was well preserved, and the patient's serum creatinine level was 0.70 mg/dL.

The father (the donor) had normal kidney ultrasound scans at 41 years of age. Contrast-enhanced abdominal CT also showed no cysts in the kidneys. An allograft biopsy at the time of transplantation showed no cystic dilatation of

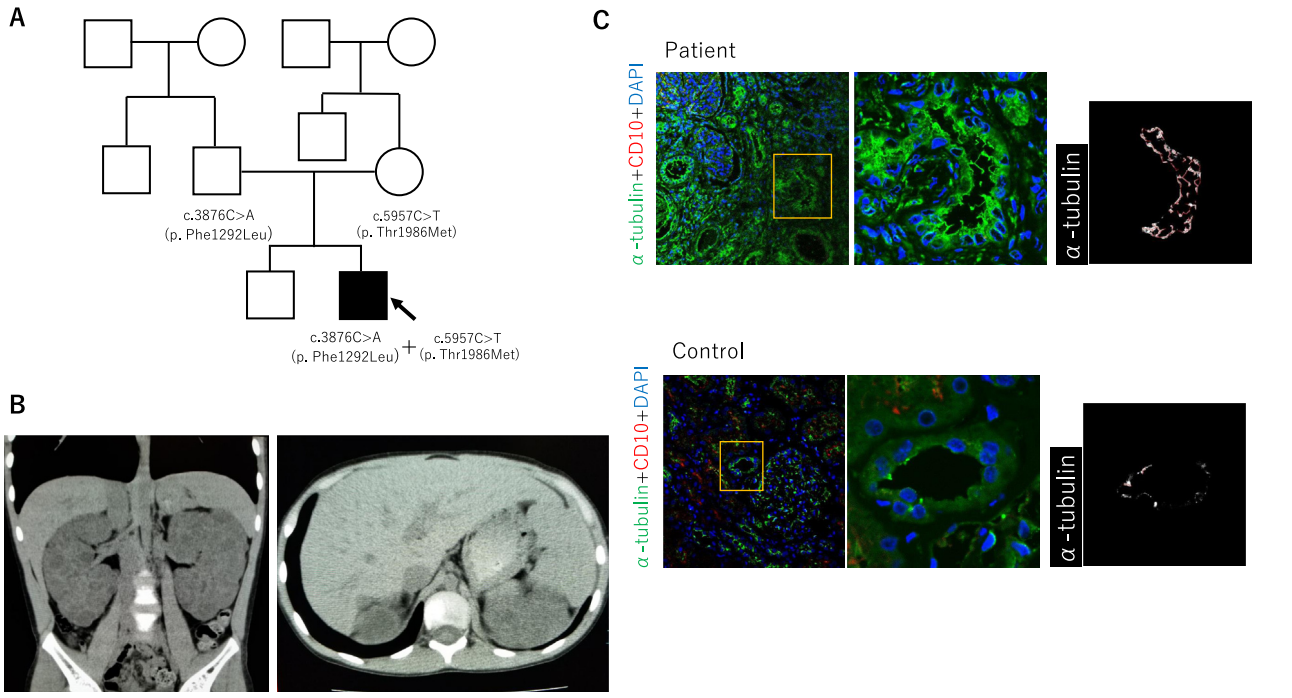

Figure 1. Family tree of the patient. (A) Family members were without kidney cysts. The father and mother each had a heterozygous variant of the *PKD1* gene. (B) The patient's abdominal computed tomography scan. Numerous small cysts were observed in both enlarged kidneys (left panel). There were no signs of hepatic fibrosis or dilated bile ducts in the liver (right panel). (C) Immunostaining for α-tubulin showed that the ciliary length in the distal tubule of the patient's kidney was 24.5 μm/1000 μm² (upper panel), which was considerably greater than the ciliary length in control kidney samples obtained from 3 patients with minimal change nephrotic syndrome (6.3 ± 0.2 μm/1000 μm², lower panel).

the tubules. The mother also reported normal kidney ultrasound scans at 38 years of age (Fig 1).

Genetics

The study protocol was approved by the Ethics Committee of Tokyo Women's Medical University (approval No.360). Panel exome sequencing targeting 70 genes associated with cystic kidney disease revealed compound heterozygous variants in the *PKD1* gene: c.3876C>A (p. Phe1292Leu) and c.5957C>T (p. Thr1986Met). The father had a heterozygous variant c.3876C>A (p. Phe1292Leu) in the *PKD1* gene, and the mother had a heterozygous variant c.5957C>T (p. Thr1986Met) in the *PKD1* gene. These variants have been previously reported in patients with ADPKD,[6,7] and were classified as likely pathogenic based on in silico analysis and the American College of Medical Genetics and Genomics guidelines (Table S1).[8] However, the pathogenicity of these variants has not been established, and the absence of kidney cysts in the parents suggests that these variants may be hypomorphic alleles.

Pathological Analysis

Pathological examination of the excised right kidney showed dilatation of the kidney tubules (Fig S1A). Immunofluorescence (IF) staining for cluster of differentiation 10 and epithelial membrane antigen showed moderate dilatation of the proximal tubules and severe dilatation of the distal tubules, respectively (Figs S1B and C). The IF staining for aquaporin-2 showed slight dilatation in the collecting ducts (Fig S1D).

Next, we examined morphologic changes in the primary cilia in the distal tubule of the excised right kidney using IF staining for α-tubulin.[9] Ciliary length was measured using the ImageJ/Fiji ridge detection plugin as previously described (Item S1).[10] As shown in Fig S1C and Fig S2, the ciliary length in the distal tubules of the excised kidney was 24.5 μm/1000 μm², which was considerably greater than the ciliary length in the control samples obtained from 3 patients with minimal change nephrotic syndrome (6.3 ± 0.2 μm/1000 μm²).

DISCUSSION

There have been several reports of children with very early-onset PKD who have inherited the compound heterozygous *PKD1* gene variants from asymptomatic parents with normal ultrasound findings.[3,4] Affected children died in utero or required intensive neonatal care for severe respiratory failure.[4] One fetus had glomerular cysts and dilatation of all areas in the nephrons, consistent with fetal ADPKD kidneys.[5] There was no collecting duct dilatation, which is usually observed in ARPKD.[5] These findings suggested that 2 hypomorphic *PKD1* alleles caused very early-onset severe PKD. Our patient, who had

asymptomatic parents, had symptoms mimicking ARPKD, including enlarged echogenic kidneys in utero and kidney failure in infancy. Histologic analysis showed moderate dilatation of the proximal tubules and severe dilatation of the distal tubules, but no collecting duct dilatation. This pathology was consistent with ADPKD rather than ARPKD. In addition, genetic analysis revealed compound heterozygous variants that were likely pathogenic based on in silico analysis and American College of Medical Genetics and Genomics standards and guidelines.[8] These findings suggest that 2 hypomorphic alleles caused very early-onset severe PKD in our patient, as previously described.[3,4] It is not uncommon for young adults with no apparent kidney cysts detected by ultrasound to develop ADPKD later in life. However, Pei et al[11] reported that the negative predictive value of ADPKD was 98.3% for those aged 30-39 years who had no kidney cyst detected on ultrasound and 100% for those aged 40-59 years who had no kidney cyst. Therefore, it is unlikely that the parents, who are ∼40 years of age, develop ADPKD later in life.

In this study, we observed a remarkable elongation of primary cilia in the patient's kidney tubules. Previous studies have analyzed ciliary length in transgenic animals. In the PCK rat, a rodent model of ARPKD, cystic cholangiocytes were reported to have short, malformed cilia that did not express fibrocystin, the protein expressed by the *Pkhd1* gene.[12] In a mouse model of maturity-onset diabetes of the young type 5, which is characterized by kidney cysts along with diabetes, there was a dramatic decrease in ciliary length in ductal cells.[13] By contrast, knockin mice with 2 hypomorphic *Pkd1* alleles, which led to reduced polycystin (PC)-1 function, caused gradual cystogenesis and primary ciliary elongation, while *Pkd1* heterozygous knockout mice were normal.[14] In addition, elongation of the kidney primary cilia has also been described in patients with ADPKD.[15,16] Therefore, elongation of the kidney primary cilia may be a consistent finding in all phenotypes of ADPKD. Furthermore, cilia were also elongated in kidneys from both *Pkd1* and *Pkd2* conditional knockout mice, and prominent, early, and sustained β-catenin activation was responsible for ciliary elongation and cystogenesis.[16] It has been shown that inhibition of PC-1 and PC-2 reduces Ca^{2+} uptake, which induces ciliary elongation by activation of cyclic AMP-dependent protein kinase A.[12,15] Together, these findings suggest that the *PKD1* variants in our patient with very early-onset PKD and remarkable elongation of primary cilia were pathogenic.

In conclusion, we described a case with 2 hypomorphic *PKD1* gene abnormalities. Pathological examination showed a remarkable elongation of the primary cilia, providing evidence that the *PKD1* gene variants identified in this patient may be pathogenic.

SUPPLEMENTARY MATERIALS

Supplementary File (PDF)

Figure S1: Pathological examinations of the patient's kidney.

Figure S2: Confocal images of immunostaining for α-tubulin of the distal tubules.

Item S1: Supplementary methods.

Table S1: PKD1 Gene Variants Identified in the Patient.

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